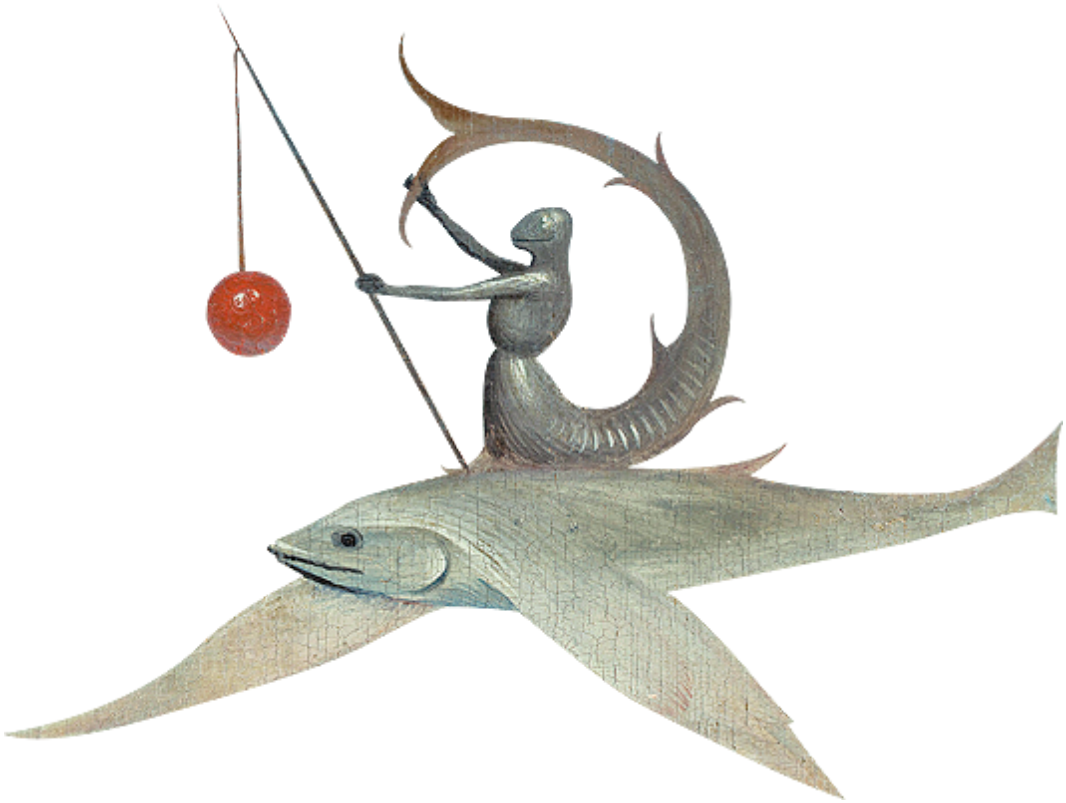
TESIS DOCTORAL DOCTORAL THESIS

REGISTROS DE PACIENTES EN ENFERMEDADES RARAS: IDENTIFICACIÓN DE PROBLEMAS ESPECÍFICOS Y SOLUCIONES POTENCIALES PARA MEJORAR LA CALIDAD DE LOS DATOS Y RESULTADOS

PATIENT REGISTRIES IN RARE DISEASES: IDENTIFYING THE SPECIFIC ISSUES AND POTENTIAL SOLUTIONS TO IMPROVE QUALITY DATA AND OUTCOMES



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Abbreviations

AE: Adverse Events

AESI: Adverse Events of Special Interest

AHRQ: Agency for Healthcare Research and Quality

ATC: Anatomic Therapeutic Classification

CTH: Clinical Trial Head

eCRF: Electronic Case Report Form

EBMT: European Society for Blood & Marrow Transplantation

ECFSPR: European Cystic Fibrosis Society Patient Registry

EDQM: European Directorate for the Quality of Medicines

EMA: European Medicines Agency

ENCePP: European Network of Centers for Pharmacoepidemiology and Pharmacovigilance

EPIRARE: European Platform for Rare Disease Registries

EU: European Union

EU PAS: The European Union electronic Register of Post-Authorization Studies

EUCERD: European Union Committee of Experts on Rare Diseases

EURD: European Union reference dates

FSH: Follicle Stimulating Hormone

GABA: Gamma Amino Butyric Acid

GDPR: General Data Protection Regulation

GVP: Good Pharmacovigilance Practice

HCP: Health Care Provider

HGVS: Human Genome Variation Society

ICD: International Classification of Diseases

ICH: International Council for Harmonization

ICF: International Classification of Functioning and Disability

KOL: Key Opinion Leaders

LAM: Lymphangioleiomyomatosis

LH: Luteinizing Hormone

MAA: Marketing Authorization Application

MAH: Marketing Authorization Holder

MedDRA: Medical Dictionary for Regulatory Activities

mTOR: mammalian Target of Rapamycin

OMIM: Online Mendelian Inheritance in Man

PAES: Post-Authorization Efficacy Study

PASS: Post-Authorization Safety Study

PI: Principal Investigator

QoL: Quality of Life

rAML: Renal Angiomyolipoma

RCT: Randomized Controlled Trial

RD: Rare Diseases

RPs: Research Projects

RWD: Real World Data

SAB: Scientific Advisory Board

SAE: Serious Adverse Event

SAP: Statistical Analysis Plan

SEGA: Subependymal Giant Cell Astrocytoma

TAND: TSC-Associated Neuropsychiatric Disorders

TOSCA: TuberOus SClerosis registry to increase disease Awareness

TSC: Tuberous Sclerosis Complex

UCUM: Unified Code for Units of Measure

UMLS: Unified Medical Language System

WC: Working Committee

WHO: World Health Organization

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Abstract

Patient registries have been recognized as an invaluable tool to gather real world data about different aspects of disease as well as the treatments. Furthermore, regulators are also being inclined towards data from patient registries as a measure of post-marketing effectiveness/safety of therapies approved. The significance of patient registries is immeasurable in rare diseases, because they offer invaluable help in understanding natural history of the disease, which is otherwise difficult, owing to the complexity of these diseases and heavily scattered geographic distribution of the patients. By gathering information of patients at one database, not only the disease manifestations can be observed in detail but also the treatment trends and economic burden.

Various efforts have been initiated to promote the patient registries in rare diseases. More recently, the EMA issued discussion paper on methodological and operational aspects of disease registries to standardize the data collection in patient registries. This paper discusses various aspects of planning and operations of a patient registry and specific measures that should be taken to ensure adequate quality of registry data, while harmonizing the registry data across all registries in same disease area. However, it is to be realized that even the EMA guidance is not specific to rare disease patient registries. Hence, many practical issues in rare disease patient registries are still unaddressed.

This thesis was conducted with the objective to identify the unmet needs in the guidance on rare disease patient registries and offer potential solutions to these issues, to help to improve the quality and sustainability in the future registries in rare diseases. Gathering a first hand experience while leading one of the largest rare disease registry, from

inception to publication, has offered intensive understanding of rare diseases registries, its specific concerns and related measures to be taken to avoid these issues.

Below are the studies published under this thesis:

1. Marques R, et al. Treatment patterns and use of resources in patients with Tuberous Sclerosis Complex: insights from the TOSCA registry. Frontiers Neurol. (2019) 10:1144. DOI: 10.3389/fneur.2019.01144.
2. Marques R, *et al.* The TOSCA Registry for Tuberous Sclerosis- Lessons learnt for future registry development in rare and complex diseases. *Frontiers Neurol*. (2019) 10:1182. DOI 10.3389/fneur.2019.01182.
3. Marques R, *et al.* TuberOus SClerosis registry to increase disease Awareness: A review on alignment of its planning, execution and publications with EMA guidelines. *Frontiers Neurol.* (2020). 11:365. DOI 10.3389/fneur.2020.00365

The results and observations recorded as a part of these publications strengthen the surmounting evidence on the significance of patient registries in rare diseases. However, these observations also highlight the unique issues that are encountered in rare disease registries and the impact of such issues on the quality of data gathered and the consequent registry outcomes. Hence, the need for guidance specific to rare disease registry is further emphasized.

This thesis research has fulfilled its primary objective identifying from an insider view the issues that emerged during the designing, development, analysis and interpretation of the results in TOSCA, one of the most complete rare disease registry (which had a core registry, PASS and research projects), determining learnings and areas for improvement.

Understanding the potential issues and steps to avoid them may help improve quality, usefulness and sustainability of future rare disease registries.

Resumen

Los registros de pacientes han sido reconocidos como una herramienta inestimable para recopilar datos, dentro de la práctica clínica habitual, sobre los diferentes aspectos de una enfermedad así como de sus tratamientos. Además, los organismos reguladores también se están inclinando cada vez más por el uso de datos obtenidos en registros de pacientes para medir la efectividad/seguridad tras la comercialización de terapias aprobadas. La importancia de los registros de pacientes en enfermedades raras es inconmensurable porque nos ofrecen una ayuda inestimable para comprender la historia natural de estas enfermedades, que de otro modo sería difícil debido a su complejidad y la muy dispersa distribución geográfica de los pacientes. Además, al reunir información de pacientes en una base de datos, estos registros nos pueden permitir no solo observar en detalle manifestaciones y características clínicas, sino también las tendencias de los tratamientos en el tiempo y la carga económica asociada a la enfermedad.

Numerosos esfuerzos se han realizado para promover registros de pacientes en enfermedades raras. Recientemente, la EMA ha emitido un documento sobre aspectos metodológicos y operativos de los registros de enfermedades con el fin de estandarizar la recopilación de datos en los registros de pacientes. Este documento analiza importantes aspectos, como son la planificación, los procesos operativos y las medidas específicas que deben tomarse para garantizar una calidad adecuada de los datos, a la vez que propone armonizar la información incluida en todos los registros de un mismo tipo de enfermedad. Sin embargo, debe tenerse en cuenta que esta guía creada por la EMA no es específica para los registros de pacientes de enfermedades raras. Por lo

tanto, muchos de los problemas que pueden aparecer en los registros de pacientes de enfermedades raras aún no se abordan.

Esta tesis ha sido realizada con el objetivo de identificar las necesidades no cubiertas en las guías sobre registros de pacientes y ofrecer posibles soluciones a estas cuestiones, para ayudar a mejorar la calidad y la sostenibilidad en futuros registros de enfermedades raras. La experiencia adquirida en primera persona al liderar uno de los mayores registros de enfermedades raras, desde su concepción hasta la publicación de los resultados, ha permitido una comprensión profunda de este tipo de registros de pacientes, sus problemas específicos y las medidas pertinentes que deben tomarse para evitarlos.

A continuación, se incluyen los estudios publicados bajo esta tesis:

1. Marques R, *et al*. Treatment patterns and use of resources in patients with Tuberous Sclerosis Complex: insights from the TOSCA registry. *Frontiers Neurol*. (2019) 10:1144. DOI: 10.3389/fneur.2019.01144.

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Los resultados y observaciones registrados como parte de estas publicaciones refuerzan la creciente evidencia de la importancia de los registros de pacientes en enfermedades raras. Pero, además, estas observaciones también resaltan los problemas únicos que se encuentran en los registros de estas enfermedades y el impacto de dichos problemas en la calidad de los datos recopilados y los consecuentes resultados del registro. Por lo tanto, se enfatiza aún más la necesidad de una orientación específica para este tipo de registros.

La investigación llevada a cabo en esta tesis ha cumplido su objetivo principal identificando desde una perspectiva intérnalos problemas aparecidos durante el diseño, desarrollo, análisis e interpretación de los resultados de TOSCA, uno de los mayores y más completos registros de pacientes de enfermedades raras (que contiene una parte central, subestudios y estudio de seguridad post autorización), determinando áreas de mejora y aprendizajes. Comprender los posibles problemas y los pasos para evitarlos puede ayudar a mejorar la calidad, la utilidad y la sostenibilidad de los futuros registros de enfermedades raras.

Section 1. Introduction

1.1 Patient Registries and their significance

The term '*registry'* refers to the act of recording or registering as well as the record or entry itself. Hence, 'registries 'may refer to both, the programs that collect and store data as well as the records henceforth created.(1)

According to the National Committee on Vital and Health Statistics, the term 'registries' in public health and medicine is described as "an organized system for the collection, storage, retrieval, analysis, and dissemination of information on individual persons who have either a particular disease, a condition (e.g., a risk factor) that predisposes [them] to the occurrence of a health-related event, or prior exposure to substances (or circumstances) known or suspected to cause adverse health effects."(2)

Registries may also be referred with other names such as clinical registries, clinical data registries, disease registries, and outcomes registries.(1)

In patient registries, the data are collected in an observational manner, meaning that the medical professional/treating physician/healthcare provider (HCP) determines the management of patients and registry protocol does not interfere with such treatment. A patient registry is generally designed to fulfiller-specified purposes and the extent of data collection and analysis is also defined beforehand. Hence, the data collection is purpose-driven and not vice-versa. Furthermore, the data elements in a registry are clearly defined and consistent across all patients. The data collection in a registry is uniform for all patients, with regards to both, the types of data and the frequency of their collection.(1)

In general, registries capture data that is recorded for clinical reference by the treating HCPs, such as history, examination, laboratory test, or patient-reported data. However, at least one data element is collected actively, i.e. some data are collected specifically for the purpose of the registry and not indirectly taken from the pre-recorded sources (such as administrative, billing, pharmacy databases, etc.). While the data collected in patient registries is used further in studies committed to address the pre-specified purpose of the registry, additional data may also be collected when some flexible studies are later incorporated into the registry.(1)

1.1.1 Purpose of patient registries

A patient registry can be a powerful tool to observe the course of disease and its manifestations; to map the treatment patterns and outcomes; to identify factors that impact prognosis and quality of life; to describe patient care pattern; to assess treatment/intervention effectiveness and safety; and to evaluate quality of care. Overall, the purposes of patient registries can be broadly described in terms of patient outcomes, as discussed below.

- *Describing Natural History of Disease*

  Patient registries help to evaluate the natural history of a disease, which includes its characteristics, management, and clinical outcomes with and/or without treatment. Natural history of a particular disease may vary according to race, region, patient subgroups, and over time. Also, natural history for some diseases is relatively less explored. Furthermore, some interventions/treatments may aid in altering the natural history of a disease.(1) For example, in patients with lysosomal storage diseases, where survival in to 20's was reported earlier, are now surviving to fourth and fifth

decades of their lives. These changes in natural history have been demonstrated in a patient registry.(3)

- *Determining Effectiveness*

Since it has been an established fact that the results of clinical trials do not necessarily reflect the real-world conditions, registries are now being increasingly utilized to evaluate the real world situation. Clinical trials often include well-defined patient groups or subgroups and their efficacy data may not be generalized to other populations or subgroups of interest. For instance, several heart failure trials had included predominantly white male patient sample, with a mean age of approximately 60 years. However, in real world practice, heart failure patients are often older, more diverse, and have a higher mortality rate than the clinical trial sample population. Hence, registry derived data have been used to fill these gaps for the decision makers. Data from registries may also be further utilized to determine effectiveness outcomes for a longer time period than clinical trials. As an example, registries for effects of growth hormone have recorded data for children until they reach adulthood and further into adulthood as well. Apart from clinical effectiveness, cost effectiveness is another important aspect that can be analyzed from registry derived data.(1)

- *Measuring or Monitoring Safety and Harm*

Patient registries can be designed to act as an active surveillance system for safety monitoring. This means that registries can be prepared to record the occurrence of unexpected or harmful events of various treatments or interventions. These events

may vary from patient-reported minor side effects to severe adverse events such as fatal drug reactions.

In the current active surveillance of drug adverse reactions, once drug is in clinical practice, the data is based on recognition of adverse events and then its active reporting to the system. The incidence rates of adverse effects cannot be determined with this system as the denominator, i.e. the total number of patients exposed is unknown.

Hence, patient registries, by a systematic data collection, offer the advantage for better active surveillance.(1)

- *Measuring Quality*

Registries may be designed to determine the quality of care and compare the different healthcare services/patient populations with the gold standards or comparative benchmarks of care. These registries may assist in identifying disparities in access to care, and determine the potential for improvements.(1)

- *Multiple Purposes*

Many registries are developed with more than one purpose. Registries, after initiation, may also be expanded to add additional objectives. While registries may have additional purposes, their design is nevertheless based on the original or primary purpose. This means that drawing conclusions for secondary purposes should be done cautiously.(1)

Hence, studies based on properly designed and implemented patient registries can provide a real-world data regarding clinical practice, patient outcomes, safety, and clinical,

comparative, and cost-effectiveness, and can thus assist in development of evidence and decision-making purposes.

1.2 Recent guidelines for patient registries: EMA Discussion Paper

In order to generate standardized and reliable data for benefit-risk evaluation of medicines from patient registries for regulatory purposes, EMA supports a systematic and standardized approach to planning and execution of all patient registries. Hence, in 2015, the EMA established the Patient Registry Initiative and the Cross-Committee Task Force on registries to identify the barriers and establish good registry practices. Consequently, in November 2018, the EMA issued a draft discussion paper on methodological and operational aspects of disease registries and made proposals on registry studies and good registry practice.(4) In this thesis, we refer to the EMA discussion paper on methodological and operational aspects of disease registries as “EMA guidance”.

The objective of the EMA guidance paper is to discuss methodological and operational aspects of patient disease registries. It aims to provide guidance to plan and implement patient registries, so as to support collection of high quality registry data, acceptable for regulatory requirements.

The EMA guidance is a reflection of recommendations based on multiple workshops and resources, including the EMA Patient Registries Workshop, the four disease-specific workshops on registries for cystic fibrosis, multiple sclerosis, CAR-T cell products and haemophilia, the Qualification opinion on the European Cystic Fibrosis Society Patient Registry (ECFSPR), the Draft qualification opinion on the Cellular therapy module of the European Society for Blood & Marrow Transplantation (EBMT) Registry, and existing

guidance published in the PARENT Joint Action Methodological Guidance and the US Agency for Healthcare Research and Quality (AHRQ)'s handbook. It is also aligned with the recommendations from the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology and the ENCePP Code of Conduct.

The EMA guidance elaborates on multiple aspects of planning and execution of patient registries. It is expected to become the gold standard for registry guidance across all patient registries including those covering small populations, pediatric indications and rare diseases. While many specific aspects of EMA guidance are presented as a part of the third publication summarized in this document, the general recommendations of this guidance are discussed below.

1.2.1 Core Concepts

The basic principles and concepts are reiterated to avoid any misunderstandings with regards to the basic terminologies and definitions, including patient registry, incident patients, prevalent patients, registry participant, registry coordinator and population registry.

This paper defines a patient registry as an "organized system that uses observational methods to collect uniform data on a patient population defined by a particular disease, exposure or condition (e.g. age, pregnancy, specific patient characteristics)", and which is followed over time. Hence, by collecting data in patients and following them over time, a registry inherently creates a cohort of patients that may be secondarily observed to find answers to other research questions using different study designs. From regulators

perspective, patient disease registries gather better insights on clinical outcomes in patients receiving different treatments than product registries, and may support a wider range of study designs. Hence, the EMA guidance is primarily applicable to disease registries, though many considerations may also be applicable to product registries.

The EMA guidance provides an emphasis on a clear distinction between a registry and a registry-based study (a *registry study*), as summarized in Table 1, and provides separate set of guidance for both.

Table 1. Important differences between a registry and a registry study.

	Registry	**Registry study**
Nature	Data collection system	Investigation of a research question or hypothesis
Timelines	Long-term, open-ended	Defined by the study objective and described in the study protocol
Patient enrolment	Exhaustive within the boundaries of the purpose of the registry (e.g. all patients diagnosed with a disease in a hospital, region or country)	Defined by research objective and described in the study protocol It may be a subset of the registry population.
Data collection	Wide range of data may be collected depending on the purpose of the registry	Restricted to what is needed by the research question including data on potential confounders and effect modifiers; Additional data collection may be required.
Analysis plan	Routine periodical data analysis; additional ad-hoc analyses	Statistical analysis plan separate from the study protocol
Collection and reporting of suspected adverse reactions	National requirements as regards the management of safety data apply. Any active data collection with involvement of a MAH must follow the regulatory framework for PASS.	National requirements may apply. Regulatory requirements to MAHs differ between studies with primary or secondary data collection.
Data quality control	Applied routinely to all data and processes	Additional quality assurance may be needed.
Regulatory status	Non-interventional	Non-interventional or interventional

Source: European Medicines Agency. Discussion paper: Use of patient disease registries for regulatory purposes-methodological and operational considerations. EMA/763513/2018. (2018)

1.2.2 Guidance for patient disease registries

- *Patient population*

  The EMA guidance recommends ensuring exhaustive enrolment of patients. A clear and concise definition of patient population and precise defined inclusion and exclusion criteria are recommended. Registries are inherently prone to selection bias, and numerous factors influence patient enrolment, which are unfortunately difficult to identify in advance and prevent. Despite this, efforts should be done to carefully plan the registry to avoid selection bias as much as possible to increase data validity.

- *Time elements*

  Recording the accurate time elements that are essential components of baseline data is crucial. The guidance proposes a list of core time elements that should generally be collected in all registries. For example, date of birth, date of death, date of first appearance of symptoms, date of first diagnosis, date of definitive diagnosis.

- *Core data elements*

  The guidance recommends a list of core data elements that should normally be collected in all patients. It also recommends to use a harmonized or mapped list of data elements across registries for a same disease condition to support regulatory evaluations and facilitate implementation of a common data quality system, data exchange, common data analysis and interpretation of results from different registries.

- *Terminologies*

  EMA guidance paper suggests using common terminologies across registries for diseases, diagnostic tests, symptoms, medicinal products, active substances, adverse events and other relevant data. Table 2 provides a list of source of terminologies for different data elements. The local or national terminologies, whenever used, should be mapped to international terminologies.

Table 2. Examples of recommended international terminologies for disease registries

Data elements	Standard(s)	Weblinks
Diseases, diagnostics, symptoms, indication for use of medicine	ICD-9, ICD-10, ICD-11 ICD-o-3 (cancers) MedDRA[1]	http://www.who.int/classifications/icd/en/ http://codes.iarc.fr/ https://www.meddra.org/
Rare disorders (disease, malformation syndrome, clinical syndrome, morphological or biological anomaly or particular clinical situation in the course of a disorder)	Orphadata (entries are cross-referenced with ICD-10, OMIM, UMLS, MeSH, MedDRA)	http://www.orphadata.org/cgi-bin/inc/product1.inc.php
Medicinal products	Article 57 database (EEA) ISO IDMP standards and related terminologies (forthcoming)	https://www.ema.europa.eu/en https://www.ema.europa.eu/en
AESI, other adverse events, suspected adverse reactions	MedDRA	https://www.meddra.org/
Routes of administration, pharmaceutical dosage forms, packaging, units of administration	EDQM Standard Terms Database	https://www.edqm.eu/en/standard-terms-database
Test results units	Unified Code for Units of Measure (UCUM)	http://unitsofmeasure.org/ucum.html
Tests	MedDRA, ICD-10, ICD-11	https://www.meddra.org/
Genetic diagnosis	International classification of mutations (HGVS)	http://www.hgvs.org/ https://www.genenames.org/
Classification of functioning/disability	International Classification of Functioning and Disability (ICF)	http://www.who.int/classifications/icf/whodasii/en/

Terminologies and formats for individual case safety reports (ICSR) specification	Code Sets and Object Identifiers based on the ICH E2BR(3) ICSR Implementation Guide	http://estri.ich.org/e2br3/index.htm
Source: European Medicines Agency. Discussion paper: Use of patient disease registries for regulatory purposes- methodological and operational considerations. EMA/763513/2018. (2018)		

- *Quality management*:

EMA guidance puts significant emphasis on quality management in order to make the registry data reliable enough for regulatory purposes. Quality management should be a part of all registries and include four activities, namely, quality planning, quality assurance, quality control and quality improvement. Measures of data quality are described in Table 3.

Table 3. Measures of data quality

Measures	Description
Data consistency	Consistency in the formats and definitions of the data entered in the registry over time and across different registries, especially across those of patients with a same disease
Data completeness	Complete information on all eligible patients, and ensure minimal missing data
Data accuracy	Data recorded in registry is verified with patient data from medical charts or records.
Data timeliness	Timely recording and reporting of data based on their intended use and in compliance with an agreed procedure

Measures to improve data quality can be implemented at two levels: management level and operational registry level. At management level, data quality measures can

be put into effect at the level of the registry coordinator (if there is a single registry with several contributing centers) or at the central level of a registry platform (for example in case several national registries collaborate to record data in a similar way for a common purpose). At the operational level, data quality measures can be implemented at a local level. Standard operating procedures and work instructions should be provided at all centers contributing to the registry. Adequate training of data managers and other involved personnel should ensure accurate data entry. Furthermore, automated data quality checks may help identify missing information.

- *Safety analysis*:

  All disease registries should follow the national requirements for the management of safety data and report all suspected adverse reactions through the local pharmacovigilance system. Any MAH-sponsored/managed active data collection system in a disease registry for a particular medicinal product must comply with the regulatory framework for PASS. While disease registries may not help detect new safety signals or their analysis, they may help in characterization of known or suspected adverse reactions. A defined list of adverse events of special interest (AESI) may be integrated in the routine data collection system.

- *Governance*:

  Most registries have an individual governance model, depending upon their design, purpose, operating procedures, regulatory environment or funding sources. The guidance proposed the responsibilities ad roles of different stakeholders in registry governance, in order to strengthen the use of registry data. An effective collaboration between all stakeholders is increasingly emphasized, which include agreement on

principles of data sharing between registries, MAAs/MAHs and regulators. For the principles of data ownership, informed consent and data security, the General Data Protection Regulation (GDPR) should be followed.

1.2.3 Guidance for registry studies

- *Regulatory context*:

  Regulators may request MAH to conduct registry studies as a legal obligation. These generally include post-authorization safety (PASS) and efficacy (PAES) studies, as described in Table 4. For such studies, the responsibility for supervision, monitoring of generated data, analysis of benefit-risk balance and communication of new information lies with the MAH.

Table 4: Major types of Registry studies for regulatory purpose.

Regulatory study	Purpose
Post-authorization efficacy studies (PAES)	to study efficacy/effectiveness in patient sub-groups defined by variables such as age, co-morbidities, use of concomitant medication or genetic factors, and to provide historical control data that could be used for comparative purposes
Post-authorization drug safety studies (PASS)	to collect safety data on adverse events using standardized data collection tools and amplify a safety signal, particularly for rare outcomes, to assess the incidence of important identified and potential risks, to compare the risk of some adverse events between relevant exposure groups or to assess the effectiveness of risk minimization measures. Registries may be particularly valuable when examining the safety of medicinal product used for an orphan disease.

- *Timelines*:

  Early initiation of the discussion of a post-authorization study is recommended and should involve the MAH(s), regulators and registry coordinators. The study proposal should be adequately descriptive for the registries and participating centers to assess whether they can participate in the study in terms of data availability and data quality requirements.

- *Study protocol*:

  The data collection method has to be an early decision. The choices include secondary data collection (i.e. where the data required is already available and can be extracted from a dataset), or primary data collection (where the data required for registry are collected prospectively directly from patients). Whenever PASS has to be included with a registry, the study protocol should be in accordance with the Good Pharmacovigilance practice (GVP) Module VIII and the technical guidance on the format and content of the protocol for non-interventional PASS

  The protocol should provide an estimated sample size and the feasibility to attain this (number of patients as well as duration of follow-up) in the concerned registry using conservative assumptions. Protocol should also provide the milestones and timelines for completing the main study phases.

- *Study population*:

  When data for study is to be extracted from routinely collected registry data, defining study population is as methodologically challenging as in secondary data collection. For registry studies intended to observe safety or effectiveness of a new treatment,

the study population will either include incident patients (i.e. newly diagnosed patients who have received their first treatment with the study drug) or the prevalent patients (i.e. patients already included in the registry to whom the study drug has been recently prescribed). The study population (i.e. incident or prevalent patients) has a significant impact on data analysis and interpretation of the results. Hence, the data collection should be thoroughly specific to be able to distinguish incident patients from prevalent patients, and also able to identify differences in their characteristics. When study/registry is enrolling patients prospectively, efforts should be made to include all eligible patients being treated in the individual centers.

- *Data collection*:

  The responsibility to collect adequate and relevant data for study purpose and also for sensitivity analysis, as well as potential confounders rests with the investigator. Additional data collection may be considered depending upon the legal status of the study.

- *Data quality*:

  The need for additional measures of data quality in a registry study depends on the extent of routine data quality management in the registry. For registry studies intended for regulatory purposes, data quality may be ensured with source data verification and periodic auditing on a minimum of 10% of randomly selected patients may be considered adequate.

- *Data analysis*:

  Statistical method for data analysis should be appropriate and applicable according to the scientific question of interest. The statistical analysis plan (SAP) in protocol should include the method and justification for handling of missing data. It should always be remembered that registry data is observational in nature, in contrast to the data generated in clinical trials, and hence, analysis of registry data should be done cautiously. The EMA guidance offers insights into the commonly encountered methodological problems and how they could be approached.

- *Safety reporting*:

  The recommendations from Module VI of the GVP should be followed, according to the mode of data collection, i.e. primary or secondary.

  For registry studies with primary data collection, the concerned MAH(s) should have a data collection or electronic system to collect, analyze and report information on HCP/patient notified adverse events. Investigators of registry studies should also be informed of the mechanisms allowing them to report at any time to the national spontaneous reporting system any adverse event or suspected adverse reaction occurring during the course of the study.

- *Reporting of study results*:

  For PASS and PAES imposed by regulatory authorities as a legal obligation, the final study report should follow the format for the Guidance for the format and content of the final study report of non-interventional post-authorization safety studies.(5) Hence, the MAHs should be able to comment on the study results and their interpretation as well

as on the format of the report. Regulatory authorities may request additional information or clarifications and the scientific aspects of the requests should be handled by the lead investigator.

The guidance paper recognizes the issue of sustainability of disease registries. Hence, a collaborative approach between all stakeholders is recommended to address adequate funding to maintain resources and infrastructure for long-term follow ups.

1.3 Introduction to rare diseases and need of patient registries in rare diseases

1.3.1 Definition of rare diseases

According to the Orphanet, a rare disease is one which affects less than 5 persons per 10,000population. Currently, between 5,000 and 8,000 rare diseases have been recognized and most of them have a genetic basis. Rare diseases are often serious and chronic, and may even be life-threatening.(6) According to an evidence based estimate on Orphanet database, population prevalence of rare diseases is 3.5-5.9%, resulting in 263-446 million people being affected with rare diseases at any time point.(7) Considering their significant burden, rare diseases are now receiving attention worldwide in terms of clinical research, development and marketing of medicinal products in different disease areas, including the use of various regulatory incentives in both the EU and the USA.(6)

1.3.2 Challenges in the management of rare diseases

The challenges faced in rare diseases are fundamentally different from the commonly encountered diseases. The fragmented knowledge regarding the disease pathology,

natural course and epidemiological data hampers the diagnosis and management of many rare diseases. Clinical development in rare diseases is complicated and cumbersome due to the widely scattered geographic distribution of small number of patients further complicated by the lack of validated biomarkers and surrogate end-points, and limited clinical expertise and expert centers.(8)

Adequate funding for both the fundamental research into the disease, as well as measures to progress the clinical research is crucial. Of equal significance is harmonization of terminologies and rare disease classification on an international level to generate reliable epidemiological data. Since rare disease market remains less attractive for pharmaceutical companies, the funding in clinical trials for orphan drugs is not as enormous as in mainstream disease areas. Hence, the long-term efficacy and safety of many orphan drugs remains uncertain.(8)

1.3.3 Significance of patient registries in rare diseases

Considering the scarcity of relevant knowledge and experience with most rare diseases, along with many practical issues, clinical research in rare diseases require special support, cooperation and infrastructure. Hence, using observational data methods, including prospective long-term patient registries, is crucial to establish a broad and comprehensive knowledge base for rare diseases. Among the important data elements that can be collected in an observational manner include the prevalence and distribution of these rare diseases and key patient, familial, and disease characteristics, including the natural history of the disease.(9)

1.3.4 Challenges in rare disease patient registries

While the basics of rare disease registries are similar to those of patient registries in general, there are several unique challenges. The limited number of patients as well as patient support groups, lack of thorough understanding of disease course and manifestations, lack of treatment guidelines in many rare diseases, as well as lack of clinical expertise, makes the planning and implementation of rare disease registries quite complicated. Added complication is an inherently different range of stakeholders, which directly affects the implementation, governance, funding, communication and as well as their level of interest and willingness to participate in the study of rare diseases.(9)

Since the basics of rare diseases are not well explored in most cases, most rare disease registries need a broad outreach to identify and recruit enough patients to fill these knowledge gaps. Hence, the scope and objectives of rare disease registries are often broader than in a typical disease registry. Further, the registries may evolve overtime in terms of scope, building as new signals for future directions are detected.(9)

Typically, the objectives of rare disease registries may be categorized under following:

- To connect affected patients, families, and clinicians
- To understand the natural history, evolution, risk, and outcomes of specific diseases
- To support research on genetic, molecular, and physiological basis of rare diseases.
- To establish a patient base for evaluating drugs, medical devices, and orphan products.

1.3.5 Rare Disease Registry Stakeholders

Different classes of stakeholders in any registry have their set agendas. The stakeholders involved in rare disease registries and their potential interests have been summarized in Table 5. In general, stakeholders may include patient advocacy groups (often multiple), regulatory agencies (especially for registries targeted for future drug development or approval issues), clinicians, scientists, pharmaceutical industry, payers, and the patients and their families. An effective collaboration between stakeholder groups is thus critical for the success of a registry.

Table 5. Potential registry stakeholders and their roles.

Stakeholder	Role in registry	Motivations for registry involvement
Patients and their families/caregivers	Participants	Increase knowledge about the disease Create community Facilitate development of new treatments.
Patient advocacy groups	Advocates, sponsors	Increase knowledge about the disease; increase access to care Support training and research in disease area Raise profile of the disease to encourage funding for more research.
Clinicians/Investigators	Data contributors	Increase knowledge about the disease; Learn from the registry community Gather data to refine complex or undefined diagnoses Develop and inform treatment guidelines.
Academia	Principal investigators, scientific advisors	Improve understanding of disease; Create data source for research in disease area.
Biopharmaceutical industry	Sponsors, developers	Understand the natural history of the disease to design better clinical trials and evaluate potential relevant clinical endpoints; Fulfill post-marketing commitment; provide patient pool for interventional studies; Determine potential market share and access patients; generate publications.

Government/regulatory agencies/payers	Sponsors, recipients of information	Increase knowledge about the disease Monitor the safety of approved products Evaluate cost-effectiveness and budget impact Evaluate evidence for reimbursement.
Source: Chapter 20. Rare Disease Registries. In: Gliklich R, D. N. (April 2014). Registries for Evaluating Patient Outcomes: A User's Guide. (Third edition). Rockville, MD: Agency for Healthcare Research and Quality		

While the data reliability in registries may not compare with those generated in clinical trials, registry based data may be the sole source of such data for the stakeholders in rare diseases. Data generated from registry may also help the stakeholders design and develop a feasible controlled clinical trial in future research. Pharmaceutical industry hence supports many rare disease patient registries, both disease-based and product-based, as sponsors and developers, particularly when clinical development program is usually brief and includes a small, relatively heterogeneous subpopulation of the disease population.

With the assessment of long-term safety and benefits of treatments, patient registries may help develop a treatment algorithm for these diseases. Hence, regulators have recognized the importance of such registries to also act as a historical comparator data as well as filling the knowledge gaps that cannot be possibly determined in clinical trials in rare diseases.

While the conflicts of interest may arise for different stakeholders, proper collaboration may ensure that the needs of all stakeholders are met. An effective collaboration amongst all stakeholders across the globe may help standardize the data for future use.(9)

1.3.6 Current status of rare disease registries

The significance of rare disease registries has been recognized and underlined by the European Union (EU), through the “EU Council Recommendation of 8 June 2009 on an action in the field of rare diseases”. In the past decade, attempts have been made to provide guidance for rare disease registries, in order to standardize the patient registry setting and implementation. In 2013, the European Union Committee of Experts on Rare Diseases (EUCERD) adopted a set of Recommendations on Rare Disease Patient Registration and Data Collection. These recommendations formalize the consensus reached and guide all stakeholders into systematic discussions on data collection and registration.(10) Furthermore, many international projects, including EPIRARE (11) and RD-CONNECT (12) have been initiated to promote international registries. Similarly, the National Center of Rare Diseases in Italy has also released recommendations for improving the quality of rare diseases registry.(13)

According to the Orphanet statistics for May 2019 (14), there were 753 registries in rare diseases in the European region. Of these 753 registries, only 69 were global registries and other 69 were European registries. More than half, that is 535of the 753 registries, were being conducted on national level, and the remaining 80 were regional registries.

1.3.7 Unmet needs in guidance for rare disease registries

Despite the guidelines offered for patient registries, the ‘rare’ case of rare disease registries still do not have a gold standard of guidelines that address the specific practical issues faced in these registries. While the EMA guidance paper does offer some direction that can be helpful for rare disease registries, some issues are still left unaddressed. One

of the studies conducted as a part of this thesis (15) aims to identify the practical issues with the largest registry conducted in TSC, one of the most complex rare diseases, the details of which are discussed in the forthcoming sections.

1.4 TOSCA- an international rare disease registry

1.4.1 Tuberous sclerosis complex

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder, affecting multiple organs and organ systems. This disorder is characterized by formation of hamartomas in multiple organ systems, resulting in diverse symptoms and severity. TSC is caused by mutations in either of TSC1 or TSC2 genes, which encode proteins hamartin (TSC1gene) and tuberin (TSC2) gene.(16,17)

Epidemiology

TSC affects approximately 1 million people worldwide. The estimated incidence of TSC at birth is 1 in 6000 newborns.(18) There are no reported gender or ethnicity based variations in TSC prevalence and incidence rates.(16)Owing to its wide phenotypic variability, the disease is often difficult to recognize and prevalence may be underestimated.

Clinical manifestations

As explained earlier, TSC is a multi-organ disease and location and extent of lesions determine the type and severity of symptoms.(19) TSC manifestations can appear at any stage in life. (Figure 1)

- Cardiac rhabdomyomas (33%-48%) affect patients from prenatal stage to childhood.(20-22)
- Neurological disorders such as epilepsy (90%), cognitive impairment (50%) and brain tumors such as SEGA (20%)also generally appear from prenatal stage to childhood.(20-23)
- Dermatological manifestations affect 90% of patients and can appear from birth to adulthood.(20-22)
- Retinal hamartomas (40%-50%) affect children up to 5 years of age.(20-22)
- Liver or renal angiomyolipomas (80%) appear from 5 years of age to adulthood.(21,23)

- Lymphangioleiomyomatosos (LAM) affects 40% of women from late childhood, adolescents to adults.(20,21,23)

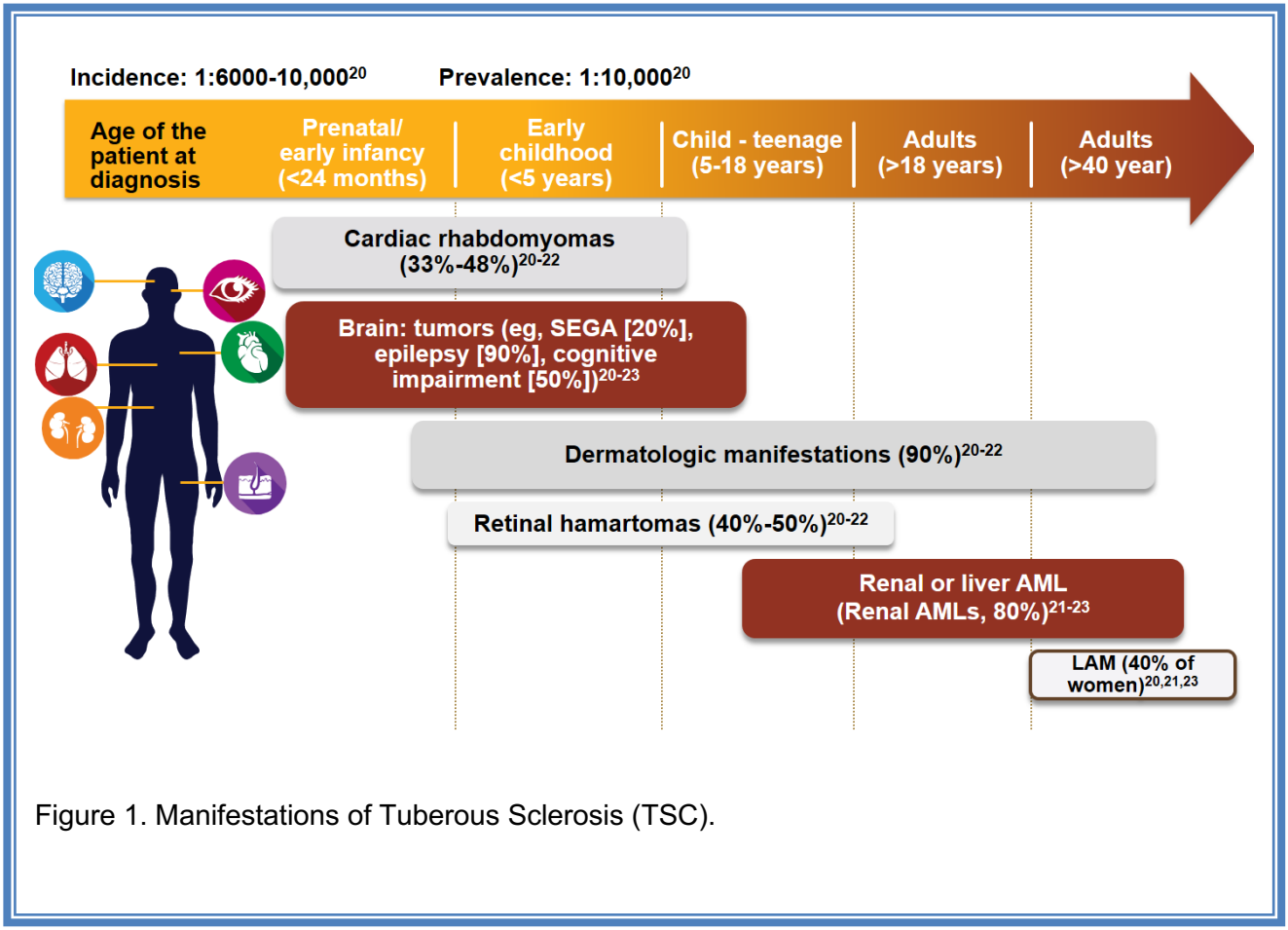

Figure 1. Manifestations of Tuberous Sclerosis (TSC).

The leading causes of morbidity and mortality in patients with TCS are the tumors of the central nervous system, followed by renal disease.(19)

Considering the wide range of clinical manifestations and significant morbidity, a deeper understanding of real life scenario for TSC is required. As generating data from clinical trials in rare diseases like TSC is impractical, patient registries provide an opportunity to fulfill the knowledge gaps and understand the unmet need is this area.

1.4.2 TOSCA: The largest patient registry in TSC

In 2011, Novartis collaborated with medical experts and patient advocates to evaluate the need for a TSC registry to improve our understanding about this condition. Online questionnaire based surveys were conducted in January and February 2011, among experts and organizations across Europe that were involved in TSC management. The survey results along with round table discussions indicated a lack of national TSC registries in many European countries. Hence, a strong need for large scale collaboration, rather than smaller studies was realized. A clear consensus regarding the need to establish a TSC registry led to the idea of TuberOus SClerosis registry to increase disease Awareness (TOSCA). While the registry was initially planned for Europe, later,

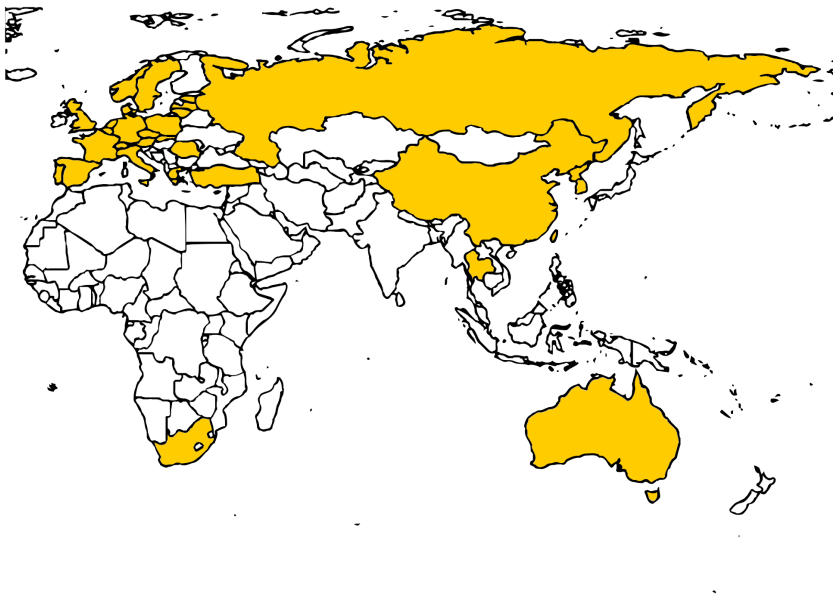

Figure 2. Countries involved in TOSCA.

Europe: Austria, Belgium, Czech Republic, Denmark, Estonia, France, Germany, Greece, Italy, Latvia, Lithuania, Netherlands, Norway, Poland, Portugal, Romania, Slovakia, Slovenia, Spain, Sweden, United Kingdom; **Australasia:**Australia; **Asia:**China, Israel, Korea, Taiwan, Thailand, Turkey; **Eurasia:**Russia; **Africa:**South Africa

some non-European countries also joined the registry, making it a truly international registry (Figure 2). (24)

Methods

TOSCA registry design

TOSCA was a multicentre, international disease registry, which gathered data regarding

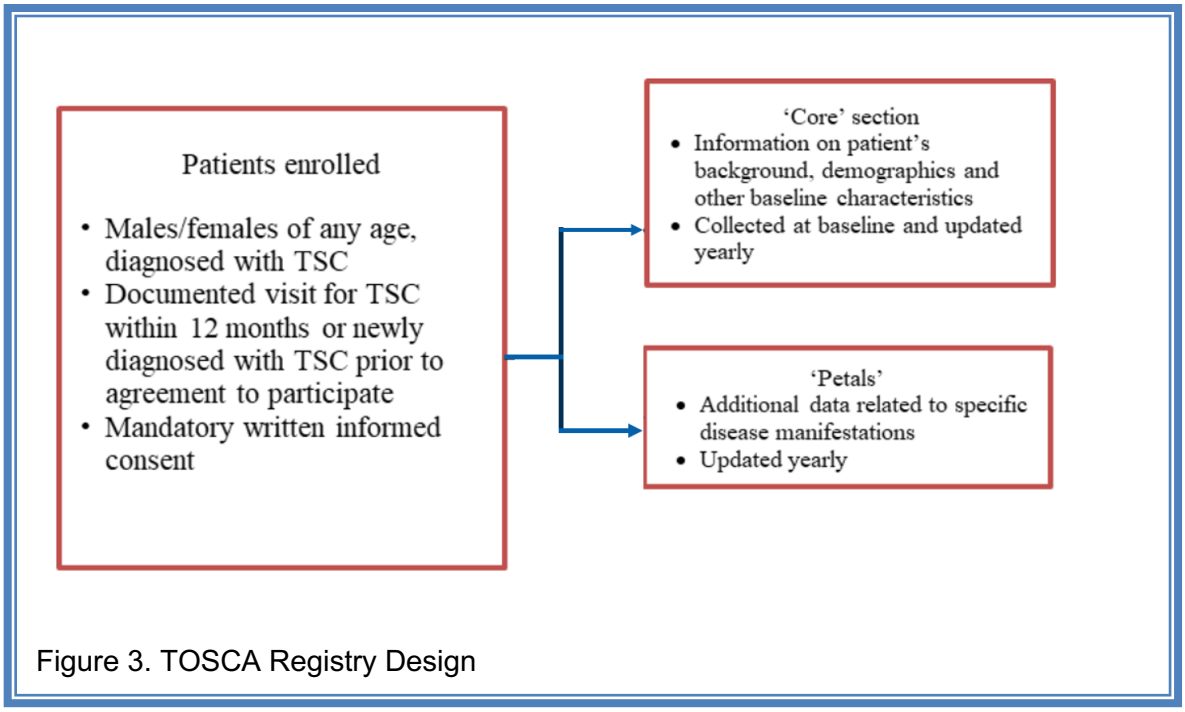

Figure 3. TOSCA Registry Design

clinical manifestations, interventions and clinical outcomes in patients with TSC (Figure3). The registry had been structured retrospectively and prospectively to collect patient and disease information.

TOSCA followed a core and petal form of data collection system. This means that the data was collected in two pre-specified sections, i.e., the main, 'core' section and subsections (also referred to as 'petals') (Figure 4).

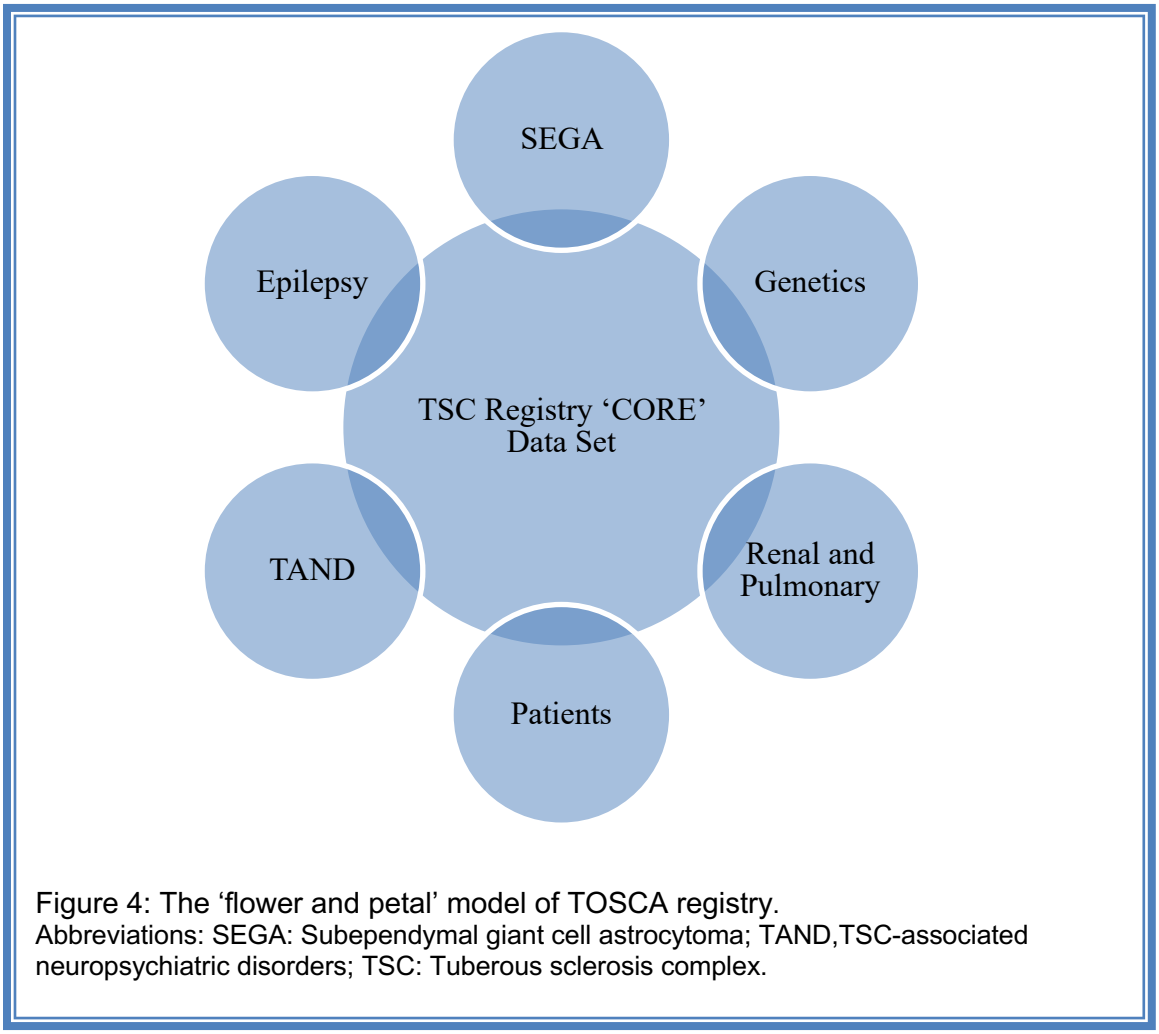

Figure 4: The 'flower and petal' model of TOSCA registry.
Abbreviations: SEGA: Subependymal giant cell astrocytoma; TAND,TSC-associated neuropsychiatric disorders; TSC: Tuberous sclerosis complex.

- ***Core section:*** In this mandatory data section for all patients, data related to a general predefined set of patient background, including demographics, family history, prenatal history, and disease features (i.e., neurological, neuropsychiatric, renal, cardiovascular, pulmonary) was collected.
- ***Petals section:*** Additional and more detailed data related to specific disease manifestations, respective to individual research projects was collected.

Baseline data was collected at the time of enrolment in the registry for all patients. Later, an annual update for all data in core section and subsections, as appropriate, were done. For data collection, hospital discharge files, clinical records, clinic visits, electronic medical records, patients' questionnaires, and ad hoc clinical databases were utilized. For the prospective follow-up visits, standard practice of the site and the treating physician's judgment were followed. All the collected data were recorded on an electronic case report form (eCRF), accessed via a secure web portal hosted by a contract research organization.

It is to be noted that since TOSCA was not designed as a population-based epidemiological registry, the outcomes do not represent incidence and prevalence data of TSC or its manifestations.

In addition to the above mentioned core and research projects, another sub-study was also undertaken, namely, the TOSCA Post-Authorization Safety Study (PASS). This drug safety study, requested by the EMA, was accommodated to obtain data to assess the long-term safety and tolerability profile of everolimus (Votubia®) in approved indications for the treatment of patients with TSC residing in the European Union.

Eligibility criteria

In TOSCA registry, patient of any age, with a diagnosis of TSC were enrolled. Each patient must have had a documented visit for TSC within the preceding 12 months, or must be newly diagnosed with TSC prior to agreement to participate in the registry. The patients enrolled in TOSCA PASS were participants from TOSCA, who were being treated with everolimus (Votubia®) for an approved TSC indication in the European Union. Informed consent form was signed before enrolment by all patients (parents or guardians, where applicable).

Study duration

The first patient was entered in the registry in August 2012 and patient enrolment was completed by 10 August 2014. Further, the baseline data was locked on 30 September 2014. The patients were followed-up for five years. The overall data was locked in August 2017. For pediatric patients enrolled in TOSCA PASS, the follow-up period is going to be extended until they reach Tanner stage V if evaluated per local routine practice, or until the age of 16 years for females and 17 years for males, regardless of whether the therapy has ended. This is being done to gather data for long-term safety and on the impact of everolimus on sexual maturation and potential fertility.

Objectives and main variables

The main objectives of TOSCA and TOSCA PASS study are summarized in Tables 6 and 7. The aim of TOSCA was to understand the course of TSC manifestations and their impact on clinical outcomes.

Table 6: Objectives and Variables in TOSCA

Objectives	Variables
To map the course of TSC manifestations and their prognostic roles	• Proportion of patients with each TSC manifestation (e.g., SEGA, angiomyolipoma, lymphangioleiomyomatosis), its complications and overall survival
To identify patients with rare symptoms and comorbidities	• Incidence and prevalence of rare symptoms and comorbidities
To record interventions and their outcomes	• Frequency of interventions by type, by sequence and by role of the treating physician, and of physician specialty and referral to site of excellence • Outcome of manifestations by type of intervention • Frequency and type of follow-up visits, imaging/tests, hospitalization, emergency room visits and surgical procedures
To contribute to creating an evidence base for disease assessment and therapy and inform research on TSC	• Identification of scientific hypotheses to be tested in preclinical and/or clinical investigations; promote observational and experimental prospective studies on specific groups of patients
To measure quality of life in patients with TSC	• Validated questionnaires on quality of life
To collect information on sexual maturation/endocrine assessments in patients with TSC, if available	• Endocrine assessments (e.g., FSH, LH, Inhibin B, estradiol, testosterone, progesterone)

Abbreviations: FSH follicle stimulating hormone, LH luteinising hormone, SEGA subependymal giant cell astrocytoma, TOSCA TuberOus SClerosis registry to increase disease Awareness, TSC, tuberous sclerosis complex.

Table 7: TOSCA PASS objectives and main variables

Objectives	Variables
To document the long-term safety and tolerability profile of everolimus in the treatment of patients with TSC residing in the European Union who are prescribed everolimus for approved indications	• Incidence of AEs, SAEs, and everolimus-related AEs in the observation period • Incidence of events of special interest (e.g., noninfectious pneumonitis, severe infections, hypersensitivity, stomatitis, secondary amenorrhea in post-adolescent females, etc.)
To collect everolimus therapeutic drug monitoring data within routine clinical practice as per the Summary of Product Characteristics	• Everolimus blood concentration, if available

Abbreviations: AE adverse event, PASS post-authorization safety study, SAE serious adverse event, TOSCA TuberOus SClerosis registry to increase disease Awareness, TSC tuberous sclerosis complex

Statistical considerations

In TOSCA, data from all enrolled patients were expected to be included in data analysis for all variables, wherever feasible. Data analysis for all variables was conducted. Continuous variables were analyzed in terms of value at the time of registration and change from baseline at subsequent examinations.

TOSCA registry organization

TOSCA registry organization (Figure 5) includes the Scientific Advisory Board (SAB), a Working Committee, and Research Groups, which work in collaboration.

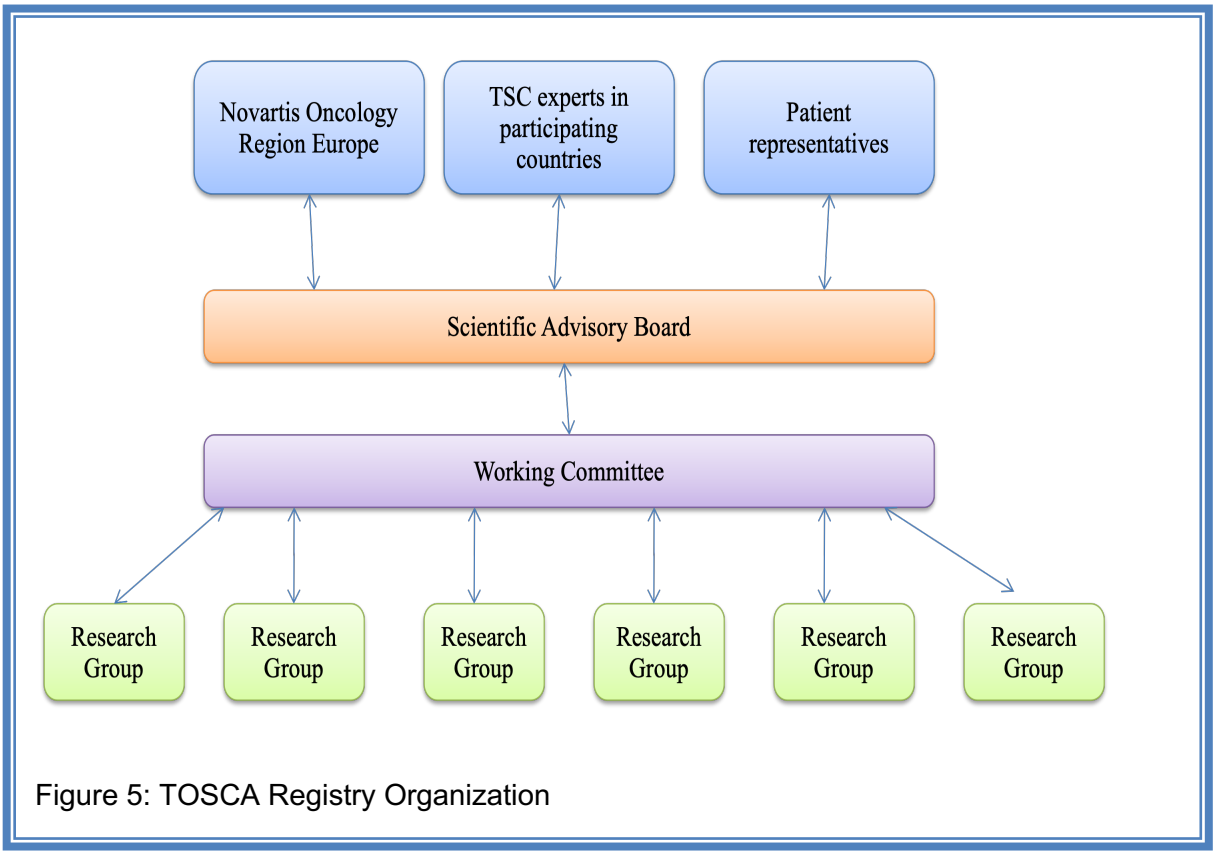

Figure 5: TOSCA Registry Organization

Scientific Advisory Board

The Scientific Advisory Board comprises of up to 30 members (TSC healthcare professionals from various specialties and patients' association group representatives), external to the sponsor and three representatives of the sponsor. This Scientific Advisory Board was responsible for the scientific principles of the registry, promotion of the use of the registry in the participating sites, publication of data in agreement with the publication policy, and approval of research projects.

Working Committee

The Working Committee was a subgroup consisting of up to 14 members from the SAB and was responsible for the registry content and coordination of all the operational

activities. It was also responsible for defining the statistical analysis plan and publication policy, and for developing and maintaining the database structure of the registry in collaboration with other members, according to their specialty and research interests.

Research Groups

Physicians from registry sites participating in individual research projects formed the research groups. Individual research groups were responsible for submission of research project proposals to the Working Committee, and for further management and analysis of those projects.

Results

First Administrative analysis

In order to evaluate the feasibility and accuracy of data being collected, the first administrative analysis was performed in March 2013 on complete 'core' data collected for first 100 patients, with a total of 469 fields of information. Regarding data completeness, it was noted that for >90% patients, information on 85% of the fields was found to be complete. Hence, this administrative analysis ensured high data quality and completeness. (24)

Baseline Analysis (25)

In TOSCA, overall 2223 patients were recruited from 170 centers across 31 countries, and 57% of these patients were recruited from neuropediatric/pediatric clinics. Since at

the cut-off date September 30, 2014, complete baseline data was gathered for 2093 patients (1009 male and 1084 female) and were hence included in this baseline analysis.

The age-wise distribution of enrolled patients is depicted in Figure 6. (63.3% of enrolled patients were ≤18 years). The median age at inclusion in TOSCA was 13 years (range, 0–71 years), while the median age at the initial TSC diagnosis was 1 year (range 0–69 years). In 124 patients (5.9%) TSC had been diagnosed prenatally. In 902 patients (43.1%), molecular testing had been performed, of which TSC1 mutations were identified in 19.7% of the patients and TSC2in 63.3% patients.

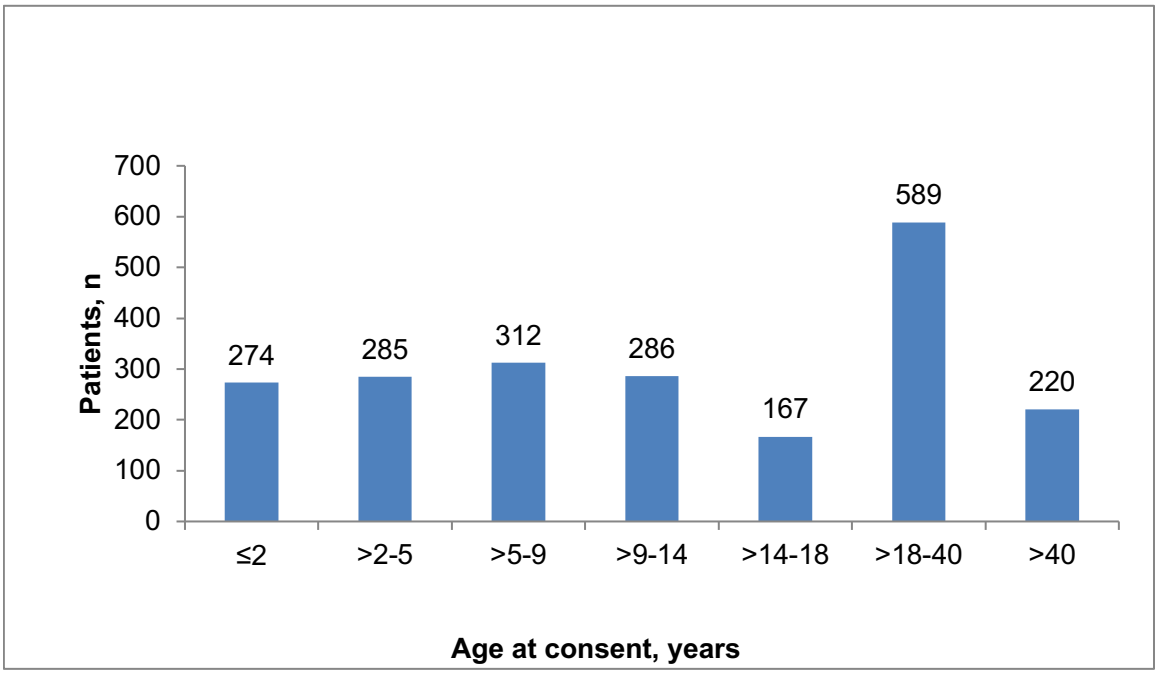

Figure 6. Patient distribution by age in TOSCA

Clinical manifestations

The summary of clinical manifestations of TSC at baseline is presented in Table 8. Of note, cortical tubers (82.2%) and subependymal nodules (78.2%) were the most frequently reported neurological manifestations. Whopping 83.5% patients (n=1748) reportedly had epilepsy, and 66.9% (n=1169) had focal seizures (median age at diagnosis of focal seizures was 1 year).

Among the patients evaluated for TSC-Associated Neuropsychiatric Disorders (TAND), 57.8% (n=682) had academic/scholastic difficulties. Furthermore, among 39.2 % (n=822) patients who had been evaluated using intelligent quotient (IQ)-type tests, mild to profound intellectual disability was reported in 54.9% (n=451) patients.

Table 8. Baseline manifestations of TSC reported in TOSCA.

Manifestations of TSC	Patients at baseline, n (%)
Neurological	
SEGA	510 (24.4)
Cortical tuber	1721 (82.2)
SEN	1636 (78.2)
Cerebral white matter radial migration lines	429 (20.5)
Renal	
Renal angiomyolipoma	987 (47.2)
Multiple renal cysts	477 (22.8)
Polycystic kidneys	73 (3.5)
Impaired renal function	43 (2.1)
Renal malignancy	24 (1.1)
Pulmonary	
Lymphangioleiomyomatosis	144 (6.9)
Cardiovascular	
Cardiac rhabdomyoma	717 (34.3)
Dermatologic	
≥ 3 hypomelanotic macules	1399 (66.8)
Facial angiofibroma	1199 (57.3)
Shagreen patch	573 (27.4)
Ungual or periungual fibromas	350 (16.7)
Forehead plaque	295 (14.1)
Confetti lesions	179 (8.6)
Ophthalmologic	
Retinal hamartoma	294 (14.0)
Epilepsy	1748 (83.5)

Among renal manifestations, the most commonly reported was renal angiomyolipomas (47.2%; n=987), diagnosed at a mean age of 17.4 years (median: 13 years). As far as pulmonary manifestations were concerned, lymphangioleiomyomatosis (LAM) were reported in 6.9% patients (n=144), and almost all of these (98.6%; n=142) were adults (>18 years). The most frequently reported cardiac manifestation was cardiac rhabdomyomas (34.3%; n=717), the mean age of diagnosis being 3.1 years. The most common dermatological manifestation was facial angiofibroma (57.3%;n=1199), the median age of onset being 6.0 years (range 0–67 years). Dental manifestations were commonly presented as randomly distributed pits in dental enamel (4.7%; n=98) and gingival fibromas (4.6%; n=96). The most frequent ophthalmological manifestation, retinal hamartomas, were seen in 14% patients (n=294), mean age at diagnosis was 8.3 years (median age 5.0 years; range 0–50 years). In 9.1% patients (n=190) liver hamartomas were reported; which were more common among females (73.7% of patients with liver hamartomas were female).

Overall, the previously ‘rarely reported’ manifestations were reported in 15.1% (n=316 patients). Amongst these were bone sclerotic foci (n=87), scoliosis (n=46), thyroid adenoma (n=15), spleen angiomyolipoma (n=5), pancreatic neuroendocrine tumor (n=5), and hemihypertrophy (abnormal growth on one side of the body), calvarial sclerosis and thickening (each in 2 patients).

Other research papers from TOSCA have presented the results of different research questions and analysis.

Conclusions

By gathering data on a large number of TSC patients across the globe, TOSCA has ensured a better understanding of the TSC and its multiple manifestations. Multiple analysis, conducted with regards to different research questions, have provided in depth knowledge about different aspects of TSC. The research projects for individual manifestations demonstrate the significant impact of these manifestations. Inadequate surveillance for TAND was an important issue that needs to be addressed in clinical practice.

Cost estimation for the use of resources in a rare but devastating disease like TCS would have been a valuable asset. However, such intended analysis needs to be included in the protocol from the planning stage itself. A careful consideration to perspectives (e.g. payer, patient, social), countries, and sample size should be given while conducting a cost analysis in these circumstances.

Section 2. Objectives

The main objective of this thesis is to identify the unmet needs in the guidance on rare disease registries and potential solutions to these issues which may benefit future registries. This was achieved by gathering a firsthand experience leading one of the largest and complete international rare disease registry (having core project, research projects and PASS), from inception to publication stage. This research aims to improve the guidance to achieve better quality, usefulness, and sustainability of future registries in rare diseases.

Specific objectives

1. Paper 1

to analyze the change in treatment patterns in patients with TSC included in the TOSCA registry over the 5 year follow-up, to identify differences in management as well as the availability of medical and non-medical health resources with respect to patients' age or country of residence.(26)

2. Paper 2

to identify issues/concerns encountered during the design, execution, and publication phases of TOSCA. This paper also reflects on lessons learnt from TOSCA registry that may guide future registries in rare and complex diseases. (15)

to compare and evaluate the deviations of the TOSCA registry from the EMA guidance and its potential impact on the registry outcomes and success. It also determines if the learnings from TOSCA can complement the EMA guidance, especially in case of rare disease registries.(27)

This research project was aimed at improving our understanding of the functioning of a rare disease registry from the ‘insider’ perspective. The better understanding of the potential challenges offers future directions for better registry planning in future registries in rare diseases.

Section 3. Publications

3.1 Paper 1

Treatment Patterns and Use of Resources in Patients With Tuberous Sclerosis Complex: Insights From the TOSCA Registry

Frontiers in Neurology. 2019;10:1144.

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Treatment Patterns and Use of Resources in Patients With Tuberous Sclerosis Complex: Insights From the TOSCA Registry

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Treatment Patterns and Use of Resources in Patients With Tuberous Sclerosis Complex: Insights From the TOSCA Registry

3.1.1 Abstract

Introduction

Tuberous Sclerosis Complex (TSC) is a rare autosomal-dominant disorder caused by mutations in the TSC1 or TSC2genes. TSC exhibits a wide array of manifestations. However, the burden of TSC and its impact on healthcare resources remains unknown. Furthermore, treatment plans are diverse, depending upon country specific clinical practice and consequently impact the use of resources. This paper is aimed to describe the use of TSC-related resources and treatment patterns within the TOSCA registry. In TOSCA, a total of 2,214 patients with TSC across 31 countries were enrolled and were followed up for up to 5 years.

Methodology

A search was conducted to identify the variables containing both medical and non-medical resource use information within TOSCA. This search was performed both at the level of the core project as well as at the level of the research projects on epilepsy, subependymal giant cell astrocytoma (SEGA), lymphangioleiomyomatosis (LAM), and renal angiomyolipoma (rAML) and TSC-Associated Neuropsychiatric Disorders (TAND) taking into account the time-points of the study, age groups, and countries. Data from the quality of life (QoL) research project were analyzed by type of visit and age at enrolment.

Results

Treatment and use of resources varied vastly, according to the clinical manifestations, time-point in the study, and age groups. GABAergics were the most prescribed drugs for epilepsy. Also, mTOR inhibitors were noted to be significantly replacing surgery in patients with SEGA, despite the current recommendations proposing both treatment options. mTOR inhibitors were also noted to be becoming common treatments in rAML and LAM patients. Of the 143 patients in the quality of life (QoL) research project, 42 (29.4%) reported inpatient stays over the last year. Data from non-medical resource use revealed a critical impact of TSC on job status and capacity. Disability allowances were more common in children than adults (51.1% vs. 38.2%). Psychological counseling, social services and social worker services were needed by <15% of the patients, regardless of age.

Conclusion

The long-term nature, together with the variability in its clinical manifestations, makes TSC a complex and resource demanding disease. The present study shows a comprehensive picture of the resource use implications of TSC.

Keywords: TSC, resource use, TOSCA, management, registry, rare diseases

3.1.2 Introduction

Tuberous sclerosis complex (TSC) is an autosomal-dominant disorder, caused by mutations in either TSC1 or TSC2genes. The condition is characterized by the formation of hamartomatous lesions in multiple organ systems.(16) It is also associated with a wide range of TSC-associated neuropsychiatric disorders, abbreviated as TAND.(17)

The condition varies widely in terms of manifestations and severity, even amongst family members, which is associated with the size, number, location and distribution of the lesions.(18,28) The most commonly affected organs include the brain, kidneys, lungs, skin, heart, and eyes.(18,24,29-31) However, none of the symptoms is necessarily found in all patients and considered absolutely pathognomonic.(30)

While many studies have evaluated usage of resources in TSC (32-39), they have mostly been conducted in individual countries and have enrolled limited number of patients further specified by age or by clinical manifestation. Hence, these studies offer specific information, which cannot be extrapolated to other countries or clinical contexts. Wide variation across countries can be expected depending on the country-specific clinical practice. Consequently, the burden of TSC and its impact on the use of healthcare resources required for its management remains largely unknown.

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) was a large scale non-interventional study in patients with TSC, started in 2012 and was conducted at 170 sites in 31 countries. This registry was founded by Novartis AG and its related clinical study protocol and final study results are disclosed on the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) portal at

http://www.encepp.eu/ (EU PAS Register Number EUPAS324). It enrolled patients of any age with TSC and followed up for up to 5 years. Patient data including demographics and information related to clinical features of TSC across all organ systems, comorbidities, and rare manifestations, were collected at baseline and at regular visits scheduled at a maximum interval of 1 year. (24)

The TOSCA registry consisted of a 'core' part and six associated research projects focusing on: epilepsy, subependymal giant cell astrocytomas (SEGA), renal angiomyolipoma (rAML)/lymphangioleiomyomatosis (LAM), genetics, quality of life (QoL), and TAND. In the 'core' part, data related to demography, family history, prenatal history, disease features, and information on treatments were collected. The research projects recorded in-depth data related to specific disease manifestations or to specific aspects of the disease. The QoL research project recorded also data on the use of medical and non-medical resources for seven European countries (Belgium, Germany, Italy, Spain, Sweden, France, and the UK). (24)

Due to its long-term follow-up (up to 5 years) and to the inclusion of patients of any age from different countries from all over the world, the TOSCA registry offered a unique opportunity to observe trends in treatment patterns for various TSC manifestations over time, and to evaluate differences in disease management depending on the age of the patients or their country of residence. In addition, results can be analyzed in context with the results from the other research projects.

The aims of the present study were to analyze how the treatment modalities in patients with TSC included in the TOSCA registry changed during the 5 years of follow-up, to

identify differences in management as well as the availability of medical and non-medical health resources with respect to patients' age or country of residence.

3.1.3 Methodology

This study was based on data obtained from the TOSCA registry, which was designed and conducted according to the Guidelines for Good Clinical Practice and ethical principles outlined in the Declaration of Helsinki.

Initially, a search was conducted to identify the variables that are associated with the use of TSC-related resources (including medical and non-medical resources). For this, an exhaustive analysis, of all the listings and tables produced as a part of the final analysis of the TOSCA registry, was conducted, which are detailed in the supplementary tables in the publication.

Data related to treatment usage (proportion of treated patients and types of treatment) were available for the overall population of patients included in the core registry. Data regarding use of other medical resources (hospitalizations, primary, and secondary care visits) and regarding use of non-medical resources (variables related to educational needs, patient or caregiver employment situation and patient support/social services needs) were available for a subset of 143 patients included in the QoL research project, which was carried out in the 7 European countries (Belgium, Germany, Italy, Spain, Sweden, France, and the UK).

Treatment patterns were analyzed using the core registry data, for four clinical manifestations (epilepsy, SEGA, LAM, and rAML), the number of visits [baseline or follow-

ups (FU1 to FU5), where FUs were conducted at intervals not longer than 12 months apart], the age group (≤2, >2 to ≤5, >5 to ≤9, >9 to ≤14, >14 to <18, ≥18 to ≤40, and >40 years), and the country of residence (for those countries included in the QoL research project). Baseline data were retrospectively collected and follow-up data were prospectively collected for up to 5 years. All the results were reported in terms of absolute and relative frequencies. The use of other medical resources and the use of non-medical resources was analyzed for the overall population included in the QoL research project. Again, all the results were reported in terms of absolute and relative frequencies.

3.1.4 Results

Baseline Characteristics of Patients

In TOSCA, 2,214 patients were enrolled across 31 countries, of which data from 2,211 eligible patients were analyzed and data for three patients were excluded from the analysis due to major protocol deviations. Of the 2,211 analyzed patients, 1,152 (52.1%) were female. The median age at enrolment was 13 years (range <1–71 years), and the median age at initial TSC diagnosis was 1 year (range <1–69 years).

The most common manifestation was epilepsy, affecting 1,879 (85.0%) patients. Among patients with epilepsy, 1,343 (71.5%) had focal seizures (FS) and 735 (39.1%) had infantile spasms (IS). Other common manifestations were hypomelanotic macules in 1,555 patients (70.3%), facial angiofibromas in 1,533 patients (69.3%), and rAML in 1,317 patients (59.6%).

Yet another significant manifestation was TAND, even though it was the most under-assessed aspect of TSC in the TOSCA registry. TAND assessment includes the evaluation of common behavioral problems, psychiatric disorders, intellectual abilities, academic performance, and neuropsychological difficulties. At baseline, only 818 out of 2,211 (37%) patients reported having at least one behavioral problem. In 319 patients (14.4%), autism spectrum disorder (ASD) and in 267 patients (12.1%), attention deficit hyperactivity disorders (ADHD) was diagnosed; and 82 (3.7%) and 132 (6.0%) patients had depressive disorders or anxiety, respectively. In addition, 736 patients (33.3%) were reported to have difficulties in academic performance. Among the 894 patients with reported TAND, normal intellectual ability (defined as full scale IQ ≥80) was reported for 44.2% (n=395/894).

Table 9. Use of treatments according to follow-up (FU) visit.

	Baseline (N=2211)	**FU1** (N=2099)	**FU2** (N=1935)	**FU3** (N=1664)	**FU4** (N=764)	**FU5** (N=147)
Patients with IS	721	151	120	91	45	14
Patients treated for IS (n, %)	698 (96.8)	145 (96.0)	113 (94.2)	85 (93.4)	44 (97.8)	14 (100.0)
Patients with FS	1261	614	544	506	236	29
Patients treated for FS (n, %)	1237 (98.1)	599 (97.6)	530 (97.4)	493 (97.4)	231 (97.9)	28 (96.6)
Patients with SEGA	553	489	468	420	208	52
Patients treated for SEGA (n, %)	221 (40.0)	187 (38.2)	188 (40.2)	181 (43.1)	101 (48.6)	22 (42.3)
Patients with rAML	1062	1067	1041	945	472	121
Patients treated for rAML (n, %)	315 (29.7)	300 (28.1)	321 (30.8)	288 (30.5)	165 (35.0)	53 (43.8)
Patients with LAM	154	157	162	149	68	21
Patients treated for LAM (n, %)	50 (32.5)	47 (29.9)	54 (33.3)	43 (28.9)	20 (29.4)	0 (0.0)

FS: Focal Seizures; IS: Infantile Spasms; LAM: lymphangioleiomyomatosis; rAML: renal angiomyolipoma; SEGA: subependymal giant cell astrocytomas

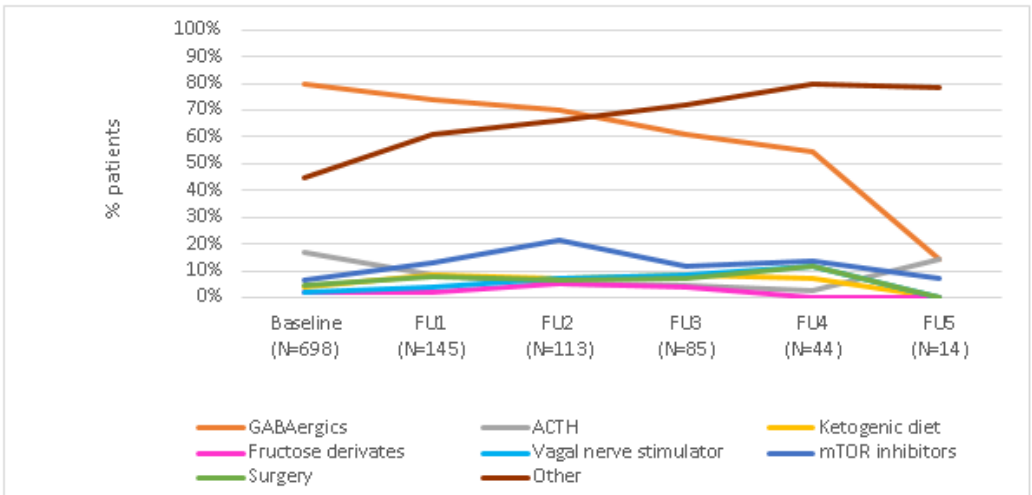
Treatments

In the TOSCA registry, the proportion of patients who received treatment varied largely depending on the clinical manifestations (Table 9), with values at baseline (patients who ever had the manifestation) ranging between 96.8% (698/721) for IS and 32.5% (50/154)

for LAM. The detailed treatment trends according to clinical manifestations are described below.

Epilepsy

As shown in Table 9, almost all patients with epilepsy received antiepileptic drug treatment throughout the follow-up. Figures 7 and 8 show the treatment trends in patients with IS and FS, respectively, during the registry duration.

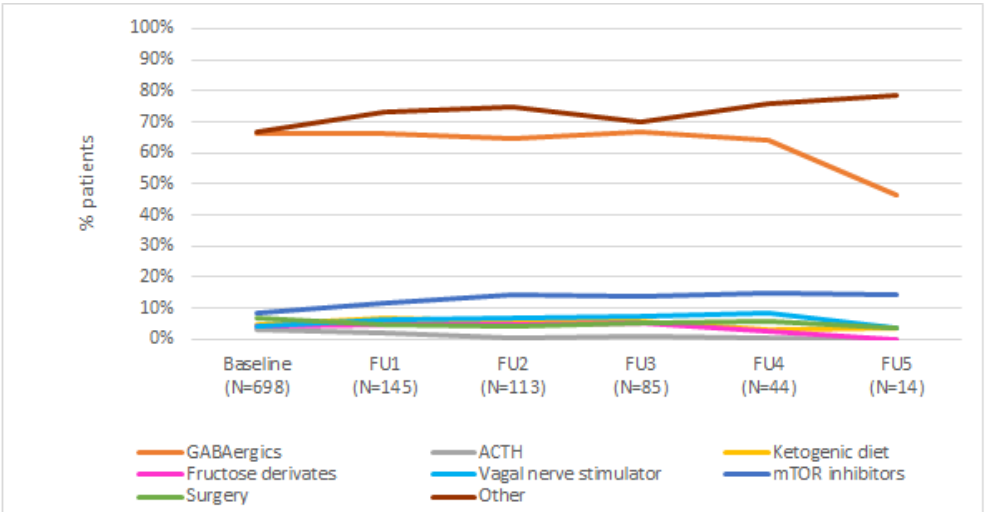


	BASELINE (N=698)	FU1 (N=145)	FU2 (N=113)	FU3 (N=85)	FU4 (N=44)	FU5 (N=14)
GABAergics	554 (79.4%)	107 (73.8%)	79 (69.9%)	52 (61.2%)	24 (54.5%)	2 (14.3%)
ACTH	119 (17.0%)	12 (8.3%)	6 (5.3%)	4 (4.7%)	1 (2.3%)	2 (14.3%)
Ketogenic diet	27 (3.9%)	12 (8.3%)	8 (7.1%)	7 (8.2%)	3 (6.8%)	0 (0.0%)
Fructose derivates	12 (1.7%)	3 (2.1%)	6 (5.3%)	3 (3.5%)	0 (0.0%)	0 (0.0%)
Vagal nerve stimulator	15 (2.1%)	6 (4.1%)	8 (7.1%)	7 (8.2%)	5 (11.4%)	0 (0.0%)
mTOR inhibitors	43 (6.2%)	19 (13.1%)	24 (21.2%)	10 (11.8%)	6 (13.6%)	1 (7.1%)
Surgery	30 (4.3%)	11 (7.6%)	7 (6.2%)	6 (7.1%)	5 (11.4%)	0 (0.0%)
Other	310 (44.4%)	88 (60.7%)	75 (66.4%)	61 (71.8%)	35 (79.5%)	11 (78.6%)

Figure 7. Treatments for Infantile Spasms in each Follow-up Visit.
Patients may receive more than one treatment. Baseline data refers to patients who 'ever had' the manifestation. Other include lamotrigine, topiramate, levetiracetam and valproate.

- At baseline, GABAergic agents (e.g. vigabatrin) were most commonly used treatment (in 79.3% patients with IS, and in 66.2% patients with FS)

- Use of GABAergic agents declined over time. By fifth FU visit, it was being used in 14.3% patients with IS and in 46.4% patients with FS.



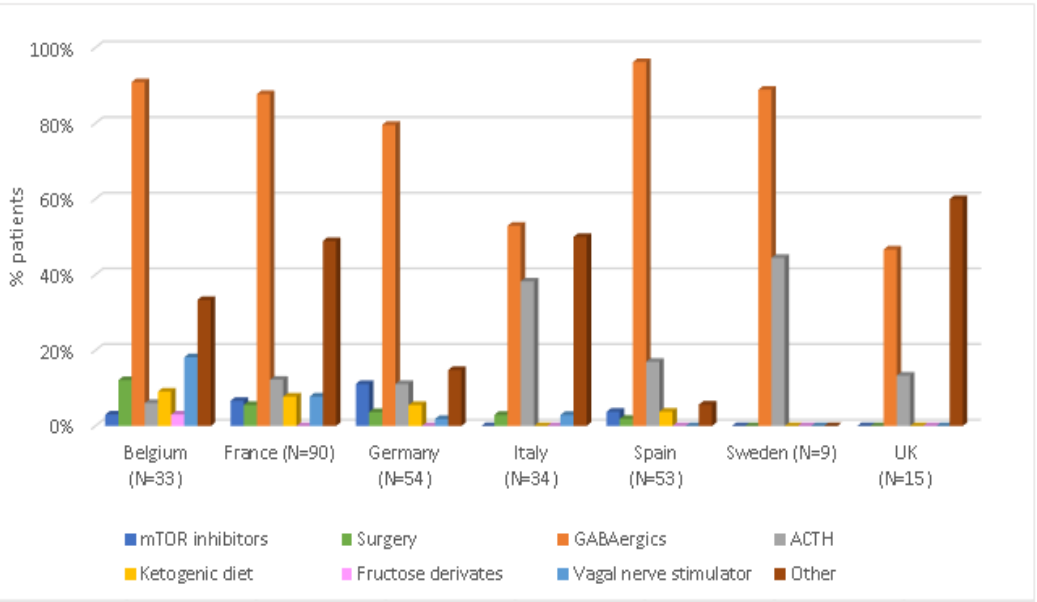
	BASELINE (N=1,237)	FU1 (N=599)	FU2 (N=530)	FU3 (N=493)	FU4 (N=231)	FU5 (N=28)
GABAergics	819 (66.2%)	398 (66.4%)	343 (64.7%)	328 (66.5%)	148 (64.1%)	13 (46.4%)
ACTH	35 (2.8%)	11 (1.8%)	3 (0.6%)	5 (1.0%)	1 (0.4%)	0 (0.0%)
Ketogenic diet	59 (4.8%)	41 (6.8%)	31 (5.8%)	27 (5.5%)	7 (3.0%)	1 (3.6%)
Fructose derivates	49 (4.0%)	28 (4.7%)	24 (4.5%)	25 (5.1%)	6 (2.6%)	0 (0.0%)
Vagal nerve stimulator	48 (3.9%)	39 (6.5%)	36 (6.8%)	37 (7.5%)	20 (8.7%)	1 (3.6%)
mTOR inhibitors	103 (8.3%)	71 (11.8%)	77 (14.5%)	67 (13.6%)	34 (14.7%)	4 (14.3%)
Surgery	84 (6.8%)	27 (4.5%)	23 (4.3%)	26 (5.3%)	13 (5.6%)	1 (3.6%)
Other	823 (66.5%)	439 (73.3%)	397 (74.9%)	346 (70.2%)	175 (75.8%)	22 (78.6%)

Figure 8. Treatments for Focal Seizures in each Follow-up Visit.
Patients may receive more than one treatment. Baseline data refers to patients who 'ever had' the manifestation.

- Other treatment options such as mammalian target of rapamycin (mTOR) inhibitors, the ketogenic diet (KD) and epilepsy surgery were used in <20% of the patients at baseline, and remained relatively stable over time.

The country-wise trends in treatment of IS and FS are depicted in Figures 9 and 10, respectively.

- GABAergics (mono- or combination therapy) were most commonly used treatment options in all countries both in patients with IS and FS.
- In all countries except Belgium, Adrenocorticotropic Hormone (ACTH) was the second most common treatment for treating IS. (Figure 9)

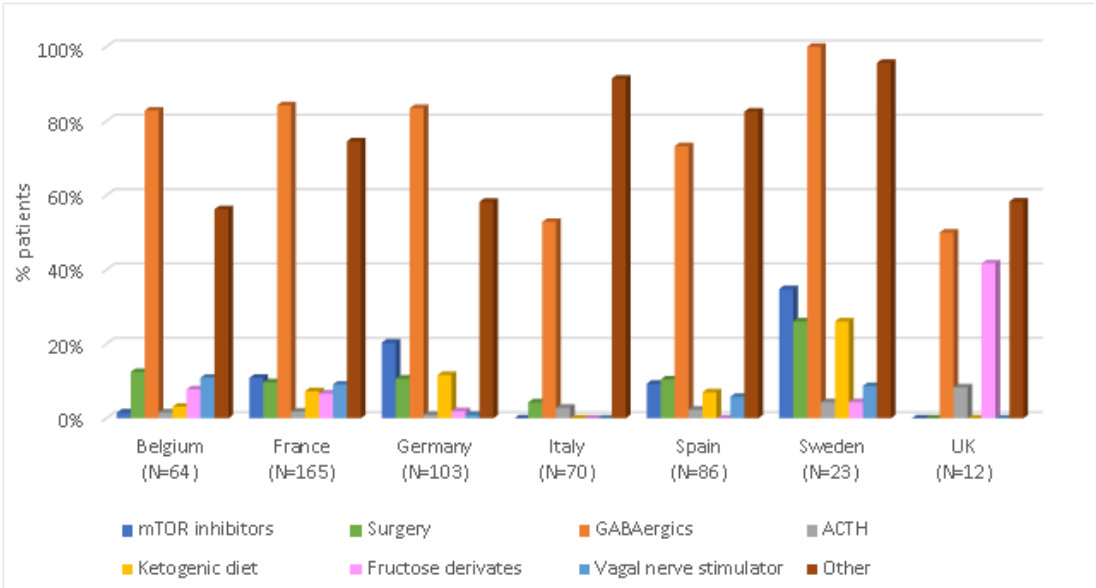


	Belgium (N=33)	France (N=90)	Germany (N=54)	Italy (N=34)	Spain (N=53)	Sweden (N=9)	UK (N=15)
mTOR inhibitors	1 (3.0%)	6 (6.7%)	6 (11.1%)	0 (0.0%)	2 (3.8%)	0 (0.0%)	0 (0.0%)
Surgery	4 (12.1%)	5 (5.6%)	2 (3.7%)	1 (2.9%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
GABAergics	30 (90.9%)	79 (87.8%)	43 (79.6%)	18 (52.9%)	51 (96.2%)	8 (88.9%)	7 (46.7%)
ACTH	2 (6.1%)	11 (12.2%)	6 (11.1%)	13 (38.2%)	9 (17.0%)	4 (44.4%)	2 (13.3%)
Ketogenic diet	3 (9.1%)	7 (7.8%)	3 (5.6%)	0 (0.0%)	2 (3.8%)	0 (0.0%)	0 (0.0%)
Fructose derivates	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vagal nerve stimulator	6 (18.2%)	7 (7.8%)	1 (1.9%)	1 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	11 (33.3%)	44 (48.9%)	8 (14.8%)	17 (50.0%)	3 (5.7%)	0 (0.0%)	9 (60.0%)

Figure 9. Treatments for Infantile Spasms by Country.
Patients may receive more than one treatment.

- In FS, the other common treatments were epilepsy surgery (in Belgium, Italy, and Spain) and mTOR inhibitors (in Sweden, Germany, and France) (Figure 10).
- In the UK, neither surgery nor mTOR inhibitors were used at all in patients with IS or FS. Similarly, these treatments were not employed in patients with IS from Sweden.

- Across all countries, more than half of treatments in patients with FS were not specified (included in “others” category). The non-specified treatments in FS were as high as >90% in Italy and Sweden. (Figure 10)



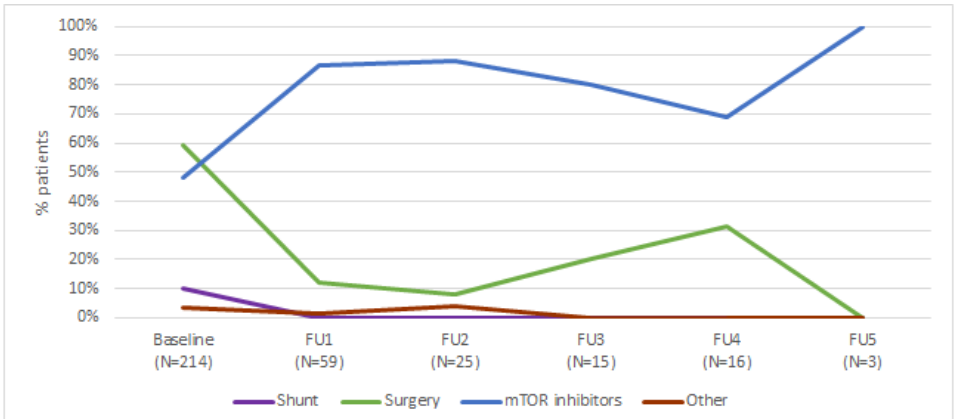
	Belgium (N=64)	France (N=165)	Germany (N=103)	Italy (N=70)	Spain (N=86)	Sweden (N=23)	UK (N=12)
mTOR inhibitors	1 (1.6%)	18 (10.9%)	21 (20.4%)	0 (0.0%)	8 (9.3%)	8 (34.8%)	0 (0.0%)
Surgery	8 (12.5%)	16 (9.7%)	11 (10.7%)	3 (4.3%)	9 (10.5%)	6 (26.1%)	0 (0.0%)
GABAergics	53 (82.8%)	139 (84.2%)	86 (83.5%)	37 (52.9%)	63 (73.3%)	23 (100.0%)	6 (50.0%)
ACTH	1 (1.6%)	3 (1.8%)	1 (1.0%)	2 (2.9%)	2 (2.3%)	1 (4.3%)	1 (8.3%)
Ketogenic diet	2 (3.1%)	12 (7.3%)	12 (11.7%)	0 (0.0%)	6 (7.0%)	6 (26.1%)	0 (0.0%)
Fructose derivates	5 (7.8%)	11 (6.7%)	2 (1.9%)	0 (0.0%)	0 (0.0%)	1 (4.3%)	5 (41.7%)
Vagal nerve stimulator	7 (10.9%)	15 (9.1%)	1 (1.0%)	0 (0.0%)	5 (5.8%)	2 (8.7%)	0 (0.0%)
Other	36 (56.3%)	123 (74.5%)	60 (58.3%)	64 (91.4%)	71 (82.6%)	22 (95.7%)	7 (58.3%)

Figure 10. Treatments for Focal Seizures by Country.
Patients may receive more than one treatment.

SEGA

In patients with SEGA, 40.0% of patients had ever received treatment for SEGA at baseline and the trends remained stable over time (Table 9).

- As shown in Figure 11, the most common treatment options in SEGA were mTOR inhibitors and surgery, which varied remarkably upon follow-up, age and the country of residence.
    - At baseline, 48.1% patients with SEGA received mTOR inhibitors, which increased over time, reaching 86.4% in the 1stFU visit and 100% by the 5th FU visit.
    - With the increasing trends towards use of mTOR inhibitors, there was a declining trend in surgery. At baseline, 59.3% patients underwent surgery, which declined to 11.9% in 1stFU visit and became nil by the 5thFU visit.

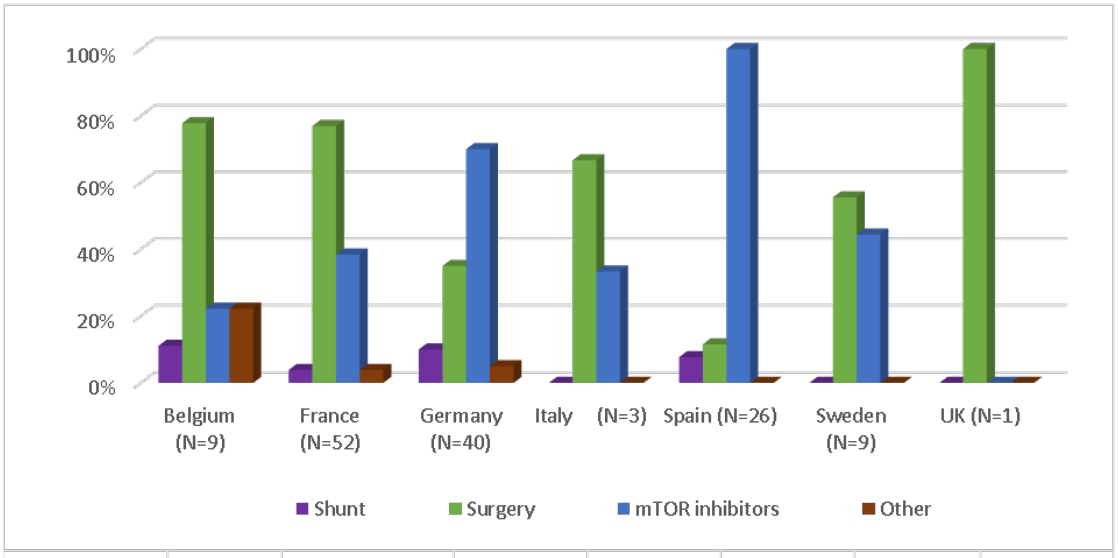


	BASELINE (N=214)	FU1 (N=59)	FU2 (N=25)	FU3 (N=15)	FU4 (N=16)	FU5 (N=3)
Shunt	22 (10.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Surgery	127 (59.3%)	7 (11.9%)	2 (8.0%)	3 (20.0%)	5 (31.3%)	0 (0.0%)
mTOR inhibitors	103 (48.1%)	51 (86.4%)	22 (88.0%)	12 (80.0%)	11 (68.8%)	3 (100.0%)
Other	7 (3.3%)	1 (1.7%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

Figure 11. Treatments for SEGA according to the Number of FU Visit.
Patients mayreceive more than one treatment. Baseline data refers to patients who 'ever had' the manifestation.

- Treatment trends in SEGA also varied with age, as shown in Figure 12. Children aged 9–14 were treated most commonly (51.0%) while children aged <2 years (15.2%) and adults aged > 40 years (29.6%) were treated least frequently.

- Treatment options used for SEGA also varied across age groups. While mTOR inhibitors were the most common treatments used in children aged ≤9 years, surgery was the most common treatment in adolescents and adults (Figure 12).



	Belgium (N=9)	France (N=52)	Germany (N=40)	Italy (N=3)	Spain (N=26)	Sweden (N=9)	UK (N=1)
Shunt	1 (11.1%)	2 (3.8%)	4 (10.0%)	0 (0.0%)	2 (7.7%)	0 (0.0%)	0 (0.0%)
Surgery	7 (77.8%)	40 (76.9%)	14 (35.0%)	2 (66.7%)	3 (11.5%)	5 (55.6%)	1 (100.0%)
mTOR inhibitors	2 (22.2%)	20 (38.5%)	28 (70.0%)	1 (33.3%)	26 (100.0%)	4 (44.4%)	0 (0.0%)
Other	2 (22.2%)	2 (3.8%)	2 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

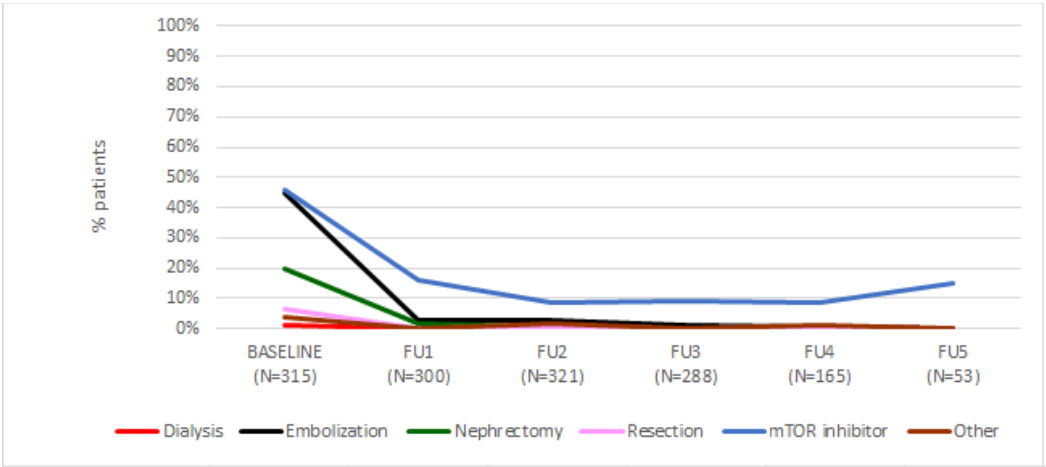
Figure 13. Treatments for SEGA by Country.
Patients may receive more than one treatment.

- Country-wise analysis of treatment of SEGA (Figure 13) revealed variations in treatments employed. In Germany and Spain, mTOR inhibitors were more often prescribed (in 70% and 100% patients with SEGA, respectively). In contrast, surgery was the most common treatment in Belgium (77.8%) and in France (76.9%). The only patient with SEGA from the UK (100%) also underwent surgery.

rAML

At baseline, 29.7% patients (n=315) with SEGA received treatment, which remained around 30% till 3rdFU visit and then escalated to 35.0% at 4thFU visit and further to 43.8% at 5thFU visit.

- As in SEGA, the most commonly used treatments in rAML were mTOR inhibitors



	BASELINE (N=315)	FU1 (N=300)	FU2 (N=321)	FU3 (N=288)	FU4 (N=165)	FU5 (N=53)
Dialysis	4 (1.3%)	1 (0.3%)	1 (0.3%)	0 (0.0%)	1 (0.6%)	0 (0.0%)
Embolization	141 (44.8%)	9 (3.0%)	9 (2.8%)	3 (1.0%)	1 (0.6%)	0 (0.0%)
Nephrectomy	63 (20.0%)	5 (1.7%)	3 (0.9%)	1 (0.3%)	1 (0.6%)	0 (0.0%)
Resection	21 (6.7%)	1 (0.3%)	2 (0.6%)	0 (0.0%)	1 (0.6%)	0 (0.0%)
mTOR inhibitors	144 (45.7%)	49 (16.3%)	28 (8.7%)	26 (9.0%)	14 (8.5%)	8 (15.1%)
Other	13 (4.1%)	1 (0.3%)	5 (1.6%)	1 (0.3%)	2 (1.2%)	0 (0.0%)

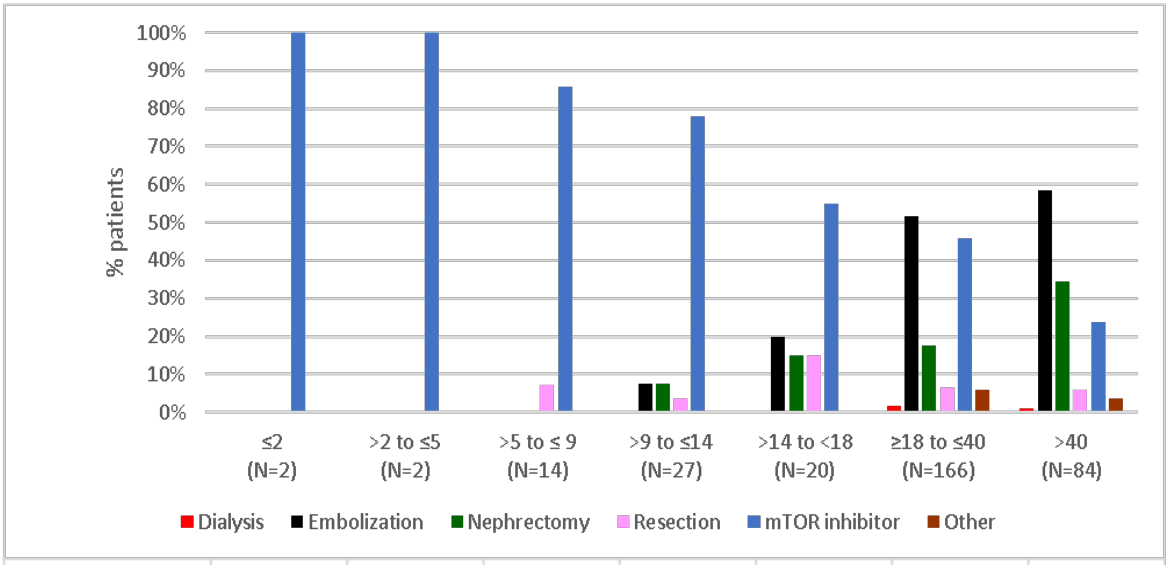
Figure 14. rAML Treatments according to Follow-Up.
Patients may receive more than one treatment. Baseline data refers to patients who 'ever had' the manifestation.

and embolization. (Figure 14) At baseline, 144 (45.7%) patients received mTOR inhibitors and 141 (44.8%) patients underwent embolization.

- Use of all treatments declined consistently over time and only 8 (15.1%) patients at 5thFU visit were receiving mTOR inhibitors. By the final visit, data on

embolizations were not available. In 4thFU visit, only one patient (0.6%) underwent embolization.

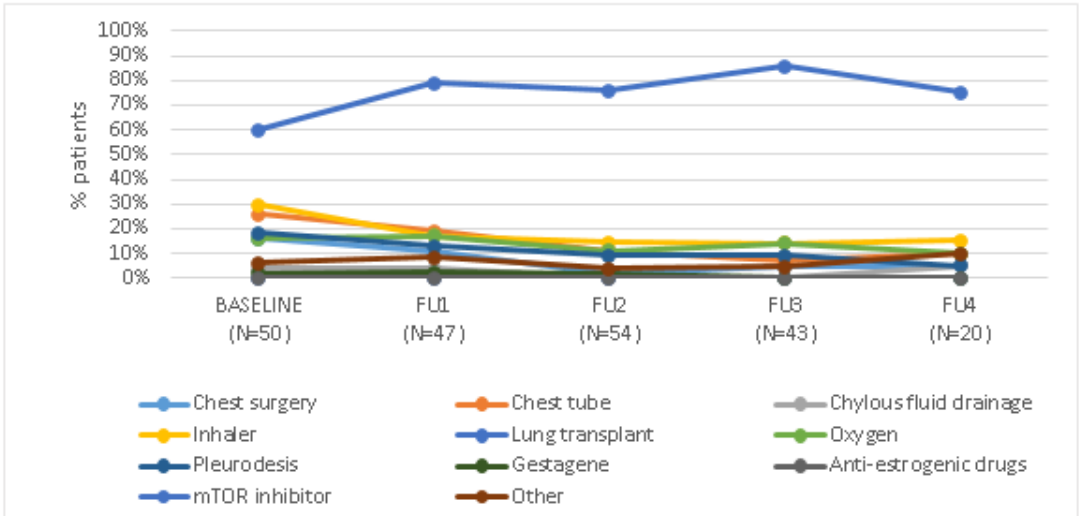
- Since rAML is an uncommon manifestation in children, most of the patients receiving treatment for rAML were adolescent and adults (Figure 15). Embolizations were rarely performed in children (7.4% in patients aged 9–14years). However, more than half (51.8%) rAML patients aged 18–40 years and older (58.3%) underwent embolization. Also, use of mTOR inhibitors was relatively higher in younger patients, which were prescribed for other TSC manifestations. (Figure 15)



	≤2 (N=2)	>2 to ≤5 (N=2)	>5 to ≤ 9 (N=14)	>9 to ≤14 (N=27)	>14 to <18 (N=20)	≥18 to ≤40 (N=166)	>40 (N=84)
Dialysis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.8%)	1 (1.2%)
Embolization	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	4 (20.0%)	86 (51.8%)	49 (58.3%)
Nephrectomy	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	3 (15.0%)	29 (17.5%)	29 (34.5%)
Resection	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (3.7%)	3 (15.0%)	11 (6.6%)	5 (6.0%)
mTOR inhibitors	2 (100.0%)	2 (100.0%)	12 (85.7%)	21 (77.8%)	11 (55.0%)	76 (45.8%)	20 (23.8%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (6.0%)	3 (3.6%)

Figure 15. rAML Treatments according to Age at Baseline.
Patients may receive more than one treatment.

- The country-wise trends in treatment utilization in rAML patients are shown in Figure 16. As evident, mTOR inhibitors were the most commonly used treatment option for rAML in all countries.



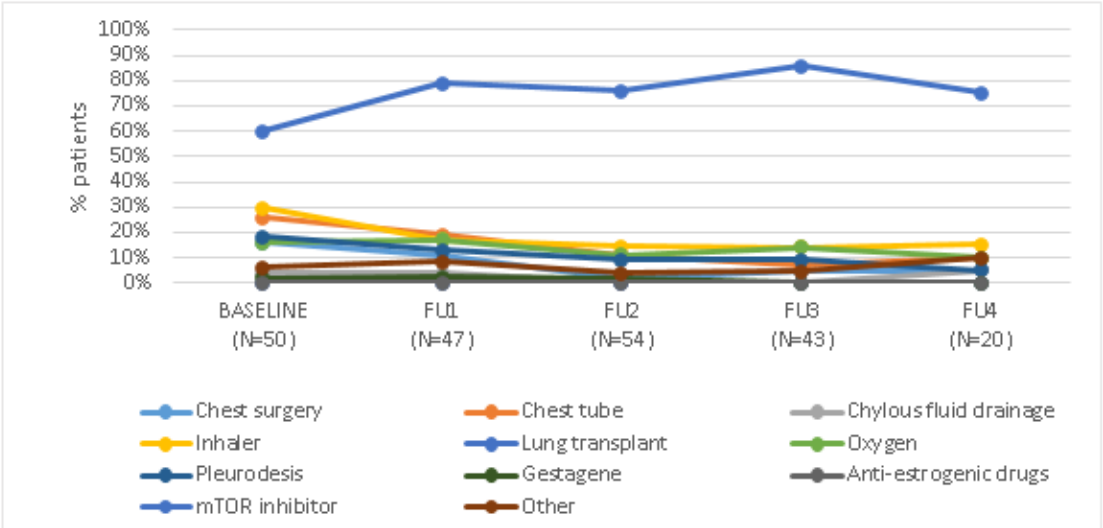
	BASELINE (N=50)	FU1 (N=47)	FU2 (N=54)	FU3 (N=43)	FU4 (N=20)
Chest surgery	8 (16.0%)	5 (10.6%)	1 (1.9%)	2 (4.7%)	1 (5.0%)
Chest tube	13 (26.0%)	9 (19.1%)	6 (11.1%)	3 (7.0%)	2 (10.0%)
Chylous fluid drainage	2 (4.0%)	2 (4.3%)	0 (0.0%)	0 (0.0%)	1 (5.0%)
Inhaler	15 (30.0%)	8 (17.0%)	8 (14.8%)	6 (14.0%)	3 (15.0%)
Lung transplant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Oxygen	8 (16.0%)	8 (17.0%)	6 (11.1%)	6 (14.0%)	2 (10.0%)
Pleurodesis	9 (18.0%)	6 (12.8%)	5 (9.3%)	4 (9.3%)	1 (5.0%)
Gestagene	1 (2.0%)	1 (2.1%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
Anti-estrogenic drugs	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
mTOR inhibitor	30 (60.0%)	37 (78.7%)	41 (75.9%)	37 (86.0%)	15 (75.0%)
Other	3 (6.0%)	4 (8.5%)	2 (3.7%)	2 (4.7%)	2 (10.0%)

Figure 16. rAML Treatment by Country.
Patients may receive more than one treatment.

LAM

The proportion of patients with LAM who received treatment declined over time. (Table 9)

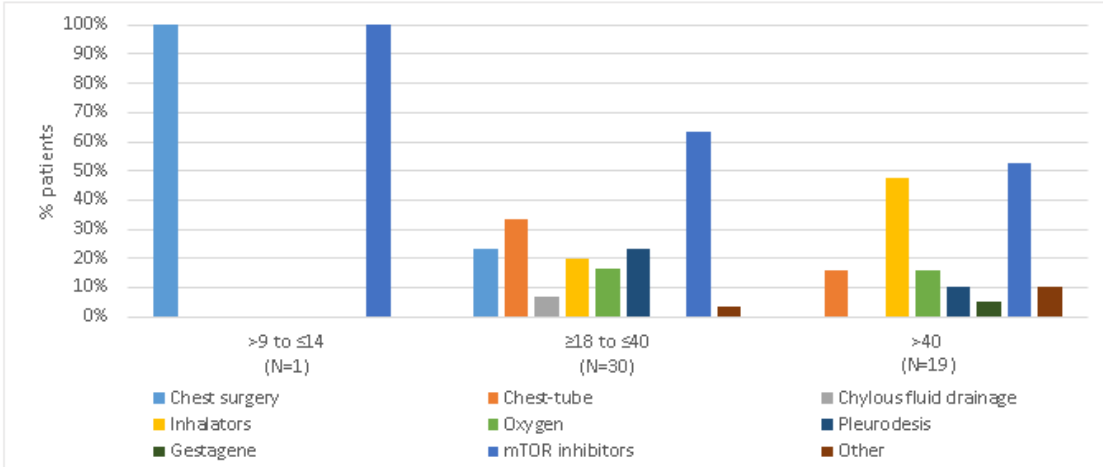
As in other manifestations, mTOR inhibitors remained the most commonly utilized treatment for LAM (60.0% at baseline) and its use increased up to 86.0% by 3rdFU visit and further to 75.0% by 4thFU visit. (Figure 17)



	BASELINE (N=50)	FU1 (N=47)	FU2 (N=54)	FU3 (N=43)	FU4 (N=20)
Chest surgery	8 (16.0%)	5 (10.6%)	1 (1.9%)	2 (4.7%)	1 (5.0%)
Chest tube	13 (26.0%)	9 (19.1%)	6 (11.1%)	3 (7.0%)	2 (10.0%)
Chylous fluid drainage	2 (4.0%)	2 (4.3%)	0 (0.0%)	0 (0.0%)	1 (5.0%)
Inhaler	15 (30.0%)	8 (17.0%)	8 (14.8%)	6 (14.0%)	3 (15.0%)
Lung transplant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Oxygen	8 (16.0%)	8 (17.0%)	6 (11.1%)	6 (14.0%)	2 (10.0%)
Pleurodesis	9 (18.0%)	6 (12.8%)	5 (9.3%)	4 (9.3%)	1 (5.0%)
Gestagene	1 (2.0%)	1 (2.1%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
Anti-estrogenic drugs	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
mTOR inhibitor	30 (60.0%)	37 (78.7%)	41 (75.9%)	37 (86.0%)	15 (75.0%)
Other	3 (6.0%)	4 (8.5%)	2 (3.7%)	2 (4.7%)	2 (10.0%)

Figure 17. LAM Treatments according to Follow-Up.
Patients may receive more than one treatment. Baseline data refers to patients who 'ever had' the manifestation.

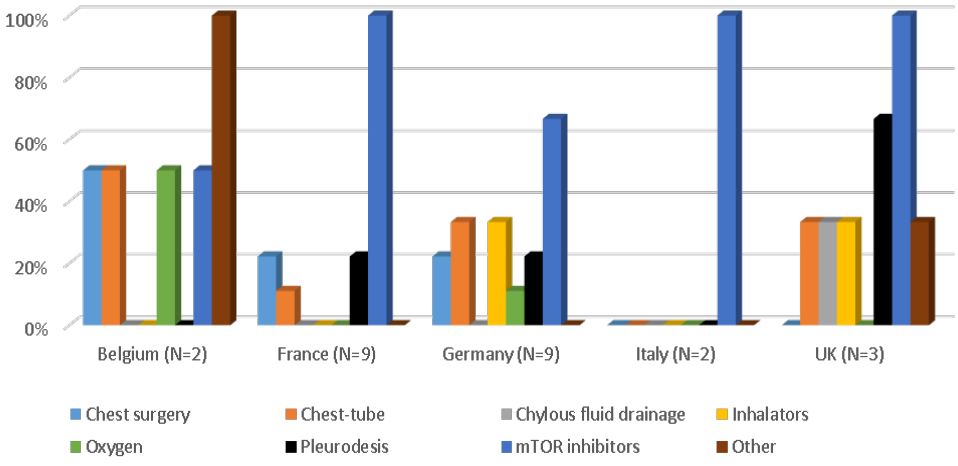
- The treatment trends in LAM patients are shown in Figure 18. No data were available for younger patients as LAM was diagnosed in patients ≥9 years. In adolescents, both chest surgery and mTOR inhibitors were used, while most adult patients were treated with mTOR inhibitors.



	>9 to ≤14 (N=1)	≥18 to ≤40 (N=30)	>40 (N=19)
Chest surgery	1 (100.0%)	7 (23.3%)	0 (0.0%)
Chest-tube	0 (0.0%)	10 (33.3%)	3 (15.8%)
Chylous fluid drainage	0 (0.0%)	2 (6.7%)	0 (0.0%)
Inhalators	0 (0.0%)	6 (20.0%)	9 (47.4%)
Oxygen	0 (0.0%)	5 (16.7%)	3 (15.8%)
Pleurodesis	0 (0.0%)	7 (23.3%)	2 (10.5%)
Gestagene	0 (0.0%)	0 (0.0%)	1 (5.3%)
mTOR inhibitors	1 (100.0%)	19 (63.3%)	10 (52.6%)
Other	0 (0.0%)	1 (3.3%)	2 (10.5%)

Figure 18. LAM Treatments according to Age at Baseline.
Patients may receive more than one treatment.

- The country-wise trends in LAM treatment are depicted in Figure 19. In France and Italy, all LAM patients received mTOR inhibitors, while the use of mTOR inhibitors in LAM patients was 66.7% in Germany, 50% in Belgium, and in 25.0% in the UK. Data regarding treatment option trends were not available from Spain and Sweden.



	Belgium (N=2)	France (N=9)	Germany (N=9)	Italy (N=2)	UK (N=3)
Chest surgery	1 (50.0%)	2 (22.2%)	2 (22.2%)	0 (0.0%)	0 (0,0%)
Chest-tube	1 (50.0%)	1 (11.1%)	3 (33.3%)	0 (0.0%)	1 (33,3%)
Chylous fluid drainage	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33,3%)
Inhalators	0 (0.0%)	0 (0.0%)	3 (33.3%)	0 (0.0%)	1 (33,3%)
Oxygen	1 (50.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0,0%)
Pleurodesis	0 (0.0%)	2 (22.2%)	2 (22.2%)	0 (0.0%)	2 (66,7%)
mTOR inhibitors	1 (50.0%)	9 (100.0%)	6 (66.7%)	2 (100.0%)	3 (100,0%)
Other	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33,3%)

Figure 19. LAM Treatments by Country.
Patients may receive more than one treatment.

Hospitalizations and Visits

For analyzing the frequency of hospitalizations and specialist visits, the subset of 143 patients included in the QoL research project was utilized. However, data from 11 patients from Spain was excluded due to data inconsistencies. Hence, this section is based on data of 132 patients in QoL research project in TOSCA. The detailed analysis in this section is provided in Supplementary materials in the publication.

Specialist visits

- Overall, 88 specialist visits were reported over 12 months during the last year.
- Half of the patients (n=69/132; 52.3%) had at least one specialist visit for TSC during the last year, and about one-fourth (n=29/132; 22.0%) had ≥3 visits.
- Non-TSC related specialist visits were reported for 34 patients (25.8%), and 14 of them (10.6%) reported ≥3 visits during the last year.
- Visits to the general practitioner (GP) were excluded from the analysis due to significant missing data.

Hospitalizations

- More than 70% patients did not have any hospitalization over 12 months during the last year.
- About one-third of patients (n=41/143; 28.7%) reported at least one hospitalization, and 6.3% (9/143) reported ≥3 hospitalizations.

Resource utilization

Information on the use of non-medical resources (education, employment, use of social services and patient support requirements) was collected within the QoL research project.

- Regarding education, 28 children (31.8%) were not attending a mainstream school, and the rest (n=57; 64.8%) were attending a mainstream school. Amongst those in mainstream schools, 64.9% (37/57) received special education within the school, and for 45.6% (26/57), the school offered special programs adequate to their condition.
- Impact on career was analyzed in a questionnaire, which was completed by 55 adult patients and 88 carers for children with TSC.
    - Less than half of the adult patients (n=23/55; 41.8%) and 65.9% of children's carers (n=58/88) reported to have a job.
    - However, a quarter of the adult patients (n=14/55; 25.5%) and 9.1% of children's carers (n=8/88) reported that they were not able to work due to TSC.
    - Half of the adult patients (n=28/55; 50.9%) and 56.8% of the children's carers (n=50.88) stated that TSC had an impact on their career.
- Half of the children (n=45/88; 51.1%) and 38.2% of the adults (n=21/55) received a disability allowance.
- Furthermore, 20% of the adults (n=11/55) received support with daily activities.
- Other services such as psychological counseling, social services, and social worker services were received by <15% of the patients, irrespective of their age.

3.1.5 Discussion

The research analyzed treatment patterns and use of medical/non-medical resources in patients enrolled in the TOSCA registry. While other single nation studies with limited number of patients of certain age-groups or specific manifestations have already been conducted,(33-39) TOSCA offered a promising opportunity to look at the larger scenario, owing to its international reach across 31 countries and patients of all ages and manifestations.

This analysis reveals that the treatment patterns vary not only with the clinical manifestation but also with the age and country. An example is the drastic difference in the use of mTOR inhibitors in patients with SEGA across countries (ranging from none in the UK to 100% in Spain), and also with the age of the patients (ranging from 70% in patients aged 5–9 to 0% in patients aged >40).

The country-wise variation in treatment utilization does not only indicate variation in clinical practice, but also access to different treatment options. This is particularly true for mTOR inhibitors, which were available at different time points for the various indications in specific countries and/or healthcare systems. For instance, the reimbursement for everolimus for FS was initiated in January 2017 in Germany and April 2018 in Sweden, but this did not happen until late 2018 or the beginning of 2019 in the rest of European countries. Similarly, in patients with SEGA, everolimus was reimbursed beginning from October 2011 in Germany and in the UK only through the Individual Funding Request (IFR) route, while it was not available until 2016 in Italy and in Belgium. Similar is the case for everolimus in rAML management.

Differences in age groups may also have translated in differences in clinical practice between pediatric and adult neurologists in those manifestations treated before the TOSCA registry and within the time horizon of the TOSCA registry (i.e., after baseline). In TOSCA, the most commonly prescribed treatment for epilepsy was GABAergics, which was in line with the current guidelines (40,41) recommending vigabatrin as a first-line antiepileptic drug treatment in patients with TSC and either IS or FS in patients <1 year. However, these results should be interpreted carefully, considering that GABAergics as a category included many antiepileptic drugs. Furthermore, there was a large proportion of treatments included in the category “others” (at baseline, 44.4% for IS and 66.5% for FS). In future studies, more attention should, therefore, be paid to the definition of treatment variables.

Another noteworthy point is that enrolment in TOSCA was started in August 2012, and data was locked in August 2017. However, everolimus received EMA approval for the treatment of drug-resistant epilepsy as late as in January 2017. Hence, the impact of this approval on treatment patterns could not be evaluated with these results. However, it is also to be noted that in TSC-associated refractory seizures, everolimus was being increasingly used by many clinicians. It may be attributed to the on-going mTOR studies in epilepsy and other TSC-associated conditions.

Our analysis also reveals an increasing use of mTOR inhibitors in wide range of TSC manifestations including SEGA, LAM, and rAML. Considering the fact that TSC is a multi-organ condition, and different manifestations can co-occur in one patient, it might not be correct to attribute the use of mTOR inhibitors to a single manifestation. For example, the

use of mTOR inhibitors in LAM might be attributed to its use for other indications in patients with TSC.

According to this analysis, the increase in use of mTOR inhibitors was simultaneous with the decline in surgery. These trends are noted over the registry duration, across countries and age groups. In age groups and countries where mTOR inhibitors are used more commonly, surgery is less commonly utilized. This is in line with current recommendations in SEGA(40,42), which indicate surgical resection for acutely symptomatic SEGA, both surgery and mTOR inhibitors for growing but asymptomatic SEGA and mTOR inhibitors for large or bilateral SEGA unfit for surgical resection.

Although one may expect economic impact of delaying or avoiding surgery on overall management costs, these changes were not evaluated in this analysis. A study (43) has demonstrated that medication and total costs after SEGA surgery in TSC patients in the post-surgery year were 1.6–4.3 times the costs in the pre-surgery year. However, economic evaluations comparing surgery and mTOR inhibitors in patients with SEGA have not been yet conducted.

The use of surgery in patients with SEGA in the TOSCA registry was lower than reported in a previous survey conducted by Rentz et al (38), which did not report use of mTOR inhibitors. Hence, comparing the use of medical resources and in particular the use of surgery depending on whether the patients receive treatment with mTOR inhibitors may offer valuable insights.

The treatment trends observed in rAML and LAM are similar to those in SEGA. These trends should be interpreted carefully, owing to the existence of multiple manifestations in the same patient.

A potential reason for increasing use of mTOR inhibitors in LAM may be attributed to their inclusion in the recent international guidelines for the diagnosis and management of LAM (44), where mTOR inhibitors were recommended for patients with abnormal or declining lung function or with problematic chylous effusions that could have affected the treatment patterns.

Since TSC is a multi-organ disease, a systemic mTOR inhibitor for one manifestation may also exert impact on surgical intervention for other manifestations. Concomitant systemic effects in patients treated with mTOR inhibitors have been reported.(45) Although these effects have directly not been yet analyzed, our analysis supports this hypothesis.

While our analysis demonstrates that patients with TSC are demanding healthcare resource users, an uneven distribution of use of resources across patients and countries is also evident. For example, while one-third of the patients included in the QoL research project did not have any TSC-associated specialist visit, a quarter of these patients had ≥3 specialist visit during the same period. Similarly, while 71% of the patients were not hospitalized at any time, up to 6.3% were hospitalized ≥3 times during the past year. Hence, identifying patients who are expected to be intense resource users might help in better resource allocation in TSC management.

Our analysis demonstrates a significant impact of TSC on education and employability. More than half of the children had special needs (either not in a mainstream school or

received special education within their school). Unemployment rates were high both in adult patients (up to 50%) and caregivers of children with TSC (34.1%). Hence, TSC diagnosis has vast economic impact. A study from France (44) that included adult patients with TSC and with a diagnosed epilepsy before 16 years old has reported that 52% of patients required special education programs and only 37% reported having a stable professional life, even though 65% of them had a salary below the minimum income threshold in France.

In our analysis, very low proportion of patients received psychological support, which was also observed in a multicenter French study.(46) This is contrary to the expected rates of TAND and suggests that the psychological needs of patients are not being addressed adequately. While lack of physicians' unawareness and clear guidelines on TAND evaluation before 2013 may have led to lower reported rates of TAND, the 2005 guidelines(47) were also not effectively implemented. Lower rates of disability allowances, social worker services and routine support suggest the lack of awareness among the TSC patients regarding availability of such support or rather lack of them in some countries.

The major strength of TOSCA was the prospective follow-up of TSC patients which allowed tracing changes in treatment patterns over time. While data at last two follow-up visits were available for much less patients (764 and 147 patients) than those at baseline (2,211), many other studies have been conducted with much lower sample populations.(35,48) Nevertheless, the results from last two visits in TOSCA should be interpreted cautiously.

Our analysis has some limitations. The major limitation is very low proportion of patients included in resource use section (<10% from TOSCA were included in QoL research project). This is in contrast with excellent data quality for the medical aspects of TSC recorded in the core study, which may be attributed to observatory but not mandatory nature of data collection and lack of site monitoring review of the QoL research project data collection. Carrying out specific studies to broaden the evidence on the use of medical resources in patients with TSC remains an interesting topic for future research.

Being an observational study, TOSCA collected only available data from standard clinical practice. Since the study sites were mostly centers with expertise in TSC, patients with milder symptoms might have been underestimated. Lack of homogeneous data collection in routine practice is another issue of concern. However, including specialists and multiple centers allowed TOSCA to enroll a significant number of TSC patients, which should be representative of real clinical practice.

Direct comparisons of resource usage in TOSCA and other studies in TSC are not feasible. This is because cost estimations in TOSCA were not performed due to its multinational nature. Furthermore, differences in study design in TOSCA and other studies make this task impractical. Another concern is that very few studies have included overall patients with TSC without specifying a certain manifestation, which is the case with TOSCA.

According to our analysis, treatment patterns varied with clinical manifestations, age, period and country. Hence, comparing these treatment patterns with individual studies,

without considering these baseline characteristics might be methodologically inappropriate.

Data regarding healthcare visits and hospitalizations, as well as about use of non-medical resources, was derived from a limited sample of patients, all of which were residing in Europe. Hence, extrapolation of these results to generalize for other parts of the world might be a realistic approach.

Comparing the use of medical resources in patients with TSC treated with or without mTOR inhibitors remains another area of interest for future research. Data regarding use medical and non-medical resources in the QoL research project was derived from a patient and caregiver survey, which may have inconsistencies due to lack of understanding or recall on the patient's part. Hence, any future studies in this domain should consider supervision of such surveys by staff to ensure data completion.

3.1.6 Conclusion

Despite the limitations mentioned, this analysis has provided insights about treatment patterns and current use of medical and non-medical resources in a large cohort of patients with TSC followed for a long period of time in seven European countries. It also demonstrated the gaining popularity of mTOR inhibitors for certain TSC related manifestations, often accompanied by reductions in surgical interventions. This analysis strengthens the fact of high consumption of medical and non-medical resources by patients with TSC. Whether the use of mTOR inhibitors impacts the use of other resources, particularly surgery and further economic impact, requires more systematic research.

3.2 Paper 2

The TOSCA Registry for Tuberous Sclerosis—Lessons Learnt for Future Registry Development in Rare and Complex Diseases

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The TOSCA Registry for Tuberous Sclerosis—Lessons Learnt for Future Registry Development in Rare and Complex Diseases

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The TOSCA Registry for Tuberous Sclerosis—Lessons Learnt for Future Registry Development in Rare and Complex Diseases

3.2.1 Abstract

Introduction

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) is an international disease registry aimed to improve the understanding about the clinical manifestations of TSC. This research was aimed to identify issues/concerns encountered during the design, execution, and publication phases of TOSCA. The research also reflects on lessons learnt from this registry that may guide future registries in rare and complex diseases.

Methods

This questionnaire based survey was conducted amongst 511 people involved in the registry, including 28 members of the Scientific Advisory Board (SAB), 162 principal investigators (PIs), and 321 employees of the sponsor belonging to the medical department or that were clinical research associate (CRA). A questionnaire (Appendix 1) specifically designed to identify the strengths, weaknesses, and issues that arose at any stage of development and implementation of the TOSCA registry, was sent by email to these participants. The questionnaire, with a total of 225 questions, was divided into 7 sections (identification of issues during registry planning, during the operation of the registry, during data analysis, during the publication of the results, other issues, assessment of lessons learnt, and additional comments). Questionnaires received within 2 months from the date of sending initial emails were included in the analysis.

Results

Overall, 53 (10.4%) questionnaires were received (64.3% for SAB members, 12.3% for PIs and 4.7% for employees of the sponsor). The overall completeness rate for closed questions was 87.6%. Among the most commonly identified concern in this registry were the limited duration of the registry (38%) and issues related to handling of missing data (32%). Furthermore, 25% of the respondents were concerned about the potential biases, which might have affected the validity of registry outcomes. Most of the respondents (>80%) agreed that TOSCA registry improved our understanding of the natural history and manifestations of TSC, increased disease awareness and also identified relevant information which may assist in further clinical research in TSC.

Conclusions

This research highlights the potential of registries as a powerful tool to increase disease awareness, gather real-world evidence, and offer directions for future research. The survey emphasizes the need to implement strategies to ensure patient retention and long-term sustainability of patient registries, to improve data quality, and to minimize potential biases.

Keywords

lessons, issues, strengths, weaknesses, TOSCA, registry, TSC

3.2.2Introduction

Patient registries are organized observational data collection systems that collect uniform data on a patient population defined by a particular disease, exposure or condition (e.g., age, pregnancy, specific patient characteristics).(4) Patient registries may provide insights into disease pathology and manifestations, human and economic burden of the disease, as well as clinical practice patterns. They may also help to identify patient subgroups for future clinical trials and to generate new research questions.(1) Hence, patient registries are instrumental in clinical research, and improving patient care and healthcare planning, particularly in the field of rare diseases. Despite such potential usefulness, patient registries have several limitations, particularly the potential biases, lack of standardization in data collection, accuracy, and comprehensiveness of the data, fragmentation of clinical data, and ethical concerns.(4) Since most registries are conducted in one or limited number of countries,(14) there generalizability is limited. Moreover, the academic initiation of many registries may limit their acceptance for pharmaceutical research. Hence, efforts to ensure data quality may improve acceptability of patient registries as scientific evidence in medical research.

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) is a multicenter, international disease registry that was designed to assess manifestations, interventions, and outcomes in patients with TSC, a rare genetic disorder characterized by growth of hamartomas in several organs.(24) TOSCA enrolled 2,216 patients with TSC at 170 sites across 30 countries worldwide between 2012 and 2014. Patients of any age diagnosed with TSC, who had a documented visit for TSC within the preceding 12 months or newly diagnosed patients were included. All enrolled patients signed an informed

consent form (ICF) approved by local ethic committee (EC)/institutional review board (IRB). Patients' data were collected at baseline visit and at five annual follow-up visits and recorded by principal investigators (PIs) in an electronic clinical database. The data was locked in 2017. (24)

The TOSCA registry design consisted of a main 'core' part and a number of sub-studies (referred to as 'research projects' or 'petal projects'). The 'core' section was designed to collect a general predefined set of patient data including demographics, family history, prenatal history, and disease features (i.e. neurological, neuropsychiatric, renal, cardiovascular, and pulmonary). Additional and more detailed data related to specific disease manifestations were collected in the research projects of the registry. Furthermore, following the EMA request (EMEA/H/C/002311/II/0004), a post approval safety study (PASS) was included to document the long-term safety and tolerability profile of Votubia® in the treatment of TSC patients. Clinical study protocol and final study results are available on ENCePP portal at http://www.encepp.eu/ (EU PAS Register Number EUPAS324). (24)

The TOSCA registry was funded, designed and managed by a pharmaceutical sponsor (Novartis), with the support of a Scientific Advisory Board (SAB), a Working Committee (WC), and Research Groups, the composition and responsibilities of these are explained below.

- The SAB consisted of up to 30 members, including TSC healthcare professionals, patient representatives and a maximum of three representatives of the sponsor (Novartis). The medical experts were selected based on the number of publications in TSC, research interests and working in reference sites for TSC in their country.

Patient representatives were included as well to ensure that their perspective is considered in the project design and execution. The SAB was responsible for the scientific principles of the registry, the promotion of the use of the registry, the publication of data, and the approval of research projects.

- The WC was a subgroup consisting of up to 14 members from the SAB and was responsible for the registry content and coordination of all the operational activities, for defining the statistical analysis plan and publication policy, and for developing and maintaining the database structure of the registry.
- Research groups were made up of physicians participating in the registry and their role consists on the submission of research project proposals to the WC, together with the subsequent management of that particular project.

TOSCA was well suited for this research for its large patient population size, worldwide scope (including European and non-European countries), involvement of experienced personnel from healthcare professionals, patient representatives and pharmaceutical industry, including research projects and a PASS sub-study, and the long-term follow-up (up to 5 years). Hence, the content and structure of TOSCA offered an excellent opportunity to identify the potential issues while conducting a patient registry and providing adequate ways to bypass these issues.

3.2.3Objective

This research had two main objectives:

- to identify issues that arose during the design and operation of the TOSCA registry as well as during the interpretation and publication of the results; and

- to identify areas for improvement and pitfalls to be avoided which may benefit future registries in rare and complex diseases.

3.2.3 Methodology

A questionnaire was developed considering the aim to identify issues that might have arisen at any stage of the TOSCA registry project from its inception to the publication of the results, and to identify its strengths and weakness, and opportunities and threats that could be of interest for the development of future registries in rare diseases. The questionnaire was developed by the TOSCA clinical trial head with contribution of patient representatives, members of SAB, and Novartis quantitative safety and epidemiology department. The questionnaire was built following a guide aimed to support the design, implementation, analysis, interpretation, and quality evaluation of registries published by Gliklich et al. (1) The questions included were prepared based on the steps to conduct a registry described in this guideline and the specific TOSCA registry project characteristics.

Overall, the questionnaire comprised of 225 questions. These questions were split into seven sections; the first five of which covered a range of aspects related to issues during the registry (planning, operation, data analysis, results publication, and other issues), and the last two were devoted to assess lessons learnt from the TOSCA registry and to gather additional comments. (Table 10)

Table 10. Structure of questionnaire

1) Identification of issues during registry planning • *Perception on the definition of the purpose and the objectives of the registry* • *Perception on the definition of the inclusion/exclusion criteria* • *Definition of the variables included in the registry* • *Definition of the size, the duration, the setting and the geographical areas* • *Identification of stakeholders, team building and establishment of a governance* • *Data access & use of data* • *Publication plan* • *Development of the protocol and related documents* • *Development of the project plan* • *Development of risk management plans & risk management during the registry*

2) Identification of issues during the operation of the registry
• *Issues related to patient recruitment or retention*
  ○ *Barriers to patient recruitment/retention*
  ○ *Evaluation of success of patient recruitment strategies*
  ○ *Evaluation of success of patient retention strategies*
  ○ *Evaluation of center/physician or patient selection bias*
• *Issues related to data collection & quality assurance*
  ○ *Issues related to data collection*
  ○ *Identification of quality issues & timing for detection*
• *Issues related to budget*
• *Issues related to project management*
  ○ *Ownership & accountability*
  ○ *Coordination*
  ○ *Estimation of the use of resources/duration/complexity*

3) Issues during data analysis • *Identification of sources of bias* • *Treatment of missing data* • *Appropriateness of time horizon & planned interim analysis* • *Appropriateness of pre-specified analyses* • *Interpretation of the results* • *Identification of issues related to data access* • *Identification of strengths & limitations of the registry*
4) Issues during the publication of the results
5) Other issues

6) Assessment of learnings
• *General learning topics*
• *Value of the registry organization*
  ○ *Inclusion of patients in the SAB and in the WC*
  ○ *Inclusion of clinicians in the SAB and in the WC*

○ *Inclusion of members from the pharmaceutical industry in the SAB and in the WC* • *Pitfalls and learning opportunities emerged from the integration of research projects within the TOSCA registry* • *Pitfalls and learning opportunities emerged from the integration of a Votubia® PASS within the TOSCA registry*
7) Additional comments

The questionnaire was sent over email to 511 people involved in TOSCA on September 7th, 2018. Among these 511 people, 28 were part of SAB, while 162 were PIs and 321 were Novartis employees not included in the SAB. Although all of 511 participants received the same questionnaire, some questions were directed only to certain people involved that particular fields. For example, participants who were not involved in budget planning, allocation and/or control, were directed to skip the subsequent questions regarding these topics. To facilitate the analysis, most questions were close-ended ("yes"/"no" or using a Likert scale). Besides, all the questions contained "N/A" (not applicable) option and a free-text field, where the participants were encouraged to justify their answers. The participants were given two months for replying and two reminders were sent. No remuneration was offered to participants.

A time period of two months from the date of initial email (cut-off date: 8th November 2018) with the questionnaire was considered for including the response of participants. All questionnaires received during this time period were considered in the analysis. All data were analyzed using Microsoft Excel. Relative and absolute frequencies were analyzed for all the questions, and whenever possible, for the groups of questions belonging to the same section or subsection.

3.2.4Results

Participants/Respondents

In total, 53 filled questionnaires were received (response rate: 10.4%). The respondents included 18/28 of SAB members including Novartis representatives (response rate: 64.3%), 20/162 of PIs not included in SAB (response rate: 12.3%) and 15/321 of other Novartis employees not included in the SAB (response rate: 4.7%). The overall rate of completion of the questionnaire was 88% for closed-ended questions. Average amount of missing data per question was 12% (range 2%–30%). The rates of missing data were 4% for SAB members, 4% for PIs and 7% for other Novartis employees.

Identification of Issues

The survey response with respect to issues faced across the main stages of TOSCA (i.e., registry planning, operation, data analysis, publication and others), along with the percentage of respondents reporting that particular issue is summarized in Figure 20. Survey questions that did not receive any rating as a potential issue are excluded from Figure 20. Such questions were pertaining to the identification of clinicians to lead the research projects or to delays in the development of the registry due to patient identification. Additionally, none of the respondents reported any issue regarding the extent of involvement of WC members in the protocol and related documents, in the documentation of protocol amendments, or timely intimation of such amendments to the respondents. Moreover, no issues were reported regarding registry oversight or the adverse event collection/reporting processes.

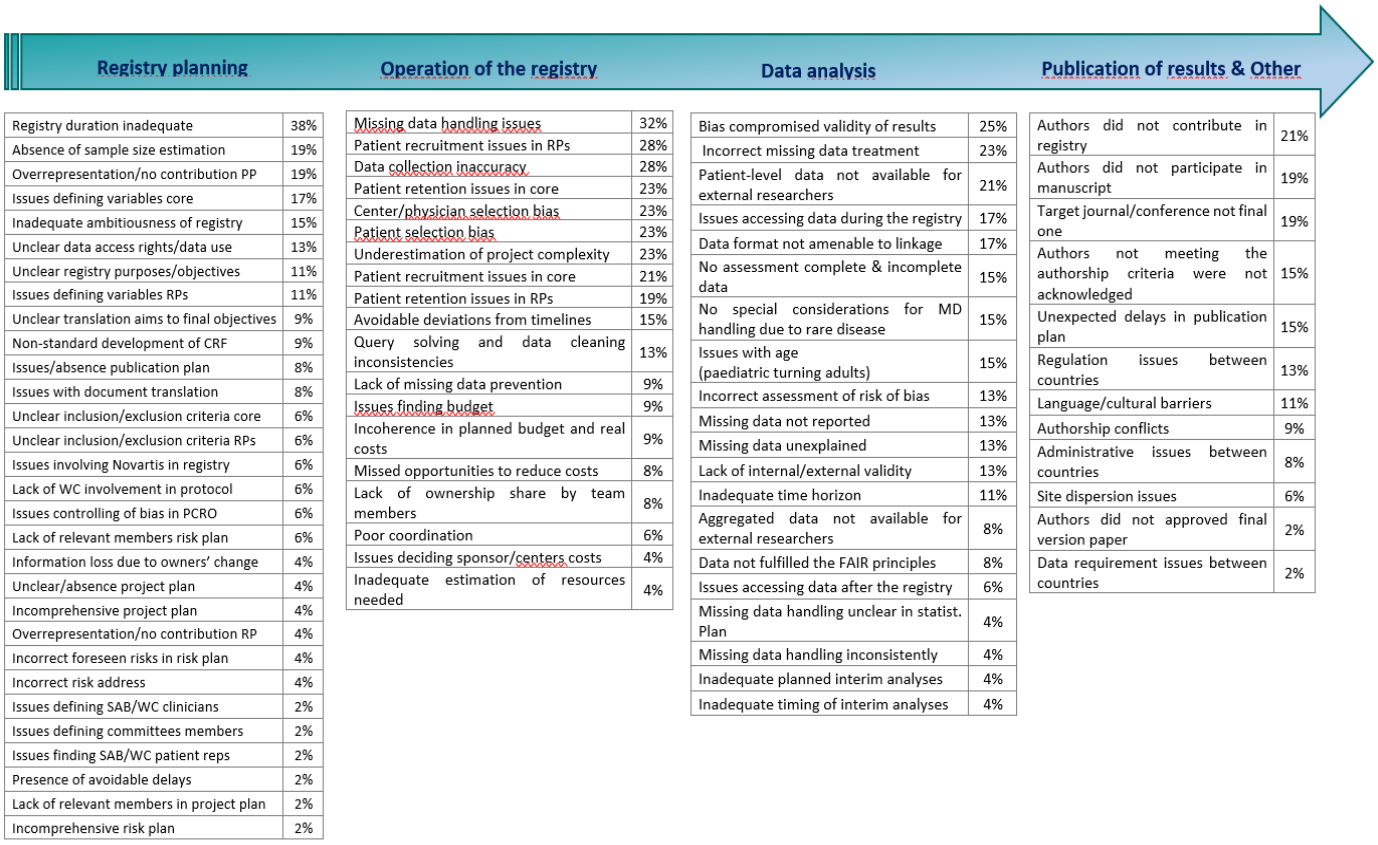

Figure 20. Typology and Weight of Issues derived from the Different Stages within the TOSCA Registry.
CRF, case report form; FAIR, Findable, Accessible, Interoperable and Reusable (data); MD, missing data; PCRO, patient-caregiver reported outcomes; PP, project plan; RP, research project; SAB,Scientific Advisory Board; WC,WorkingCommittee.

Registry Planning

The most common issue highlighted in the survey was the limited duration of the registry (up to 5 years) and 38% respondents reported this concern. Respondents agreed regarding the need for a long-term registry and some respondents stated that a longer follow-up might have captured a more realistic impact of the disease. Constraints,

including budget limitations, affected the data collection and lead to significant missing data from follow-up 3.

Respondents considered the registry too ambitious in terms of recruitment, duration or compliance and its long-term sustainability unrealistic. Among the lowest-rated issues with registry planning were timeline delays, risk, and project plan problems and issues when defining SAB-WC members.

Operation of the Registry

With regards to registry operation, missing data were the most rated by respondents (32%) as an issue of concern. However, it is noteworthy that most data missing were related to TSC-Associated Neuropsychiatric Disorders (TAND), which may be attributed to the lack of knowledge of these TSC manifestations by the physicians, or patient/caregiver reported outcomes. Few respondents reported issues related to resources and costs, which were mostly due to budget restraints, particularly toward the research projects.

Data Analysis

The most reported issues in data analysis were the effect of bias on the results validity (25%), and inappropriate handling of missing data (23%). Majority of the respondents (51%) agreed on the presence of some type of bias, either selection bias, information bias and/or measurement bias. Potential solutions to these issues suggested by respondents included involvement of statisticians for the whole registry from its conception, budget extensions or further monitoring during data collection. Among the

least reported issues were related to interim analyses (4%) and inconsistency in missing data handling (4%).

Publication of Results and Other Issues

The most commonly reported issues were the lack of contribution of authors in the TOSCA registry itself (21%) and the lack of author participation in manuscripts (19%). Issues related to data requirements across different countries and lack of final author approval of publications were considered to be least important issues (2% respondents for each issue). Overall, authorship conflicts were reported only by 9% respondents.

Assessment of Lessons Learnt From TOSCA Registry

The contributions of the TOSCA registry to the field of TSC, along with the agreement rates of respondents to these contributions, have been summarized in Table 11. Overall, the rates of completeness for this section of questionnaire were high, with an average rate of missing data of 5% per question (range 2–15%), which was attributed to either lack of recall or access to data. More than 80% of the survey respondents agreed that TOSCA improved the knowledge on the natural history and manifestations of TSC, increased the awareness of the disease and helped to identify information relevant to clinical research. Despite the overall consensus in positive contribution of TOSCA, one respondent stated that the contribution was relatively small considering the cost and time spent in the registry.

Most respondents considered the inclusion of different groups (TSC experts [reported by 84%], the pharmaceutical industry [reported by 75%] and patient representatives [reported by 59%]) in the SAB and the WC as either important or very important.

Overall, more than 75% of the respondents considered the inclusion of patient representatives to be valuable in facilitating communication. However, some respondents reported concerns with inclusion of patient representatives such as ethical issues (reported by 6%) or confidentiality issues (reported by 6%). Interestingly, 17% respondents indicated the wish to increase the number of patient representatives in the SAB/WC, especially if they had medical background.

There was a clear consensus (>90% respondents) regarding inclusion of TSC experts in the SAB and the WC, especially to provide interpretation of results, to propose the collection of variables and analyses of medical interest and to improve the quality of publications. However, respondents considered the overall number of TSC experts to be too high in both in the WC and SAB.

More that 80% respondents reported inclusion of members of pharmaceutical industry in the SAB and the WC as important or very important, especially to provide technical, and/or financial support in the dissemination and publication of the results. However, few respondents indicated the inclusion of different pharmaceutical companies as well as members with more specific skills (e.g., statistics, medical, operational, data management) necessary.

Table 11. Assessment of lessons learnt derived from the TOSCA registry (N=53)

TOSCA registry contributions	Yes	No		Missing	N/A
		It was intended	It was unintended		
Improvement of knowledge on the natural history of TSC and its manifestations	47 (89%)	3 (6%)	0 (0%)	1 (2%)	2 (4%)
Increase disease awareness	46 (87%)	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Identification of useful information for the development of clinical research in TSC	44 (83%)	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Trigger research questions/developing hypothesis for new research in TSC	41 (77%)	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Improvement of epidemiological knowledge of TSC	40 (75%)	2 (4%)	7 (13%)	1 (2%)	3 (6%)
Foster the communication between TSC experts and Novartis	40 (75%)	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Improvement of knowledge on the clinical management of the disease in different countries	38 (72%)	3 (6%)	7 (13%)	1 (2%)	4 (8%)
Provision of data on quality of life	38 (72%)	3 (6%)	8 (15%)	2 (4%)	2 (4%)
Identification of useful information for the development of studies involving large/diverse geographic areas	38 (72%)	3 (6%)	6 (11%)	2 (4%)	3 (6%)

Foster the communication between TSC experts	38 (72%)	3 (6%)	7 (13%)	3 (6%)	2 (4%)
Provision of data on the effectiveness & efficiency of interventions in the real world	37 (70%)	7 (13%)	5 (9%)	2 (4%)	2 (4%)
Improvement of clinical practice	37 (70%)	4 (8%)	7 (13%)	2 (4%)	3 (6%)
Quantification of the use of resources and the burden of the disease	37 (70%)	7 (13%)	3 (6%)	2 (4%)	4 (8%)
Identification of centers/physicians treating patients with TSC	35 (66%)	5 (9%)	7 (13%)	4 (8%)	2 (4%)
Identification of useful information for the development of studies in pediatric patients	34 (64%)	3 (6%)	10 (19%)	2 (4%)	3 (6%)
Foster the communication between TSC experts and patients	34 (64%)	4 (8%)	7 (13%)	4 (8%)	3 (6%)
Assessment of the agreement between clinical practice and guidelines	33 (62%)	7 (13%)	9 (17%)	2 (4%)	2 (4%)
Provision of data on the safety of the interventions in patients with TSC in the real world	31 (58%)	7 (13%)	10 (19%)	3 (6%)	2 (4%)
Improvement of health care planning & resource allocation	31 (58%)	9 (17%)	8 (15%)	2 (4%)	3 (6%)
Development of new clinical practice guidelines	30 (57%)	8 (15%)	9 (17%)	3 (6%)	3 (6%)
Identification of patients with TSC that might benefit from certain interventions	30 (57%)	8 (15%)	9 (17%)	3 (6%)	3 (6%)

or might be included in future clinical trials					
Identification of useful information for the development of clinical research in other rare diseases	28 (53%)	6 (11%)	10 (19%)	4 (8%)	4 (8%)
Foster the communication between TSC patients and Novartis	24 (45%)	7 (13%)	12 (23%)	6 (11%)	3 (6%)
Facilitation of market access for Votubia®	23 (43%)	6 (11%)	10 (19%)	8 (15%)	4 (8%)

Pitfalls and Lessons Learnt From the Integration of Research Projects within the TOSCA Registry

Overall, 57% respondents agreed that inclusion of research projects in TOSCA registry was appropriate. These research projects ensured extensive data collection and were multidisciplinary in nature, hence allowing in depth analysis of specific TSC manifestations and areas. However, respondents also considered research projects to be complex, and burdensome. It was agreed that instead of including research projects as a protocol amendment at a later stage, they should be considered at the registry planning stage. Other reported pitfalls included the lack of publications and statistical plans, along with budget constraints and patient retention. While 38% of respondents agreed with separation of the core from the research projects, 17% respondents also stated such separation to cause delays and agreed that both the core and the research projects should have been done simultaneously. The efficiency in resource management in

research projects was controversial. While 28% respondents considered the management efficient, 23% reported it was not so.

There was lack of agreement regarding the contents of the core and the research projects, and whether some variables in the core registry should have been included in the research projects, and vice versa. Similarly, no consensus was reached regarding extent of missing data in core and research projects. On similar lines, whether the number of participants in each research projects was adequate to answer clinically relevant questions, was debatable. However, 38% of the respondents reported that results from the research projects could be extrapolated to all the respondents in the core registry, and 43% stated that results from the research projects would be representative of real world. Finally, 17% respondents agreed that research projects provided striking or relevant results while 13% did not agree. Among those who stated that the research projects provided relevant findings, these were related to the impact on renal angiomyolipoma (rAML), the effects of subependymal giant cell astrocytoma (SEGA) in adults, the results obtained in TAND and aspects related to quality of life.

Pitfalls and Lessons Learnt From the Integration Everolimus, Votubia® PASS (Post Authorization Safety Study) Within the TOSCA Registry

The survey questionnaire also included some questions related to the Votubia® PASS study, which was conducted to evaluate the long-term safety profile of everolimus, an orphan drug directed to treat SEGA, rAML and seizures that did not respond to other treatments. About half of the respondents (43%) considered appropriate to integrate the PASS study within the TOSCA registry, mainly due to efficiency gains such as better

surveillance, retention, recruitment, and long-term effects of adverse events. However, some pitfalls also emerged from this integration, as the extra workload imposed by PASS within TOSCA design, the characterization of PASS as a sub-study of TOSCA and the important differences between both studies (e.g., administrative, reporting, regulatory requirements).

Approximately 30% (range 26–34%) of respondents agreed on the convenience of separating the elaboration, data collection, and approval of both the PASS and TOSCA, and 32% of the respondents considered that there was a good management of time and resources in PASS.

About 19% respondents stated that there were no variables in PASS that should have been collected in the core registry or vice versa. Data quality and completeness was considered worse in TOSCA than in PASS by 21% respondents. Whether the number of participants in PASS was adequate was another matter of discrepancy among respondents; 13% of respondents reported they were sufficient vs. 9% who considered the sample unrepresentative (60% said “N/A”, 17% were missing). About 17% respondents considered the results in PASS representative of the whole TOSCA population and 25% respondents considered it to be translatable into real world.

Respondents had mixed opinions regarding the dissemination of results (11% said ‘yes’, 8% said ‘no’, 62% reported ‘N/A’, 19% were missing). No consensus was reached regarding the potential benefit on the TOSCA registry derived by the interaction of health authorities during the PASS.

3.2.5. Discussion

This questionnaire based survey identified the main issues that arose during TOSCA registry from its inception to the publication of the results. It also identified the lessons that could be relevant to the design and development of future registries in rare and complex diseases.

The most desirable aspect of TOSCA, as agreed by all respondents was involvement of a range of stakeholders (including TSC experts, members from industry, and patients). This ensured considerations of different perspectives, and hence, the registry was able to incorporate variables of interest for physicians, pharmaceutical industry, as well as the patients.

In TOSCA, patient representatives in the SAB were considered instrumental in facilitating communication of the results to advocacy groups, and also in increasing public awareness on the disease. Many other successful registries have had an active participation of patient representatives in its design, governance and/or operation. Some such examples are the Improve Care Now network for inflammatory bowel disease in the United States (49), the Parkinson Net Approach in the Netherlands (50), and the TREAT-NMD European network for neuromuscular disorders (51).

Adopting standard operating procedures helped successful operations of TOSCA registry, as no issues were reported regarding registry oversight, adverse event collection/reporting processes (only related to the PASS sub-study), or project management. Such approach may also help avoid issues in future registries.

Another highlight in TOSCA was high patient recruitment rate in the core project. Different recruitment strategies were adopted across countries which included phone contacts, proposal of participation in scheduled visits, exploitation of local patient databases, targeted mailing and newsletters to the investigators, virtual investigator meetings and the contacts with local patients' associations and family groups.

On the contrast, patient retention was relatively poor. After 3 years follow up, some sites stopped reporting data on a regular basis and a high number of patients (93.5%) discontinued. This occurred despite about one-third of the respondents preferring the TOSCA registry to have a longer duration or even to be permanent. The contrast between the low retention rates and the high expectations highlights the need for realistic goals when setting up a registry, but also the need for continuous motivation, adequate budget, and close oversight for registries that are expected to last longer than one or 2 years. Since patient discontinuation and long term sustainability are commonly encountered issues in all the registries, strategies to reduce losses to follow-up are urgently needed.

Missing data collection was the most common issue, especially in the last follow-up visits. Conducting a pilot study may help ensure optimal question design and minimize missing data. Other strategies to minimize missing data or handling are describe detailed mechanisms for identifying and collecting missing data in the registry protocol, to distinguish between nice-to-have, and essential data (as in TOSCA study management document like the CRF manual and monitoring plan) and to describe the handling of missing data in the statistical analysis plan (as in TOSCA study management documents). (24)

Despite being an international registry across 31 countries, language related issues did not arise in TOSCA. These issues were minimized by provision of study oversight and site support in local languages including the discussion of the protocol and the electronic case report forms (eCRFs) requirements. However, it was suggested that in future multinational projects, agreeing, and defining each term or concept with representatives from each country and language may further avoid any issue related to a mistranslation.

The most common issue of concern in data analysis was bias. Registries, in general, are prone to many biases. In TOSCA, several respondents stated that disease burden might be overestimated due to selection bias towards patients with more severe manifestations in large hospitals and referral centers. Furthermore, an overrepresentation of pediatric neurologists might have introduced selection bias. However, it is to be noted that the eCRF had some specialty-specific questions, which could not be answered properly by all the participants. Hence, data collection for some specialties such as dermatology or ophthalmology was not completely reliable.

It is imperative that future registries ensure an adequately homogeneous patient enrolment. Also, the investigators should be a representative sample of the physicians treating that condition, and able to properly assess all the variables. Hence, reducing bias requires the participation of statisticians during registry planning, a careful site and PI selection across countries and also an increased and continuous support at site level to understand study requirements and eCRF questions. All the publications of TOSCA have mentioned that TOSCA is not an epidemiological study, but a very large cohort study.

In addition to potential biases and missing data issues, data access was another area where some difficulties were faced, as many believed that the terms of data access was not clearly described. Hence, further involvement of all stakeholders while defining terms of data access should be encouraged.

According to the EMA guidance, “clarity is needed regarding data ownership, including patients’ wishes regarding the use of their data”. (4) None of the previous registries have analyzed issues during the data publication process. In this research, 9% of the respondents reported authorship conflicts, the most common of which was poor involvement of some authors in the manuscripts or the lack of acknowledgment for all the contributors. Hence, the authorship criteria should be based on actual contribution rather than pre-signed agreements. Furthermore, the significant deviations between the planned and the expected journals for the publication suggest that delay in publications may be avoided by setting a realistic target journal. In addition, difficulties faced during compiling publications from annual follow-up visits should also be considered while devising a publication plan.

While most of respondent believed that addition of research projects in the main registry was beneficial in terms of the knowledge gained, the lack of statistical and publication plans as well as financial restraints, the results of research projects might have been limited. Also, most respondents believed that the research projects were not well-handled. Hence, better planning of registry based studies should be ensured, including detailed budget planning in all project proposals. The EMA guidance states a clear distinction between registry and registry study and necessitates separate protocols for all registry studies. (4)

On another point, most respondents considered that the data quality and completeness were worse in the TOSCA registry than in the PASS. While it is true that the aims of a PASS study are completely different from those in the TOSCA registry, a better integration of the TOSCA registry and the PASS could have been exploited to improve the data quality in TOSCA.

The research has some limitations. This research is primarily based on a single registry experience in patients with a one disease. However, these issues seem to be applicable to registries in other diseases as well. Also, very limited number of patient representatives had filled the questionnaire, owing to a lower percentage of patient representatives in the SAB. Furthermore, a high representation of SAB members in respondents group might have overestimated the related issues. Low response rates of the PIs and Novartis employees may be attributed to the perception on the burdensomeness of the questionnaire, the lack of economic compensation for the participants, a decreasing interest in the study or a lack of belief in the interest of such questionnaire.

A pilot questionnaire in future studies may help assess the validity and reliability of the questionnaire and to improve response rates. Finally, the questionnaire was designed and sent one year after the completion of the registry, which might have introduced recall biases. However, a retrospective analysis helped in obtaining a complete picture about the difficulties arisen throughout the project.

3.2.6 Conclusion

This analysis has provided the insights to the issues identified in TOSCA registry, which will help to foresee and prevent issues in the design and development of future

multinational registries in rare diseases. Meticulous planning, adequate monitoring and sufficient budget allocation are the key elements to the success of registries. However, continuous efforts to improve data quality, reduce biases, avoid access related issues, and ensure patient retention and long-term sustainability. Finally, this analysis further strengthens the impact of registries as a powerful tool to increase disease awareness, and provide a real-world view of clinical practice. The registry, however, has some limitations. Maintaining a balance between ambition, pragmatism, and costs while designing and operating a registry, is difficult but crucial.

3.3 Paper 3

TuberOus SClerosis registry to increase disease Awareness: A review on alignment of its planning, execution and publications with EMA guidelines

TuberOus SClerosis registry to increase disease Awareness: A review on alignment of its planning, execution and publications with EMA guidelines

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TuberOus SClerosis registry to increase disease Awareness: A review on alignment of its planning, execution and publications with EMA guidelines

3.3.1 Abstract

While patient registries are a powerful tool to gather real-world data, particularly in rare diseases, most of the guidelines issued do not address issues specific to rare disease patient registries. In November 2018, the European Medicines Agency (EMA) released a draft discussion paper on methodological and operational aspects of disease registries and made proposals on good registry practice. This highly anticipated guidance is expected to be gold standard for good registry practice and will encourage overall registry use in regulatory decision making.

TuberOus SClerosis registry to increase disease Awareness (TOSCA) was an international, multicenter patient registry to assess the manifestations, interventions and outcomes in patients with TSC. This registry was conducted (planning, patient enrolment, data collection and locking as well as final baseline analysis) before the abovementioned EMA guidance was released. Extensive feedback and lessons learnt from the TOSCA registry have provided insights into rare disease registry planning and operations.

In this paper, we tested the recommendations from the EMA guidance on TOSCA, in terms of compliance and deviations on a point-by-point basis. Close observation revealed that in most aspects, TOSCA complied well with the EMA guidance. However, there were several practical issues identified in TOSCA, which deviated from EMA guidance. These deviations highlight certain issues that have to be carefully considered in rare disease registries and deviations from EMA guidance do not necessarily compromise registry

quality. Despite multiple deviations of TOSCA from the EMA guidance, TOSCA was able to meet its objectives to enhance our understanding of TSC and its manifestations.

Keywords:

Tuberous sclerosis complex, rare disease, rare disease registry, patient registry, TOSCA

3.3.2Introduction

Role of patient registries in rare diseases

With limited number of patients who are geographically scattered and further phenotype diversity, rare diseases lack the typical disease based research for its basics as well as clinical drug development process.(52,53)These disease collectively pose a huge challenge and healthcare burden across the world.

Patient registries are a window to the real-world data (RWD) about the disease, and the treatment approaches.(52)The EMA frequently relies on patient registries to gather RWD on the risks and benefits of a particular product, as a condition to monitor post-marketing safety and efficacy, and as a condition for approval.(54)

Rare disease patient registries have a significant role in improving understanding of natural history, evolution, and manifestations of the diseases with a very limited number of patients. Not only they improve genetic and molecular research of rare diseases, they also help connect the patients and families who are facing similar challenges as well as clinicians working in the same disease area. These registries also help establish a patient

base for the evaluation of orphan drugs, and other such therapies, by serving as an active participation or as a historical control.(9)

The recognition of patient registries in rare diseases by the “EU Council Recommendation of 8 June 2009 on an action in the field of rare diseases” (55) demonstrates their importance. With such acknowledgements, the number of rare disease registries has been on a rise in the recent years.(13)According to the Orphanet Report Series Rare Disease Registries in Europe, May 2019, there are 69 global rare disease registries, 69 rare disease registries in Europe, 535 rare disease registries at National level and further at regional level.(14)

While the rare disease registries are being encouraged, there is a lack of harmonization in terms of the objectives, patient inclusion and exclusion criteria, the core data elements and overall data quality and completeness. Hence, a practical guidance with detailed consideration to all aspects of planning and execution is crucial for setting up a successful rare disease registry.(54)Furthermore, rare disease registries present unique challenges and issues different from patient registries in commonly prevalent diseases. Resolving the hurdles and limitations during planning and execution of rare disease registries may help stakeholders.

In general, several efforts have been made to standardize the patient registry setting and implementation. The European Union Committee of Experts on Rare Diseases (EUCERD) adopted a set of Recommendations on Rare Disease Patient Registration and Data Collection, in 2013.(10) Furthermore, many international projects, including EPIRARE and RD-CONNECT have been initiated to promote international registries.

Orphanet provides direct online access to an inventory and encyclopedia of rare diseases.(14) Similarly, the National Center of Rare Diseases in Italy have also released recommendations for improving the quality of rare diseases registry.(13)

In an attempt to expand the overall use of patient disease registries across all populations in the benefit-risk evaluation of medicines for regulatory purposes, the EMA supports a more systematic and standardized approach to planning and execution of all patient registries. In November 2018, the EMA issued a draft discussion paper on methodological and operational aspects of disease registries and made proposals on registry studies and good registry practice.(4)This EMA guidance is a reflection of recommendations based on multiple workshops and resources and is also aligned with the recommendations from the European Network of Centres for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology and the ENCePP Code of Conduct. The EMA guidance elaborates on multiple aspects of planning and execution of patient registries.(4)While this guidance does not specifically address issues in rare disease registries, it is expected to become the gold standard for registry guidance across all patient registries including those covering small populations, pediatric indications and rare diseases.

Overview of TOSCA

Tuberous sclerosis complex is a rare, autosomal dominant disorder, characterized by formation of hamartomas in multiple organ systems and originates from genetic mutations in either one or both TSC1 and TSC2 genes. In most patients, it manifests as dermatological, renal or neurological abnormalities, though any organ system can be

affected.(56) Owing to multiple knowledge gaps in this disease area, TSC consensus panel acknowledged that the current TSC recommendation guidelines are not based on high levels of evidence and requires additional information about TSC to improvise management strategies.(24)

The lack of a large TSC registry was highlighted in the round table discussions and surveys conducted by Novartis among medical experts and patient advocates. The need for a large scale collaboration led to the conceptualization of the TuberOus SClerosis registry to increase disease Awareness i.e. TOSCA registry.(24) TOSCA is a multicenter, international disease registry to collect data to assess the manifestations, interventions and their outcomes in patients with TSC.(24) The baseline data of 2093 patients in TOSCA has been already been published.(25)

Systematic collection and dissemination of lessons learnt from TOSCA

As a first multinational registry for a TSC, TOSCA had several issues, predominantly in its planning and implementation. Such issues were highlighted in a questionnaire based survey was conducted among the members of SAB, PIs and sponsor employees involved in this registry. This survey identified key strengths and limitations regarding planning and implementation in TOSCA.(15) The practical lessons learnt from TOSCA may supplement the EMA guidance for future rare disease registries. The TOSCA survey paper (15) is referred here as “TOSCA lessons paper”.

Rationale

The EMA guidance paper for good registry practice was released in November 2018, and by then, the TOSCA registry was reaching the stage of final data analysis. Hence, this paper is an attempt to compare and evaluate how the TOSCA registry differs from the EMA recommendations on a point-by-point basis and whether such deviations may have affected the registry outcomes. If and how the learnings from TOSCA can complement the EMA guidance, especially in case of rare disease registries, is also analyzed. The observations in this paper also incorporate the experiences and perspectives of the Clinical Trial Head (CTH) of the TOSCA registry, and hence, also offer insights regarding practical issues during the conduction of the registry.

3.3.3. Observations

The suggestions derived from EMA are divided into four categories: registry planning, operations of registry, data analysis and publication of results. The recommendations from the EMA guidance are summarized under each subheading, followed by the TOSCA methodology, along with the relevant issues, if identified, in TOSCA. The point-wise comparison and/or compliance of TOSCA and EMA guidance is summarized in the Table 12.

Table 12. Summary of TOSCA compliance with EMA guidance

Topic (corresponding EMA guidance chapter)	Recommendations from EMA guidance	Procedure adopted in TOSCA Registry	TOSCA compliance with EMA guidance
Registry planning			
Protocol preparation (5.1., 6.3.)	• Meticulous predefined design and SAP in protocol • Protocol changes to be included as formal protocol amendments • Separate protocol for registry studies (e.g. PASS) • Protocol to meet ENCePP checklist	• Meticulous planning with KOLs and the other stakeholders • Six research projects included in protocol amendment • No separate protocol for registry studies (Votubia® PASS) • PASS enlisted with ENCePP	Partial
Terminologies (5.5.)	• Standard Orphadata, along with ICH-9, 10 and 11, MedDRA	• MedDRA • WHO Drug Reference List, based on ATC classification system	Complete
Data collection/Data elements/ Time elements (5.3., 5.4., 6.5.)	• Wide range of data depending on registry objectives • Use “Set of common data elements for RD registration” on EURD Platform • Core list of dates to be collected	• Core (compulsory) and subsections (petals) design of data elements • Additional safety information collected for PASS • Dates collected for pre-defined relevant variables	Complete
Duration/Timelines (3.3, 5.1., 6.2.)	• Long-term follow-up dictated by schedules for data collection • Registry study to follow up to achieve study objective	• 5 years follow-up • Extended follow-up for PASS	Partial

Operations of the registry			
Patient enrolment (5.2., 6.4.)	• Clear conceptual and operational definition of target population • Exhaustive patient enrolment • Registry study a subset of the registry population or enroll additional patients, if required	• Documented visit for TSC within the preceding 12 months or newly diagnosed • Retrospective as well as prospective data collection from 170 sites across 31 countries. • 2214 patients enrolled in TOSCA registry,571 in 6 RPs and 179 patients in PASS.	Complete
Informed Consent (5.8.4.)	• Patients are aware: why/what data is collected, how/ by whom it will be used, and at what level of details	• Patient Information Brochure and informed consent form	Complete
Quality management (5.6., 6.6.)	• Quality management inconsistency, completeness, accuracy and timelines (5.6.2., 5.6.3.) • Use data quality indicators to ensure data quality (5.6.4.)	• Routine measures for quality maintenance deployed on a site and registry level flagging inconsistency, completeness, accuracy. • 5 yearly interim analyses conducted to assess data quality	Partial
Data Sharing (5.8.3.)	• Data sharing is encouraged, at least on an aggregated and ideally on an anonymized patient-level	• Participants of TOSCA were able to submit research requests that enabled data-access • Data-access requests can be filed with data-owner upon completion of registry	Complete
Data Security (5.8.5)	• Security measures should be implemented to maintain the privacy of patients	• Overseen and managed by neutral 3rd party (CRO) and clarified in contract	Complete

Data analysis			
Data analysis (5.6.3., 5.7., 6.7.)	• Subjective to registry purpose • Registry study to have separate SAP	• Due to exploratory registry purpose mainly descriptive analysis • PASS with yearly interim analysis but no separate SAP	Partial
Safety analysis (5.7., 6.8.)	• Reporting of AEs • Monitoring of AESI • Aggregated analysis of AEs	• AE reporting at site level according to national regulations • AESI assessed in sub-population in the context of a PASS • No analysis of all AEs planned in the objectives of the registry	Partial
Publications			
Publication policy(6.9.)	• Lead investigator retains authority to prepare publication of registry results. • MAH discuss final results and interpretation, if required.	• WC, with the approval of SAB developed publication strategy. • WC responsible for preparation and coordination of all presentations and publication activities. • Sponsor data owner • MAH not involved	Complete

* until they reach Tanner stage V or age of 16 years in females and 17 years in males.

ATC: Anatomic Therapeutic Classification; CRO: Clinical Research Organization; ENCePP: European Network of Centres for Pharmacoepidemiology and Pharmacovigilance; EURD: European Platform on Rare Diseases Registration; ICH: International Council for Harmonisation; KOL: Key Opinion Leaders; MAH: Marketing Authorization Holder; MedDRA: Medical Dictionary for Regulatory Activities; PASS: Post-Authorization Safety Study; RD: Rare Diseases; RPs: Research projects; SAB: Scientific Advisory Board; SAP: Statistical Analysis Plan; TOSCA: TuberOus SClerosis registry to increase disease Awareness; WC: working Committee; WHO: World Health Organization

Registry planning

Design and governance of registry

The EMA guidance suggests meticulous planning, including statistical analysis plan and other details, including those for research projects. It also emphasizes the effective collaboration between all involved parties and explicitly describes the role of different stakeholders such as registry coordinators, Pharmaceutical companies and Regulatory authorities.(4) Also, EMA makes a clear distinction between registry and the registry studies and provides a separate section for guidance for registry study. EMA recommends a separate protocol for registry study, including a detailed statistical analysis plan. Furthermore, the EMA guidance also recommends the use of the ENCePP checklist for the creation and evaluation of registry study protocols. Additionally, the protocol should follow all applicable national and regional regulations such as the GVP Module VIII, if appropriate. Any changes in either registry or study protocol should be recorded as formal protocol amendments.(4)

While planning TOSCA, all efforts were made to thoroughly plan the registry, and to achieve its objectives through a systematic and reliable data collection system. Such an intricate and detailed planning was feasible with the involvement of key experts from different areas, including TSC medical healthcare experts, representatives from pharmaceutical sponsor as well as patient representatives in the SAB and WC.(24)The SAB and WC collectively ensured a thorough planning and systematic implementation of the registry protocol.

Involvement of patient representatives was instrumental in patient enrolment and further facilitated the communication with patients. Since patient representatives generally have a better understanding of patient journey within a disease, the collaboration with patient advocacy groups significantly helped and overall facilitated the research project analyzing quality of life outcomes.

After the approval of Votubia®, the EMA requested (EMEA/H/C/002311/II/0004) a Post-Authorization Safety Study (PASS) in TCS, which was subsequently included in the TOSCA registry.(24) Contrary to the recommendations of the later released EMA guidance, the TOSCA PASS did not have a separate protocol, but was incorporated in the registry protocol as a protocol amendment. The registry study protocol was furthermore listed in the ENCePP list (CRAD001MIC03- ENCePP number 3247) and The European Union electronic Register of Post-Authorisation Studies (EU PAS Register) (EUPAS3247).

After the successful set-up of TOSCA, six additional research projects were added to TOSCA as protocol amendments. These research projects aimed to answer certain research questions pertaining to a deeper understanding of TSC. However, in the TOSCA lessons paper, it was realized that although research projects were crucial, lack of adequate planning as well as finances for such complex projects rendered them burdensome for PIs and sponsor, which in turn, might have hampered their potential to provide new insights for different manifestations of TSC.(15)

Registry duration and follow-up

EMA acknowledges that though long term follow-ups are always desirable but not practical. Hence, the timelines are usually dictated by financing and schedules for data collection.(4)This is particularly true in rare disease and small populations, where budget restrictions usually strongly impact registry duration, registry data quality and registry data completeness.

In the TOSCA registry, the planned duration of follow-up, once a patient was enrolled in the registry, was up to 5 years. However, in Votubia® PASS, for pediatric patients in the EU region, it was agreed to continue the follow-up till they reach Tanner stage V or until 16 years of age for females and 17 years for males. Consequently, some patients were expected to be followed-up until 2027, to ensure a more thorough evaluation of long-term effect of Votubia®.(24)

According to the TOSCA lessons paper, 38% respondents considered a 5-year follow up in the main registry to be short to holistically assess the real life impact of the disease. A longer follow-up would definitely be more helpful for a rare disease, especially when there are multiple manifestations.(15)

Operational aspects

Patient enrolment

In general, registries are prone to selection bias, pertaining to multiple confounding factors. Hence, EMA suggests having a clear conceptual definition of target population, which can be further translated into operational definition. Comprehensive patient enrolment requires a meticulous process to exhaustively enroll patients fulfilling the

operational definition, to avoid selection bias. A voluntary and informed consent with detailed information regarding the purpose and extent of data collection, as well as its further use/sharing to external parties, is mandatory during patient enrolment. Informed consent should comply with General Data Protection Regulation (GDPR). Patients also need to be informed about their potential to restrict consent as well as their withdrawal at any time.(4)

The TOSCA registry was structured to retrospectively and prospectively collect data from patients with TSC. In order to gather a large multinational cohort of TSC patients, TOSCA aimed for exhaustive recruitment, as recommended by the EMA guidance, overall enrolling 2214 patients from 170 sites across 31 countries. Such commendably high recruitment rates in a rare disease of mostly pediatric involvement was achieved through the close collaboration with all stakeholders as well as using the recommended clear conceptual and operational definition of target population. Aligned with the EMA recommendations (refer Table 12), all patients enrolled in TOSCA signed a voluntary informed consent. Separate informed consents were signed for research projects as well as PASS study.(24,25)

Site/database management and quality control

The validity and reliability in registry data is considered skeptically due to the uncertainties in data quality. Data quality issues in post-authorization registry studies may even impact the marketing authorization. EMA suggests four main activities for quality management, which include quality planning, quality assurance, quality control and quality improvement. Maintaining data quality comprises four major components: data

consistency, data completeness, data accuracy and data timelines. Measures to continuously assure data quality should be in place at management level as well as operational level of the registry. The EMA guidance also suggests using indicators of data quality to regularly measure and improve data quality.(4)

In TOSCA, suitable measures were taken for adequate site management and data quality. High data quality was ensured with thorough training of all personnel at the participating sites, use of a fully validated software, and validation of data completeness and accuracy with an international clinical research organization (CRO). Additionally, quality assurance reviews, audits and evaluation of registry progress were conducted at regular intervals by authorized representatives from Novartis and regulatory agencies.

While there were no specific data quality indicators used (refer Table 12), maintenance of data quality and accuracy was evaluated in the first administrative analysis of the data for first 100 patients in the registry. It revealed that of the total of 469 fields of information, the information on at least 85% of the fields was in more than 90% patients. Hence, a high degree of accuracy, and optimum quality of data collection was ensured.(24) In total, five annual interim analysis were conducted to ensure data quality and take corrective measures, where needed.

In the TOSCA lessons paper, 25% of the respondents had concerns regarding the presence of some form of bias, which may be selection bias, information bias or measurement bias. These biases may have compromised the validity of collected data. It was recommended that further efforts must be made to minimize biases which are particularly likely to occur in registries and further, more likely in a rare disease setting.

Involvement of a statistician from the planning stage itself may help avoid the potential biases in future registries.(15)

Data handling

Data elements

The EMA guidance suggests the use of harmonized core data and core time elements collected in a pre-defined format across all patient registries for the same disease to facilitate a common data quality system, data exchange and further interpretation and comparison of results from different registries. Lack of harmonization leads to a time- and resource intensive process when mapping data elements of multiple sources. (4)

A list of core data elements comprises of ‘crucial’ and ‘should have’ data elements. The crucial data elements are the mandatory data to be collected in all patients and hence require greater resource allocation to ensure completeness, standardization, data quality and verification of the information. The ‘should have’ data and time elements are additional data and time elements, which may be important for some stakeholders or in some sub-population, but not essential to all.(4)

The core data and time elements for disease registries can be identified with intensive discussions amongst clinicians, disease experts, patient representatives and if required, regulatory authorities. A standard set of core data elements for rare diseases has been developed as “Set of common data elements for RD registration” on the European Platform on Rare Diseases Registration (EU RD Platform).(57) Furthermore, some disease-specific lists of core data elements are also available.(58-61)

The details pertaining to the data and time elements in the TOSCA registry have already been published earlier.(24) In brief, TOSCA followed a flower and petal model of data elements. The main 'core' section was designed to collect a general predefined set of patient background data including demographics, family history, prenatal history, and disease features including the corresponding dates, where relevant. Additional and more detailed data related to specific disease manifestations were collected in the 'petal segments' i.e. subsections of the registry which may have only taken place in certain countries, sites or sub-populations.

Furthermore, it is to be noted that the data elements used in TOSCA registry may form a sample list of identified data elements for future registries in TSC, especially when unlike cystic fibrosis, there is a lack of standard set of core data elements in TSC.

Terminologies

In order to internationally harmonize various registries across same diseases, it is recommended to use international terminologies for diseases, diagnostic tests, symptoms, medicinal products and adverse events. When national or local terminologies are used, mapping to international terminologies is recommended.(4)

The EMA guidance recommends use of standard Orphadata (62) for terminologies associated with rare diseases, along with ICH-9, 10 and 11, Medical dictionary for regulatory activities (MedDRA) (63) for standardizing terminologies. MedDRA is also internationally acceptable for adverse event classification for regulatory purposes.

As per the TOSCA protocol, medical history/current medical conditions were coded using the MedDRA.(63) Additionally, the World Health Organization (WHO) Drug Reference List(64), which employs the Anatomical Therapeutic Chemical (ATC) classification system were used to code the concomitant medications.

Data analysis

EMA suggests that a statistical method, suitable to justify the individual research question and variables in individual registry, should be pre-defined for registry and registry studies in their respective protocols, and should include the method of handling the missing data. Data analysis should be performed based on pre-defined time schedules. Any changes in the statistical analysis plan should be recorded as formal protocol amendments. EMA guidance also suggests the reporting of adverse events (AEs), the monitoring of AEs of special interest, and the aggregated analysis of AEs.(4)

Being an exploratory study, the data in TOSCA registry underwent descriptive analysis for relevant variables. In the absence of a definitive statistical analysis plan, adequate attempts were made to open-endedly analyze and interpret data and identify any potential correlations. Further data analysis while manuscript preparation ensured the identification of interesting insights regarding different manifestations of TSC. Furthermore, missing data were not imputed, in general. For partially missing data, the values were imputed for analysis purpose.

In multi-national registries, it is essential to follow the local requirements on AE reporting. Accordingly, in TOSCA, various sites reported the AEs to their corresponding national authorities. The AEs of special interest were pre-defined and assessed as a part of

Votubia® PASS. A detailed analysis of reported AEs was out of scope of TOSCA objectives and hence, was not attempted.

In the TOSCA lessons paper, 32% respondents had concerns related to the handling of missing data. However, it is also to be noted that variables with the most missing data were related to a particular manifestation, i.e. TSC-Associated Neuropsychiatric Disorders (TAND). This may be attributed to the lack of knowledge of TAND-related manifestations investigated through the physicians- or patient/caregiver reported outcomes. For other manifestations, the missing data were minimal, reflecting an overall good quality data collection.(15)

Publication

EMA states that regardless of the funding source, the lead investigator retains primary authority to independently prepare publications of the study results. If applicable, the marketing authorization holder (MAH) co-funding the registry study, is entitled to view the final results and interpretations prior to submission for publication. The MAH may also share their views regarding the study results and interpretation, in advance of submission within a reasonable time limit, e.g. one month, and without unjustly delaying the publication. EMA also entitles the MAH to request change in presentation of results to delete confidential information.(4)

Since TOSCA was not aimed for a drug dossier submission approval, the MAH did not participate in the publication process. Instead, only the Novartis Medical department (Medical affairs) was involved in publication preparation and review.

Initially, the publication policy was not clearly defined and the need for a publication plan was soon realized after the first manuscript was prepared. Hence, a detailed publication policy was released in January 2015. The publication strategy was developed by the WC, with the approval from SAB. The responsibility to ensure the implementation of the publication strategy and perform all coordination was with the WC.

A clear protocol was prepared with regards to the process of developing presentations and publications. A kick-off meeting (face-to-face or teleconference) with all authors and reviewers was suggested to discuss all details i.e. timelines, journal and relevant topics regarding the manuscript before the initiation of manuscript writing. SAB retained the final authority regarding authorship and order or authorship. This publication policy and the planned information dissemination was clearly in-line with the EMA guidance.

The publication policy stated that at least one manuscript would be published following each interim analysis. Secondary manuscripts and abstracts were planned to communicate the results and knowledge to a wider audience. Translations of posters presented at International Congresses were encouraged to be presented in local languages at National Congresses.

The results of the TOSCA registry analyses were presented as posters/presentations on the main TSC, or specific manifestations, congresses. So far, nine publications from the TOSCA registry study have been released. (15,17,24,25,27,65-68) A robust publication plan for data derived from the main registry, research projects and the TOSCA PASS study is in place and it is expected to be achieved by 2020. Furthermore, 15 oral presentations and 27 posters have been presented at International Congresses. Of these

5 oral presentations and 8 posters have been further translated and presented in National and Local Congresses. Additionally, three posters with country-specific data have been presented at National Congresses. In the future, data collected in TOSCA may be used for performing new analysis to address specific research questions on the basis of retrospective observations. In-detailed analysis of specific data will further help the clinicians to have a better understanding of TSC and its manifestations.

Sustainability

Sustainability issues, after the initial phase of funding, are common in registries. Sustainable funding is required throughout the registry duration for multiple reasons including maintenance of core registry features, additional staff hiring for specific studies etc. In a Patient Registry Workshop, EMA recommended to consider the learnings from existing successful registries to improve sustainability in the future registries. It is suggested that instead of aiming for a short-term funding support, registry holders should engage with public agencies and define/clarify the long-term role of industry. Sustainability may be improved with a clear development strategy, appropriate management and the clear stakeholder partnership.(69) A cost-sharing collaboration between stakeholders may also help improve sustainability.

The TOSCA registry was solely sponsored by Novartis, and with initial registry planning, no funding issues were expected. However, later addition of the six research projects, which lacked adequate time and resource planning, had budget constraints. Such issues could have been addressed better with inclusion of research projects at the registry planning stage. Nevertheless, despite the issues, research projects captured important

information regarding the diverse manifestations of TSC, which will enhance the understanding about the disease and its manifestations.

3.3.4 Conclusion

Despite being conducted before the EMA guidance was released, TOSCA was mostly in accordance with the EMA guidance, although some deviations were also noted (refer Table 12). Meticulous planning with involvement of multiple stakeholders, careful implementation ensuring valuable and high quality data collection, definition of core and extended data elements, inclusion of research projects and registry studies in TOSCA were adequately appropriate. With these measures, TOSCA was able to achieve the desired objectives, particularly in improving our understanding about TSC and its manifestations, as well as increasing the awareness about this rare disease. Another commendable achievement in TOSCA was a high recruitment rate across all geographic regions, which was only possible with a strong collaboration among stakeholders. The most important takeaway points from this paper, which may help future registries, are to include research projects in the initial protocol and to have separate protocol for registry study (PAES/PASS).

The EMA guidance on Good Registry Practice offers valuable guidance for future registries and registry studies. These guidelines will help harmonize disease based databases established across different registries. However, some of the EMA recommendations are practically not feasible in rare disease registries. For instance, collecting a very large number of variables open-endedly in a small population may be difficult owing to the burden on patients. Additionally, ensuring adequate financial

resources to collect high quality data in open-ended registries for each rare disease may be difficult. The contribution of patient communities in rare disease, if properly engaged, can be instrumental to ensure high accrual and minimal loss to follow up. Adopting additional measures to address the issues specific to rare disease registry is thus suggested for optimal outcomes.

Section 4 Additional Publications and International Conference Presentations

From the observations and data collected from TOSCA, below papers have been published with University affiliation (Institute of Biomedicine (IBIOMED), University of Leon, León, Spain)

1. The TOSCA Registry for Tuberous Sclerosis—Lessons Learnt for Future Registry Development in Rare and Complex Diseases. Marques *et al*. *Frontiers in Neurology* (2019) 10:1182. DOI:10.3389/fneur.2019.01182.
2. Treatment Patterns and Use of Resources in Patients With Tuberous Sclerosis Complex: Insights From the TOSCA Registry. *Marques et al*. *Frontiers in Neurology* (2019) 10:1144 DOI:10.3389/fneur.2019.01144.
3. TuberOus SClerosis registry to increase disease Awareness: A review on alignment of its planning, execution and publications with EMA guidelines. *Marques et al. Frontiers Neurology*. (2020).11:365 DOI 10.3389/fneur.2020.00365
4. Newly Diagnosed and Growing Subependymal Giant Cell Astrocytoma in Adults With Tuberous Sclerosis Complex: Results From the International TOSCA Study. Jansen *et al. Frontiers in Neurology* (2019) 10:0821 DOI:10.3389/fneur.2019.00821
5. Clinical Characteristics of Subependymal Giant Cell Astrocytoma in Tuberous Sclerosis Complex. Jansen *et al*. *Frontiers in Neurology*(2019) 10:0705 DOI:10.3389/fneur.2019.00705

6. Epilepsy in tuberous sclerosis complex: Findings from the TOSCA Study. Nabbout *et al. Epilepsia Open*(2018) 4:73-84 DOI:10.1002/epi4.12286
7. TSC-associated neuropsychiatric disorders (TAND): findings from the TOSCA natural history study. De Vries *et al. Orphanet Journal of Rare Diseases* (2018) 13:157 DOI:10.1186/s13023-018-0901-8
8. Renal angiomyolipoma in patients with tuberous sclerosis complex: findings from the TuberOus SClerosis registry to increase disease Awareness. Kingswood *et al. Nephrology, Dialysis and Transplantation Journal* (2019) 34: 502–508 DOI:10.1093/ndt/gfy063
9. Tuberous Sclerosis Complex-Associated Neuropsychiatric Disorders (TAND): New Findings on Age, Sex, and Genotype in Relation to Intellectual Phenotype. de Vries *et al. Frontiers in Neurology* (2020) 11:603. doi: 10.3389/fneur.2020.00603
10. Renal Manifestations of Tuberous Sclerosis Complex: Key Findings From The Final Analysis of The TOSCA Study Focusing Mainly on Renal Angiomyolipomas. Kingswood *et al. Frontiers in Neurology* (2020) 11: 972. doi: 10.3389/fneur.2020.00972
11. Burden of Illness and Quality of Life in Tuberous Sclerosis Complex: Findings from the TOSCA Study. Jansen et al. *Frontiers in Neurology* (2020) 11:904. doi: 10.3389/fneur.2020.00904
12. Natural Clusters of Tuberous Sclerosis Complex (TSC)-Associated Neuropsychiatric Disorders (TAND): New Findings from the TOSCA TAND

Research Project. De Vries *et al. Journal of Neurodevelopmental Disorders* (2020) 12:24. https://doi.org/10.1186/s11689-020-09327-0

Furthermore, below papers have been planned or in preparation process:

1. Rare manifestations and malignancies in tuberous sclerosis complex: Findings from TuberOus SClerosis registry to increAse disease awareness (TOSCA). Sauter *et al. Frontiers in Neurology.* (2020) Planned
2. Epilepsy in Tuberous Sclerosis Complex: Results from TOSCA Main Study and Epilepsy Research Project. Nabbout *et al. Frontiers in Neurology* (2020) Planned.
3. Genotype-Phenotype Correlation in Tuberous Sclerosis Complex: Findings from TOSCA Registry. Boronat *et al. Frontiers in Neurology* (2020). Under preparation.
4. TuberOus SClerosis registry to increAse disease awareness (TOSCA) post-authorization safety study of everolimus in patients with tuberous sclerosis complex. Kingswood *et al. Frontiers in Neurolgy* (2020) Under preparation.
5. TOSCA–TuberOus SClerosis registry to increAse disease awareness: Final Analysis Exploring Clinical Manifestations Associated with Tuberous Sclerosis Complex in 2211 Patients. Kingswood et al. Frontiers in Neurology (2020) Under preparation.

In addition to the publications, below is the list of presentations at various international conferences:

1. Burden of Illness and Quality of Life Among Patients with Tuberous Sclerosis Complex: Assessed as Part of the International TOSCA Study. Jansen et al. 10th European Conference for Rare Diseases and Orphan Products. Virtual 2020
2. Tuberous Sclerosis Complex (TSC)-Associated Neuropsychiatric Disorders (TAND): New Findings on Age, Gender and Genotype in Relation to Intellectual Phenotype. de Vries et al. TSC international research conference. Tokyo 2018
3. Natural Clusters of Tuberous Sclerosis Complex (TSC)-Associated Neuropsychiatric Disorders (TAND): New Findings from the TOSCA TAND Research Project. de Vries et al. TSC international research conference. Tokyo 2018
4. Tuberous Sclerosis Complex (TSC) -Associated Epilepsy: Final Results from TOSCA Study as oral communication. Nabbout et al. TSC international research conference. Tokyo 2018
5. Burden of Illness and Quality of Life Among Patients with Tuberous Sclerosis Complex: Assessed as Part of the International TOSCA Study. Jansen et al. TSC international research conference. Tokyo 2018
6. Characteristics of Subependymal Giant Cell Astrocytoma in Adults Patients with Tuberous Sclerosis Complex: Findings from the Final Analysis of the TOSCA Study. Jansen et al. TSC international research conference. Tokyo 2018
7. Renal Manifestations of Tuberous Sclerosis Complex: Key Findings from Final Analysis of TOSCA Study. Kingswood et al. TSC international research conference. Tokyo 2018

8. TuberOus SClerosis registry to increase disease awareness: Final Analysis Exploring Clinical Manifestations Associated with Tuberous Sclerosis Complex in 2211 Patients. Oral communication. Kingswood et al. TSC international research conference. Tokyo 2018.
9. Efficacy and safety of long-acting pasireotide (LA-PAS) in patients with uncontrolled acromegaly: Results from the prospective cohort of European observational ACRONIS study. Pivonello et al. European Society of Endocrinology. Virtual 2020

Section 5 Conclusion

The results and observations in the three publications from this thesis research conclude that patient registries are an invaluable tool to describe a rare disease and further expand our knowledge about lesser understood aspects of these diseases

The first paper summarizes the overall scientific outcomes of TOSCA registry that has provided better understanding of a complex rare disease, tuberous sclerosis, with high quality relevant data recorded and analyzed with proper planning and implementation of this registry. These results highlight the changes in treatment trends across the multiple TSC manifestations and countries over time, which is a direct reflection of availability of therapies as well as country-specific clinical practices, along with various other factors. Furthermore, it proposed an adequate planning of cost-estimation in future rare diseases patient registries that may help to identify the economic burden.

Conducting a questionnaire based survey (second paper) about the functioning of this rare disease registry from the 'insiders' (i.e. those who were involved in the planning and operations) has allowed us to identify the lapses that could arise in rare disease patient registries and also the measures that can be taken to avoid such issues from emerging. An important pointer to be considered for future patient registries in rare diseases is proper operational and financial planning of the research projects included in the registry, which can improve the quality and reliability of the data and results generated.

A head-to-head observation of TOSCA with EMA guidance on patient registries (third paper) provides valuable discernment on different aspects of patient registries in rare diseases. While some of the EMA suggestions such as an adequately detailed study

protocol and statistical analysis plan for registry studies (PASS/PAES) will help in future rare disease patient registries, few of them are practically not feasible in context of rare disease registries, considering the scattered geographic demography and lack of adequate understanding about these diseases. Hence, careful planning for future rare disease registries is recommended to best utilize the available resources.

Being involved in a rare disease patient registry from inception to conclusion had offered an extensive as well as intensive insight at every step of a registry. This comprehensive and thorough discernment will help avoid the bumps and smoothen the road while planning and conducting patient registries in rare diseases in the future.

Conclusión

Los resultados y las observaciones de las tres publicaciones incluidas en esta tesis concluyen que los registros de pacientes son una herramienta inestimable para describir una enfermedad rara y ampliar aún más nuestro conocimiento sobre aspectos menos entendidos de estas enfermedades.

El primer manuscrito resume los resultados científicos que nos han permitido conocer mejor la esclerosis tuberosa, una enfermedad tan compleja como desconocida, por medio de los datos relevantes y sólidos obtenidos gracias a la adecuada planificación e implementación del registro TOSCA. Estos resultados destacan los cambios en las tendencias de tratamiento en las múltiples manifestaciones de TSC a lo largo del tiempo, lo que principalmente es un reflejo directo de la disponibilidad de terapias así como de las prácticas clínicas específicas de cada país. Además, nos revela que la adecuada planificación de las estimaciones de costos es necesaria para identificar la carga económica del estudio, importante punto a tener en cuenta en futuros registros.

La realización de un cuestionario (segundo manuscrito) específico sobre este registro de enfermedades raras por parte desus "expertos" (es decir, aquellos que participaron tanto en la planificación como en la parte operativa) nos ha permitido identificar los fallos que pueden surgir en los registros de pacientes de enfermedades raras y también las medidas que se pueden tomar para evitar que aparezcan tales problemas. Especialmente, hay que tener en cuenta que una planificación operativa y financiera adecuada de los proyectos de investigación/subestudios incluidos en estos registros puede mejorar la calidad y la fiabilidad de los datos y resultados generados.

Una comparación directa de TOSCA con las guías de la EMA sobre registros de pacientes (tercer manuscrito) proporciona un discernimiento valioso sobre los diferentes aspectos de los registros de pacientes en enfermedades raras. Si bien seguir algunas de las sugerencias de la EMA, como preparar detalladamente el protocolo y un plan de análisis estadístico para los registros de estudio (PASS/PAES), ayudarán en futuros registros de enfermedades raras, otras pocas recomendaciones son prácticamente imposibles de implementar en el contexto de los registros de enfermedades raras, considerando la demografía geográfica dispersa y la falta de comprensión adecuada de estas enfermedades. Por lo tanto, una planificación cuidadosa de futuros registros de enfermedades raras es necesaria para poder utilizar de la forma más eficiente los recursos disponibles.

Dirigir un registro de pacientes de enfermedades raras de inicio a fin nos ha ofrecido una visión amplia y profunda de cada una de sus partes en cada uno de sus pasos. Compartir este entendimiento completo y exhaustivo podrá ayudara evitar golpes y allanar el camino en la planificacióny ejecución de futuros registros de pacientes de enfermedades raras.

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Appendix

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Treatment Patterns and Use of Resources in Patients With Tuberous Sclerosis Complex: Insights From the TOSCA Registry

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Tuberous Sclerosis Complex (TSC) is a rare autosomal-dominant disorder caused by mutations in the *TSC1* or *TSC2* genes. Patients with TSC may suffer from a wide range of clinical manifestations; however, the burden of TSC and its impact on healthcare resources needed for its management remain unknown. Besides, the use of resources might vary across countries depending on the country-specific clinical practice. The aim of this paper is to describe the use of TSC-related resources and treatment patterns within the TOSCA registry. A total of 2,214 patients with TSC from 31 countries were enrolled and had a follow-up of up to 5 years. A search was conducted to identify the variables containing both medical and non-medical resource use information within TOSCA. This search was performed both at the level of the core project as well as at the level of the research projects on epilepsy, subependymal giant cell astrocytoma (SEGA), lymphangioleiomyomatosis (LAM), and renal angiomyolipoma (rAML) taking into

account the timepoints of the study, age groups, and countries. Data from the quality of life (QoL) research project were analyzed by type of visit and age at enrollment. Treatments varied greatly depending on the clinical manifestation, timepoint in the study, and age groups. GAB Aergics were the most prescribed drugs for epilepsy, and mTOR inhibitors are dramatically replacing surgery in patients with SEGA, despite current recommendations proposing both treatment options. mTOR inhibitors are also becoming common treatments in rAML and LAM patients. Forty-two out of the 143 patients (29.4%) who participated in the QoL research project reported inpatient stays over the last year. Data from non-medical resource use showed the critical impact of TSC on job status and capacity. Disability allowances were more common in children than adults (51.1% vs 38.2%). Psychological counseling, social services and social worker services were needed by <15% of the patients, regardless of age. The long-term nature, together with the variability in its clinical manifestations, makes TSC a complex and resource-demanding disease. The present study shows a comprehensive picture of the resource use implications of TSC.

Keywords: TSC, resource use, TOSCA, management, registry, rare diseases

INTRODUCTION

Tuberoussclerosis complex (TSC) is an autosomal-dominant disorder characterized by the formation of hamartomatous lesions in multiple organ systems (1) and the association with a wide range of TSC-associated neuropsychiatric disorders, abbreviated as TAND (2).

TSC is caused by mutations in either *TSC1* or *TSC2* genes. The proteins encoded by these two genes—hamartin and tuberin—form a complex that inhibits the mammalian target of rapamycin (mTOR) complex 1, which is involved in the regulation of cell growth and proliferation (1).

The manifestations and the severity of the disease are variable, even between relatives, and depend on size, number, location and distribution of the lesions (3, 4). Common locations include the brain, kidneys, lungs, skin, heart, and eyes (4–8). However, no single symptom is observed in all patients, and none of the symptoms can be considered as absolutely pathognomonic (6).

The use of resources and the costs of managing patients with TSC have been estimated in several studies carried out in Sweden (9), the United Kingdom (UK) (10–12), the Netherlands (13), the United States (US) (14, 15), and Canada (16). All of them have been developed on a national-basis in European countries or in North America, and most of them have been carried out in a limited number of patients filtered by age or by clinical manifestation. Therefore, the information coming from these studies is specific and cannot be completely extrapolated to other countries or clinical contexts. High variations across countries can appear depending on the country-specific clinical practice. As a consequence, the burden of TSC and its impact on the use of healthcare resources required for its management remain unknown.

TABLE 1 | Use of treatments according to follow-up visit.

	Baseline (*N* = 2211)	**FU1 (*N* = 2099)**	**FU2 (*N* = 1935)**	**FU3 (*N* = 1664)**	**FU4 (*N* = 764)**	**FU5 (*N* = 147)**
Patients with IS	721	151	120	91	45	14
Patients treated for IS (*n*, %)	698 (96.8)	145 (96.0)	113 (94.2)	85 (93.4)	44 (97.8)	14 (100.0)
Patients with FS	1,261	614	544	506	236	29
Patients treated for FS (*n*, %)	1,237 (98.1)	599 (97.6)	530 (97.4)	493 (97.4)	231 (97.9)	28 (96.6)
Patients with SEGA	553	489	468	420	208	52
Patients treated for SEGA (*n*, %)	221 (40.0)	187 (38.2)	188 (40.2)	181 (43.1)	101 (48.6)	22 (42.3)
Patients with rAML	1,062	1,067	1,041	945	472	121
Patients treated for rAML (*n*, %)	315 (29.7)	300 (28.1)	321 (30.8)	288 (30.5)	165 (35.0)	53 (43.8)
Patients with LAM	154	157	162	149	68	21
Patients treated for LAM (*n*, %)	50 (32.5)	47 (29.9)	54 (33.3)	43 (28.9)	20 (29.4)	0 (0.0)

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) was a large scale non-interventional study in patients with TSC, started in 2012 and was conducted at 170 sites in 31 countries. TOSCA registry was totally founded by Novartis AG and its related clinical study protocol and final study results are disclosed on the ENCePP portal at http://www.encepp.eu/ (EU PAS Register Number EUPAS324) (17). The design and methodology of TOSCA were published previously (8). In short, patients of any age with TSC were enrolled and followed-up for up to 5 years. Patient data including demographics and information related to clinical features of TSC across all organ systems, comorbidities, and rare manifestations, were collected at baseline and at regular visits scheduled at a maximum interval of 1 year.

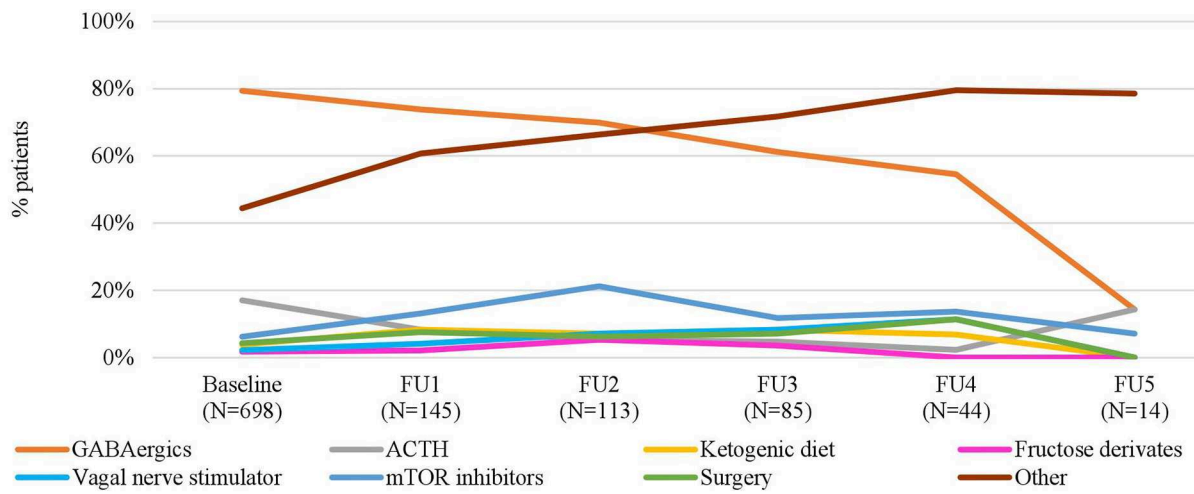
The registry consisted of a "core" part and six associated research projects focusing on: epilepsy, subependymal giant cell astrocytomas (SEGA), renal angiomyolipoma (rAML)/lymphangioleiomyomatosis (LAM), genetics, quality of life (QoL), and TSC-associated neuropsychiatric disorders (TAND); the "core" part collected demographic data, family history, prenatal history, disease features, and information on treatments, whereas the research projects recorded in-depth data related to specific disease manifestations or to specific aspects of the disease (8). One of the research projects (research project on QoL) recorded data on the use of medical and non-medical resources for seven European countries (Belgium, Germany, Italy, Spain, Sweden, France, and the UK).

Due to its long-term follow-up (up to 5 years) and to the inclusion of patients of any age from different countries from all over the world, the TOSCA registry offered a unique opportunity to observe how treatment patterns for the manifestations of TSC changed over time, and to evaluate differences in disease management depending on the age of the patients or their country of residence. In addition, results can be analyzed in context with the results from the other research projects.

The aims of the present study were to analyse how the treatment modalities in patients with TSC included in the TOSCA registry changed during the 5 years of follow-up, to identify differences in management as well as the availability of medical and non-medical health resources with respect to patients' age or country of residence.

METHODS

This study was based on data obtained from the TOSCA registry. The TOSCA registry was a non-interventional clinical study founded by Novartis AG, designed and conducted according to the Guidelines for Good Clinical Practice and ethical principles



	Baseline (N=698)	FU1 (N=145)	FU2 (N=113)	FU3 (N=85)	FU4 (N=44)	FU5 (N=14)
GABAergics	554 (79.4%)	107 (73.8%)	79 (69.9%)	52 (61.2%)	24 (54.5%)	2 (14.3%)
ACTH	119 (17.0%)	12 (8.3%)	6 (5.3%)	4 (4.7%)	1 (2.3%)	2 (14.3%)
Ketogenic diet	27 (3.9%)	12 (8.3%)	8 (7.1%)	7 (8.2%)	3 (6.8%)	0 (0.0%)
Fructose derivates	12 (1.7%)	3 (2.1%)	6 (5.3%)	3 (3.5%)	0 (0.0%)	0 (0.0%)
Vagal nerve stimulator	15 (2.1%)	6 (4.1%)	8 (7.1%)	7 (8.2%)	5 (11.4%)	0 (0.0%)
mTOR inhibitors	43 (6.2%)	19 (13.1%)	24 (21.2%)	10 (11.8%)	6 (13.6%)	1 (7.1%)
Surgery	30 (4.3%)	11 (7.6%)	7 (6.2%)	6 (7.1%)	5 (11.4%)	0 (0.0%)
Other	310 (44.4%)	88 (60.7%)	75 (66.4%)	61 (71.8%)	35 (79.5%)	11 (78.6%)

FIGURE 1 | Treatments for Infantile Spasms in each Follow-up Visit. Patients may receive more than one treatment. Baseline data refers to patients who "ever had" the manifestation. Other include lamotrigine, topiramate, levetiracetam and valproate.

outlined in the Declaration of Helsinki (18, 19). After appropriate approval by central and local research ethics committees, written informed consent was obtained from all patients, parents, or guardians, prior to enrollment.

The first step for the present manuscript was a search for variables that could be of interest for the purpose of a study on the use of TSC-related resources (including medical and non-medical resources), and an exhaustive analysis of all the listings and tables produced as part of the final analysis of the TOSCA registry, in order to identify relevant outcomes and analyses for each variable. The variables and potential analyses are detailed in the **Tables S1, S2**.

Data on use of treatments (proportion of treated patients and types of treatment) were available for the overall population of patients included in the core registry. Data on the use of other medical resources (hospitalizations, primary, and secondary care visits) and on the use of non-medical resources (variables related to education needs, patient or caregiver employment situation and patient support/social services needs) were available for a subset of 143 patients included in the QoL research project, which was carried out in 7 European countries (Belgium, Germany, Italy, Spain, Sweden, France, and the UK).

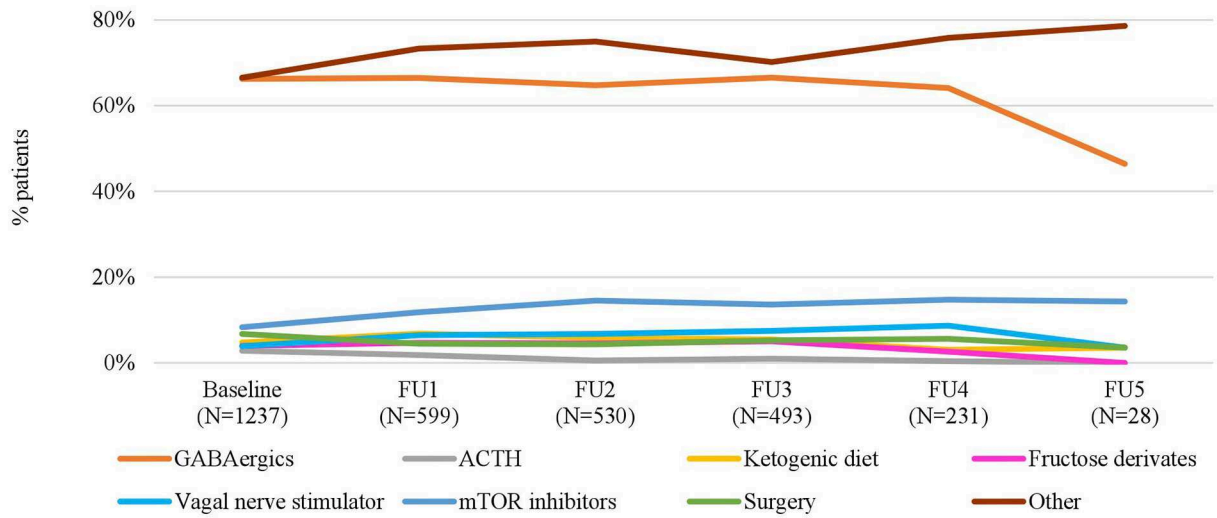
Treatment patterns were analyzed using the core registry data according to 4 clinical manifestations (epilepsy, SEGA, LAM, and rAML), the number of visits [baseline or follow-ups (FU1 to FU5), where FUs were conducted at intervals not longer than 12 months apart], the age group ($\leq$2, >2 to $\leq$5, >5 to $\leq$9, >9 to $\leq$14, >14 to <18, $\geq$18 to $\leq$40, and >40 years), and the country of residence (for those countries included in the QoL research project; i.e., Belgium, Germany, Italy, Spain, Sweden, France, and the UK). Baseline data were retrospectively collected and FU data were prospectively collected up to 5 years. All the results were reported in terms of absolute and relative frequencies.

The use of other medical resources and the use of non-medical resources was analyzed for the overall population included in the QoL research project. Again, all the results were reported in terms of absolute and relative frequencies.

RESULTS

Baseline Characteristics of Patients

The baseline characteristics of patients enrolled in TOSCA registry were analyzed in detail. In brief, a total of 2,214 patients from 31 countries worldwide were enrolled into the study. Data from 2,211 eligible patients were analyzed as part of the TOSCA clinical study report delivered to Health Authorities by Novartis AG. Data of 3 patients were excluded from the analysis because of major protocol deviations. Of the analyzed patients, 1,152



	Baseline (N=1237)	FU1 (N=599)	FU2 (N=530)	FU3 (N=493)	FU4 (N=231)	FU5 (N=28)
GABAergics	819 (66.2%)	398 (66.4%)	343 (64.7%)	328 (66.5%)	148 (64.1%)	13 (46.4%)
ACTH	35 (2.8%)	11 (1.8%)	3 (0.6%)	5 (1.0%)	1 (0.4%)	0 (0.0%)
Ketogenic diet	59 (4.8%)	41 (6.8%)	31 (5.8%)	27 (5.5%)	7 (3.0%)	1 (3.6%)
Fructose derivates	49 (4.0%)	28 (4.7%)	24 (4.5%)	25 (5.1%)	6 (2.6%)	0 (0.0%)
Vagal nerve stimulator	48 (3.9%)	39 (6.5%)	36 (6.8%)	37 (7.5%)	20 (8.7%)	1 (3.6%)
mTOR inhibitors	103 (8.3%)	71 (11.8%)	77 (14.5%)	67 (13.6%)	34 (14.7%)	4 (14.3%)
Surgery	84 (6.8%)	27 (4.5%)	23 (4.3%)	26 (5.3%)	13 (5.6%)	1 (3.6%)
Other	823 (66.5%)	439 (73.3%)	397 (74.9%)	346 (70.2%)	175 (75.8%)	22 (78.6%)

FIGURE 2 | Treatments for Focal Seizures in each Follow-up Visit. Patients may receive more than one treatment. Baseline data refers to patients who "ever had" the manifestation.

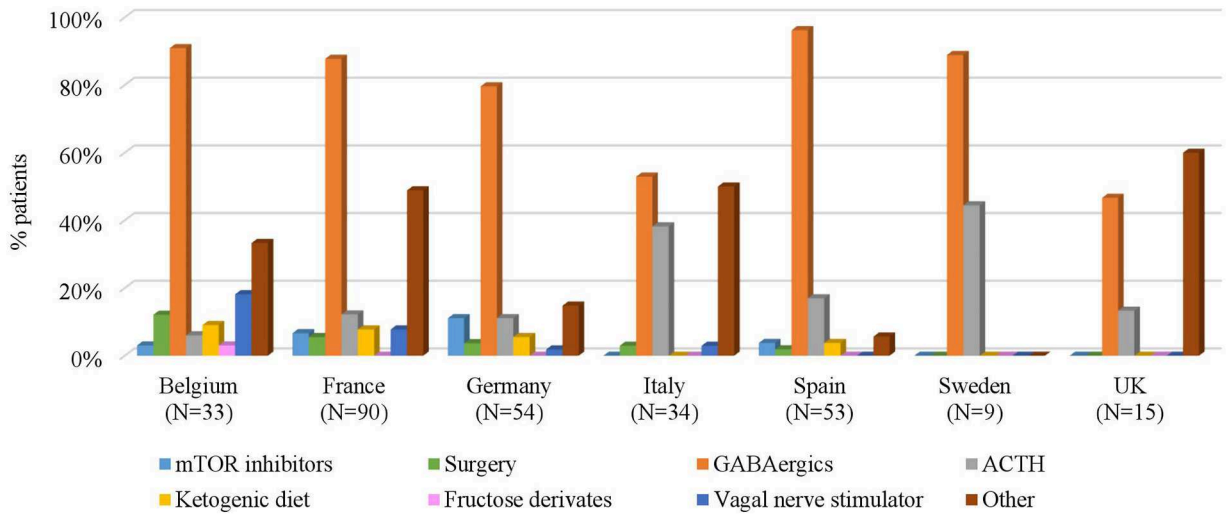
(52.1%) were female. The median age at enrolment was 13 years (range <1–71), and the median age at first TSC diagnosis was 1 year (range <1–69 years). The most common manifestation was epilepsy occurring in 1,879 (85.0%) of patients. Among patients with epilepsy, 1,343 (71.5%) had focal seizures (FS) and 735 (39.1%) had infantile spasms (IS). Other common manifestations were hypomelanotic macules in 1,555 patients (70.3%), facial angiofribromas in 1,533 patients (69.3%), and rAML in 1,317 patients (59.6%).

Another important manifestation was TAND, even though it was the most underassessed aspect of TSC in the registry. TAND assessment includes the evaluation of common behavioral problems, psychiatric disorders, intellectual abilities, academic performance, and neuropsychological difficulties. At baseline, only 818 out of 2,211 (37%) patients reported to have at least one behavioral problem, in 319 (14.4%) patients autism spectrum disorder (ASD) and in 267 (12.1%) patients attention deficit hyperactivity disorders (ADHD) was diagnosed, and 82 (3.7%) and 132 (6.0%) patients had depressive disorders or anxiety, respectively. In addition, 736 patients (33.3%) were reported to have difficulties in academic performance. Among the 894 patients with reported TAND, normal intellectual ability (defined as full scale IQ ≥80) was reported for 44.2% (395/894).

Treatments

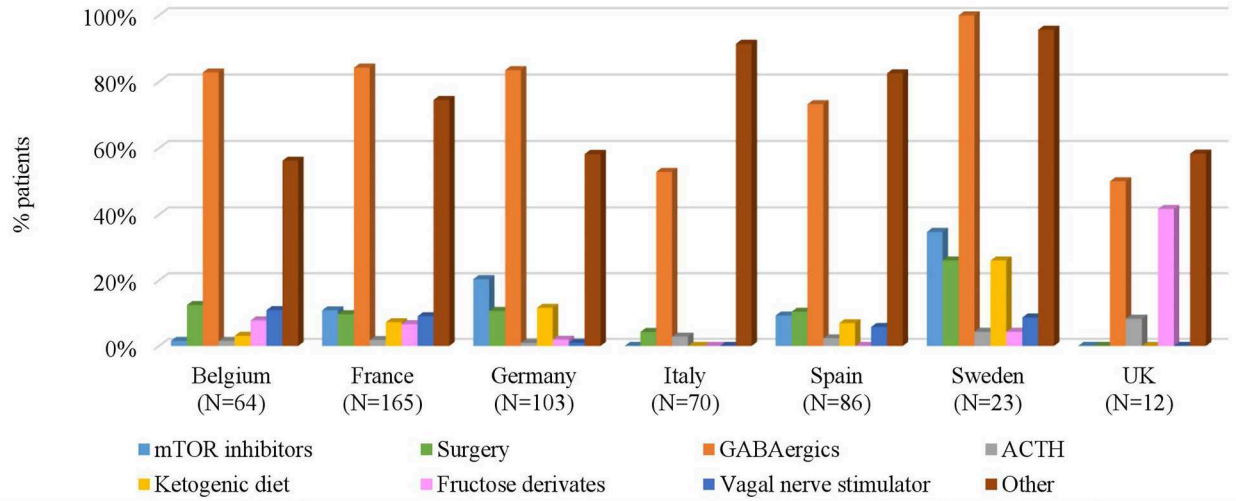
In the TOSCA registry, the proportion of patients who received treatment varied largely depending on the clinical manifestations (**Table 1**), with values at baseline (patients who ever had the manifestation) ranging between 96.8% (698/721) for IS and 32.5% (50/154) for LAM. Almost all patients with epilepsy received antiepileptic drug treatment without relevant variations throughout the study (**Table 1**). At baseline, the most common treatments were GABAergic agents (e.g., vigabatrin), both in mono- and combination therapy), which were used in 79.3% of treated patients with IS, and in 66.2% of treated patients with FS (**Figures 1**, **2**).

However, the use of GABAergic agents decreased over time, reaching a minimum of 14.3% in the fifth FU visit for the IS patients and 46.4% for FS patients. Other treatment options such as mammalian target of rapamycin (mTOR) inhibitors, the ketogenic diet (KD) and epilepsy surgery were used in <20%



	Belgium (N=33)	France (N=90)	Germany (N=54)	Italy (N=34)	Spain (N=53)	Sweden (N=9)	UK (N=15)
mTOR inhibitors	1 (3.0%)	6 (6.7%)	6 (11.1%)	0 (0.0%)	2 (3.8%)	0 (0.0%)	0 (0.0%)
Surgery	4 (12.1%)	5 (5.6%)	2 (3.7%)	1 (2.9%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
GABAergics	30 (90.9%)	79 (87.8%)	43 (79.6%)	18 (52.9%)	51 (96.2%)	8 (88.9%)	7 (46.7%)
ACTH	2 (6.1%)	11 (12.2%)	6 (11.1%)	13 (38.2%)	9 (17.0%)	4 (44.4%)	2 (13.3%)
Ketogenic diet	3 (9.1%)	7 (7.8%)	3 (5.6%)	0 (0.0%)	2 (3.8%)	0 (0.0%)	0 (0.0%)
Fructose derivates	1 (3.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Vagal nerve stimulator	6 (18.2%)	7 (7.8%)	1 (1.9%)	1 (2.9%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Other	11 (33.3%)	44 (48.9%)	8 (14.8%)	17 (50.0%)	3 (5.7%)	0 (0.0%)	9 (60.0%)

FIGURE 3 | Treatments for Infantile Spasms by Country. Patients may receive more than one treatment.



	Belgium (N=64)	France (N=165)	Germany (N=103)	Italy (N=70)	Spain (N=86)	Sweden (N=23)	UK (N=12)
mTOR inhibitors	1 (1.6%)	18 (10.9%)	21 (20.4%)	0 (0.0%)	8 (9.3%)	8 (34.8%)	0 (0.0%)
Surgery	8 (12.5%)	16 (9.7%)	11 (10.7%)	3 (4.3%)	9 (10.5%)	6 (26.1%)	0 (0.0%)
GABAergics	53 (82.8%)	139 (84.2%)	86 (83.5%)	37 (52.9%)	63 (73.3%)	23 (100.0%)	6 (50.0%)
ACTH	1 (1.6%)	3 (1.8%)	1 (1.0%)	2 (2.9%)	2 (2.3%)	1 (4.3%)	1 (8.3%)
Ketogenic diet	2 (3.1%)	12 (7.3%)	12 (11.7%)	0 (0.0%)	6 (7.0%)	6 (26.1%)	0 (0.0%)
Fructose derivates	5 (7.8%)	11 (6.7%)	2 (1.9%)	0 (0.0%)	0 (0.0%)	1 (4.3%)	5 (41.7%)
Vagal nerve stimulator	7 (10.9%)	15 (9.1%)	1 (1.0%)	0 (0.0%)	5 (5.8%)	2 (8.7%)	0 (0.0%)
Other	36 (56.3%)	123 (74.5%)	60 (58.3%)	64 (91.4%)	71 (82.6%)	22 (95.7%)	7 (58.3%)

FIGURE 4 | Treatments for Focal Seizures by Country. Patients may receive more than one treatment.

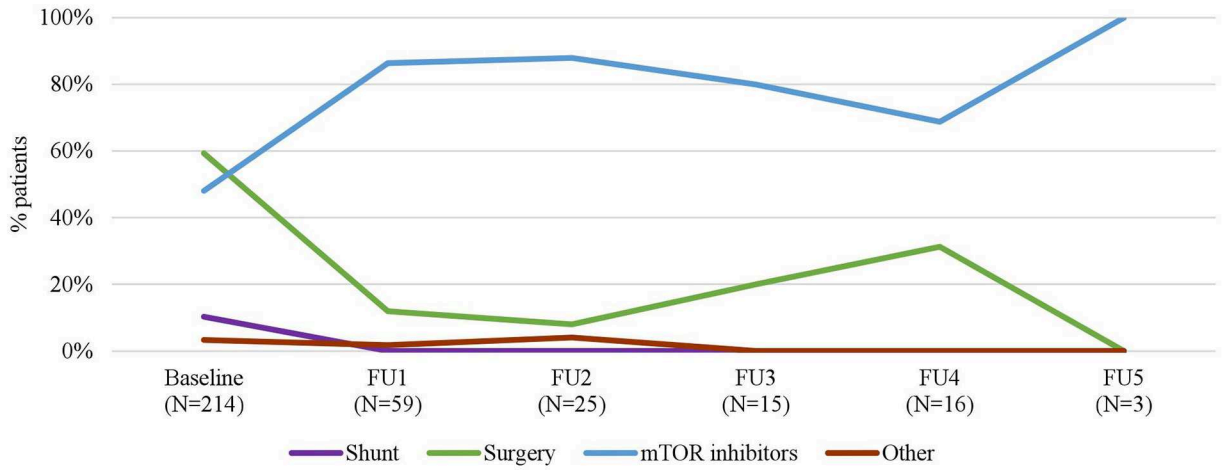
of the patients at baseline, and remained relatively stable over time (**Figures 1**, **2**).

When analyzing the types of treatment by country, GABAergics alone or in combination were by far the most common treatment options in all countries both in patients with IS (ranging between 46.7% in the UK and 96.2% in Spain) and in patients with FS (ranging between 50% in the UK and 100% in Sweden) (**Figures 3**, **4**). Adrenocorticotropic Hormone (ACTH) was the second most common treatment for treating IS in all countries except in Belgium. Other common treatments for treating FS were epilepsy surgery (in Belgium, Italy, and Spain) and mTOR inhibitors (in Sweden, Germany, and France) (**Figure 4**). Of note, both surgery and mTOR inhibitors were not used at all in patients with IS or FS from the UK, and in patients with IS from Sweden. More than 50% of the treatments in patients with FS were not specified (included in "others" category) in all countries, even more than 90% in Italy and Sweden (**Figure 4**).

At baseline, 40.0% of patients had ever received treatment for SEGA and this proportion remained stable over time (**Table 1**). mTOR inhibitors and surgery were the most common procedures in patients with SEGA with marked differences depending on follow-up, age and the country of residence (**Figure 5**). At baseline, mTOR inhibitors were administered in 48.1% of the patients who received treatment for SEGA, but their use increased over time (reaching 86.4% of patients in the 1st FU visit and 100% in the 5th). In contrast, 59.3% patients received surgery at baseline, but the proportion of patients undergoing surgery decreased over time as the use of mTOR inhibitors increased (reaching 11.9% of patients in the 1st FU visit and no patients in the 5th) (**Figure 5**).

The proportion of patients treated for SEGA also varied depending on the age at baseline. Children aged 9–14 were treated most commonly [50 (51.0%) patients received treatment] while children aged <2 years and adults aged more than 40 years were treated least frequently [7 (15.2%) and 8 (29.6%) of patients, respectively]. Likewise, the types of treatment varied across age groups. While mTOR inhibitors were the most common treatments used in children aged 9 or less [reaching a peak (70%) in those aged between 5 and 9], surgery was the most common treatment in adolescents and adults [reaching a peak (87.5%) in those aged more than 40] (**Figure 6**).

Regarding the use of treatments for SEGA by country, mTOR inhibitors were more often prescribed in Germany (70% of the



	Baseline (N=214)	FU1 (N=59)	FU2 (N=25)	FU3 (N=15)	FU4 (N=16)	FU5 (N=3)
Shunt	22 (10.3%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Surgery	127 (59.3%)	7 (11.9%)	2 (8.0%)	3 (20.0%)	5 (31.3%)	0 (0.0%)
mTOR inhibitors	103 (48.1%)	51 (86.4%)	22 (88.0%)	12 (80.0%)	11 (68.8%)	3 (100.0%)
Other	7 (3.3%)	1 (1.7%)	1 (4.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

FIGURE 5 | Treatments for SEGA in each Follow-up Visit. Patients may receive more than one treatment. Baseline data refers to patients who "ever had" the manifestation.

patients) and Spain (100% of the patients) than in the rest of the participating countries (**Figure 7**). In contrast, surgery was the most common treatment in Belgium (77.8%) and in France (76.9%). The only patient from the UK (100%) also underwent surgery.

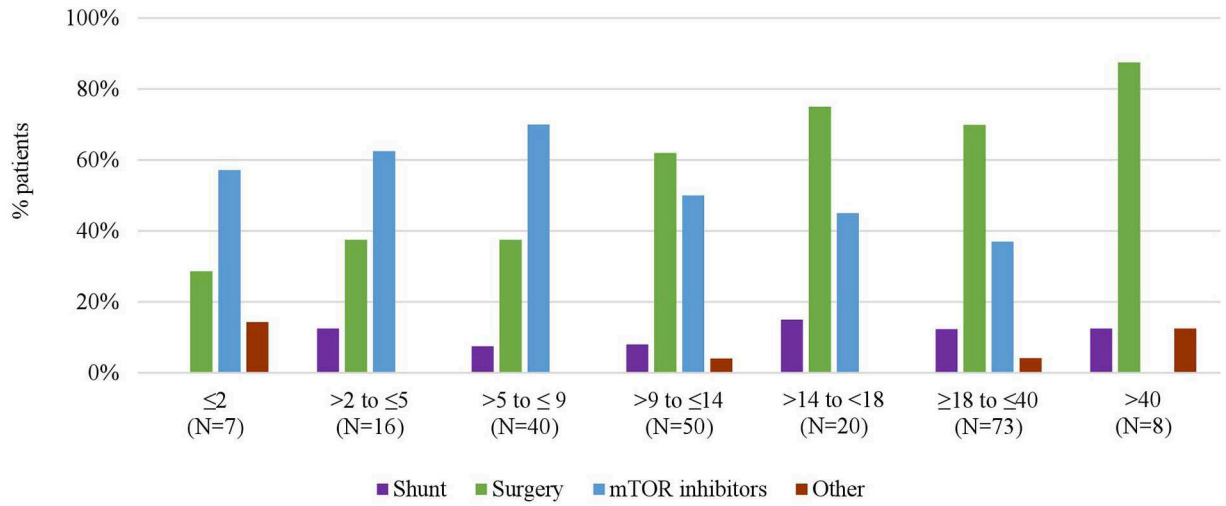
With respect to rAML, the number of patients treated was 315 (29.7%) at baseline, kept at around 30% up to FU 3 and increased in FU 4 (35.0%) and FU 5 (43.8%) (**Table 1**). Similarly to SEGA, mTOR inhibitors and embolization were the most common treatments for rAML patients (**Figure 8**). At baseline, 144 (45.7%) patients received mTOR inhibitors and 141 (44.8%) patients underwent embolization; however, the use of all treatments consistently decreased with time with only 8 (15.1%) patients in FU 5 receiving mTOR inhibitors. Data on embolizations were not available for any patient at the end of the period and only one patient (0.6%) underwent this procedure in FU 4 (**Figure 8**). rAML is an uncommon manifestation in children. Therefore, most of the patients receiving treatment for rAML were adolescent and adults (**Figure 9**). Embolizations were rare in children (only 7.4% of patients aged 9–14 had undergone this procedure) whereas more than half of rAML patients aged 18–40 (51.8%) and older (58.3%) underwent this procedure. In contrast, there was a high use of mTOR inhibitors for rAML in these young patients, which certainly was prescribed for other TSC manifestations, which decreased for older patients (**Figure 9**). The distribution of treatments by country is shown in **Figure 10**. It can be observed that mTOR inhibitors were the most commonly used treatment option for rAML in all countries (**Figure 10**).

As for LAM, the number of treated patients generally decreased with time (**Table 1**). Again, mTOR inhibitors were the most common treatment for this condition (60.0% of LAM patients received mTOR inhibitors at baseline) and its use increased up to 86.0% in FU 3 and 75.0% in FU4 (**Figure 11**). Since, as expected, LAM was only diagnosed in patients aged ≥9 years, no data were available for younger patients. Adolescents were treated with both chest surgery and mTOR inhibitors, while most patients treated during adulthood received mTOR inhibitors (**Figure 12**).

mTOR inhibitors were used for LAM treatment in all patients in France and in Italy, in 66.7% in Germany, 50% in Belgium, and in 25.0% in the UK. No data on the type of treatments used in patients with LAM were available for Spain and Sweden (**Figure 13**).

Hospitalizations and Visits

The frequency of hospitalizations was analyzed in the subset of patients of the TOSCA registry included in the QoL research project ($N = 143$). Regarding visits to the specialist, the same subset was analyzed. Subjects from Spain ($N = 11$) were excluded from the analysis because of data inconsistencies in these patients. As a result, healthcare visits were analyzed in 132



	≤2 (N=7)	>2 to ≤5 (N=16)	>5 to ≤ 9 (N=40)	>9 to ≤14 (N=50)	>14 to <18 (N=20)	≥18 to ≤40 (N=73)	>40 (N=8)
Shunt	0 (0.0%)	2 (12.5%)	3 (7.5%)	4 (8.0%)	3 (15.0%)	9 (12.3%)	1 (12.5%)
Surgery	2 (28.6%)	6 (37.5%)	15 (37.5%)	31 (62.0%)	15 (75.0%)	51 (69.9%)	7 (87.5%)
mTOR inhibitors	4 (57.1%)	10 (62.5%)	28 (70.0%)	25 (50.0%)	9 (45.0%)	27 (37.0%)	0 (0.0%)
Other	1 (14.3%)	0 (0.0%)	0 (0.0%)	2 (4.0%)	0 (0.0%)	3 (4.1%)	1 (12.5%)

FIGURE 6 | Treatments for SEGA according to Age at Baseline. Patients may receive more than one treatment.

patients. A total of 88 visits to the specialists were reported over 12 months during the last year. Half of the patients (69/132; 52.3%) visited the specialist due to TSC at least once during the last year, and a quarter (29/132; 22.0%) had 3 or more visits. Visits to the specialist for reasons other than TSC were reported for 34 patients (25.8%), and 14 of them (10.6%) reported 3 or more visits during the last year (**Table S3**). Visits to the general practitioner (GP) were discarded from the analysis because of missing data (information was missing or unknown for more than 50% of the patients).

No hospitalizations were reported for 70.6% of the patients over 12 months during the last year. A third of the patients (41/143; 28.7%) reported at least one hospitalization, and 6.3% (9/143) reported 3 or more hospitalizations (**Table S4**).

Information on the use of non-medical resources (education, employment, use of social services and patient support requirements) was collected within the QoL research project, and this is summarized in **Table S5**.

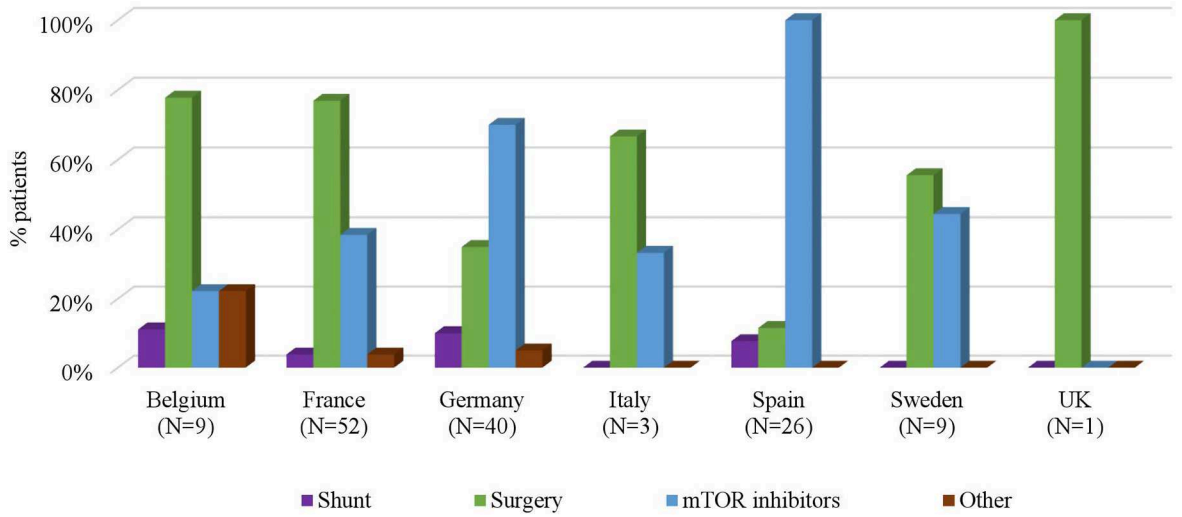
Regarding education, 28 children (31.8%) were not in a mainstream school, and the rest (N = 57; 64.8%) were educated in a mainstream school. Of those who attended a mainstream school, 64.9% received special education within the school, and for 45.6% (26/57) the school offered special programs adequate to their condition (**Table S5**).

In the questionnaire used for data collection into this research project, 55 adults with TSC who were able to complete the questionnaire themselves and 88 carers for children with TSC reported their work experience. Only half of the individuals [41.8% (23/55) adult patients and 65.9% (58/88) children's carers] reported to have a job. A quarter of the adult patients (14/55; 25.5%) reported that they were not able to work due to TSC and half (28/55; 50.9%) stated that TSC had an impact on their career. The corresponding figures for these two items in children's carers were 9.1% (8/88) and 56.8% (50/88) (**Table S5**).

Besides, half of the children (45/88; 51.1%) and 38.2% (21/55) of the adults received a disability allowance, and 20% (11/55) of the adults received support with daily activities. Other services such as psychological counseling, social services, and social worker services were received by <15% of the patients irrespective of their age (**Table S5**).

DISCUSSION

The present work investigated treatment patterns and use of medical/non-medical resources in patients enrolled into the TOSCA registry. Compared to other studies carried out in single countries including a limited number of patients of certain age-groups or with specific manifestations (9–16, 20),



	Belgium (N=9)	France (N=52)	Germany (N=40)	Italy (N=3)	Spain (N=26)	Sweden (N=9)	UK (N=1)
Shunt	1 (11.1%)	2 (3.8%)	4 (10.0%)	0 (0.0%)	2 (7.7%)	0 (0.0%)	0 (0.0%)
Surgery	7 (77.8%)	40 (76.9%)	14 (35.0%)	2 (66.7%)	3 (11.5%)	5 (55.6%)	1 (100.0%)
mTOR inhibitors	2 (22.2%)	20 (38.5%)	28 (70.0%)	1 (33.3%)	26 (100.0%)	4 (44.4%)	0 (0.0%)
Other	2 (22.2%)	2 (3.8%)	2 (5.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

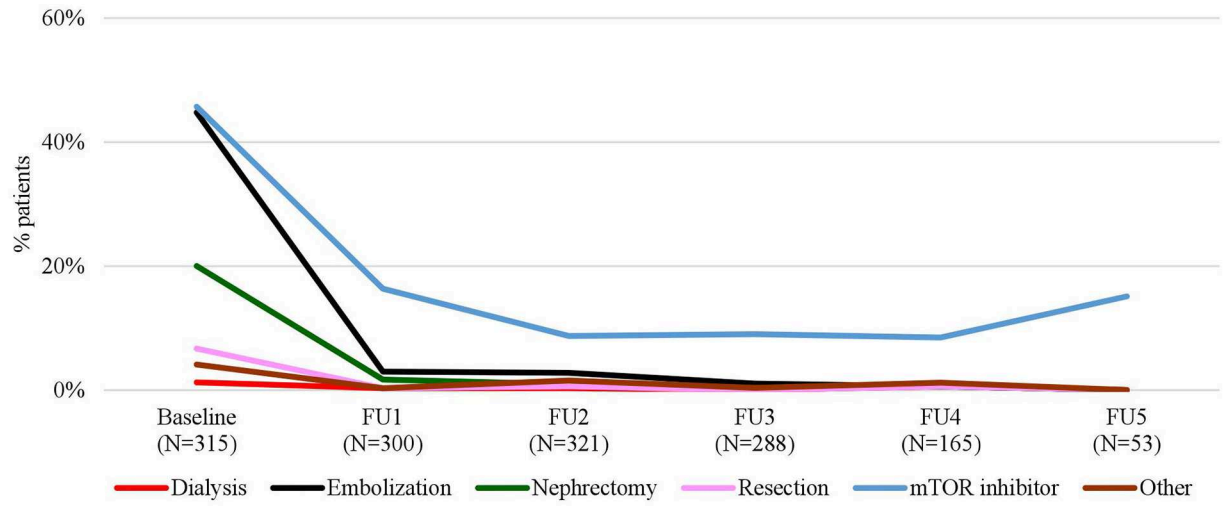
FIGURE 7 | Treatments for SEGA by Country. Patients may receive more than one treatment.

the TOSCA registry represented a unique opportunity to analyse the treatment patterns and use of resources in a large cohort of pediatric and adult patients with a wide range of clinical manifestations who had been diagnosed and treated in different countries over a 5-year observation period. This strengthens the external validity of the results and provides clues on how treatment patterns have changed over time and across regions.

One of the purposes of the 2012 International TSC Consensus Conference was to provide recommendations for standardized diagnostic criteria, management and surveillance of TSC regardless of age (21). This study shows that treatment patterns mostly depend on the clinical manifestations of the disease but also that they depend on the age and the country of residence of the patients. For instance, there are important variations in the use of mTOR inhibitors in patients with SEGA throughout countries (ranging from none in the UK to 100% in Spain), and on the age of the patients (ranging from 70% in patients aged 5–9 to 0% in patients aged >40).

The differences between countries reflect not only the effect of clinical practice, but also the effect of access barriers due to different time points at which mTOR inhibitors were available for the various indications in specific countries and/or healthcare systems. For instance, everolimus was reimbursed for patients with FS in January 2017 in Germany and April 2018 in Sweden, but was not made available until late 2018 or the beginning of 2019 in the rest of European countries (June 2018 in Spain, September 2018 in Italy, December 2018 in Belgium, and in the UK, and January 2019 in France). For patients with SEGA, everolimus was reimbursed in October 2011 in Germany and in the UK only through the Individual Funding Request (IFR) route, while it was not available until 2016 in Italy and in Belgium. Another example is the availability of mTOR inhibitors for patients with rAML as everolimus was reimbursed in the UK in October 2011, in Germany in November 2012, and in France in April 2014, even though it was not available in Spain until April 2015 and in Belgium until August 2016, and it is still not yet reimbursed in Italy.

In addition, the differences in age groups might reflect differences in clinical practice between pediatric and adult neurologists in those manifestations treated before the TOSCA registry and within the time horizon of the TOSCA registry (i.e., after baseline). In line with the current guidelines (21, 22), which recommend the use of vigabatrin as a first-line antiepileptic drug treatment in patients with TSC and either IS or FS before the age of 1 year, the most prescribed drugs were GABAergics. In any case, these results must be interpreted with caution due to the large proportion of treatments included in the category "others" (at baseline, 44.4% for IS and 66.5% for FS) and to the fact that the category "GABAergics" included a large number of different AEDs. In future studies,



	Baseline (N=315)	FU1 (N=300)	FU2 (N=321)	FU3 (N=288)	FU4 (N=165)	FU5 (N=53)
Dialysis	4 (1.3%)	1 (0.3%)	1 (0.3%)	0 (0.0%)	1 (0.6%)	0 (0.0%)
Embolization	141 (44.8%)	9 (3.0%)	9 (2.8%)	3 (1.0%)	1 (0.6%)	0 (0.0%)
Nephrectomy	63 (20.0%)	5 (1.7%)	3 (0.9%)	1 (0.3%)	1 (0.6%)	0 (0.0%)
Resection	21 (6.7%)	1 (0.3%)	2 (0.6%)	0 (0.0%)	1 (0.6%)	0 (0.0%)
mTOR inhibitors	144 (45.7%)	49 (16.3%)	28 (8.7%)	26 (9.0%)	14 (8.5%)	8 (15.1%)
Other	13 (4.1%)	1 (0.3%)	5 (1.6%)	1 (0.3%)	2 (1.2%)	0 (0.0%)

FIGURE 8 | rAML Treatments according to Follow-Up. Patients may receive more than one treatment. Baseline data refers to patients who "ever had" the manifestation.

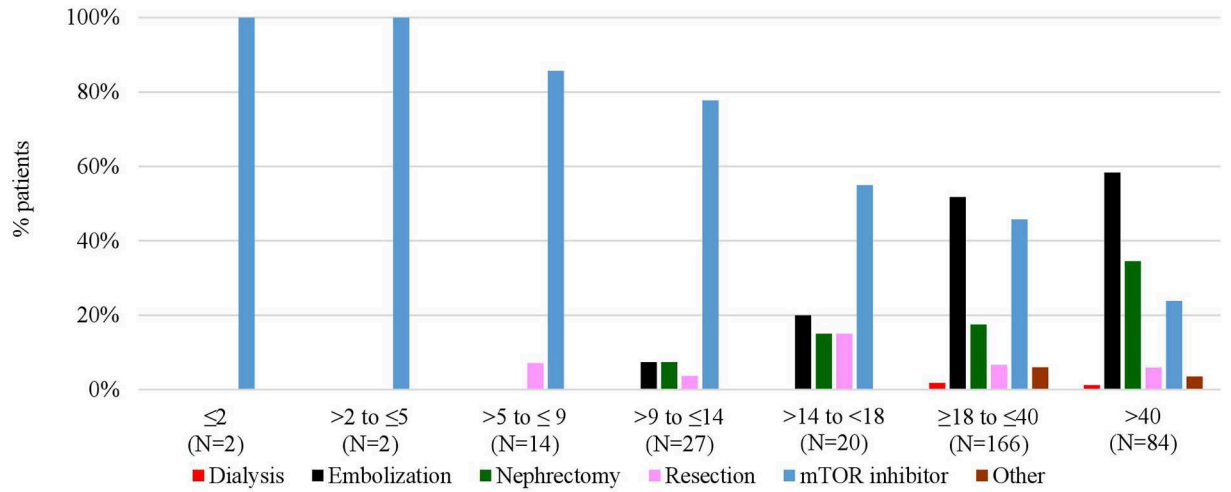
more attention should therefore be paid to the definition of treatment variables.

Besides, one has to take into consideration, that TOSCA enrollment started in August 2012, and last data entry was in August 2017. Everolimus, was approved by European Medicines Agency (EMA) for the treatment of drug-resistant epilepsy as late as in January 2017. It was therefore not possible to evaluate the consequences of the approval of this mTOR inhibitor on the treatment patterns of patients with TSC-associated epilepsies. Despite this, physicians struggling to treat TSC-associated seizures that had proved refractory to conventional AED treatment had already started using everolimus with increasing frequency. We hypothesize that this use was due to other TSC-associated conditions and on-going mTOR studies in epilepsy.

This study shows how mTOR inhibitors have become common treatments for a variety of manifestations in patients with TSC such as SEGA, LAM, and rAML. However, since more than one manifestation might co-occur in a single patient, it may not be correct to attribute the use of mTOR inhibitors to a single manifestation. An example of this is the use of mTOR inhibitors in patients with LAM as a consequence of the growing use of mTOR inhibitors for other indications in patients with TSC.

In patients with SEGA, current recommendations propose the use of surgical resection for acutely symptomatic SEGA, the use of both surgery and mTOR inhibitors for growing but asymptomatic SEGA and the use of mTOR inhibitors for patients with large or bilateral SEGA that are not amenable to surgical resection (21, 23). In line with the recommendations, the analyses on the use of treatment according to FU visits, countries, and age groups in the patients included in the TOSCA registry show that the increases in the use of mTOR are often accompanied by decreases in the use of surgery. For instance, it is particularly striking to observe how the increasing use of mTOR inhibitors registered in the different FU visits (**Figure 5**) is almost a mirror image of the decreasing use of surgery, and to observe how in age groups and countries where mTOR inhibitors are used the most, surgery is used the least and vice versa (**Figures 6, 7**).

The exact economic cost of these changes was not possible to evaluate from this dataset. However, the potential reductions and delays in the use of surgery may have economic implications not only at the time of treatment initiation, but also in the follow-up of the patients. In this regard, a study comparing pre-surgery and post-surgery costs in TSC patients with SEGA surgery carried out in the US (24) found that medication and total costs in the post-surgery year were 1.6–4.3 times the costs in the pre-surgery year. Unfortunately, no formal economic evaluations comparing



	≤2 (N=2)	>2 to ≤5 (N=2)	>5 to ≤ 9 (N=14)	>9 to ≤14 (N=27)	>14 to <18 (N=20)	≥18 to ≤40 (N=166)	>40 (N=84)
Dialysis	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	3 (1.8%)	1 (1.2%)
Embolization	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	4 (20.0%)	86 (51.8%)	49 (58.3%)
Nephrectomy	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (7.4%)	3 (15.0%)	29 (17.5%)	29 (34.5%)
Resection	0 (0.0%)	0 (0.0%)	1 (7.1%)	1 (3.7%)	3 (15.0%)	11 (6.6%)	5 (6.0%)
mTOR inhibitors	2 (100.0%)	2 (100.0%)	12 (85.7%)	21 (77.8%)	11 (55.0%)	76 (45.8%)	20 (23.8%)
Other	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	10 (6.0%)	3 (3.6%)

FIGURE 9 | rAML Treatments according to Age at Baseline. Patients may receive more than one treatment.

surgery and mTOR inhibitors in patients with SEGA have been carried out.

Interestingly, the use of surgery in patients with SEGA was lower in the TOSCA registry (**Figure 5**) than in a previous survey study carried out by Rentz et al. (15). This study included 676 patients -or caregivers- and reported surgery in 31 and 47% of pediatric and adult patients, respectively, but did not report any use of mTOR inhibitors in any of the groups. Comparing the use of medical resources, and in particular the use of surgery depending on whether the patients receive treatment with mTOR inhibitors is an area of major interest that remains largely unexplored.

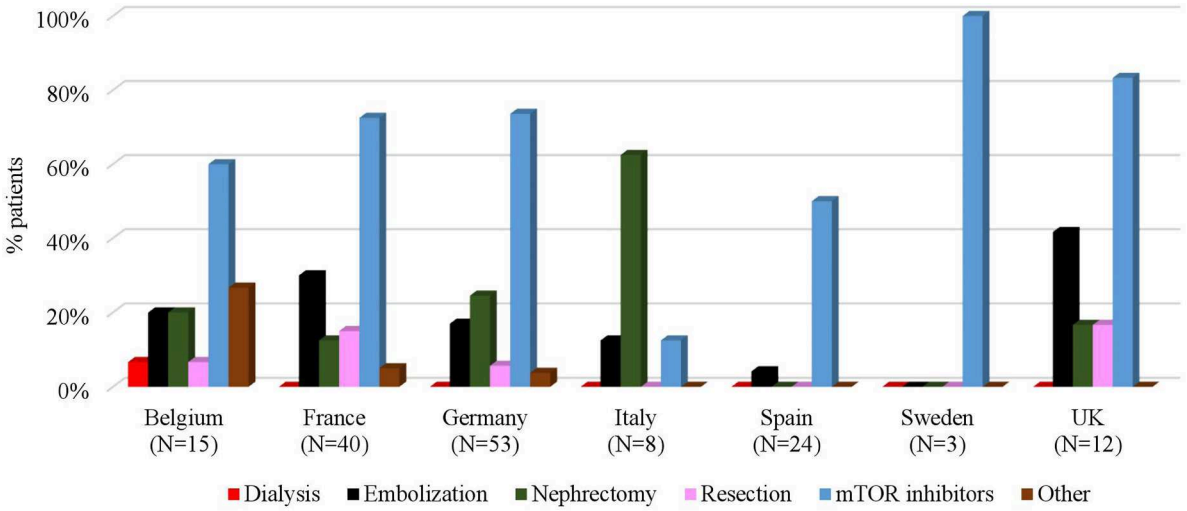
The results observed in rAML and LAM are in line with those observed in patients with SEGA. However, as stated above, since we are considering a population with co-occurring manifestations it is difficult to determine if mTOR inhibitors were used to treat these particular manifestations. It is worth commenting that in Sweden, where 100% of patients with rAML who received treatment received mTOR inhibitors, no patients had nephrectomy surgery; by contrast, in Italy, where only 12.5% of the patients who received treatment for this manifestation were treated with mTOR inhibitors, 62.5% had nephrectomy surgery (**Figure 10**).

While these results might also be influenced by the age of the patients in each country at baseline, it is important to emphasize that embolization surgery in rAML and chest surgery in LAM are rescue therapies in urgent situations, but mTOR inhibitors are the only available treatment that both modifies the disease and improves the outcomes (21, 25, 26).

A reason for the increased use of mTOR inhibitors in patients with LAM might be its inclusion in the recent international guidelines published for the diagnosis and management of LAM, in which mTOR inhibitors were recommended for patients with abnormal or declining lung function or with problematic chylous effusions, that could have affected the treatment patterns (27).

Given that TSC is a multi-organ disease, treatment of a certain manifestation with a systemic mTOR inhibitor will probably result in reductions of the use of surgical interventions for other manifestations as well. Concomitant systemic effects in patients treated with mTOR inhibitors have been reported (28). The impact of these effects on the use of other treatments or other medical resources have not yet been analyzed and is an interesting topic for future research. The consistent reductions in the use of surgery observed for all the manifestations in the present study support this hypothesis.

Similar to other studies (11, 15, 20), this study shows that patients with TSC are demanding healthcare resource users, but it also shows that the use of resources is not evenly distributed across patients and countries. In this regard, while a third of the patients included in the QoL research project did not attend



	Belgium (N=15)	France (N=40)	Germany (N=53)	Italy (N=8)	Spain (N=24)	Sweden (N=3)	UK (N=12)
Dialysis	1 (6.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Embolization	3 (20.0%)	12 (30.0%)	9 (17.0%)	1 (12.5%)	1 (4.2%)	0 (0.0%)	5 (41.7%)
Nephrectomy	3 (20.0%)	5 (12.5%)	13 (24.5%)	5 (62.5%)	0 (0.0%)	0 (0.0%)	2 (16.7%)
Resection	1 (6.7%)	6 (15.0%)	3 (5.7%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (16.7%)
mTOR inhibitors	9 (60.0%)	29 (72.5%)	39 (73.6%)	1 (12.5%)	12 (50.0%)	3 (100.0%)	10 (83.3%)
Other	4 (26.7%)	2 (5.0%)	2 (3.8%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

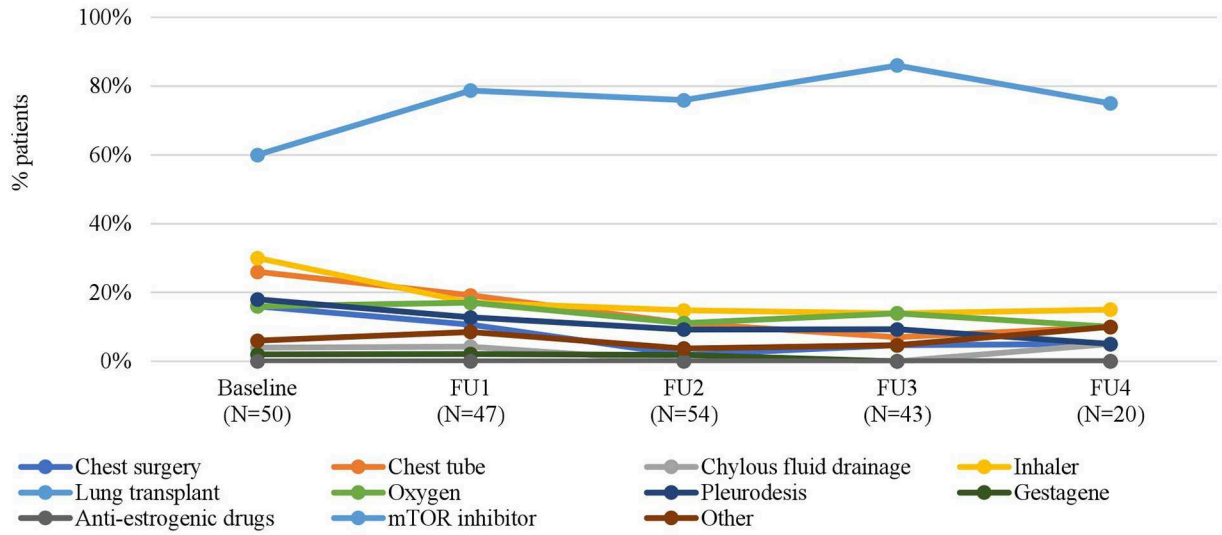
FIGURE 10 | rAML Treatment by Country. Patients may receive more than one treatment.

any specialist due to TSC during the past year, a quarter of the patients had three or more visits in the same period. Likewise, while 71% of the patients were not hospitalized at any time, up to 6.3% were hospitalized three or more times during the past year. In future studies, it would be interesting to identify the clinical features of the patients who are likely to be more intense resource users in order to provide a better allocation of resources for the management of the disease.

The present study also shows that the impact of TSC on education and on employability is high. More than half of the children had special needs (were not in a mainstream school or received special education within their school), and unemployment rates were high both in patients and caregivers of children with TSC (34.1% in children's caregivers, and up to 50% in adults with TSC). Therefore, the economic impact of a TSC diagnosis is high for the patients and for their families. In line with these results, a multicenter French study that included adult patients with TSC and with a diagnosed epilepsy before 16 years old found that 52% of patients required special education programs and only 37% reported having a stable professional life, even though 65% of them had a salary below the minimum income threshold in France (29).

The rate of patients receiving psychological support was reportedly low both for adults and children. The same low rates were observed in the multicenter French study, where 35% of children and 13% of adults had a regular psychological follow-up (29). This contrasts with the expected rates of TAND and suggests that the psychological needs of patients are not being addressed properly. Of note, physicians' unawareness and no clear guidelines on TAND evaluation before 2013 might have led to more missing data, underestimating TAND difficulties. However, a set of consensus guidelines for the evaluation of neuropsychiatric problems had already been published in 2005 (30), suggesting that there was a lack of implementation of existing guidelines. Likewise, the proportion of patients receiving disability allowances was higher in children (51.1%) than adults (38.2%), the use of social worker services was reportedly lower in both children and adults (8.0% in children and 1.8 % in adults), and <10% of patients (5.7% of children and 3.6% of adults) reported to have received help while completing benefit applications. Altogether, these results indicate that many patients with TSC might be unaware of the possibility of receiving social services or that these services are not available in all the countries.

A strength of the TOSCA registry was the prospective follow-up of patients, which allowed to trace changes in treatment patterns over time. However, data from the two last follow-up visits (after 4 and 5 years) were available, for only 764 and 147 patients out of 2,211, respectively. Hence, caution is required when drawing conclusions from the last two visits. Although



	Baseline (N=50)	FU1 (N=47)	FU2 (N=54)	FU3 (N=43)	FU4 (N=20)
Chest surgery	8 (16.0%)	5 (10.6%)	1 (1.9%)	2 (4.7%)	1 (5.0%)
Chest tube	13 (26.0%)	9 (19.1%)	6 (11.1%)	3 (7.0%)	2 (10.0%)
Chylous fluid drainage	2 (4.0%)	2 (4.3%)	0 (0.0%)	0 (0.0%)	1 (5.0%)
Inhaler	15 (30.0%)	8 (17.0%)	8 (14.8%)	6 (14.0%)	3 (15.0%)
Lung transplant	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Oxygen	8 (16.0%)	8 (17.0%)	6 (11.1%)	6 (14.0%)	2 (10.0%)
Pleurodesis	9 (18.0%)	6 (12.8%)	5 (9.3%)	4 (9.3%)	1 (5.0%)
Gestagene	1 (2.0%)	1 (2.1%)	1 (1.9%)	0 (0.0%)	0 (0.0%)
Anti-estrogenic drugs	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
mTOR inhibitor	30 (60.0%)	37 (78.7%)	41 (75.9%)	37 (86.0%)	15 (75.0%)
Other	3 (6.0%)	4 (8.5%)	2 (3.7%)	2 (4.7%)	2 (10.0%)

FIGURE 11 | LAM Treatments according to Follow-Up. Patients may receive more than one treatment. Baseline data refers to patients who "ever had" the manifestation.

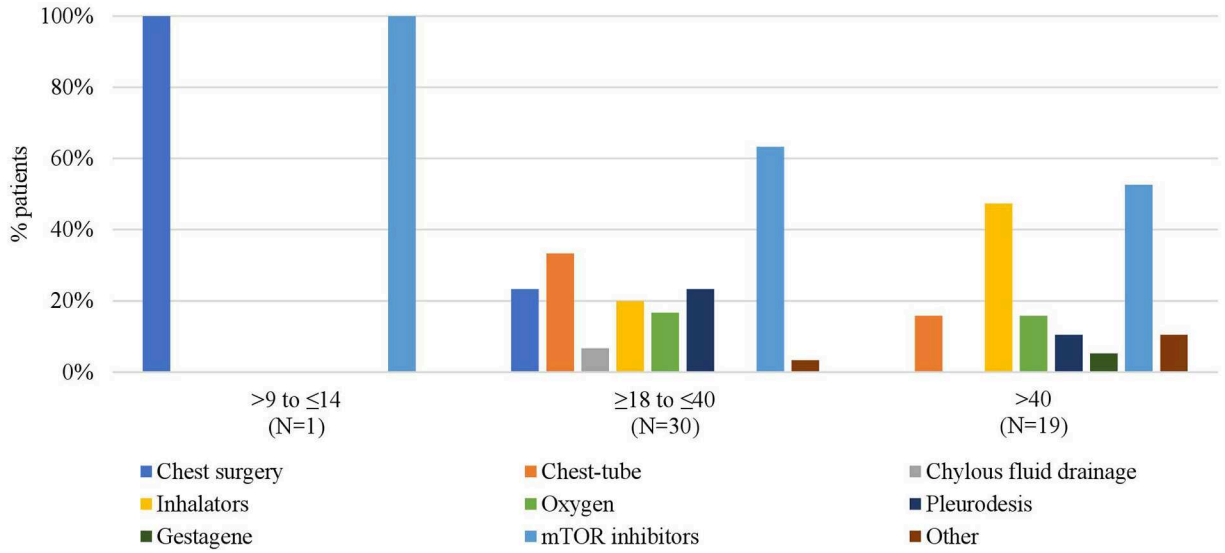
the number of patients in the last follow-up is relatively low compared to the patients for whom data was available at baseline, other studies on use of resources in patients with TSC have been carried out in patient cohorts with a smaller sample size. For instance, a study carried out by Skalicky et al. (20) included 116 patients and another study carried out by Lennert et al. (12) included only 95 patients.

The present study has some limitations. The main caveat was that data relating resource use from the QoL research project was collected for <10% of the patients included in the registry, which is in contrast with excellent data quality for the medical aspects of TSC recorded in the core study. This might be due to the fact that data collection of data into the QoL research study was not mandatory, due to the observative nature of the registry, or might be due to the absence of site monitoring review of the QoL research project data collection. Carrying out specific studies to broaden the evidence on the use of medical resources in patients with TSC remains an interesting topic for future research.

Also, the observational nature of the TOSCA registry meant that only available data from standard clinical practice was supposed to collected. As recruitment was made through centers with expertise in TSC, where mainly moderate-severe TSC manifestations are seen, milder cases could have been underestimated. Getting data from routine practice also meant discrepancies in some variables, as the way information is collected within centers is not homogeneous. In any case, the involvement of various centers and specialists has helped inclusion of a significant number of TSC patients, which should be representative of real clinical practice.

Unlike in other studies evaluating the costs of managing TSC manifestations carried out in a single country (10, 11, 13, 14, 16), costs estimations could not be performed given that the analyses were conducted using data from 31 countries with different healthcare systems.

Furthermore, there are differences between the design of this study and that of previous studies evaluating the use of resources in TSC patients (10–16, 20), which limits the conclusions that can be drawn when comparing our results. Besides the differences in geographical areas and timeframes, while the TOSCA registry included patients with proven TSC, but regardless of specific manifestations, only three of the studies published so far (11, 14, 15) were carried out in an overall TSC population (i.e., not



	>9 to ≤14 (N=1)	≥18 to ≤40 (N=30)	>40 (N=19)
Chest surgery	1 (100.0%)	7 (23.3%)	0 (0.0%)
Chest-tube	0 (0.0%)	10 (33.3%)	3 (15.8%)
Chylous fluid drainage	0 (0.0%)	2 (6.7%)	0 (0.0%)
Inhalators	0 (0.0%)	6 (20.0%)	9 (47.4%)
Oxygen	0 (0.0%)	5 (16.7%)	3 (15.8%)
Pleurodesis	0 (0.0%)	7 (23.3%)	2 (10.5%)
Gestagene	0 (0.0%)	0 (0.0%)	1 (5.3%)
mTOR inhibitors	1 (100.0%)	19 (63.3%)	10 (52.6%)
Other	0 (0.0%)	1 (3.3%)	2 (10.5%)

FIGURE 12 | LAM Treatments according to Age at Baseline. Patients may receive more than one treatment.

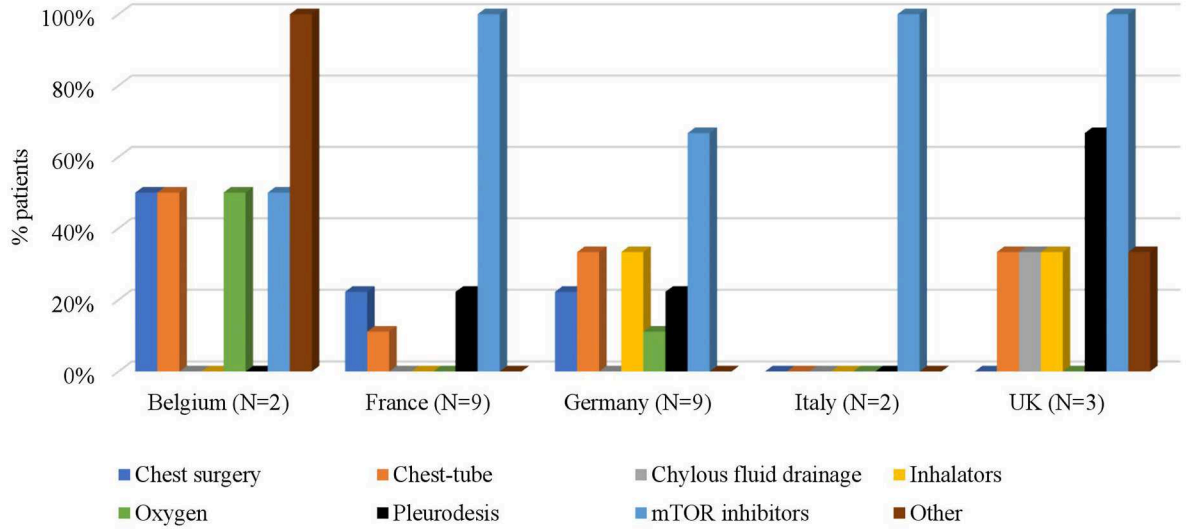
defined by a specific manifestation), while the rest included only patients with epilepsy (10, 12), SEGA (20), LAM (16), or kidney involvement (13).

Our results show that the use of treatments for specific conditions greatly differed depending on the clinical manifestations and the specialists caring for the patients, the period analyzed, as well as their ages and the countries of residence. Therefore, comparing the results of the patients included in the TOSCA registry with those observed in other studies without paying attention to their baseline characteristics might be methodologically inappropriate.

Information about healthcare visits and hospitalizations, as well as about use of non-medical resources, was only available for a cohort of 143 patients from the 7 European countries included in the QoL research project. The fact that all the patients included in this project were treated in European countries limits the ability to extrapolate the conclusions to other continents. Also, some data inconsistencies were found regarding specialist visits in Spanish patients and the information regarding primary care (GP visits) was missing or unknown for half of the patients (50.3% for TSC-related visits and 53.9% for visits for other reasons). Future studies should incorporate monitoring strategies during data collection in order to minimize these issues.

Comparing the use of medical resources in patients with TSC treated with or without mTOR inhibitors remains another area of interest for future research. In addition, the information on medical and non-medical resources in the QoL research project was provided by the patient itself or a caregiver. Although this has been a common methodology in similar studies (10, 11, 15), there can be inconsistencies or missing data if patients do not remember the answers or do not understand the questions. Future research should pay attention to this point, involving specific staff to supervise data completion.

In conclusion, in spite of the limitations indicated above, this study has provided more detailed information about treatment patterns and current use of medical and non-medical resources in a large cohort of patients with TSC followed for a long period of time in seven European countries. It shows how mTOR inhibitors have become common treatments for certain TSC-related manifestations, often accompanied by reductions in the use of surgery. In addition, it confirms that the use of medical



	Belgium (N=2)	France (N=9)	Germany (N=9)	Italy (N=2)	UK (N=3)
Chest surgery	1 (50.0%)	2 (22.2%)	2 (22.2%)	0 (0.0%)	0 (0,0%)
Chest-tube	1 (50.0%)	1 (11.1%)	3 (33.3%)	0 (0.0%)	1 (33,3%)
Chylous fluid drainage	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33,3%)
Inhalators	0 (0.0%)	0 (0.0%)	3 (33.3%)	0 (0.0%)	1 (33,3%)
Oxygen	1 (50.0%)	0 (0.0%)	1 (11.1%)	0 (0.0%)	0 (0,0%)
Pleurodesis	0 (0.0%)	2 (22.2%)	2 (22.2%)	0 (0.0%)	2 (66,7%)
mTOR inhibitors	1 (50.0%)	9 (100.0%)	6 (66.7%)	2 (100.0%)	3 (100,0%)
Other	2 (100.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (33,3%)

FIGURE 13 | LAM Treatments by Country. Patients may receive more than one treatment.

and non-medical resources in patients with TSC is high. Further research is needed to determine the impact of mTOR inhibitors on the use of other resources, and in particular, to quantify the economic consequences of potential reductions in the use of other treatments, primarily surgery.

DATA AVAILABILITY STATEMENT

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each center: National Hospital Organization Central Ethics Committee, Gazi University Clinical Research Ethics Committee, Independent Multidisciplinary Committee on Ethical Review of Clinical Trials, Peking Union Medical College Hospital, Commissie Medische Ethiek UZ Brussel, CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé), Comité Etico Investigación Clínica de Euskadi (CEIC-E), Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía, Research Ethics Committee of the University of Tartu (UT REC), Ethikkommission der Medizinischen Universität Graz, North Wales REC – West, Regionala Etikprövningsnämnden i Göteborg, REK – Regionale komiteer for medisinsk og helsefaglig forskningsetikk, Komisja Bioetyczna przy Instytucie Pomnik Centrum Zdrowia Dziecka, Ethikkommission bei der Ludwig-Maximilians-Universitat München, Hokkaido University Hospital Independent clinical research Institutional Ethics Committee, Medical Juntendo University Institutional Ethics Committee, National Center for Chile Health and Deveropment of IRB, Osaka University Hospital of IRB, Ethics Committee at Moscow Institute of

Pediatrics and Pediatric Surgery, Peking University First Hospital, Sanbo Brain Hospital Capital Medical University, Tianjin Children's Hospital, Childrens Hospital Of Fudan University, Zhongshan Hospital Fudan University, Fudan University Shanghai Cancer Center, The Second Affiliated Hospital of Guangzhou Medical University, The First Affiliated Hospital, Sun Yan-Sen University, The First Affiliated Hospital Of Guangzhou Medical University, Shenzhen Children's Hospital, West China Hospital, Sichuan University, Xijing Hospital, Children's Hospital of Chongqing Medical University, Wuhan Children's Hospital, The Second Affiliated Hospital of Xi'an Jiaotong University, Guangdong 999 Brain Hospital, Seoul National University Hospital Institutional Review Board, National Taiwan University Hospital (NTUH) Research Ethics Committee (REC), Institutional Review Board of the Taichung Veterans General Hospital, Institutional Review Board of Chung Shan Medical University Hospital, Institutional Review Board, Tungs' Taichung MetroHarbor Hospital, Institutional Review Board of National Cheng Kung University Hospital, Metro South Human Research Ethics Committee, Sydney Children's Hospital Network Human Research Ethics Committee, St Vincents Hospital Human Research Ethics Committee, Royal Melbourne Hospital Human Research Ethics Committee, Siriraj Institutional Review Board, The Institutional Review board, Faculty of Medicine, Chulalongkorn University, 3rd Floor, Ananthamahidol Building, King Chulalongkorn Memorial Hospital, The committee on Human Rights Related to Research Involving Human Subjects, Institutional Review board, Royal Thai Army Medical Department IRB RTA, 5th Floor, Phramongkutklaowejvitya Building, Phramongkutklao College of Medicine, Research Ethics Committee, Faculty of Medicine, Chiang Mai University, Research and Development, Queen Sirikit National Institute of Child Health, Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town, Shaare Zedek Meidcla Center Helsinki Comittee, Sheba Medical Center Helsinki Comittee, Tel Aviv Sourasly Medical Center Helsinki Comittee, General University Hospital of Patras Ethics Committee, Pendeli Children's Hospital Ethics Committee, General University Hospital of Athens G. Gennimatas Ethics Committee, Evaggelismos General Hospital Ethics Committee, General University Hospital of Thessaloniki AHEPA Ethics Committee, General University Hospital of Ionnina Ethics Committee, METC UMC Utrecht, Direcció General de Regulació, Planificació i Recursos Sanitaris, Comité Ético de Investigación Clínica del Hospital Universitario Vall d'Hebron de Barcelona, Generalitat de Catalunya. 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AUTHOR CONTRIBUTIONS

RM contributed to designing the study, data analysis, data interpretation, and drafting, revising, final review, and approval of the manuscript. EB, MB, PC, MD, JF, MF, CH, SJ, JL, AM, RN, VS, MS, RT, BZ, JK, and AJ were responsible for designing the study, patient accrual, clinical care, data interpretation, and drafting, revising, final review, and approval of the manuscript. TC, VC, Gd'A, PV, CF, FO'C, JQ, YT, and SY were responsible

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.01144/full#supplementary-material

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The TOSCA Registry for Tuberous Sclerosis—Lessons Learnt for Future Registry Development in Rare and Complex Diseases

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Introduction: The TuberOus SClerosis registry to increase disease Awareness (TOSCA) is an international disease registry designed to provide insights into the clinical characteristics of patients with Tuberous Sclerosis Complex (TSC). The aims of this study were to identify issues that arose during the design, execution, and publication phases of TOSCA, and to reflect on lessons learnt that may guide future registries in rare and complex diseases.

Methods: A questionnaire was designed to identify the strengths, weaknesses, and issues that arose at any stage of development and implementation of the TOSCA registry. The questionnaire contained 225 questions distributed in 7 sections (identification of issues during registry planning, during the operation of the registry, during data analysis, during the publication of the results, other issues, assessment of lessons learnt, and

additional comments), and was sent by e-mail to 511 people involved in the registry, including 28 members of the Scientific Advisory Board (SAB), 162 principal investigators (PIs), and 321 employees of the sponsor belonging to the medical department or that were clinical research associate (CRA). Questionnaires received within the 2 months from the initial mailing were included in the analysis.

Results: A total of 53 (10.4%) questionnaires were received (64.3% for SAB members, 12.3% for PIs and 4.7% for employees of the sponsor), and the overall completeness rate for closed questions was 87.6%. The most common issues identified were the limited duration of the registry (38%) and issues related to handling of missing data (32%). In addition, 25% of the respondents commented that biases might have compromised the validity of the results. More than 80% of the respondents reported that the registry improved the knowledge on the natural history and manifestations of TSC, increased disease awareness and helped to identify relevant information for clinical research in TSC.

Conclusions: This analysis shows the importance of registries as a powerful tool to increase disease awareness, to produce real-world evidence, and to generate questions for future research. However, there is a need to implement strategies to ensure patient retention and long-term sustainability of patient registries, to improve data quality, and to reduce biases.

Keywords: lessons, issues, strengths, weaknesses, TOSCA, registry, TSC

INTRODUCTION

Patient registries are organized systems that use observational study methods to collect uniform data on a patient population defined by a particular disease, exposure or condition (e.g., age, pregnancy, specific patient characteristics), and which is followed over time (1). Patient registries may also help to understand the natural history of the disease, to estimate the human and economic burden of the disease, to monitor clinical practice patterns, to identify patients' subgroups that might be included in future clinical trials and to generate new research questions (2).

Therefore, patient registries are a key instrument to develop clinical research, and to improve patient care and healthcare planning, particularly in the field of rare diseases. In spite of its usefulness, patient registries do have several limitations arising from biases, lack of standardization in data collection, accuracy, and comprehensiveness of the data, fragmentation of clinical data, and ethical concerns (2). Most registries are carried out in a small number of centers belonging to a single country or, at best, in a limited number of countries (3), which constitutes an important limitation for the generalizability of the results. The fact that many registries are initiated in the field of academia might also limit their use for pharmaceutical research. In addition to academic initiatives on registries, there are different initiatives worldwide for patients' group registries where the accuracy of the data can be questioned.

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) is a multicenter, international disease registry that was designed to assess manifestations, interventions, and outcomes in patients with Tuberous Sclerosis Complex (TSC), a rare genetic disorder characterized by growth of hamartomas in several organs (4). This registry, designed as an observational clinical study, enrolled from 2012 to 2014 a total of 2,216 patients in 170 sites in 31 countries worldwide. Patients of any age diagnosed with TSC having a documented visit for TSC within the preceding 12 months or newly diagnosed patients (4) were enrolled after signing an inform consent form (ICF) approved by local ethic committee (EC)/institutional review board (IRB). Patients' data were collected at baseline visit and at 5 yearly follow-up visits and recorded by principal investigators (PIs) in an electronic clinical database. The registry clinical database lock occurred in 2017.

The TOSCA registry design consisted of a main "core" part and a number of sub-studies (referred to as "research projects" or "petal projects") (4). The "core" section was designed to collect a general predefined set of patient background data including demographics, family history, prenatal history, and disease features (i.e., neurological, neuropsychiatric, renal, cardiovascular, pulmonary). Additional and more detailed data related to specific disease manifestations were collected in the sub-studies/research projects of the registry. Additionally, the TOSCA registry included a sub-study designed as post approval safety study (PASS), following the European Medicines Agency's (EMA) request (EMEA/H/C/002311/II/0004), to document the long-term safety and tolerability profile of Votubia® in the treatment of TSC patients residing in the European Union for the licensed indications and collect everolimus therapeutic drug monitoring (TDM) data within routine clinical practice as per SmPC. Clinical study protocol and final study results are available on ENCePP portal at http://www.encepp.eu/ (EU PAS Register Number EUPAS324) (5).

The TOSCA registry was funded, designed and managed by a pharmaceutical sponsor (Novartis) with the support of a Scientific Advisory Board (SAB), a Working Committee (WC), and Research Groups (4):

- The SAB consisted of up to 30 members, including TSC healthcare professionals, patient representatives and a maximum of three representatives of the sponsor (Novartis). The medical experts were selected based on the number of publications in TSC, research interests and working in reference sites for TSC in their country. Patient representatives were included as well to ensure that their perspective is considered in the project design and execution. The chair and co-chair were selected by vote of all members. The SAB was responsible for the scientific principles of the registry, the promotion of the use of the registry, the publication of data, and the approval of research projects. All the details of SAB constitution, rules and goals are reported in a SAB charter.
- The WC was a subgroup consisting of up to 14 members from the SAB and was responsible for the registry content and coordination of all the operational activities, for defining the statistical analysis plan and publication policy, and for developing and maintaining the database structure of the registry. All the details of WC constitution, rules, and goals were reported in a WC charter.
- Research groups were made up of physicians participating in the registry and their role consists on the submission of research project proposals to the WC, together with the subsequent management of that particular project.

Apart from being the largest registry in patients with TSC, the TOSCA registry has noteworthy features, including its worldwide scope (including European and non-European countries), its nature as a large-scale cooperation effort between healthcare professionals, patient representatives and pharmaceutical industry, the inclusion of a large number of patients, the design as a core minimal set of data and the more detailed data collection on specific aspects (research projects), the long-term follow-up (up to 5 years), and the inclusion of a PASS sub-study (4). For this reason, both in terms of contents and structure, the TOSCA registry offers an excellent opportunity to assess what lesson can be learnt from a registry, which issues should be addressed and what pitfalls can be avoided when setting up and managing an international registry in a rare disease.

OBJECTIVE

The aim of this analysis was 2-fold: firstly, to identify issues that arose during the design and operation of the TOSCA registry and during the interpretation and publication of the results; secondly, it aimed to identify areas for improvement and pitfalls that can be useful for the development of successful future registries in rare and complex diseases.

This paper is structured as follows. Section Methods describes the methodology and the instruments employed to extract the information. Section Results describes the issues encountered by each group of stakeholders in every domain of the registry; it also outlines the pitfalls and lessons learnt from the integration of the research projects and the everolimus sub-study PASS within the TOSCA registry. Finally, section Discussion contains a discussion of the results and provides recommendations for future registries in rare, multisystemic, and complex diseases.

METHODS

A questionnaire was designed to identify issues that might have arisen at any stage of the TOSCA registry project from its inception to the publication of the results, and to identify its strengths and weakness, and opportunities and threats that could be of interest for the development of future registries in rare diseases. It was developed by the TOSCA clinical trial head with contribution of TOSCA patient representatives steering committee members and Novartis quantitative safety and epidemiology department. The questionnaire was built following a guide aimed to support the design, implementation, analysis, interpretation, and quality evaluation of registries published by Gliklich et al. (2). The questions included were prepared based on the steps to conduct a registry described in this guideline and the specific TOSCA registry project characteristics.

The questionnaire contained 225 questions split into seven sections (**Supplementary Material**); the first five sections covered a range of aspects related to issues during the registry (planning, operation, data analysis, results publication, and other issues), and the last two were devoted to assess lessons learnt from the TOSCA registry and to gather additional comments (**Table 1**).

On September 7th 2018 the questionnaire was sent by e-mail to the 511 people who had been involved in the TOSCA registry. Twenty-eight of them were part of the SAB, while 162 were principal investigators (PIs) and 321 were Novartis employees not included in the SAB. All the receptors of the questionnaire (henceforth "participants") received the same document, but some questions precluded respondents to answer subsequent parts of the questionnaire (for instance, if participants responded that were not involved in budget planning, allocation and/or control, they were invited to skip the subsequent questions regarding these topics). To facilitate the analysis, most questions were close-ended ("yes"/"no" or using a Likert scale). Besides, all the questions contained "N/A" (not applicable) option and a free-text field where the participants were encouraged to justify their answers. The participants were given 2 months for replying and two reminders were sent. No remuneration was offered to respondents.

The analysis was carried out on the completed questionnaires received in the 2 months following the initial mailing (cut-off date: November 8th 2018). All data were analyzed using Microsoft Excel. Relative and absolute frequencies were analyzed for all the questions, and whenever possible, for the groups of questions belonging to the same section or subsection.

RESULTS

By the cut-off date (November 8th 2018), a total of 53 questionnaires were received (53/511; 10.4%). The response

TABLE 1 | Structure of the Questionnaire.

1) Identification of issues during registry planning
- Perception on the definition of the purpose and the objectives of the registry
- Perception on the definition of the inclusion/exclusion criteria
- Definition of the variables included in the registry
- Definition of the size, the duration, the setting and the geographical areas
- Identification of stakeholders, team building and establishment of a governance
- Data access & use of data
- Publication plan
- Development of the protocol and related documents
- Development of the project plan
- Development of risk management plans & risk management during the registry

2) Identification of issues during the operation of the registry
- Issues related to patient recruitment or retention
  - Barriers to patient recruitment/retention
  - Evaluation of success of patient recruitment strategies
  - Evaluation of success of patient retention strategies
  - Evaluation of center/physician or patient selection bias
- Issues related to data collection & quality assurance
  - Issues related to data collection
  - Identification of quality issues & timing for detection
- Issues related to budget
- Issues related to project management
  - Ownership & accountability
  - Coordination
  - Estimation of the use of resources/duration/complexity

3) Issues during data analysis
- Identification of sources of bias
- Treatment of missing data
- Appropriateness of time horizon & planned interim analysis
- Appropriateness of pre-specified analyses
- Interpretation of the results
- Identification of issues related to data access
- Identification of strengths & limitations of the registry

4) Issues during the publication of the results

5) Other issues

6) Assessment of learnings
- General learning topics
- Value of the registry organization
  - Inclusion of patients in the SAB and in the WC
  - Inclusion of clinicians in the SAB and in the WC
  - Inclusion of members from the pharmaceutical industry in the SAB and in the WC
- Pitfalls and learning opportunities emerged from the integration of research projects within the TOSCA registry
- Pitfalls and learning opportunities emerged from the integration of a Votubia® PASS within the TOSCA registry

7) Additional comments

SAB, Scientific Advisory Board; WC, Working Committee; TOSCA, TuberOus SClerosis registry to increase disease Awareness; PASS, post approval safety study.

rates per type of participant who filled the questionnaire in (hereafter referred to as "respondents") were 64.3% (18/28) for members of the SAB including Novartis representatives, 12.3% (20/162) for PIs not included in the SAB and 4.7% (15/321) for other Novartis employees not included in the SAB.

The overall rate of completion of the questionnaire (i.e., answered questions/total questions) was 88% for closed questions (of the amount of missing data per question was 12% on average, range 2–30%); the rates of missing data according to the type of respondent were 4% for members of the SAB, 4% for PIs and 7% for other Novartis employees.

Identification of Issues

A summary of all the issues reported by the survey respondents in relation to TOSCA is shown in **Figure 1**. This figure represents the main stages of the TOSCA registry *(registry planning, operation, data analysis, publication, and other)* and the issues encountered by the respondents in each of these stages. Percentages in brackets are related to the proportion of respondents who reported each issue. Questions from the survey which were not rated as an issue by any of the respondents were not included in **Figure 1**. These non-issue questions mainly relate to the identification of clinicians to lead the research projects or to delays in the development of the registry due to patient identification. All respondents also agreed that no issues arose neither on the grade of involvement of WC members in the protocol and related documents, nor in the documentation of protocol amendments, nor whether the information about these amendments was provided in a timely manner to respondents. Finally, no issues were reported regarding registry oversight or the adverse event collection/reporting processes.

Registry Planning

The limited duration of the registry (up to 5 years) was considered the most common issue amongst the survey respondents (38%). There was a consensus amongst those answering the questionnaire on the appropriateness of having a long-term registry and some respondents stated that a longer follow-up would have been good in order to capture the impact of the disease in a more realistic way; however, constraints, such as budget limitations, were impactful leading to substantial amounts of missing data from follow-up 3. Respondents considered the registry too ambitious in terms of recruitment, duration or compliance and its long-term sustainability unrealistic. Conversely, timeline delays, risk, and project plan problems and issues when defining SAB-WC members were the lowest-rated complications associated with registry planning.

Operation of the Registry

Missing data were the main complication stated by respondents in relation to the operational domain of the registry (32%) (**Figure 1**). Variables with the most data missing were related to TSC-Associated Neuropsychiatric Disorders (TAND)—for reasons such as the lack of knowledge of these TSC manifestations by the physicians—or patient/caregiver reported outcomes, whereas those with fewer missing data were associated to physical signs and symptoms of the patients. A low proportion of respondents stated issues related to resources and costs and there were mainly related to budget limitations, especially toward the research projects.

Data Analysis

The effect of bias on the validity of the results was considered as the main issue related to data analysis by the respondents

Registry planning	
Registry duration inadequate	38%
Group overrepresentation/no contribution PP	19%
Absence of sample size estimation	17%
Issues defining variables core	15%
Inadequate ambitiousness of registry	15%
Unclear data access rights/data use	13%
Unclear registry purposes/objectives	11%
Unclear translation of purpose to objectives	9%
Issues defining variables RPs	9%
Non-standard development of CRF	9%
Issues/absence publication plan	8%
Issues with document translation	8%
Unclear inclusion/exclusion criteria core	6%
Unclear inclusion/exclusion criteria RPs	6%
Lack of WC involvement in protocol	6%
Issues controlling of bias in PCRO	6%
Lack of relevant members risk plan	6%
Issues involving Novartis members in registry	6%
Information loss due to change in owners	4%
Unclear/absence project plan	4%
Incomprehensive project plan	4%
Overrepresentation/lack of contribution RP	4%
Incorrect foreseen risks in risk plan	4%
Incorrect risk address	4%
Issues defining SAB/WC clinicians	2%
Issues defining roles of committees participants	2%
Issues finding SAB/WC patient reps	2%
Presence of avoidable delays	2%
Lack of relevant members in project plan	2%
Incomprehensive risk plan	2%

Operation of the registry	
Missing data handling issues	32%
Patient recruitment issues in RPs	28%
Data collection inaccuracy	28%
Patient retention issues in core	23%
Center/physician selection bias	23%
Patient selection bias	23%
Underestimation of project complexity	23%
Patient recruitment issues in core	21%
Patient retention issues in RPs	19%
Avoidable deviations from timelines	15%
Query solving/data cleaning inconsistencies	13%
Lack of missing data prevention	9%
Issues finding budget	9%
Incoherence in planned budget and real costs	9%
Missed opportunities to reduce costs	8%
Lack of ownership share by team members	8%
Poor coordination	6%
Issues deciding sponsor/centers costs	4%
Inadequate estimation of resources needed	4%

Data analysis	
Bias compromised validity of results	25%
Incorrect missing data treatment	23%
Patient-level data not available for external researchers	21%
Issues accessing data during the registry	17%
Data format not amenable to linkage	17%
No assessment complete & incomplete data	15%
No special considerations for MD handling due to rare disease	15%
Issues with age (paediatric turning adults)	15%
Incorrect assessment of risk of bias	13%
Missing data not reported	13%
Missing data unexplained	13%
Lack of internal/external validity	13%
Inadequate time horizon	11%
Aggregated data not available for external researchers	8%
Data not fulfilled the FAIR principles	8%
Issues accessing data after the registry	6%
Missing data handling unclear in statist. Plan	4%
Missing data handling inconsistently	4%
Inadequate planned interim analyses	4%
Inadequate timing of interim analyses	4%

Publication of results & Other issues	
Authors did not contribute in registry	21%
Authors did not participate in manuscript	19%
Target journal/conference not final one	19%
Authors not meeting the authorship criteria were not acknowledged	15%
Unexpected delays in publication plan	15%
Regulation issues between countries	13%
Language/cultural barriers	11%
Authorship conflicts	9%
Administrative issues between countries	8%
Site dispersion issues	6%
Authors did not approved final version paper	2%
Data requirement issues between countries	2%

FIGURE 1 | Typology and Weight of Issues derived from the Different Stages within the TOSCA Registry. CRF, case report form; FAIR, Findable, Accessible, Interoperable and Reusable (data); MD, missing data; PCRO, patient-caregiver reported outcomes; PP, project plan; RP, research project; SAB, Scientific Advisory Board; WC, Working Committee.

(25%), together with the incorrect treatment of missing data (stated by 23% of the respondents). More than half of the respondents (51%) agreed on the presence of some type of bias, either selection bias (e.g., unclear inclusion-exclusion criteria or registry population as a non-random selection from the target population), information bias (e.g., selective recall, inconsistent data collection, or wrong-inexact data recording) and/or measurement bias (e.g., faulty-inaccurate measurements or misclassification of outcomes). The involvement of statisticians throughout the whole project from its conception, budget extensions or further monitoring during data collection were considered as potential solutions to these issues by the respondents. Issues related to interim analyses and missing data handling were amongst the least reported by the respondents (4% of the respondents each issue) (**Figure 1**) in this section and mainly related to the desire of making these analyses longer and the missing data present in the final follow-ups (follow-up 4 and follow-up 5).

Publication of Results and Other Issues

Regarding publication of the results and other issues, the lack of contribution to the TOSCA registry and the lack of participation in manuscripts were the issues most rated by the respondents in the survey (21 and 19%, respectively), whereas questions related to data requirements between countries and final approval of publications were considered the less important complications related to the registry (2% of the respondents each issue). Overall, respondents felt that no authorship conflicts (e.g., issues related to the inclusion of all authors and/or the order in which some authors appeared in publications) happened during the publication process (<10% of respondents stated this type of issue).

Assessment of Lessons Learnt From TOSCA Registry

Table 2 shows contributions of the TOSCA registry to the field of TSC and the rate of agreement of the respondents with these contributions. These contributions were classified into the ones finally accomplished by TOSCA registry and those not accomplished, either because it was not achieved even though it was intended or because it was not intended (**Table 2**). Overall, the rates of completeness were high in this section of the questionnaire, with an average rate of missing data of 5% per question (range 2–15%) mainly due to the fact that they did not remember the data or did not have access to it.

More than 80% of the survey respondents perceived that TOSCA improved the knowledge on the natural history and manifestations of TSC, increased the awareness of the disease and helped to identify information relevant to clinical research. Thus, overall there was a convergence that the TOSCA registry positively contributed to make progress into the knowledge of TSC, although one respondent considered this progress as small given the cost and time spent in the registry. The lowest consensus was reached on the items "the registry contributed

TABLE 2 | Assessment of lessons learnt derived from the TOSCA registry ($N = 53$).

TOSCA registry contributions	Yes	No, but it was intended	No, but it was not intended	Missing	N/A
Improvement of knowledge on the natural history of TSC and its manifestations	47 (89%)	3 (6%)	0 (0%)	1 (2%)	2 (4%)
Increase disease awareness	46 (87%)	2 (4%)	1 (2%)	1 (2%)	3 (6%)
Identification of useful information for the development of clinical research in TSC	44 (83%)	2 (4%)	1 (2%)	3 (6%)	3 (6%)
Trigger research questions/developing hypothesis for new research in TSC	41 (77%)	2 (4%)	4 (8%)	3 (6%)	3 (6%)
Improvement of epidemiological knowledge of TSC	40 (75%)	2 (4%)	7 (13%)	1 (2%)	3 (6%)
Foster the communication between TSC experts and Novartis	40 (75%)	3 (6%)	4 (8%)	3 (6%)	2 (4%)
Improvement of knowledge on the clinical management of the disease in different countries	38 (72%)	3 (6%)	7 (13%)	1 (2%)	4 (8%)
Provision of data on quality of life	38 (72%)	3 (6%)	8 (15%)	2 (4%)	2 (4%)
Identification of useful information for the development of studies involving large/diverse geographic areas	38 (72%)	3 (6%)	6 (11%)	2 (4%)	3 (6%)
Foster the communication between TSC experts	38 (72%)	3 (6%)	7 (13%)	3 (6%)	2 (4%)
Provision of data on the effectiveness & efficiency of interventions in the real world	37 (70%)	7 (13%)	5 (9%)	2 (4%)	2 (4%)
Improvement of clinical practice	37 (70%)	4 (8%)	7 (13%)	2 (4%)	3 (6%)
Quantification of the use of resources and the burden of the disease	37 (70%)	7 (13%)	3 (6%)	2 (4%)	4 (8%)
Identification of centers/physicians treating patients with TSC	35 (66%)	5 (9%)	7 (13%)	4 (8%)	2 (4%)
Identification of useful information for the development of studies in pediatric patients	34 (64%)	3 (6%)	10 (19%)	2 (4%)	3 (6%)
Foster the communication between TSC experts and patients	34 (64%)	4 (8%)	7 (13%)	4 (8%)	3 (6%)
Assessment of the agreement between clinical practice and guidelines	33 (62%)	7 (13%)	9 (17%)	2 (4%)	2 (4%)
Provision of data on the safety of the interventions in patients with TSC in the real world	31 (58%)	7 (13%)	10 (19%)	3 (6%)	2 (4%)
Improvement of health care planning & resource allocation	31 (58%)	9 (17%)	8 (15%)	2 (4%)	3 (6%)
Development of new clinical practice guidelines	30 (57%)	8 (15%)	9 (17%)	3 (6%)	3 (6%)
Identification of patients with TSC that might benefit from certain interventions or might be included in future clinical trials	30 (57%)	8 (15%)	9 (17%)	3 (6%)	3 (6%)
Identification of useful information for the development of clinical research in other rare diseases	28 (53%)	6 (11%)	10 (19%)	4 (8%)	4 (8%)
Foster the communication between TSC patients and Novartis	24 (45%)	7 (13%)	12 (23%)	6 (11%)	3 (6%)
Facilitation of market access for Votubia®	23 (43%)	6 (11%)	10 (19%)	8 (15%)	4 (8%)

TOSCA, TuberOus SClerosis registry to increase disease Awareness; TSC, Tuberous Sclerosis Complex.

to facilitate market access for Votubia®" and "the registry contributed to foster the communication between TSC patients and Novartis", agreed by <50% of the respondents.

The items where TOSCA made no contribution to the fields of rare diseases registries or TSC were classified in those where the registry was not meant to contribute and those where the contribution was intended but not accomplished (**Table 2**). Fewer than 20% of respondents stated items where the contribution was intended but not accomplished, mainly in improving healthcare planning and resource allocation (17%) or developing new guidelines (15%). The items from which the contribution was not accomplished but also not intended were mainly

related to foster the communication between TSC patients and Novartis (23%).

Most respondents considered the inclusion of different groups (TSC experts [reported by 84%], the pharmaceutical industry [reported by 75%] and patient representatives [reported by 59%]) in the SAB and the WC as either important or very important, despite some respondents were concerned that including patient representatives would create issues, such as ethical issues (reported by 6%) or confidentiality issues (reported by 6%). Overall, more than 75% of the respondents considered the inclusion of patient representatives to be good in facilitating communication—about the registry's purpose and value to patient advocacy groups—and to furthermore increase public awareness of the disease. Seventeen percent of the respondents also stated that they would have increased the number of patient representatives in the SAB/WC, especially if they had medical background.

There was a clear convergence regarding the importance of including TSC experts in the SAB and the WC, especially to provide interpretation of results, to propose the collection of variables and analyses of medical interest and to improve the quality of publications (more than 90% of respondents rated the inclusion of TSC experts as relevant or very relevant for these items). However, respondents considered the overall number of TSC experts to be too high in both in the WC and SAB. There was also agreement about the importance of including members of the pharmaceutical industry in the SAB and the WC, especially to provide technical, and/or financial support in the dissemination and publication of the results (rated as important or very important by more than 80% of respondents). However, the inclusion of different pharmaceutical companies as well as members with more specific skills (e.g., statistics, medical, operational, data management) was felt necessary by few respondents (9 and 2%, respectively).

Pitfalls and Lessons Learnt From the Integration of Research Projects Within the TOSCA Registry

More than half of the respondents (57%) considered appropriate to include research projects within the structure of the TOSCA registry. Further benefits derived from the projects were the extensive data collection and its multidisciplinary nature, which would have allowed a deep analysis of specific areas of TSC resulting in better knowledge of the disease, and furthermore the procurement of patient reported outcomes, such as burden of illness or quality of life.

On the other hand, respondents also stated that research projects were complex, burdensome and should have been considered at the registry planning stage (as they were included as study protocol amendments). The absence of publications and statistical plans together with the lack of budget (for aspects such edit checks on collected data or PI reimbursement for data entry) and patient retention were other pitfalls stated in the survey.

On average, 38% of respondents considered that separating the core from the research projects was a good idea; conversely, 17% of the respondents on average stated that this separation caused delays and agreed that both the core and the research projects should have been done simultaneously.

No consensus was reached regarding the efficiency in resource management for the research projects (28% of respondents considered the management efficient, whereas 23% thought it was not).

Regarding the contents of the core and the research projects, there were mixed opinions on whether some variables in the core registry should have been included in the research projects, and vice versa (21% said "yes" vs. 23% said "no," 43% said "N/A," 13% were missing). Regarding the amount of missing data, there was also an absence of consensus regarding whether the proportion of missing data was similar between the core and the research projects; missing data appeared to be reported similar between the core and the research projects by 18% of the respondents who provided a valid answer (e.g., yes, no or N/A), while considered different by 25%. The opinions reflected in the answers on whether the number of respondents in the research projects was sufficient to answer questions of clinical relevance were heterogeneous (19% said "yes" vs. 26% said "no"; 38% said "N/A", 15% were missing). More consensus was obtained on the representativeness of the results, as 38% of the respondents providing a valid response stated that results from the research projects could be extrapolated to all the respondents in the core registry, and 43% stated that results from the research projects would be representative of real world.

Finally, more respondents agreed that research projects provided striking or relevant results (17% said "yes" vs. 13% said "no," 51% "N/A", 19% were missing) while there was uncertainty on whether new projects emerged from the research projects (13 vs. 11% said "yes" and "no," respectively; 58% reported "N/A", 17% were missing). Of those who stated that the research projects provided relevant findings, these were related to the impact on renal angiomyolipoma (rAML), the effects of subependymal giant cell astrocytoma (SEGA) in adults, the results obtained in TAND and aspects related to quality of life. Appropriateness in the dissemination of results was uncertain (19% said "yes", 19% said "no", 42% said "N/A", 21% were missing).

Pitfalls and Lessons Learnt From the Integration Everolimus, Votubia® PASS (Post Authorization Safety Study) Within the TOSCA Registry

Some questions in the survey were related to the PASS study, which was embedded in the TOSCA registry to evaluate the long-term safety profile of everolimus (commercially known as Votubia®) an orphan drug directed to treat SEGA, rAML and seizures that did not respond to other treatments. Almost half of the respondents (43%) considered appropriate to integrate the PASS study within the TOSCA registry, mainly due to efficiency gains such as better surveillance, retention, recruitment, and long-term effects of adverse events. However, some pitfalls also emerged from this integration, as the extra workload imposed by PASS within TOSCA design, the characterization of PASS as a sub-study of TOSCA and the important differences between both studies (e.g., administrative, reporting, regulatory requirements).

Approximately 30% (range 26–34%) of respondents agreed on the convenience of separating the elaboration, data collection, and approval of both the PASS and TOSCA, and 32% of the

respondents considered that there was a good management of time and resources in PASS.

Conversely to what happened with the research projects, more respondents considered that there were no variables in PASS that should have been collected in the core registry or vice versa (9 vs. 19%, on average). Twenty-one percent of the respondents considered data quality and completeness was worse in the TOSCA registry than in the PASS. There were discrepancies between respondents regarding the number of patients in PASS, with 13% of respondents thinking they were sufficient vs. 9% who considered the sample unrepresentative (60% said "N/A", 17% were missing). A bigger proportion of the respondents considered the results in PASS representative of the whole TOSCA population (17%) and translatable into real world (25%) that those who did not (8 and 2%, respectively). Importantly, none of the respondents perceived that new projects emerged from the PASS study, although there was an important degree of uncertainty surrounding this item (19% said "no," 62% reported "N/A," 19% were missing).

Regarding the dissemination of results, respondents had mixed opinions (11% said "yes", 8% said "no", 62% reported "N/A", 19% were missing). No consensus was reached regarding the potential benefit on the TOSCA registry derived by the interaction of health authorities during the PASS, again with important levels of uncertainty (8% said "yes", 8% said "no", 68% wrote "N/A", 17% were missing).

DISCUSSION

The analyses performed here identified the main issues that arose during TOSCA registry from its inception to the publication of the results, and the take-home messages and lessons that could be relevant to the design and development of future registries in rare and complex diseases.

All the respondents agreed that one of the most positive aspects of the TOSCA registry was the involvement of a range of stakeholders (including TSC experts, members from industry, and patients). By involving people with different perspectives and profiles, the study analyzed variables that were of interest to physicians, to the pharmaceutical industry, and most importantly, to patients.

There is a growing emphasis on patient-focused registries (6) and, in this particular case, patients' representative in the SAB were considered a key element to facilitate communication of the results to advocacy groups, and to increase public awareness on the disease. Other successful examples of registries with an active participation of patients in its design, governance and/or operation are the ImproveCareNow network for inflammatory bowel disease in the United States (7), the ParkinsonNet Approach in the Netherlands (8), and the TREAT-NMD European network for neuromuscular disorders (9).

In the TOSCA registry, no issues were reported regarding registry oversight, adverse event collection/reporting processes (only related to the PASS sub-study), or project management, which means that these aspects worked particularly well. The use of standard operational procedures may have helped to prevent this type of issues and is highly advised for the development of future registries.

Another aspect that was rated positively was the high recruitment in the core project. The recruitment strategies varied among the enrolling countries and included phone contacts, proposal of participation in scheduled visits, exploitation of local patient databases, targeted mailing and newsletters to the investigators, virtual investigator meetings and the contacts with local patients' associations and family groups.

By contrast, patient retention was poor in TOSCA registry; after 3 years follow up, some sites stopped reporting data in a constant manner and a high number of patients discontinued (93.5%). Patient discontinuation is a common issue in all the registries. Therefore, strategies to reduce losses to follow-up are urgently needed, especially when taking into account that approximately a third of the respondents answered that they would have preferred the TOSCA registry to have a longer duration or even to be permanent.

The contrast between the low retention rates and the high expectations highlights the need for realistic goals when setting up a registry, but also the need for continuous motivation, adequate budget, and close oversight for registries that are expected to last longer than one or 2 years. Unfortunately, long-term sustainability is an important issue for most registries (1).

Issues related to missing data collection were among the most common difficulties during the operation of the registry and during data analysis, especially in the last follow-up visits. According to one of the respondents, carrying out a pilot study would have been useful to make sure questions were formulated in the most optimal way, and to reduce the amount of missing data. Other strategies related to missing data reduction or handling are to detail mechanisms to identify and collect missing data in the protocol, to distinguish between nice-to-have, and essential data (as in TOSCA study management document like the CRF manual and of monitoring plan) and to describe the handling of missing data in the statistical analysis plan (also part of TOSCA study management documents) (1).

Issues related to language translations were not observed in the TOSCA registry, which can be considered a success in a project involving 31 countries. Within the TOSCA registry, the impact of translation issues was minimized by several actions, such as the study oversight and site support provided in local languages including the discussion of the protocol and the electronic case report forms (eCRFs) requirements. In spite of this, one of the respondents mentioned that in any future multinational project, agreeing, and defining each term or concept with representatives from each country and language would be important to avoid any issue related to a mistranslation. These solutions might be useful for future multinational registries.

During data analysis, the most important issues were related to biases. Due to its observational nature, registries are prone to many biases. In this case, several respondents concluded that, due to selection bias toward patients with severe manifestations recruited in large hospitals and reference centers, the burden of the disease might have been overestimated. Another reason for selection bias was the overrepresentation

of pediatric neurologists. Despite of the biases, the TOSCA registry provided relevant information about the presence of clinical manifestations on TSC patients such epilepsy that was useful from an epidemiological point of view. Besides, the eCRF included some specific questions for some specialties that could not be answered properly by all the participants; therefore, data collection for some specialties such as dermatology or ophthalmology was not completely reliable. Future studies should ensure that the sample is sufficiently homogeneous and representative of the population to be analyzed, that the investigators are a representative sample of the physicians treating that condition, and that all the variables can be properly assessed by the investigators involved in the study. Reducing bias therefore requires the participation of statisticians when planning the project, a careful site and PI selection across countries and also an increased and continuous support at site level to understand study requirements and eCRF questions. This issue was always specified in the different results and publications of the TOSCA registry, where it was emphasized that this is not an epidemiological study, but a very large cohort study.

Apart from potential biases and missing data issues, there were difficulties related to data access. In spite of the existence of a definition of the terms for data access, one TSC expert believed that the data access rights favored too much the sponsor and others thought that they were not clear enough. Therefore, more efforts are required to involve all the stakeholders in the definition of data access terms. In this respect, a discussion paper elaborated by the EMA Cross-Committee Task Force on Patient Registries goes even further, and acknowledges that "clarity is needed regarding data ownership, including patients' wishes regarding the use of their data" (1).

Issues during the publication of data from other registries have not been previously analyzed. Authorship conflicts were reported by 9% of the respondents. The most frequent issues were related to the poor involvement of some authors in the manuscripts or the lack of acknowledgment for all the contributors. This highlights the need for authorship criteria based on real contribution instead of pre-signed agreements.

Another conclusion resulting from analyzing the deviations between the planned and the expected journals for the publication is that setting unrealistic target journals might be an important cause for delays during the publication process. The difficulties related to publishing results from yearly follow-ups should also be taken into account when devising a publication plan.

According to most respondents, it was positive to carry out research projects besides the TOSCA registry because they allowed to carry out detailed analyses of specific manifestations in patients with TSC or provided additional information on the burden of the disease. However, due to insufficient funding and to the lack of specific statistical and publication plans, the validity and dissemination of the results from the research projects were scarce. In addition, most respondents considered that the research projects were not well-handled and that the implication from the investigators was not sufficient. This might be seen as a lost opportunity, but also as a need for better planning for studies emerging from registries, and highlights the need to include detailed budget planning within all project proposals. Interestingly, the EMA provided very clear guidance on this matter stating the importance of differentiating between registries (including their periodic analyses) and registry studies. In line, protocols are meant to be completely separate, meaning the addition of research projects as amendments are not in line with the Good Registry Practice and should be considered as almost separate studies with their own budget, management, monitoring, etc. (1).

Conversely, most respondents considered data quality and completeness were worse in the TOSCA registry than in the PASS. While it is true that the aims of a PASS study are completely different from those in the TOSCA registry, a better integration of the TOSCA registry and the PASS could have been exploited to increase the quality of the TOSCA registry.

The analysis of the lessons from TOSCA might also have some limitations. First, it is only based on one single registry experience in patients with a single disease. However, most of the issues are applicable to registries in other diseases. The second limitation is associated to the low number of TSC patients' representatives who were able to fill this questionnaire. This might be due to the low percentage of patient representatives in the SAB. Thirdly, a major limitation was the high percentage of the SAB in the respondents' group. Some reasons for the low response rates of the PIs and Novartis employees could be the perception on the burdensomeness of the questionnaire, the lack of economic compensation for the participants, a decreasing interest in the study or a lack of belief in the interest of such questionnaire. In future studies, a pilot of the questionnaire should be performed in a small sample of the population before being distributed further in order to test the validity and reliability of the questionnaire and to improve response rates.

Finally, the questionnaire was designed and sent 1 year after the completion of the registry, and this may have resulted in recall biases. In any case, we believe that by performing the analysis retrospectively, we could obtain a complete view on the difficulties arisen throughout the project.

In conclusion, this analysis has contributed to foresee and prevent issues in the design and development of future multinational registries in rare diseases. Careful planning, adequate monitoring and sufficient budget allocation are key elements for the success of registries. By contrast, there is a need to improve data quality, to reduce biases, to avoid access-related issues, and to ensure patient retention and long-term sustainability. Finally, this analysis also shows that registries are a powerful tool to increase disease awareness, and to produce a real-world view of clinical practice, but they have many limitations too. When designing and carrying out a registry, keeping a balance between ambition, pragmatism, and costs is a difficult task.

DATA AVAILABILITY STATEMENT

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting

patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each centre. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

RM designing the study, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. EB, MB, PC, MD, JF, MF, CH, SJ, JL, AM, RN, VS, MS, RT, BZ, JK, and AJ designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. TC, GB, VC, PV, CF, FO'C, JQ, YT, and SY designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. LD'A designing the study, trial management, data collection, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. SS designing the study, trial statistician, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2019.01182/full#supplementary-material

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REVIEW
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TuberOus SClerosis Registry to Increase Disease Awareness: A Review on Alignment of Its Planning, Execution, and Publications With European Medicines Agency Guidelines

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Patient registries offer a powerful and practical means of real-world data collection system for rare diseases. Many guidelines have been released to standardize patient registries, although most of them do not address issues specific to rare disease patient registries. In November 2018, the European Medicines Agency (EMA) released a draft discussion paper on methodological and operational aspects of disease registries and made proposals on good registry practice (henceforth referred to as EMA guidance). This guidance was highly anticipated by all stakeholders with a strong interest toward governance, operationalization, and study conduct in registries. With improved clarity toward conduct of patient registries, this guidance will encourage overall registry use in regulatory decision making. TuberOus SClerosis registry to increase disease Awareness (TOSCA) was an international, multicenter patient registry to assess the manifestations, interventions, and outcomes in patients with tuberous sclerosis complex (TSC). The planning of TOSCA was initiated in 2011, patient enrolment commenced in August 2012, and final analysis database was locked in August 2017, long before the EMA guidance was released. Moreover, initial publications of TOSCA, such as first interim analysis, had also been published before the release of the EMA guidance. Extensive feedback and lessons learned from the TOSCA registry have provided insights into rare disease registry planning and operations. In this paper, we tested the recommendations from the EMA guidance on a rare disease registry, that is, the TOSCA registry. We elaborated the compliance and deviations of the TOSCA registry from the EMA guidance on a point-by-point basis. A careful observation revealed that in most aspects, TOSCA was in compliance with EMA. However, there were several practical issues identified in TOSCA, which deviated from EMA guidance. These issues demonstrate that deviations

from EMA guidance, particularly in rare disease registries, do not signify compromised registry quality and can be somewhat expected in small populations. Despite multiple deviations of TOSCA from the EMA guidance, TOSCA was able to meet its objectives to enhance our understanding of TSC and its manifestations.

Keywords: tuberous sclerosis complex, rare disease, rare disease registry, patient registry, tuberous sclerosis registry to increase disease awareness

INTRODUCTION

Role of Patient Registries in Rare Diseases

Rare diseases, owing to the limited number of patients and phenotype diversity, often lack a thorough research in terms of underlying pathology of the disease, as well as the course of disease, its manifestations, and the outcomes (1, 2). Although the impact of an individual rare disease may appear limited, the collective burden of rare diseases on public health is enormous. Moreover, the awareness and knowledge about rare diseases among primary care physicians is limited.

The real-world data (RWD) collected in patient registries offer valuable insights on the disease itself, the effectiveness, and safety of particular therapies and play a crucial role in health-care decision making (1). Patient registries aid the understanding of natural history, evolution, risk, and outcomes of specific diseases. They support the research on genetic, molecular, and physiological bases of rare diseases. Furthermore, rare disease registries often fill a social gap as well, by connecting patients and families who are facing similar challenges as well as clinicians working in the same disease area. They may also establish a patient base for the evaluation of drugs, medical devices, and orphan products and may be used as historical controls to further accelerate research in areas of high unmet medical need (3). The European Medicines Agency (EMA) frequently relies on patient registries to gather RWD on the risks and benefits of a particular product, as a condition to monitor post-marketing safety and efficacy and as a condition for approval (4). Hence, patient registries offer a powerful opportunity to further the clinical research in rare diseases and improve patient care as well as health-care planning (1).

Abbreviations: AEs, adverse events; AHRQ, Agency for Healthcare Research and Quality; ATC, Anatomical Therapeutic Chemical; CTH, Clinical Trial Head; EBMT, European Society for Blood and Marrow Transplantation; ECFSPR, European Cystic Fibrosis Society Patient Registry; EMA, European Medicines Agency; ENCePP, European Network of Centers for Pharmacoepidemiology and Pharmacovigilance; EPIRARE, European Platform for Rare Disease Registries; EU, European Union; EU PAS, The European Union electronic Register of Post-Authorisation Studies; EUCERD, European Union Committee of Experts on Rare Diseases; EURD, European Union reference dates; GDPR, General Data Protection Regulation; GVP, Good Pharmacovigilance Practice; ICH, International Council for Harmonization; KOLs, key opinion leaders; MAH, Marketing Authorisation Holder; MedDRA, Medical Dictionary for Regulatory Activities; PAES, Post-Authorization Efficacy Study; PASS, Post-Authorization Safety Study; PIs, principal investigators; RCT, randomized controlled trial; RDs, rare diseases; RPs, research projects; RWD, real-world data; SAB, Scientific Advisory Board; SAP, statistical analysis plan; SEGA, subependymal giant cell astrocytoma; TAND, TSC-associated neuropsychiatric disorders; TOSCA, TuberOus SClerosis registry to increase disease Awareness; TSC, tuberous sclerosis complex; WC, working committee; WHO, World Health Organization.

The importance of rare disease registries has been recognized and underlined by the European Union (EU), through the "EU Council Recommendation of 8 June 2009 on an action in the field of rare diseases (5)." Through strengthening and acknowledging the valuable role of patient registries, there has been a significant boost in the number of rare disease patient registries in the recent years (6). According to the Orphanet Report Series Rare Disease Registries in Europe, May 2019, there are 69 global rare disease registries, 69 rare disease registries in Europe, and 535 rare disease registries at the national level and further at the regional level (7). However, these patient registries are diverse in terms of the objectives, patient inclusion and exclusion criteria, the core data elements, and overall data quality and completeness. Hence, for setting up a successful rare disease registry, a practical guidance with detailed consideration to all aspects of planning and execution is crucial (4). As more patient registries in rare diseases are being launched, more issues are being identified, regarding the hurdles and limitations during planning and execution of these registries. Resolving such issues and offering appropriate guidance to standardize the data elements across the registries is desired by all stakeholders and has hence received adequate emphasis in the EMA guidance.

Several efforts have been made to standardize the patient registry setting and implementation. The European Union Committee of Experts on Rare Diseases (EUCERD) adopted a set of Recommendations on Rare Disease Patient Registration and Data Collection in 2013. These recommendations formalize the consensus reached and guide all stakeholders into systematic discussions on data collection and registration (8). Furthermore, many international projects, including EPIRARE and RD-CONNECT, have been initiated to promote international registries (9). Orphanet provides direct online access to an inventory and encyclopedia of rare diseases (7). Similarly, the National Center of Rare Diseases in Italy has also released recommendations for improving the quality of rare diseases registry (6).

Patient registries are furthermore a tool frequently used in pediatric research and drug development to better understand diseases, as historical controls and as a mean to follow up patients over long periods of time. Children cannot be considered "small adults," as age and developmental maturation vastly affect the pharmacokinetics and pharmacodynamics of many drugs. Hence, it is imperative to assess dosing, efficacy, safety, and long-term benefit/risks of any therapeutic treatment by following a dedicated pediatric drug development process, which needs careful consideration while setting up pediatric trials. Furthermore, pediatric clinical trials have to follow

stricter regulations, require in-depth ethical consideration, and usually have longer follow-up periods with a smaller patient pool (10). Additionally, the need for frequent long distance travel to study sites and later switch from pediatric to adult care, including re-consent during a long-term follow-up, often results in loss of follow-up. High rates of lost follow-up in pediatric trials, such as a 55% lost follow-up in a US pediatric diabetes trial, after a median of 1.3 years from enrolment, are not uncommon (11). This makes integration of pediatric trials into routine clinical care valuable but challenging.

In an attempt to expand the overall use of patient disease registries across all populations in the benefit–risk evaluation of medicines for regulatory purposes, the EMA supports a more systematic and standardized approach to planning and execution of all patient registries. In 2015, the EMA established the Patient Registry Initiative and the Cross-Committee Task Force on registries to identify the barriers and establish good registry practices. In November 2018, the EMA issued a draft discussion paper on methodological and operational aspects of disease registries and made proposals on registry studies and good registry practice (12). In this paper, we refer to the EMA discussion paper on methodological and operational aspects of disease registries as "EMA guidance."

The EMA guidance is a reflection of recommendations based on multiple workshops and resources, including the EMA Patient Registries Workshop, the four disease-specific workshops on registries for cystic fibrosis, multiple sclerosis, CAR-T cell products and hemophilia, the Qualification opinion on the European Cystic Fibrosis Society Patient Registry (ECFSPR), the Draft qualification opinion on the Cellular therapy module of the European Society for Blood and Marrow Transplantation (EBMT) Registry, and existing guidance published in the PARENT Joint Action Methodological Guidance and the US Agency for Healthcare Research and Quality (AHRQ)'s handbook. It is also aligned with the recommendations from the European Network of Centers for Pharmacoepidemiology and Pharmacovigilance (ENCePP) Guide on Methodological Standards in Pharmacoepidemiology and the ENCePP Code of Conduct.

The EMA guidance elaborates on multiple aspects of planning and execution of patient registries (12). Although this guidance is not specific for rare disease registries, it is expected to become the gold standard for registry guidance across all patient registries including those covering small populations, pediatric indications, and rare diseases. This shift in mindset is reflected in national health authorities enforcing the implementation of good registry practice through legal framework and national registry initiatives. For instance, the German Ministry of Health has passed the "Gesetz für mehr Sicherheit in der Arzneimittelversorgung" (13) (GSAV, Law for More Safety in the Supply of Medicines) and IQWiG (14), outlining registry use as part of the report on scientific concepts for the generation of routine practice data and their analysis for the benefit assessment of drugs.

Overview of TuberOus SClerosis Registry to Increase Disease Awareness

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder, characterized by formation of hamartomas in multiple organ systems. This rare disorder originates from genetic mutations in either *TSC1* or *TSC2* gene. In most patients, it manifests as dermatological, renal, or neurological abnormalities, although any organ system can be affected (15). This seriously debilitating disease is rare, with an estimated prevalence between 1/6,800 and 1/15,000 population. The disease is diverse in terms of age of onset, its manifestations, and its severity (16). It can be diagnosed at any point in life, even prenatally, depending on the location of tumors. The age of onset and hence diagnosis can further vary, depending on access to clinical and genetic testing. The average age of diagnosis has been reported to be around 5 years; however, it is likely that TSC is frequently underdiagnosed depending on manifestations and access to health care (17). Despite several advances made over the years, there are still gaps in the understanding of TSC. Considering the rare prevalence and diverse clinical implications, various aspects of TSC have not been documented and published adequately to assist our understanding of the condition. Moreover, many treatment options have not been monitored long term to gather high level of disease insights. This issue is also reflected in the TSC consensus panel, which acknowledged that the current TSC recommendation guidelines are not based on high levels of evidence. Hence, more information is required about TSC to improvise management strategies (16).

In order to address these existing gaps, in 2011, Novartis collaborated with medical experts and patient advocates to evaluate the need for a TSC registry. A subsequent survey highlighted that in many European countries, there were no national TSC registries or any systematic data collection for TSC. It was realized that instead of solely relying on the fragmented evidence obtained from a limited number of patients, a larger collaboration was more desirable. This consensus regarding the need to establish a TSC registry helped conceptualize TuberOus SClerosis registry to increase disease Awareness (TOSCA) (16).

Although TOSCA was initiated in Europe, some non-European countries joined the registry later, further expanding its reach. TOSCA is a multicenter, international disease registry to collect data to assess the manifestations, interventions, and their outcomes in patients with TSC. The detailed description of registry design and structure has been published earlier by Kingswood et al. (16). The baseline data of 2,093 patients in TOSCA have been already been published (18).

Systematic Collection and Dissemination of Lessons Learned From TuberOus SClerosis Registry to Increase Disease Awareness

As TOSCA was the first multinational registry for TSC, there were various issues, predominantly in its planning and implementation. In an attempt to characterize these issues and in order to disseminate future registries in rare diseases, a questionnaire-based survey was conducted among the members

of steering committee, principal investigators (PIs), and sponsor employees involved in the TOSCA registry. This survey identified key strengths and limitations regarding planning and implementation in TOSCA (19). The practical experiences in TOSCA and the lessons learned can be used to supplement the EMA guidance for future registries in rare diseases. In this paper, we refer to the TOSCA survey (19) as "TOSCA lessons paper."

Rationale

As stated, the drafted EMA guidance regarding good registry practice was released in November 2018; by then, the TOSCA registry was reaching the stage of final data analysis. Hence, with this paper, we strive to compare and evaluate how the TOSCA registry differs from the EMA recommendations on a point-by-point basis and whether such deviations may have affected the registry outcomes. We also analyze how the learning from TOSCA can complement the EMA guidance, especially in case of rare disease registries. The observations in this paper also incorporate the experiences and perspectives of the Clinical Trial Head (CTH) of the TOSCA registry and, hence, also offer insights regarding practical issues during the conduct of the registry.

OBSERVATIONS

The suggestions derived from EMA are divided into four categories: registry planning, operations of registry, data analysis, and publication of results. The recommendations from the EMA guidance are summarized under each subheading, followed by the TOSCA methodology, along with the relevant issues, if identified, in TOSCA. The point-wise comparison and compliance of TOSCA and EMA guidance have been summarized in **Table 1**.

Registry Planning

Design and Governance of Registry

The EMA guidance recognizes patient disease registries, particularly in rare diseases, as an important source of information derived from clinical practice. Although randomized controlled trials (RCTs) are the gold standard for gathering evidence in clinical development, patient registries are more practical and offer the best platform when conducting RCTs is not feasible or ethical, for example, when using historical control data, where comparable standard of care is lacking. It is also noteworthy that a registry is not initiated and guided by a single research question or hypothesis. Rather, it is driven with the aim to describe a disease/therapeutic treatment/patient population as a whole. The EMA guidance suggests meticulous planning, including statistical analysis plan and other details, including those for research projects. It also emphasizes the effective collaboration between all involved parties and explicitly describes the role of different stakeholders such as registry coordinators, pharmaceutical companies, and regulatory authorities (12).

Furthermore, the EMA guidance treats registry studies as a separate entity and presents a dedicated section regarding guidance for registry studies. It states that, in addition to the registry protocol, each registry study should have a stand-alone protocol with detailed description of study design, patient population, data collection, and detailed statistical analysis plan. As an aid, the EMA guidance recommends the use of the ENCePP checklist for the creation and evaluation of registry study protocols. Additionally, the protocol should follow all applicable national and regional regulations such as the Good Pharmacovigilance Practice (GVP) Module VIII, if appropriate. Any changes in either registry or study protocol should be recorded as formal protocol amendments (12).

Although TOSCA was planned and initiated much before the EMA guidance was released, all efforts were made to thoroughly plan the registry and to achieve its objectives through a systematic and reliable data collection system. The TOSCA registry organization involved key experts from different areas, including TSC medical health-care experts, representatives from pharmaceutical sponsor, as well as patient representatives in the "Scientific Advisory Board" (SAB) and "Working Committee" (WC) (16). Expert opinions and views gathered in a meeting with different stakeholders ensured careful planning of the registry prior to its launch. The SAB was responsible for the general oversight of the scientific principles and conduct of the registry and also for appropriately promoting the use of the registry in the participating sites. Furthermore, the SAB advised the WC on the implementation and development of the registry. It was also responsible to review and approve the individual research projects. The SAB furthermore covered the essential mandate on publication policy and planning. The WC was responsible for the registry content and for the coordination of all the operative activities after the registry implementation. Additionally, the WC decided on the approval/rejection of requests for registry data access from those involved in the ongoing registry study or external parties. It also reviewed the core data for quality assurance purposes, including quality control analyses.

Involvement of patient representatives was instrumental in patient enrolment and further facilitated the communication with patients. Because patient representatives generally have a better understanding of patient journey within a disease, the collaboration with patient advocacy groups significantly helped and overall facilitated the research project analyzing quality of life outcomes.

After the approval of Votubia®, the EMA requested (EMEA/H/C/002311/II/0004) a Post-Authorization Safety Study (PASS) in TSC, which was subsequently included in the TOSCA registry (16). Contrary to the recommendations of the later-released EMA guidance, the TOSCA PASS did not have a separate protocol but was incorporated in the registry protocol as a protocol amendment (refer to **Table 1**). The registry study protocol was furthermore listed in the ENCePP list (CRAD001MIC03-ENCePP number 3247) and The European Union electronic Register of Post-Authorisation Studies (EU PAS Register) (EUPAS3247).

The successful setup of TOSCA allowed for additional six research projects to take place in TOSCA, which were also incorporated in the registry protocol, as protocol amendments. These research projects aimed to answer certain research questions pertaining to a deeper understanding of TSC. However, in the TOSCA lessons paper, it was realized that although research projects were crucial, lack of adequate planning as well

TABLE 1 | Summary of TOSCA compliance with EMA guidance.

Topic (corresponding EMA guidance chapter)	Recommendations from EMA guidance	Procedure adopted in TOSCA registry	TOSCA compliance with EMA guidance
REGISTRY PLANNING			
Protocol preparation (5.1, 6.3)	• Meticulous predefined design and SAP in protocol • Protocol changes to be included as formal protocol amendments • Separate protocol for registry studies (e.g., PASS) • Protocol to meet ENCePP checklist	• Meticulous planning with KOLs and the other stakeholders • Six research projects included in protocol amendment • No separate protocol for registry studies (Votubia® PASS) • PASS enlisted with ENCePP	Partial
Terminologies (5.5)	• Standard Orphadata, along with ICH-9, 10 and 11, MedDRA	• MedDRA • WHO Drug Reference List, based on ATC classification system	Complete
Data collection/data elements/time elements (5.3, 5.4, 6.5)	• Wide range of data depending on registry objectives • Use "Set of common data elements for RD registration" on EURD Platform • Core list of dates to be collected	• Core (compulsory) and subsections (petals) design of data elements • Additional safety information collected for PASS • Dates collected for pre-defined relevant variables	Complete
Duration/timelines (3.3, 5.1, 6.2)	• Long-term follow-up dictated by schedules for data collection • Registry study to follow up to achieve study objective	• 5 years follow-up • Extended follow-up for PASS	Partial
OPERATIONS OF THE REGISTRY			
Patient enrolment (5.2, 6.4)	• Clear conceptual and operational definition of target population • Exhaustive patient enrolment • Registry study a subset of the registry population or enroll additional patients, if required	• Documented visit for TSC within the preceding 12 months or newly diagnosed • Retrospective as well as prospective data collection from 170 sites across 31 countries. • 2,214 patients enrolled in TOSCA registry, 571 in 6 RPs and 179 patients in PASS.	Complete
Informed consent (5.8.4.)	• Patients are aware: why/what data is collected, how/by whom it will be used, and at what level of details	• Patient Information Brochure and informed consent form	Complete
Quality management (5.6, 6.6)	• Quality management inconsistency, completeness, accuracy and timelines (5.6.2, 5.6.3) • Use data quality indicators to ensure data quality (5.6.4)	• Routine measures for quality maintenance deployed on a site and registry level flagging inconsistency, completeness, accuracy. • 5 yearly interim analyses conducted to assess data quality	Partial
Data sharing (5.8.3)	• Data sharing is encouraged, at least on an aggregated and ideally on an anonymized patient-level	• Data access is enabled for investigators with specific research question, upon approval by SAB. • TOSCA investigators could request for access to self-recorded data on eCRF after the completion of registry data collection (August 2017)	Complete
Data security (5.8.5)	• Security measures should be implemented to maintain the privacy of patients	• Overseen and managed by neutral 3rd party (CRO) and clarified in contract	Complete
DATA ANALYSIS			
Data analysis (5.6.3, 5.7, 6.7)	• Subjective to registry purpose • Registry study to have separate SAP	• Due to exploratory registry purpose mainly descriptive analysis • PASS with yearly interim analysis but no separate SAP	Partial
Safety analysis (5.7, 6.8)	• Reporting of AEs • Monitoring of AESI • Aggregated analysis of AEs	• AE reporting at site level according to national regulations • AESI assessed in sub-population in the context of a PASS • No analysis of all AEs planned in the objectives of the registry	Partial
PUBLICATIONS			
Publication policy (6.9)	• Lead investigator retains authority to prepare publication of registry results. • MAH discuss final results and interpretation, if required.	• WC, with the approval of SAB developed publication strategy. • WC responsible for preparation and coordination of all presentations and publication activities. • Sponsor data owner • MAH not involved	Complete

**Until they reach Tanner stage V or age of 16 years in females and 17 years in males.*

ATC, Anatomic Therapeutic Classification; CRO, Clinical Research Organization; eCRF, Electronic case report forms; ENCePP, European Network of Centers for Pharmacoepidemiology and Pharmacovigilance; EURD, European Platform on Rare Diseases Registration; ICH, International Council for Harmonization; KOL, Key Opinion Leaders; MAH, Marketing Authorization Holder; MedDRA, Medical Dictionary for Regulatory Activities; PASS, Post-Authorization Safety Study; RD, Rare Diseases; RPs, Research projects; SAB, Scientific Advisory Board; SAP, Statistical Analysis Plan; TOSCA, TuberOus SClerosis registry to increase disease Awareness; WC, working Committee; WHO, World Health Organization.

as finances for such complex projects rendered them burdensome for PIs and sponsor, which in turn, might have hampered their potential to provide new insights for different manifestations of TSC (19).

Registry Duration and Follow-Up

EMA acknowledges that while theoretically registries are open-ended data collection systems to gather abundant information regarding a disease and its manifestations, the practical timelines are usually dictated by financing and schedules for data collection (12). This is particularly true in rare disease and small populations, where budget restrictions usually strongly impact registry duration, registry data quality, and registry data completeness.

In the TOSCA registry, the planned duration of follow-up, once a patient was enrolled in the registry, was up to 5 years. However, in Votubia® PASS, for pediatric patients in the EU region, it was agreed to continue the follow-up till they reach Tanner stage V or until 16 years of age for females and 17 years for males. Consequently, some patients are expected to be followed up until 2027, to ensure a more thorough evaluation of long-term effect of Votubia® (16).

According to the TOSCA lessons paper, 38% participants (members of SAB, PIs, and employees of sponsor involved in registry) considered a 5-year follow-up in the main registry to be short in order to holistically assess the real-life impact of the disease. A longer follow-up would definitely be more helpful for a rare disease, especially when there are multiple manifestations (19).

Operational Aspects

Patient Enrolment

While registries are prone to selection bias, pertaining to multiple confounding factors, all attempts should be made to avoid selection bias as much as possible. EMA suggests keen attention toward defining and enrolling patient population. A clear conceptual definition of target population, which can be further translated into operational definition, is suggested. Comprehensive patient enrolment requires a meticulous process to exhaustively enroll patients fulfilling the operational definition, to avoid selection bias. Voluntary and informed consent with detailed information regarding the purpose and extent of data collection, as well as its further use/sharing to external parties, is mandatory during patient enrolment. Informed consent should comply with General Data Protection Regulation (GDPR). Patients also need to be informed about their potential to restrict consent as well as their withdrawal at any time (12).

The TOSCA registry was structured to retrospectively and prospectively collect data from patients with TSC. In order to gather a large multinational cohort of TSC patients, TOSCA aimed for exhaustive recruitment, as recommended by the EMA guidance, overall enrolling 2,214 patients from 170 sites across 31 countries. Such high recruitment rates, particularly for a rare and predominantly pediatric disease registry like TOSCA, is commendable. This may only have been achieved through the close collaboration with all stakeholders as well as using the recommended clear conceptual and operational definition of target population. Aligned with the EMA recommendations (refer **Table 1**), all patients who are enrolled in TOSCA signed a voluntary informed consent form. Separate informed consent forms were issued for research projects as well as PASS study (16, 18).

Site/Database Management and Quality Control

Frequently, uncertainties in data quality impact the confidence in validity and reliability of data quality in registries. Such issues are particularly critical for post-authorization registry studies, where data quality may have a significant impact on marketing authorization. EMA suggests four main activities for quality management, namely, quality planning, quality assurance, quality control, and quality improvement. Maintaining data quality comprises four major components: data consistency, data completeness, data accuracy, and data timelines. Measures to continuously assure data quality should be in place at management level as well as operational level of the registry. The EMA guidance also suggests using indicators of data quality to regularly measure and improve data quality (12).

In TOSCA, suitable measures were taken for adequate site management and data quality. Before site activation, the participating personnel at registry sites underwent thorough training and detailed protocol review with designated representatives from Novartis to ensure high data quality. Only trained and designated registry staff were allowed data entry into the Novartis-provided electronic case report form, using fully validated software that complied with the regulatory requirements for electronic data capture. Additionally, the international clinical research organization responsible for management of the web-based system was also responsible for reviewing the collected data for completeness and accuracy. Online validation checks minimized data entry errors and hence any queries. The physicians participating in the registry were responsible for ensuring timely and accurate data collection. Quality assurance reviews, audits, and evaluation of registry progress were conducted at regular intervals by authorized representatives from Novartis and regulatory agencies.

Although there were no specific data quality indicators used (refer to **Table 1**), maintenance of data quality and accuracy was evaluated in the first administrative analysis of the registry data. This included the data for the first 100 patients, where a total of 469 fields of information were evaluated for each of the 100 patients. In more than 90% of patients, the information on at least 85% of the fields was found to be complete. This analysis demonstrated a high degree of accuracy, hence ensuring optimum quality of data collection (16). In total, five annual interim analysis were conducted. During further planned annual interim analyses for data quality, any inconsistencies, if found, were traced back to the source site, and adequate measures were taken for its in-site modification.

In the TOSCA lessons paper, 25% of the respondents had concerns regarding the presence of some form of bias, which may be selection bias, information bias (subjected to selective recall and inconsistent data collection), or measurement bias (misclassification of outcomes). These biases may have

compromised the validity of collected data. It was recommended that further efforts must be made to minimize biases, which are particularly likely to occur in registries and, further, more likely in a rare disease setting. Involvement of a statistician from the planning stage itself may help minimize the potential for biases in future registries (19).

Data Handling

Data Elements

The EMA guidance suggests the use of harmonized core data and core time elements collected in a predefined format across all patient registries for the same disease to assure interoperability and comparability. Harmonization to international standards further facilitates the implementation of a common data quality system, data exchange, and further interpretation and comparison of results from different registries. Lack of harmonization leads to a time-intensive and resource-intensive process, when mapping data elements of multiple sources (12).

A list of core data elements and corresponding dates is ideally composed of "crucial" and "should have" data elements. The crucial data elements are defined as those important data and time elements that have to be collected in all registries and hence require greater resource allocation to ensure completeness, standardization, data quality, and verification of the information. The "should have" data and time elements are additional data and time elements, which are of interest and important for some stakeholders or in some subpopulation, but not essential to all (12).

Core data and time elements for a particular registry should be identified with intensive discussions among clinicians, disease experts, patient representatives, and, if required, regulatory authorities. A standard set of core data elements for rare diseases has been developed as "Set of common data elements for RD registration" on the European Platform on Rare Diseases Registration (EU RD Platform) (20). Furthermore, some disease-specific lists of core data elements are available, for example, those for cystic fibrosis (21), multiple sclerosis (22), CAR-T cell products (23), and hemophilia (24), and have been agreed upon at multi-stakeholder workshops organized and published through the EMA.

The details pertaining to the data and time elements in the TOSCA registry have already been published earlier (16). In brief, TOSCA followed a flower-and-petal model of data elements. The main "core" section was designed to collect a general predefined set of patient background data including demographics, family history, prenatal history, and disease features (i.e., neurological, neuropsychiatric, renal, cardiovascular, and pulmonary) including the corresponding dates, where relevant. This mandatory section ensured that at least a minimum amount of essential information on each patient was collected across all countries to allow meaningful analyses. Additional and more detailed data related to specific disease manifestations were collected in the "petal segments," that is, subsections of the registry that may have only taken place in certain countries, sites, or subpopulations.

Furthermore, it is to be noted that the data elements used in TOSCA registry may form a sample list of identified data elements for future registries in TSC, especially when unlike cystic fibrosis, there is a lack of standard set of core data elements in TSC.

Terminologies

In order to internationally harmonize various registries across same diseases, it is recommended to use international terminologies for diseases, diagnostic tests, symptoms, medicinal products, and adverse events (AEs). When national or local terminologies are used, mapping to international terminologies is recommended (12).

The EMA guidance recommends use of standard Orphadata (25) for terminologies associated with rare diseases, along with ICH-9, 10, and 11 and Medical Dictionary for Regulatory Activities (MedDRA) (26) for standardizing terminologies. MedDRA is also internationally acceptable for AE classification for regulatory purposes.

As per the TOSCA protocol, medical history/current medical conditions were coded using the MedDRA (26). Additionally, the World Health Organization (WHO) Drug Reference List (27), which employs the Anatomical Therapeutic Chemical (ATC) classification system, was used to code the concomitant medications.

Data Analysis

EMA suggests using appropriate statistical method to justify the individual research question and variables in individual registry. Data analysis should be performed based on predefined time schedules. The handling of missing data should be described in the statistical analysis plan. The statistical plan for registry study should be different from the registry itself. Hence, a clearly defined statistical analysis plan for the registry studies should be provided and may be stand-alone or elaborated in detail as part of the registry study protocol. Furthermore, any changes in the statistical analysis plan should be recorded as formal protocol amendments (12).

As a part of the data analysis, the EMA guidance suggests the reporting of AEs, the monitoring of AEs of special interest, and the aggregated analysis of AEs. It is, however, to be noted that in multinational registries, following the local requirements on AE reporting is essential. Hence, in TOSCA, various sites reported the AEs to their corresponding national authorities. The AEs of special interest were predefined and assessed as a part of Votubia® PASS in the specifically described subpopulation. Because the objective of TOSCA was inclined toward describing the multitude of TSC manifestations, a detailed analysis of reported AEs was not attempted. However, specific AEs may be analyzed in the context of individual patient subgroups and contextualized with a particular manifestation.

Considering the exploratory nature of the TOSCA registry, and in the absence of a specific hypothesis put to test, the demographic and clinical parameters underwent descriptive analysis for relevant variables. Furthermore, missing data were not imputed, in general. For partially missing data, the values were imputed for analysis purpose. For example, in a renal angiomyolipoma patient, whose data regarding diagnosis and

epidemiology are available but treatment details were missing, the patient's data was included in the analysis.

In the TOSCA lessons paper, 32% of respondents had concerns related to the handling of missing data. In fact, a major challenge for the TOSCA registry was to ensure that data about all the disease manifestations, for each patient, were reported, even though the different sites involved did not always follow patients for all disease manifestations in the same way, as part of routine clinical care. Noteworthy is that variables with the most missing data were related to a particular manifestation, that is, TSC-associated neuropsychiatric disorders (TAND). This may be attributed to the lack of knowledge of TAND-related manifestations investigated through the physician-reported or patient/caregiver-reported outcomes. For other manifestations, the missing data were minimal, reflecting an overall good quality data collection (19).

Although there was no definitive statistical analysis plan, adequate attempts were made to open-endedly analyze and interpret data and identify any potential correlations. Further data analysis during manuscript preparation ensured the identification of interesting insights regarding different manifestations of TSC.

Data Ownership and Data Sharing

EMA guidance clearly states that the control on the use of data lies with the patients, who may decide to consent or not consent for the use of their data for clinical or research purpose and may also withdraw the previous consent.

EMA guidance dictates that the registry centers and coordinators should ensure the use and sharing of data in accordance with the EU GDPR and the patient-signed informed consent form. When contractual sharing of data with Marketing Authorisation Holder (MAH) is required, the agreement should clearly describe the extent of data access, the intellectual property rights arising from the data usage, and results dissemination.

As EMA guidance suggests, all patients, before their enrolment in TOSCA, were informed about their rights regarding the generation and usage of their data. Consequently, separate informed consent forms were signed for inclusion into main registry, PASS, and individual research projects. Hence, patients had a control for the use of their data in individual studies. They were also informed about their right to withdraw consent at any time.

Members of SAB and WC had access to the consolidated and detailed data along with the results of every interim analysis. Furthermore, appropriate data access was given to investigators who submitted a research request after endorsement by the SAB. For such purposes, a contract stating the extent of data access and intellectual property rights arising from use of data was signed to avoid any conflicts. PIs had also access to self-recorded data after the completion of data collection (i.e., August 2017). The final ownership of data generated in the registry was with the sponsor.

Publication

EMA states that regardless of the funding source, the lead investigator retains primary authority to independently prepare publications of the study results. If applicable, the MAH co-funding the registry study is entitled to view the final results and interpretations prior to submission for publication. The MAH may also share their views regarding the study results and interpretation, in advance of submission within a reasonable time limit, for example, 1 month, and without unjustly delaying the publication. EMA also entitles the MAH to request change in presentation of results to delete confidential information (12).

Because TOSCA was not aimed for a drug dossier submission approval, the MAH did not participate in the publication process. Instead, only the Novartis medical department (medical affairs) was involved in publication preparation and review.

In the initial stages of the registry, the publication policy was not well-defined. After the first manuscript, the need for a thorough publication policy and plan was realized, and the issue was rectified through a detailed publication policy released in January 2015. The WC, in turn, was responsible to develop publication strategy, which was further approved by SAB. The WC was further deemed responsible for the development and coordination of presentations and publications activities according to the publication policy. This publication policy and the planned information dissemination were clearly in line with the EMA guidance and contributed to the increased awareness of TSC.

The publication policy stated that at least one manuscript would be published following each interim analysis. Secondary manuscripts and abstracts to publications were planned to communicate the results and knowledge to a wider audience. In a further attempt to reach a broader audience, translations of posters presented at International Congresses were encouraged to be presented in local languages at National Congresses. This extension of audience reached complemented the primary objective of TOSCA: to increase awareness about this rare disease and its manifestations. A clear protocol was prepared with regard to the process of developing presentations and publications. A kick-off meeting (face-to-face or teleconference) with all authors and reviewers was suggested to discuss all details, that is, timelines, journal, and relevant topics regarding the manuscript before the initiation of manuscript writing. SAB retained the final authority regarding authorship and order or authorship.

The results of the TOSCA registry analyses were presented as posters/presentations on the main TSC, or specific manifestations, congresses. So far, nine publications from the TOSCA registry study have been released (16, 18, 19, 28–33), including its methodology, baseline analysis from second interim analysis, epilepsy, renal angiomyolipoma, subependymal giant cell astrocytoma (SEGA), and TAND from third interim analysis, SEGA in adults from final analysis, treatment patterns, and use of resources in TOSCA and learning from TOSCA. A robust publication plan for data derived from the main registry as well as research projects and the TOSCA PASS study is in place, and it is expected to be achieved by 2020. Furthermore, 15 oral presentations and 27 posters have been presented at International Congresses. Of these, five oral presentations and eight posters have been further translated and presented in National and Local Congresses. Additionally, three posters with country-specific data have been presented at National Congresses. In the future,

data collected in TOSCA may be used for performing new analysis to address specific research questions on the basis of retrospective observations. In-depth analysis of specific data will further help the clinicians to have a better understanding of TSC and its manifestations.

SUSTAINABILITY

EMA recognizes that most patient registries face sustainability issues after the initial phase of funding for initiation of registry. Throughout the registry duration, sustainable funding is required for multiple reasons including maintenance of core registry features, adaption to changes in legal requirements, additional staff hiring for specific studies, and provision of funds to local centers, as necessary. In a Patient Registry Workshop, EMA recommended to consider the learning from existing successful registries to inform the sustainability component in the planning of new registries. Registry holders should engage with public agencies and define/clarify the long-term role of industry, instead of aiming for a short-term funding support. A clear development strategy, appropriate management, and the clear stakeholder partnership may help improve sustainability (34). Furthermore, EMA suggests the collaborations to have cost-sharing agreement, indicating that a registry be co-founded by multiple partners and coordinated through an "independent third party," for example, a disease association.

The TOSCA registry was solely sponsored by Novartis, and the budget was ensured at the stage of planning of the registry. Even after the completion of data collection in the main registry in August 2017, the publication plan is being implemented with Novartis sponsorship.

With the initial registry planning, no funding issues were expected. However, six research projects were added later as protocol amendment. These research projects lacked adequate time and resource planning and had budget constraints, as they were not of primary interest in the context of any compound. Despite these issues, the research projects were able to capture important information regarding the diverse manifestations of TSC, which will enhance the understanding about the disease and its manifestations. Including research projects at the registry planning stage would ensure a more robust data collection and also improve the outcomes achieved.

CONCLUSION

Comparing the EMA guidance on Good Registry Practice with TOSCA protocol and implementation course, it appears that TOSCA did not completely comply with all aspects of the EMA guidance (refer to **Table 1**). However, on most important aspects, the TOSCA registry is definitely in accordance with the EMA guidance. This is especially noticeable on the meticulous planning with involvement of multiple stakeholders, careful implementation ensuring valuable and high-quality data collection, definition of core and extended data elements, inclusion of research projects, and registry studies. Hence, despite partial compliance and multiple deviations from EMA guidance, TOSCA was able to successfully achieve the desired outcomes and fulfill its objectives, particularly in improving our understanding about TSC and its manifestations, as well as increasing the awareness about this rare disease. It is furthermore particularly commendable that the TOSCA registry managed to recruit such a large number of patients across all geographic regions, which would not have been possible without such a strong collaboration between stakeholders. More compliance with certain aspects of EMA guidance, such as inclusion of research projects in the initial protocol and developing a separate protocol for PASS, might have avoided some issues in TOSCA and hence should be considered in future rare disease patient registries.

The EMA guidance on Good Registry Practice offers valuable guidance for future registries and registry studies. These guidelines will also help harmonize the databases established across different registries in same disease areas. It is, however, to be noted that some of the expectations are simply not feasible in the context of rare diseases. For instance, collecting a very large number of variables open-endedly in a small population may be difficult owing to the burden on patients. Additionally, it cannot be expected that adequate financial means for open-ended registries with high data quality and completeness is available for each rare disease. The contribution of patient communities in rare disease, if properly engaged, can be instrumental to ensure high accrual and minimal loss to follow-up. Adopting additional measures to address the issues specific to rare disease registry is thus suggested for optimal outcomes.

AUTHOR CONTRIBUTIONS

RM performed the conceptualization and design of the manuscript, drafting, revising, final review, and provided approval of the manuscript to be published. HT performed the design of the manuscript, drafting, revising, final review, and provided approval of the manuscript to be published. JR performed the conceptualization and design of the manuscript, drafting, revising, final review, and provided approval of the manuscript to be published.

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Newly Diagnosed and Growing Subependymal Giant Cell Astrocytoma in Adults With Tuberous Sclerosis Complex: Results From the International TOSCA Study

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The onset and growth of subependymal giant cell astrocytoma (SEGA) in tuberous sclerosis complex (TSC) typically occurs in childhood. There is minimal information on SEGA evolution in adults with TSC. Of 2,211 patients enrolled in TOSCA, 220 of the 803 adults (27.4%) ever had a SEGA. Of 186 patients with SEGA still ongoing in adulthood, 153 (82.3%) remained asymptomatic, and 33 (17.7%) were reported to ever have developed symptoms related to SEGA growth. SEGA growth since the previous scan was reported in 39 of the 186 adults (21%) with ongoing SEGA. All but one patient with

growing SEGA had mutations in *TSC2*. Fourteen adults (2.4%) were newly diagnosed with SEGA during follow-up, and majority had mutations in *TSC2*. Our findings suggest that surveillance for new or growing SEGA is warranted also in adulthood, particularly in patients with mutations in *TSC2*.

Keywords: mTOR, registry, SEGA, TOSCA, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder characterised by hamartomas in multiple organs, with the brain being the most commonly affected organ (1, 2). Subependymal giant cell astrocytoma (SEGA) occurs at the foramen of Monro, with a reported lifetime prevalence between 5 and 24% (3, 4). Although SEGAs are generally benign and non-infiltrative, these may grow, and obstruct cerebrospinal fluid (CSF) flow, thereby increasing intracranial pressure. Typical symptoms of growing SEGA include headaches, blurred vision, nausea, vomiting, worsening of seizure control or new-onset seizures, and sudden death from acute hydrocephalus (3, 5).

Diagnosis of SEGA has changed from pathology-based to imaging-based (6, 7), but formal diagnostic criteria have only been available since 2012, when an expert panel at the International Tuberous Sclerosis Complex Consensus Conference defined SEGA as a lesion at the caudothalamic groove with a size of >1 cm in any direction or a subependymal lesion at any location which has shown serial growth on consecutive imaging regardless of size (7). All SEGA-related studies performed before 2012 have been based on variable criteria, thus limiting the value of comparison (8).

Onset and growth of SEGA has been reported most commonly in the first two decades of life (9). In two of the largest series of operated SEGAs, the mean age of surgical intervention was 9.7 years (10), and 11.6 years, (11) suggesting that growth is most common at this age. SEGA have been reported in neonates (9). Data on SEGA prevalence and growth in adults are scarce. A retrospective case series of 16 patients with TSC who required SEGA surgery, highlighted that SEGA can still become symptomatic later in life (12).

Present guidelines recommend that patients with asymptomatic SEGA diagnosed during childhood should continue to be imaged periodically as adults to ensure that there is no growth (13). Patients with large or growing SEGA or with SEGA causing ventricular enlargement that are still asymptomatic, should undergo MRI (magnetic resonance imaging) scans more frequently, and such patients and their families should be educated regarding the symptoms of raised intracranial pressure (7).

Surgical resection (occasionally VP shunt alone) is the recommended intervention for acutely symptomatic individuals, while either surgical resection or medical therapy with mammalian/mechanistic target of rapamycin (mTOR) inhibitors can be effective for individuals with growing asymptomatic SEGA (13). Treatment decisions should be based on multiple factors such as the patient's clinical condition, anatomic considerations specific to SEGA, surgeon's experience, experience of the centre regarding use of mTOR inhibitors, prior history of SEGA resection, other TSC-related comorbidities, and patient/parental preference (7).

This is the first study evaluating prevalence, growth, symptoms, and treatment patterns in a large prospective cohort of adults with TSC-associated SEGA.

METHODS

TOSCA, a large-scale non-interventional study in patients with TSC, was conducted at 170 sites in 31 countries. The study design and methodology of TOSCA has been published previously (14). The study enrolled patients of any age with TSC between August 2012 and November 2014 and followed for up to 5 years. Patient data, including demographics, and information related to clinical features of TSC across all organ systems, comorbidities and rare manifestations, were collected at baseline and at regular visits scheduled at a maximum interval of 1 year.

In this study, designed prior to the 2012 imaging-based consensus, prevalence, and growth of SEGA were defined as per clinical practice of the participating centres. We evaluated SEGA manifestations among adult patients (>18 years) enrolled into the TOSCA study. SEGA-related questions included in the case report form (CRF) were presence of single or multiple SEGA, newly diagnosed SEGA, SEGA growth, clinical signs, and symptoms associated with SEGA and information regarding SEGA treatment. In addition, possible associations of SEGA prevalence with genotype were analysed using a Chi-square test. Statistical significance was set at p-value < 0.05.

Statistics were descriptive considering the exploratory nature of this study. Categorical data were reported as frequencies and percentages, and continuous variables were expressed as mean ($\pm$ standard deviation) or as median (range), unless stated otherwise.

The study was designed and conducted in accordance with the Good Clinical Practice principles, the Declaration of Helsinki and all local regulations. The institutional review board or ethics committee at each participating site approved required TOSCA-related documents. Written informed consent was obtained from all patients, parents or guardians before enrolment.

RESULTS

A total of 2,214 patients with TSC were enrolled in TOSCA study, and data were analysed for 2,211 patients. In the

TABLE 1 | Demographics of adult patients with SEGA.

Characteristics	Patients with SEGA			
	All adults ($n = 220$)	**>18 to ≤25 years ($n = 91$)**	**>25 to ≤40 years ($n = 96$)**	**>40 years ($n = 33$)**
Age at diagnosis of TSC, years; median (range)	4.0 (<1–48)	1.0 (<1–24)	4.0 (<1–37)	15.0 (<1–48)
Gender, *n* (%)				
Male	98 (44.5)	35 (38.5)	46 (47.9)	17 (51.5)
Female	122 (55.5)	56 (61.5)	50 (52.1)	16 (48.5)
Patients with molecular testing, *n* (%)	96 (43.6)	40 (44.0)	41 (42.7)	15 (45.5)
Genetic Testing, *n* (%)				
No mutation identified	12 (12.5)	6 (15.0)	3 (7.3)	3 (20.0)
TSC1 mutation	12 (12.5)	2 (5.0)	5 (12.2)	5 (33.3)
TSC2 mutation	69 (71.9)	31 (77.5)	31 (75.6)	7 (46.7)
Results not available*	5 (5.2)	1 (2.5)	1 (2.4)	0
Variation Type, *n* (%)				
Pathogenic mutation	59 (61.5)	22 (55.0)	27 (65.9)	10 (66.7)
Variant of unknown significance	5 (5.2)	4 (10.0)	1 (2.4)	0
Both pathogenic mutation and variant of unknown significance	2 (2.1)	0	2 (4.9)	0
Results not available*	30 (31.3)	14 (35)	11 (26.8)	5 (33.3)
Patients with prenatal diagnosis, *n* (%)	1 (0.5)	1 (1.1)	0	0

*Values are expressed as n (%), unless otherwise specified. *Include missing data and those results not made available due to legal/medical confidentiality statements. SEGA, subependymal giant cell astrocytoma. TSC, tuberous sclerosis complex.*

final analysis performed on data collected until August 2017, a history of SEGA was reported in 30.3% (671/2,211; 332 males and 339 females) of patients. Other neuroimaging features reported included cerebral white matter radial migration lines in 25.5, cortical tubers in 87.2, and subependymal nodules 82.9%.

Of the 803 adult patients included in the final analysis, a history of SEGA was reported in 220 patients (27.4%). The demographic of the adult patients with SEGA are shown in **Table 1**. SEGA were ongoing during study in 186 (84.5%) patients. Of these, multiple and bilateral SEGA were reported in 66 (35.5%), and 61 (32.8%) patients, respectively. SEGA growth since previous scan was reported in 39 (21%). The median age at SEGA diagnosis in this adult cohort was 20 years (range, <1–57 years), as compared to 7 years (range, <1–57 years) in the entire TOSCA cohort.

The median interval between consecutive scans was 1 year (range <1–34 years). During the study period (up to 5 years), 14 new diagnoses of SEGA were made (2.4% of total adults minus those with history of SEGA). The oldest patient with a newly reported SEGA was 57 years. Of the 186 adults with ongoing SEGA, 153 (82.3%) remained asymptomatic, and 33 (17.7%) were reported to ever have developed symptoms related to SEGA growth in the past, including primarily increase in seizure frequency (15.6%), behavioural disturbance (13.4%), and headache (10.8%), either alone or in combination with other symptoms (**Table 2**). Over time, SEGA had been treated with surgery in 55 out of 117 patients (47.0%) and with mTOR-inhibitors in 46 out of 117 patients (39.3%). Nine patients (7.7%) required a shunt for the management of hydrocephalus.

SEGA were significantly more frequent in adults with a *TSC2* mutation compared to those with a *TSC1* mutation (35.2 vs. 15.6%, $p < 0.0004$). However, there was no significant difference in multiple ($p = 0.1158$), bilateral ($p = 0.1062$), or growing SEGA ($p = 1.0000$), and presence of SEGA-related symptoms ($p = 0.2598$) between those with *TSC1* and *TSC2* mutation. The median age at SEGA diagnosis was higher in patients with *TSC1* mutations (29 years, range 9–51) compared to patients with *TSC2* mutations (21 years, range <1–49), but this difference was non-significant (**Table 3**). Furthermore, 12 of 14 adults with newly diagnosed SEGA had mutations in *TSC2* gene, while two had no mutation identified.

DISCUSSION

To our knowledge, this is the first study to evaluate SEGA prevalence, growth, symptoms, and current treatment modalities in adults with TSC-associated SEGA. The international TOSCA study allowed us to evaluate data from 803 adults (age >18 years), 220 of whom had SEGA (27.4%). During the 5 years follow-up period of the study, 23.2% of adults reported that the SEGA was still ongoing.

The occurrence of new SEGA after the age of 18 years was relatively low (2.4%) but more common than previously thought (7). In this cohort, age at SEGA diagnosis was as late as 57 years. Newly diagnosed SEGA were associated with mutations in *TSC2* in the large majority of cases (85.7%). Other risk factors such as contrast enhancement of SEN in the caudo-thalamic groove were beyond the scope of this study.

Another key finding was that SEGA growth since previous scan (mean time of 1.5–2.3 years between previous scan

TABLE 2 | Clinical characteristics of SEGA.

	Overall TOSCA population ($n = 2211$)	Adult patients			
		All adults ($n = 803$)	>18 to ≤25 years ($n = 235$)	>25 to ≤40 years ($n = 344$)	>40 years ($n = 224$)
Patients with history of SEGA	671 (30.3)	220 (27.4)	91 (38.7)	96 (27.9)	33 (14.7)
No. of patients with ongoing SEGA during the study, *n*	579	186	71	87	28
Multiple	240 (41.5)	66 (35.5)	24 (33.8)	33 (37.9)	9 (32.1)
Bilateral	236 (40.8)	61 (32.8)	21 (29.6)	30 (34.5)	10 (35.7)
Growing SEGA since previous scan*#	208 (35.9)	39 (21.0)	19 (26.8)	17 (19.5)	3 (10.7)
Signs and symptoms					
None	476 (82.2)	153 (82.3)	57 (80.3)	72 (82.8)	24 (85.7)
Increase in seizure frequency	98 (16.9)	29 (15.6)	14 (19.7)	13 (14.9)	2 (7.1)
Behavioural disturbance	77 (13.3)	25 (13.4)	8 (11.3)	16 (18.4)	1 (3.6)
Regression/loss of cognitive skills	51 (8.8)	16 (8.6)	5 (7.0)	10 (11.5)	1 (3.6)
Headache	47 (8.1)	20 (10.8)	7 (9.9)	10 (11.5)	3 (10.7)
Ventriculomegaly	32 (5.5)	8 (4.3)	5 (7.0)	3 (3.4)	0
Increased intracranial pressure	24 (4.1)	10 (5.4)	6 (8.5)	2 (2.3)	2 (7.1)
Sleep disorder	22 (3.8)	7 (3.8)	1 (1.4)	6 (6.9)	0
Eye movement abnormalities	16 (2.8)	6 (3.2)	4 (5.6)	2 (2.3)	0
Visual impairment	10 (1.7)	4 (2.2)	3 (4.2)	1 (1.1)	0
Papilloedema	8 (1.4)	4 (2.2)	2 (2.8)	1 (1.1)	1 (3.6)
Neuroendocrine dysfunction	8 (1.4)	4 (2.2)	0	3 (3.4)	1 (3.6)
Other	28 (4.8)	7 (3.8)	4 (5.6)	3 (3.4)	0

*Values are expressed as n (%), unless otherwise specified. *Median time from previous scan to last assessment was 1 year. #Growing of SEGA since previous scan was measured among those with ongoing SEGA during the study. SEGA, subependymal giant cell astrocytoma.*

and last assessment) was observed in 21% of our adult patients. Although not negligible, this is less frequent compared with children. In a cohort of 58 patients (33 children, 25 adults), Tsai et al. reported similar results, with SEGA growth in children being significantly higher than in adults (75.6 vs. 16.5%) (15).

The fact that SEGA may still grow during adulthood emphasises the need for continuous surveillance even after the age of 25 years. This was highlighted in the current guidelines that recommend that patients with asymptomatic SEGA diagnosed in childhood should continue to undergo periodical imaging as adults to ensure that there is no growth. This highlights the need for continued multidisciplinary follow-up, also at adult age. Although newly occurring SEGA during adulthood seem relatively rare and do not warrant systematic screening, physicians should keep this possibility in mind when symptoms potentially related to SEGA growth occur. Special attention should be paid to adults with mutations in *TSC2* since they seem to be at a higher risk for newly occurring SEGA and SEGA growth in adulthood as well as to individuals with intellectual disability who might not be able to verbally express SEGA-related symptoms. Importantly, certain SEGA-related symptoms (especially early symptoms) are not limited to signs of increased intracranial pressure, and therefore, parents and patients should be informed about all relevant symptoms which require referral for medical evaluation, particularly sudden behavioural changes such as acute-onset and unexplained aggression, academic difficulties or any other acute and unexplained manifestations of TSC-associated neuropsychiatric disorders (TAND) (16–18).

We acknowledge the limitations intrinsic to a large-scale, international, non-interventional/observational study. These included the fact that participants were recruited from expert TSC centres around the world and the fact that data on SEGA diagnosis, growth and SEGA-related symptoms were collected as reported per clinical practice. However, these limitations are, at least in part, offset by the large-scale and "real-world" nature of the cohort across multiple centres and countries. Being an observational study, detailed information on the treatment initiated for SEGA at adult age were not collected. The very low number of missing data for SEGA reflects good quality of data collection for this specific manifestation.

CONCLUSION

Findings from this large international study highlight the need for continued monitoring for SEGA growth in adults with ongoing SEGA. Clinicians and adults with TSC should be aware of the potential new onset SEGA in adults with SEGA-related symptoms, especially in the presence of mutations in *TSC2*.

TABLE 3 | Clinical characteristics of SEGA in adults with mutations in *TSC1* vs. *TSC2*.

	Adults with *TSC1* mutation ($n = 77$)	Adults with *TSC2* mutation ($n = 196$)	*p*-value
Patients with history of SEGA	12 (15.6)	69 (35.2)	0.0004
Median (range) age at SEGA diagnosis, years	29 (9–51)	21 (<1–49)	0.0599
No. of patients with ongoing SEGA during the study	8 (66.7)	61 (88.4)	0.1317
Multiple	5 (62.5)	19 (31.1)	0.1158
Bilateral	5 (62.5)	18 (29.5)	0.1062
Growing SEGA since previous scan	1 (12.5)	13 (21.3)	1.0000
Signs and Symptoms			
None	5 (62.5)	49 (87.5)	0.3580
Increase in seizure frequency	3 (37.5)	15 (28.3)	0.6243
Behavioural disturbance	1 (12.5)	14 (26.4)	1.0000
Headache	1 (12.5)	10 (18.9)	0.5753
Regression/loss of cognitive skills	0	5 (9.4)	1.0000
Ventriculomegaly	0	4 (7.5)	1.0000
Increased intracranial pressure	1 (12.5)	3 (5.7)	1.0000
Papilloedema	1 (12.5)	3 (5.7)	1.0000
Sleep disorder	0	2 (3.8)	1.0000
Eye movement abnormalities	0	2 (3.8)	1.0000
Visual impairment	0	2 (3.8)	1.0000
Neuroendocrine dysfunction	1 (12.5)	2 (3.8)	0.2408
Other	1 (12.5)	3 (5.7)	0.3098
Patients received treatment	8 (66.7)	37 (53.6)	0.0716

Values are expressed as n (%), unless otherwise specified.
SEGA, subependymal giant cell astrocytoma.

DATA AVAILABILITY

Novartis supports publication of scientifically rigorous analysis which is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymised patient-level data, respecting patient informed consent, by contacting study sponsor authors. The protocol can be accessed through the EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each centre: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); CEIC-E (Comité Etico Investigación Clínica de Euskadi; Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; UT REC (Research Ethics Committee of the University of Tartu); Ethikkommission der Medizinischen Universität Graz; North Wales REC–West; Regionala Etikprövningsnämnden i Göteborg; REK–Regionale komiteer for medisinsk og helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie Pomnik Centrum Zdrowia Dziecka; Ethikkommission bei der Ludwig-Maximilians-Universitat München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Development of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Childrens Hospital Of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yan-Sen University; The First Affiliated Hospital of Guangzhou Medical University; Shenzhen Children's Hospital; West China Hospital, Sichuan University; Xijing Hospital; Children's Hospital of Chongqing Medical University; Wuhan Children's Hospital; The second affiliated hospital of Xi'an jiaotong university; Guangdong 999 brain hospital; Seoul National University Hospital Institutional Review Board; National Taiwan University Hospital (NTUH) Research Ethics Committee (REC); Institutional Review Board of the Taichung Veterans General Hospital; Institutional Review Board of Chung Shan Medical University Hospital; Institutional Review Board, Tungs' Taichung MetroHarbor Hospital; Institutional Review Board of National Cheng Kung University Hospital; Metro South Human Research Ethics Committee; Sydney Children's Hospital Network Human Research Ethics Committee; St Vincents Hospital Human Research Ethics Committee; Royal Melbourne Hospital Human Research Ethics Committee; Siriraj Institutional Review Board; The Institutional Review board, Faculty of Medicine, Chulalongkorn University, 3rd Floor, Ananthamahidol Building, King Chulalongkorn Memorial Hospital; The committee on Human Rights Related to Research Involving Human Subjects; Institutional Review board, Royal Thai Army Medical Department IRB RTA, 5th Floor, Phramongkutklaowejvitya Building, Phramongkutklao College of Medicine; Research Ethics Committee, Faculty of Medicine, Chiang Mai University; Research and Development, Queen Sirikit National Institute of Child Health; Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town; Shaare Zedek Medical center Helsinki committee; Sheba Medical center Helsinki committee; Tel Aviv Sourasly Medical center Helsinki committee; General University Hospital

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Clinical Characteristics of Subependymal Giant Cell Astrocytoma in Tuberous Sclerosis Complex

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Background: This study evaluated the characteristics of subependymal giant cell astrocytoma (SEGA) in patients with tuberous sclerosis complex (TSC) entered into the TuberOus SClerosis registry to increase disease Awareness (TOSCA).

Methods: The study was conducted at 170 sites across 31 countries. Data from patients of any age with a documented clinical visit for TSC in the 12 months preceding enrollment or those newly diagnosed with TSC were entered.

Results: SEGA were reported in 554 of 2,216 patients (25%). Median age at diagnosis of SEGA was 8 years (range, <1–51), with 18.1% diagnosed after age 18 years. SEGA growth occurred in 22.7% of patients aged ≤ 18 years and in 11.6% of patients aged > 18 years. SEGA were symptomatic in 42.1% of patients. Symptoms included increased

seizure frequency (15.8%), behavioural disturbance (11.9%), and regression/loss of cognitive skills (9.9%), in addition to those typically associated with increased intracranial pressure. SEGA were significantly more frequent in patients with *TSC2* compared to *TSC1* variants (33.7 vs. 13.2 %, $p < 0.0001$). Main treatment modalities included surgery (59.6%) and mammalian target of rapamycin (mTOR) inhibitors (49%).

Conclusions: Although SEGA diagnosis and growth typically occurs during childhood, SEGA can occur and grow in both infants and adults.

Keywords: mTOR, registry, SEGA, TOSCA, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder characterized by growth of hamartomas in several organs, including the brain, kidneys, lungs, heart, eyes, and skin (1). Subependymal giant cell astrocytomas (SEGA) are benign, non-infiltrative brain lesions classified by the World Health Organization as grade I, characteristically observed in patients with TSC (2, 3). They are typically slow-growing tumours composed of different cell lineages and are not purely astrocytic in nature (4). Historically, SEGA diagnosis was based on histology (5), but over time, diagnosis became imaging based. In 2013, an international panel of experts defined the imaging characteristics of SEGA as a lesion at the caudothalamic groove with either a size of >1 cm in any direction or a subependymal lesion at any location that has shown serial growth on consecutive imaging regardless of size. Most SEGA show clear enhancement after contrast administration. However, a growing subependymal lesion even in the absence of enhancement should be considered a SEGA (6). The prevalence of SEGA was previously reported to range from 4 to 20% (2, 7–11). The studies mentioned were based on relatively small patient numbers. In the largest series by Adriaensen et al. evaluating 214 patients with TSC, SEGA was defined as a subependymal lesion near the foramen of Monro showing contrast enhancement after administration of intravenous gadolinium. SEGA occurred in 20% of individuals in this study and average maximum SEGA size was 11.4 mm (range, 4–29 mm) (2).

Although SEGA are histologically benign, their location near the foramen of Monro and their tendency to grow can lead to obstructive hydrocephalus with consecutive substantial morbidity and mortality (12). Symptoms associated with growing SEGA include those typically associated with raised intracranial pressure (headaches, photophobia, diplopia, ataxia, seizures) and/or detrimental effects on cognition and/or increased seizure burden, learning, or behaviour (13). SEGA typically appear in the first 2 decades of life, with a mean age at presentation below 18 years (14). However, there have been reports of SEGA detection prenatally (as early as at 19 weeks gestation) (15–17), as well as new diagnoses after 20 years of age (2, 18). There have been prior reports suggesting that SEGA occur at a younger age in patients with *TSC2* mutations compared with those with *TSC1* mutations (8, 19).

Currently, surgical resection and mammalian target of rapamycin (mTOR) inhibitors are the recommended treatment options for SEGA associated with TSC. Surgical resection should be considered for acutely symptomatic SEGA, while either surgical resection or medical treatment with mTOR inhibitors may be considered for growing, but not acutely symptomatic SEGA (20). However, surgical resection may be associated with preoperative and postoperative complications, and incompletely resected SEGA often tend to regrow (6, 14, 21). Everolimus, an inhibitor of mTOR, the central pathway involved in the pathophysiology of TSC, has been approved by the Food and Drug Administration (FDA) and European Medicines Agency (EMA) for patients with TSC-associated SEGA who require therapeutic intervention, but are not candidates for surgical resection (14). mTOR inhibitors have also shown improvements in the other manifestations of TSC including renal angiomyolipomas, epilepsy, lymphangioleiomyomatosis, and facial angiofibromas (22–25).

Although substantial progress has been made in our understanding of the biological and genetic basis of TSC in the past decade, several questions, especially those related to the natural history of the disease, remain unanswered. To address this gap, the TOSCA (**T**uber**O**us **SC**lerosis registry to increase disease **A**wareness) registry was designed with the aim of providing deeper insights into the manifestations of TSC and its management. The baseline core data of the TOSCA registry published previously provided understanding of the overall manifestations and natural history of TSC (26). Here, we present the clinical characteristics of SEGA in children and adults.

PATIENTS AND METHODS

TOSCA is a non-interventional, multicenter, international natural history study conducted at 170 sites across 31 countries. The study design and methodology of TOSCA have been described in detail previously (27). In brief, between August 2012 and August 2014, patients of any age with a documented clinic visit for TSC in the 12 months preceding enrollment or those newly diagnosed with TSC were enrolled. General information on patient background, such as demographic data, family history, genotype, vital signs, prenatal history, clinical features of TSC across all organ systems, comorbidities, and rare manifestations, was collected at baseline and at regular visits scheduled at a maximum interval of 1 year. Follow-up visits were scheduled according to the standard practice of the site and as per the treating physician's best judgement. The data were recorded on an electronic case report form (eCRF) that was accessed via a secure web portal hosted by a contract research organization.

Input of data was carried out by local investigators or their deputies, and then independently checked by a network of clinical research associates for accuracy and consistency using the original local case records. The web portal has an explanatory manual to guide the investigators.

Data collected specific to SEGA included tumour characteristics such as presence of single or multiple SEGA, clinical signs and symptoms associated with SEGA, and management. Characteristics of SEGA according to the age at consent were evaluated. The study also assessed the association between genotype (*TSC1* vs. *TSC2*) and SEGA characteristics using Chi-square test or fisher exact test, and median test. Since baseline data were collected prior to the 2013 international consensus on SEGA definition, no specific inclusion criteria were defined. The TOSCA cohort therefore reflects worldwide clinical practice.

Given that the natural history study is exploratory in nature, background and clinical parameters were reported with descriptive statistics only. All eligible patients enrolled in the TOSCA registry were considered for analysis. Categorical data were reported as frequencies and percentages, and continuous variables were expressed as mean (± standard deviation) or as median (range), unless stated otherwise.

TOSCA was designed and conducted according to the Guidelines for Good Clinical Practice and ethical principles outlined in the Declaration of Helsinki (28, 29). After appropriate approval by central and all local human research ethics committees, written informed consent was obtained from all patients, parents, or guardians prior to enrollment.

RESULTS

As of September 30, 2015, 2,216 patients (1,154 females and 1,062 males) with TSC were enrolled in the TOSCA registry from 170 sites across 31 countries. The demographic and clinical characteristics of the enrolled patients are shown in **Table 1**. The majority of these patients (70%) were enrolled by pediatric or adult neurologists.

Overall, SEGA were reported in 554 patients (25%); 275 (49.6%) were males and 279 (50.4%) were females. Of these, SEGA were present at baseline in 463 patients (83.6%), resolved with treatment before baseline in 80 patients (14.4%), and were reported to have resolved spontaneously in 10 patients (1.8%), the latter possibly due to measurement errors in small lesions. Detailed information was lacking for one patient. The median age at SEGA diagnosis was 8 years (range, <1–51 years). SEGA were diagnosed before 2 years of age in 26.6%, before 18 years in 81.9% of patients, and after 18 years in 18.1% patients (**Figure 1**). The oldest patient diagnosed with SEGA in the TOSCA cohort was 51 years.

Of the 463 patients with SEGA at baseline, 209 (45.1%) had multiple SEGA and in 208 patients (44.9%) SEGA were present bilaterally (**Table 2**). Among patients with SEGA present at the at the time of baseline visit, SEGA growth was observed in 68 out of 300 patients aged ≤ 18 years (22.7%) and 19 out of 163 patients aged > 18 years (11.6%). In total, 87 out of 463 patients showed SEGA growth since previous scan (18.8%). Of these, 7 patients (8%) were aged < 2 years, 68 patients (78.2%) were aged ≤ 18 years, while 19 patients (21.8%) were aged > 18 years. The median time between consecutive scans was 1 year (mean 1.5 years, range <1–18). At the time of assessment, 321 patients (69.3%) were asymptomatic. Of these, 29 (9.0%) were aged <2 years, 175 (54.5%) were > 2 years and ≤ 18 years, and 117 (36.4%) were aged > 18 years (**Table 3**). One or more symptoms (alone or in combination) assigned to SEGA in our cohort were observed in 233 patients (50.3%). The most frequent symptoms were increased seizure frequency in 73 patients (15.8%), behavioural disturbance in 55 (11.9%), regression/loss of cognitive skills in 46 (9.9%), and headache in 39 (8.4%) (**Table 2**).

TABLE 1 | Demographics and clinical characteristics of participants in the TOSCA study ($N = 2{,}216$).

Characteristics	Baseline data
Age at diagnosis of TSC, years; median (range)	1 (<1–69)
Gender, *n* (%)	
Male	1,062 (47.9)
Female	1,154 (52.1)
Patients with molecular testing, *n* (%)	1,000 (45.1)
Genetic testing, *n* (%)[a]	
No mutation identified	144 (14.4)
TSC1 mutation[b]	197 (19.7)
TSC2 mutation[b]	644 (64.4)
Both *TSC1* and *TSC2* mutations	6 (0.6)
Variation type, *n* (%)[c]	
Pathogenic mutation	678 (67.8)
Variant of unknown significance	66 (6.6)
Patients with prenatal diagnosis, *n* (%)	144 (6.5)

TSC, tuberous sclerosis complex; TOSCA, TuberOus SClerosis registry to increase disease Awareness.

[a] *Information on the type of mutation was missing for 9 patients.*

[b] *The count (n) includes 6 patients who had both TSC1 and TSC2 mutations.*

[c] *The count (n) includes 23 patients who had both variation types.*

The characteristics of SEGA associated with mutations in *TSC1* and *TSC2* are shown in **Table 2**. SEGA were significantly more frequently observed in patients with a *TSC2* mutation compared to those with a *TSC1* mutation (33.7 vs. 13.2%, $p < 0.0001$). However, there was no significant difference with respect to SEGA diagnosis before 2 years of age ($p = 0.3812$), multiple ($p = 0.8368$), bilateral ($p = 0.9550$) or growing SEGA ($p = 0.3302$), and presence of SEGA-related symptoms ($p > 0.05$) in patients with mutations in *TSC1* compared to *TSC2* (**Table 2**). A total of 208 patients received at least one treatment after SEGA diagnosis with a median time from SEGA diagnosis to treatment of 319 days (range, 1–5517 days). The most common treatment modalities included surgical resection (124 patients, 59.6%), mTOR inhibitors (102 patients, 49%), and ventriculoperitoneal shunt (22 patients, 10.6%), used alone or in combination.

DISCUSSION

Together with cortical tubers, white matter radial migration lines, and subependymal nodules, SEGA represent one of the three major central nervous system features in the diagnostic criteria for TSC (30). Although benign and slow growing, SEGA are potentially lethal and can cause serious neurological

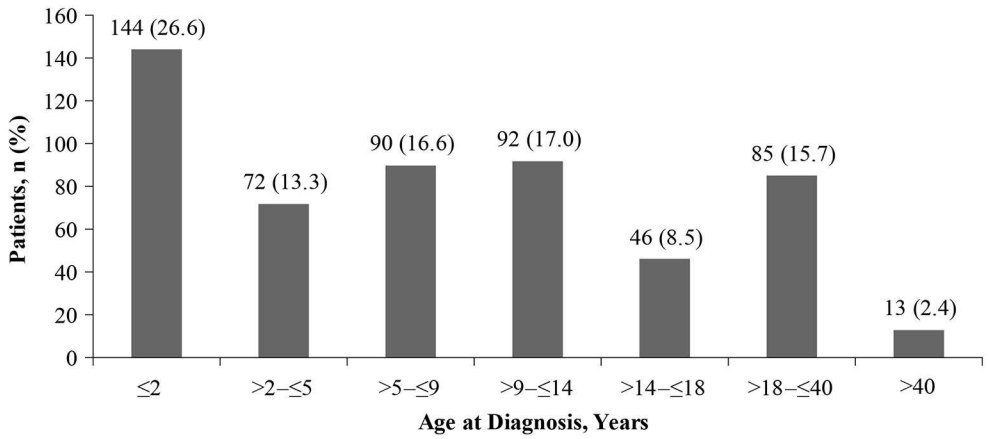

FIGURE 1 | Proportion of patients with SEGAs according to age at SEGA diagnosis ($n = 542$).

TABLE 2 | Clinical characteristics of SEGA at baseline visit in overall population and according to mutation type.

	Overall ($N = 2,216$)	Patients with *TSC2* mutation ($n = 644$)	Patients with *TSC1* mutation ($n = 197$)	*p*-value[c]
Patients with a history of SEGA[a]	554 (25.0)	217 (33.7)	26 (13.2)	<0.0001
Median age at diagnosis, years[b]; median (range)	8 (<1–51)	7.0 (<1–49)	7.0 (<1–51)	0.6167
No. of patients diagnosed with SEGA at <age 2 years[a]	144 (26.6)	67 (31.2)	5 (20.8)	0.3812
No. of patients with SEGA present at the time of visit, *n*[a]	463	185	20	0.2472
Multiple	209 (45.1)	90 (48.6)	8 (40.0)	0.8368
Bilateral	208 (44.9)	84 (45.4)	7 (35.0)	0.9550
Growing SEGA since previous scan	87 (18.8)	35 (18.9)	1 (5.0)	0.3302
Signs and symptoms assigned to SEGA[a]				
None	321 (69.3)	125 (67.6)	11 (55.0)	0.1960
Increase in seizure frequency	73 (15.8)	38 (20.5)	4 (20.0)	1.0000
Behavioural disturbance	55 (11.9)	25 (13.5)	3 (15.0)	0.7311
Regression/loss of cognitive skills	46 (9.9)	20 (10.8)	1 (5.0)	0.6996
Headache	39 (8.4)	15 (8.1)	4 (20.0)	0.0854
Ventriculomegaly	25 (5.4)	9 (4.9)	1 (5.0)	1.0000
Increased intracranial pressure	24 (4.6)	8 (4.3)	3 (15.0)	0.0710
Sleep disorder	14 (3.0)	7 (3.8)	0	1.0000
Eye movement abnormalities	13 (2.8)	6 (3.2)	1 (5.0)	0.5028
Visual impairment	8 (1.7)	4 (2.2)	0	1.0000
Papilledema	8 (1.7)	5 (2.7)	1 (5.0)	0.4498
Neuroendocrine dysfunction	6 (1.3)	3 (1.6)	0	1.0000
Other	14 (3.0)	5 (2.7)	2 (10.0)	0.1313

[a] *Chi-square or Fisher exact test.*
[b] *Median test showing comparison of SEGA characteristics between those with TSC1 mutations and TSC2 mutations.*
[c] *TSC1 vs. TSC2 at baseline.*
SEGA, subependymal giant cell astrocytoma.

complications including raised intracranial pressure due to obstructive hydrocephalus (7). However, to date, studies on the natural history of SEGA and TSC have been sparse, smaller in scale, and typically from a single centre (6). The TOSCA disease registry has collected disease information on the largest cohort of patients with TSC to date.

In the current study, SEGA was reported in 25% of patients with TSC enrolled in the study; of whom, ∼45% had bilateral SEGA. Most studies have reported lower rates of SEGA in patients with TSC ranging from 4 to 20% (2, 7–11). The method used for diagnosis of SEGA in these studies varied substantially. The highest rates reported to date came from a case series of 214 patients with TSC, which reported SEGA in 20% of their patients (2). In this study, SEGA was defined as a subependymal lesion near the foramen of Monro showing contrast enhancement after administration of intravenous gadolinium. No specifications on

TABLE 3 | Clinical characteristics of SEGA at baseline visit according to age categories.

	Age at TOSCA consent, years						
	≤2 (*n* = 283)	>2–≤5 (*n* = 301)	>5–≤9 (*n* = 335)	>9–≤14 (*n* = 307)	>14–≤18 (*n* = 184)	>18–≤40 (*n* = 579)	>40 (*n* = 227)
Patients with a history of SEGA	43 (15.2)	51 (16.9)	98 (29.3)	98 (31.9)	68 (37.0)	167 (28.8)	29 (12.8)
No. of patients with SEGA present at the time of visit, *n*	41 (14.5)	45 (15.0)	82 (24.5)	78 (25.4)	54 (29.3)	139 (24.0)	24 (10.6)
Multiple	14 (4.9)	13 (4.3)	35 (10.4)	31 (10.1)	20 (10.9)	53 (9.2)	6 (2.6)
Bilateral	13 (4.6)	13 (4.3)	33 (9.9)	31 (10.1)	20 (10.9)	51 (8.8)	9 (4.0)
Growing SEGA since previous scan	7 (2.5)	9 (3.0)	19 (5.7)	19 (6.2)	14 (7.6)	19 (3.3)	0
Signs and symptoms							
None	29 (10.2)	37 (12.3)	61 (18.2)	48 (15.6)	29 (15.8)	97 (16.8)	20 (8.8)
Increase in seizure frequency	8 (2.8)	7 (2.3)	10 (3.0)	13 (4.2)	12 (6.5)	22 (3.8)	1 (0.4)
Behavioural disturbance	3 (1.1)	3 (1.0)	13 (3.9)	10 (3.3)	5 (2.7)	20 (3.5)	1 (0.4)
Regression/loss of cognitive skills	5 (1.8)	3 (1.0)	6 (1.8)	8 (2.6)	9 (4.9)	14 (2.4)	1 (0.4)
Headache	0	1 (0.3)	3 (0.9)	8 (2.6)	10 (5.4)	15 (2.6)	2 (0.9)
Ventriculomegaly	3 (1.1)	0	4 (1.2)	7 (2.3)	4 (2.2)	7 (1.2)	0
Increased intracranial pressure	0	1 (0.3)	2 (0.6)	5 (1.6)	6 (3.3)	8 (1.4)	2 (0.9)
Sleep disorder	2 (0.7)	2 (0.7)	0	6 (2.0)	0	4 (0.7)	0
Eye movement abnormalities	1 (0.4)	1 (0.3)	1 (0.3)	3 (1.0)	2 (1.1)	5 (0.9)	0
Visual impairment	0	0	2 (0.6)	1 (0.3)	1 (0.5)	4 (0.7)	0
Papilledema	0	0	1 (0.3)	1 (0.3)	2 (1.1)	3 (0.5)	1 (0.4)
Neuroendocrine dysfunction	0	0	2 (0.6)	0	1 (0.5)	3 (0.5)	0
Other	0	0	2 (0.6)	6 (2.0)	1 (0.5)	5 (0.9)	0

Percentages were calculated using number of patients in each age group as denominator. SEGA, subependymal giant cell astrocytoma.

size or growth were taken into consideration, which is in line with the TOSCA cohort. Most of the patients in TOSCA were enrolled from specialist neurology centres, which might have influenced the number of patients with SEGA included in TOSCA. We also have no data on the number of patients who declined to participate in TOSCA. It cannot be excluded that patients with milder disease were less likely to participate. In addition, patient with milder disease might be less likely to have SEGA, potentially contributing to selection bias.

Published data reported a preponderance of SEGA in children and adolescents (2, 4, 7, 10). In TOSCA, most SEGA were indeed diagnosed in childhood, with a median age at SEGA diagnosis of 8 years. Importantly, 26.6% of patients were diagnosed with SEGA before 2 years of age (**Figure 1**), and growing SEGA were observed in 2.5% of patients aged <2 years (**Table 3**), highlighting the need for early monitoring. The potential occurrence of early SEGA growth has been highlighted previously. The study reported SEGA surgery before the age of 3 years in 9.4% of total 57 children enrolled in the study (31).

Prior reports of SEGA growth after the age of 25 years have been very rare (32). Surprisingly, we identified growing SEGA in 19 patients (2.4%) beyond the age of 18 years. This underlines the need to remain vigilant in adult patients with known SEGA as pointed out in the international recommendations for the surveillance and management of TSC (6, 20). The international consensus panel recommended performing brain imaging every 1–3 years until the age of 25 years. In TOSCA, the median time between scans for SEGA follow-up was 1 year (range, 0–18 years), which is in line with the international recommendations (6, 20). The frequency of scans within the recommended range of every 1–3 years needs to be determined based on clinical grounds, with scans performed more frequently in asymptomatic SEGA patients who are younger, whose SEGA are larger or growing, or who have developmental delays or intellectual disability. Individuals without SEGA by the age of 25 years seem not to need continued imaging (20). For those with SEGA at age 25 years, follow-up MRI intervals may be increased provided the patient remains clinically stable.

New onset of symptoms related to raised intracranial pressure as well as increase in seizure frequency or change in neurological status and behaviour or loss of skills (especially in patients with intellectual disability) should trigger an earlier scan. Similarly, a growing SEGA should prompt a more frequent clinical and radiological follow-up. Parents and patients should be educated regarding relevant symptoms that should prompt referral to medical evaluation (6). The TOSCA data suggest that SEGA-related symptoms (especially early symptoms) are not exclusively limited to signs of increased intracranial pressure.

Previous studies suggested that *TSC2* mutations are associated with a more severe clinical phenotype (8, 19). Findings from TOSCA confirmed that SEGA were present more frequently in patients with mutations in *TSC2* compared to *TSC1*.However, differences in age at onset, SEGA growth or SEGA-related symptoms were not significant. The reason for this observation remains unclear.

In the current study, surgical resection (59.6%) and mTOR inhibitor (49%) were the most common treatment modalities at baseline. Current international recommendations propose the use of surgical resection for acutely symptomatic SEGAs. For growing but asymptomatic SEGA, both surgical resection and mTOR inhibitors are potential treatments. In determining the best option, discussion of the complication risks, adverse effects, cost, length of treatment, family preference, surgical expertise in SEGA, and potential impact on TSC-associated comorbidities should be included in the decision-making process (20, 33). mTOR inhibitors have been shown to be effective in the treatment of other TSC manifestations including epilepsy, renal angiomyolipoma, and lymphangioleiomyomatosis (22–25). Hence, the treatment with mTOR inhibitors may be preferred over surgery in patients with multiple organ involvement or with a combination of mTOR inhibitor-responsive lesions. mTOR inhibitors are also recommended for patients with large or bilateral SEGA that are not amenable to surgical resection (33). SEGA are likely to regrow in case of incomplete resection. This was illustrated in a study of 57 patients with TSC who underwent a total of 64 SEGA surgeries. Gross total resection was performed in 58 cases with no regrowth, while 5 out of 6 children who underwent partial resection showed tumour regrowth within 3–12 months (31). It is also important to consider that long-term mTOR inhibitor treatment may be required, as discontinuation of mTOR inhibitors is typically associated with regrowth of tumours (21).

The median time from SEGA diagnosis to treatment initiation was 319 days. This likely reflects a watch and wait approach to document growth and the need for intervention.

The current study has the following limitations: firstly, the observational nature allowed collection of only those data that were already available from clinical practice and hence reflects "real world" data. Secondly, a major challenge for this registry was to ensure that data about all the disease manifestations for each patient were reported although the sites involved in the registry did not always follow patients for all disease manifestations in the same way. However, the low number of missing data for SEGA (4.7%) reflects good quality of data collection.

CONCLUSION

In summary, the study highlights that the rates of SEGA in patients with TSC might be higher than previously reported. Increase in seizure frequency, behavioural disturbance, regression/loss of cognitive skills were identified as frequent symptoms associated with SEGA, over and above headaches, typically associated with raised intracranial pressure. SEGA may already be present and grow at a very young age. Although SEGA mostly occur in childhood, it is important to be vigilant in adults as well, since SEGA growth does occur also in these age groups.

DATA AVAILABILITY

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each centre: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); Comité Etico Investigación Clínica de Euskadi (CEIC-E); Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; Research Ethics Committee of the University of Tartu (UT REC); Ethikkommission der Medizinischen Universität Graz; North Wales REC—West; Regionala Etikprövningsnämnden i Göteborg; REK—Regionale komiteer for medisinsk og helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie Pomnik Centrum Zdrowia Dziecka; Ethikkommission bei der Ludwig-Maximilians-Universitat München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Deveropment of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Childrens Hospital Of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yan-Sen University; The First Affiliated Hospital Of Guangzhou Medical University; Shenzhen Children's Hospital; West China Hospital, Sichuan University; Xijing Hospital; Children's Hospital of Chongqing Medical University; Wuhan Children's Hospital; The second affiliated hospital of Xi'an jiaotong university; Guangdong 999 brain hospital; Seoul National University Hospital Institutional Review Board; National Taiwan University Hospital (NTUH) Research Ethics Committee (REC); Institutional Review Board of the Taichung Veterans General Hospital; Institutional Review Board of Chung Shan Medical University Hospital; Institutional Review Board, Tungs' Taichung MetroHarbor Hospital; Institutional Review Board of National Cheng Kung University Hospital; Metro South Human Research Ethics Committee; Sydney Children's Hospital Network Human Research Ethics Committee; St. Vincents Hospital Human Research Ethics

Committee; Royal Melbourne Hospital Human Research Ethics Committee; Siriraj Institutional Review Board; The Institutional Review board, Faculty of Medicine, Chulalongkorn University, Third Floor, Ananthamahidol Building, King Chulalongkorn Memorial Hospital; The committee on Human Rights Related to Research Involving Human Subjects; Institutional Review board, Royal Thai Army Medical Department IRB RTA, Fifth Floor, Phramongkutklaowejvitya Building, Phramongkutklao College of Medicine; Research Ethics Committee, Faculty of Medicine, Chiang Mai University; Research and Development, Queen Sirikit National Institute of Child Health; Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town; Shaare Zedek Meidcla center Helsinki comittee; Sheba Medical center Helsinki comittee; Tel Aviv Sourasly Medical center Helsinki comittee; General University Hospital of Patras Ethics Committee; Pendeli Children's Hospital Ethics Committee; General University Hospital of Athens 'G. Gennimatas Ethics Committee; Evaggelismos General Hospital Ethics Committee; General University Hospital of Thessaloniki AHEPA Ethics Committee; General University Hospital of Ionnina Ethics Committee; METC UMC Utrecht; Direcció General de Regulació, Planificació i Recursos Sanitaris; Comité Ético de Investigación Clínica del Hospital Universitario Vall d'Hebron de Barcelona, Generalitat de Catalunya.Departament de Salut; Comité Ético de Investigación Clínica Hospital Universitario La Paz; Dirección General de Ordenación e Inspección, Consejería de Sanidad Comunidad de Madrid, Servicios de Control Farmacéutico y Productos Sanitarios; Comité Etico Investigación Clínica del Hospital Universitario y Politécnico de La Fe; Dirección General de Farmàcia i Productes Sanitaris, Generalitat de Valencia; Comité de Ética de la Investigación de Centro de Granada; Instituto Aragonés de Ciencias de la Salud (IACS); Comité Etico Investigación Clínica Regional del Principado de Asturias; Comité Etico Investigación Clínica Hospital 12 de Octubre; Comité Etico Investigación Clínica Hospital Universitario Virgen de la Arrixaca; Sección de Ordenación e Inspección Farmacéutica Departamento de Salud; Comité Ético de Investigación Clínica del Hospital Universitario del Río Hortega de Valladolid; Comissão de Ética para a Saúde (CES), Centro Hospitalar de Lisboa Ocidental, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar do Porto, E.P.E; Comissão de Ética para a Saúde (CES), Centro Hospitalar Lisboa Central, EPE; Comissão de Ética para a Saúde (CES), Hospital Garcia de Orta, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar de São João, EPE; Comissão de Ética para a Saúde (CES), Hospital Professor Doutor Fernando Fonseca, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar do Algarve, EPE (Unidade de Faro); LUHS Kaunas Regional Biomedical Research Ethics Committee; Paula Stradiņa klīniskās universitātes slimnīcas, Attīstības biedrības Klīniskās izpētes Ētikas komiteja, Ethics Committee for Clinical Research; Komisija Republike Slovenije za medicinsko etiko; Comitato Etico Indipendente Presso La Fondazione Ptv Policlinico Tor Vergata Di Roma; Comitato Etico Regione Calabria Sezione Centro c/o A.O.U. Mater Domini Di Catanzaro; Comitato Etico Azienda Ospedaliera Universitaria Di Cagliari; Comitato Etico Cardarelli-Santobono c/o Ao Cardarelli; Comitato Etico Per La Sperimentazione Clinica Delle Province Di Verona E Rovigo, Presso Aoui Verona; Eticka Komise Fn Brno; Eticka Komisia Dfnsp Bratislava; Eticka Komisia Pri Dfn Kosice; Eticka Komisia Bratislavskeho Samospravneho Kraja; Comisia Națională de Bioetică a Medicamentului și a Dispozitivelor Medicale; Comitato Etico Milano area 1 c/o ASST FBF Sacco—P.O. L. Sacco; Comité de Ética de la Investigación de Centro Hospital Universitario Virgen del Rocío; Comité Ético de Investigación Clínica Fundació Sant Joan de Déu Generalitat de Catalunya. Departament de Salut; Comité Ético de Investigación Clínica Hospital Infantil Universitario Niño Jesús; Consejería de Sanidad Dirección General de Salus Pública Junta de Castilla León; Dirección General de Asistencia Sanitaria, Consejería de Sanidad Gobierno del Principado de Asturias; Dirección General de Planificación, Ordenación Sanitaria y Farmacéutica e Investigación, Consejeria de Sanidad y Política Social Región de Murcia; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Paula Stradiņa klīniskās universitātes slimnīcas, Attīstības biedrības Klīniskās izpētes Ētikas komiteja, Ethics Committee for Clinical Research; The First Affiliated Hospital of The Fourth Military Medical University; Zhongshan Hospital Fudan University.

AUTHOR CONTRIBUTIONS

AJ, EB, MB, PC, MD, JF, MF, CH, SJ, JL, AM, RN, VS, MS, RT, BZ, and JK: designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. TC, VC, GBdA, PdV, CF, FO, JQ, YT, and SY: designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. LD: designing the study, trial management, data collection, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. RM: designing the study, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. SS: designing the study, trial statistician, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript.

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FULL-LENGTH ORIGINAL RESEARCH

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Epilepsy in tuberous sclerosis complex: Findings from the TOSCA Study

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Summary

Objective: To present the baseline data of the international TuberOus SClerosis registry to increase disease Awareness (TOSCA) with emphasis on the characteristics of epilepsies associated with tuberous sclerosis complex (TSC).

Methods: Retrospective and prospective patients' data on all aspects of TSC were collected from multiple countries worldwide. Epilepsy variables included seizure type, age at onset, type of treatment, and treatment outcomes and association with genotype, seizures control, and intellectual disability. As for noninterventional registries, the study protocol did not specify any particular clinical instruments, laboratory investigations, or intervention. Evaluations included those required for diagnosis and management following local best practice.

Results: Epilepsy was reported in 83.6% of patients (1852/2216) at baseline; 38.9% presented with infantile spasms and 67.5% with focal seizures. The mean age at diagnosis of infantile spasms was 0.4 year (median <1 year; range <1-30 years) and at diagnosis of focal seizures was 2.7 years (median 1 year; range <1-66 years). A total of 1469 patients (79.3%) were diagnosed with epilepsy <2 years. The rate of infantile spasms was higher in patients with a *TSC2* mutation than in patients with a *TSC1* mutation (47.3% vs 23%). γ-aminobutyric acid (GABA)ergic drugs were the most common treatment modality for both infantile spasms (78.7%) and focal seizures (65.5%). Infantile spasms and focal seizures were controlled in 76.3% and 58.2% of patients, respectively. Control of seizures was associated with lower rates of intellectual disability in both groups.

Significance: This registry reports the largest international cohort of patients with TSC. Findings confirmed the typical onset pattern of infantile spasms and other focal seizures in the first 2 years of life, and the high rates of infantile spasms in patients with *TSC2* mutation. Our results underscored the occurrence of focal seizures at all ages, including an onset that preceded emergence of infantile spasms. Seizure control was shown to be associated with lower rates of intellectual disability but did not preclude the presence of intellectual disability.

KEYWORDS
epilepsy, registry, TOSCA, tuberous sclerosis complex

1 | INTRODUCTION

Epilepsy is one of the most common neurologic symptoms in patients with tuberous sclerosis complex (TSC), with reported prevalence from 62% to 93%.[1–3] It is also a significant cause of morbidity and mortality in patients with TSC.[2,4] Epilepsy usually begins during the first months of life and in the majority before the first year.[5] Early onset epilepsy often presents as focal seizures initially and can precede, coexist with, or evolve into infantile spasms.[5,6] However, patients with TSC can present with almost all seizure types such as tonic, atonic, or tonic-clonic seizures.[6] There are several therapeutic options available for the treatment of focal seizures and infantile spasms associated with TSC including antiepileptic drugs (AEDs), hormonal therapy, epilepsy surgery, ketogenic diet, and vagus nerve stimulation. However, about two-thirds of patients develop treatment refractory epilepsies, associated with increased rates of intellectual disability and other TSC-associated neuropsychiatric disorders (TAND).[6,7]

The natural history of epilepsy in TSC has been evaluated in only a handful of studies.[1,2,8,9] Most of these studies were retrospective in nature, reported a single-center cohort, and had relatively small sample size. Only one large cohort was reported by Jeong et al, who evaluated the natural history of epilepsy in a cohort of patients with TSC enrolled from the United States and Belgium (n = 1816; 81.8% had history of focal seizures and 49.2% had infantile spasms).[2] The TuberOus SClerosis registry to increase disease Awareness (TOSCA) is an international study that enrolled patients from 170 centers across 31 countries worldwide. The baseline core data of TOSCA provided understanding of the overall TSC manifestations. The study showed epilepsy in 83.5% of patients, subependymal giant cell astrocytoma in 24.4%, and renal angiomyolipomas in 47.2%.[10] Herein we report baseline data from TOSCA with the aim of describing the characteristics of epilepsy among this large cohort of patients with TSC.

Key Points

- Epilepsy was reported in 1852 patients (83.6%) at baseline; of these, there were focal seizures in 67.5% and infantile spasms in 38.9% patients
- Epilepsy was diagnosed before 2 years of age in approximately 79% of patients
- The rate of infantile spasms was higher in patients with *TSC2* mutation than in patients with *TSC1* mutation

2 | METHODS

The methods of the TOSCA study have been described in detail previously.[11] In short, TOSCA is a multicenter, international disease study designed to collect data, retrospectively and prospectively, on patients with TSC from several countries worldwide. Sites with specialists in managing one or more aspects of TSC (in children and adults) were included in the registry. Centers were dedicated mainly to epilepsy care but with almost 30% of patients (mainly adult) enrolled from other specialties as well.

The registry consists of a "core" section and 6 subsections ("petals"). In the "core" section, general information on patient background such as demographic data, family history, genotype, vital signs, prenatal history, clinical features of TSC across all organ systems, comorbidities, and rare manifestations were reported. Data were collected retrospectively and prospectively at baseline (first inclusion visit) and interim analysis was performed on this data collection. A prospective follow-up observation period was up to 5 years, with regular visits scheduled at a minimum interval of 1 year to ensure an ongoing data stream. These follow-up data were not included in this article. Subsections ("petals") represent specific research projects to record in-depth data related to specific disease manifestations. Given that this is an international noninterventional study, evaluations included were those required for disease diagnosis and management according to the local best practice. The study protocol, therefore, did not specify any particular clinical instruments or laboratory investigations.

Patients of any age who fulfilled clinical criteria for TSC diagnosis were eligible if they had at least 1 documented visit for TSC within the previous 12 months or were newly diagnosed with TSC before participating in the registry. Variables were obtained on the basis of the most recent data collected during the last visit and included seizure type (focal seizures, infantile spasms, other seizures), age at onset of epilepsy, type of epilepsy treatment (grouped as γ-aminobutyric acid (GABA) ergics, hormonal therapy, ketogenic diet, fructose derivatives, vagus nerve stimulator, mammalian target of rapamycin (mTOR) inhibitors, surgery, other modalities) and treatment outcome (eg, epilepsy resolved spontaneously, was controlled with treatment, or was not controlled with treatment). In addition, we compared the characteristics of epilepsy between the overall epilepsy cohort and those with epilepsy diagnosed before 2 years of age (early onset seizure group). The association between seizure type and genotype as well as between seizure control and intellectual ability was evaluated. Intellectual ability was evaluated by clinician or by formal neuropsychological test and categorized as normal (IQ > 70), mild intellectual disability (ID) (IQ 51–70), moderate ID (IQ 36–50), severe ID (IQ 20-35), and profound ID (IQ < 20).

All eligible patients enrolled in the TOSCA study were considered in the analysis. Continuous variables were

analyzed in terms of value (number of patients, mean, standard deviation, median, minimum and maximum), whereas categorical variables (eg, presence/absence of a condition or manifestation) were analyzed in terms of frequency distribution at baseline. Missing data were not imputed.

This study was designed, implemented, and reported in accordance with the Good Clinical Practice and the ethical principles specified in the Declaration of Helsinki.[12] The protocol was approved by the local ethics committee at each center before patient enrollment.

3 | RESULTS

3.1 | Demographics and clinical characteristics

As of September 30, 2015 (data cutoff date for the third interim analysis), 2216 eligible patients from 170 sites across 31 countries worldwide were enrolled in the TOSCA study (Table 1). The third interim analysis included baseline data for all the patients enrolled in the study.

Baseline patient demographics and clinical characteristics are summarized in Table 2. Baseline data were available for 1154 female (52.1%) and 1,062 male (47.9%) patients; 806 patients (36.4%) were adult (>18 years) and 1410 (63.6%) were children or adolescents. The median age at consent was 13 years (range < 1-71). The mean age at TSC diagnosis was 7.0 years (median age 1 year; range < 1-69). Molecular testing for genetic mutations was performed for 1000 patients who met clinical criteria for TSC (45.1%). Of these, 638 patients (63.8%) had a *TSC2* mutation, 191 patients (19.1%) had a *TSC1* mutation, and 6 patients (0.6%) had both *TSC1* and *TSC2* mutations. No further molecular details were requested for this study. Of patients who had genetic molecular testing performed, no TSC mutation was identified in 144 patients (14.4%), whereas test results were not available for 9 patients (0.9%). Prenatal diagnosis of TSC was reported in 144 patients (6.5%) and 500 patients (22.6%) had relatives affected with TSC.

TABLE 1 Patients enrolled from different countries in TOSCA (N = 2216)

Countries	Number of patients, n (%)
Europe	
France	228 (10.3)
The Netherlands	224 (10.1)
Germany	162 (7.3)
Spain	119 (5.4)
Belgium	110 (5.0)
Italy	97 (4.4)
Portugal	54 (2.4)
Austria	52 (2.3)
Poland	52 (2.3)
United Kingdom	32 (1.4)
Greece	30 (1.4)
Slovakia	26 (1.2)
Norway	24 (1.1)
Sweden	23 (1.0)
Romania	21 (0.9)
Latvia	18 (0.8)
Estonia	12 (0.5)
Lithuania	11 (0.5)
Slovenia	8 (0.4)
Czech Republic	7 (0.3)
Denmark	4 (0.2)
Outside Europe	
China	252 (11.4)
Taiwan	140 (6.3)
Australia	101 (4.6)
Japan	98 (4.4)
Turkey	91 (4.1)
Russia	60 (2.7)
Israel	59 (2.7)
Thailand	50 (2.3)
South Africa	31 (1.4)
Korea	20 (0.9)

3.2 | Characterization of epilepsy

Epilepsy was reported at the baseline visit in 1852 patients (83.6%) (overall epilepsy cohort). In this overall epilepsy cohort, a history of focal seizures was reported in 1250 patients (67.5%) and infantile spasms in 720 patients (38.9%). The co-occurrence of focal seizures and epileptic spasms was reported in 380 patients (20.5%; Table 3). Of these, epileptic spasms occurred before focal seizures in 242 patients (13.1%) and focal seizures occurred first in 63 patients (3.4%). In 75 patients (4%), focal seizures and epileptic spasms were reported as starting concomitantly. The mean age at diagnosis of focal seizures was 2.7 years (median age 1 year; range < 1-66 years), whereas mean age at diagnosis of spasms was 0.4 years (median age < 1 year; range < 1-30 years). In 691 patients (95.6%), infantile spasms were reported within the typical age range (before 2 years). In 22 patients (3%), it occurred between 2 and 5 years and in 10 patients (1.4%) at an older age. The differences in the occurrence rate and age at diagnosis of focal seizures and epileptic spasms (alone or in combination with other types) among patients with *TSC1* mutation and *TSC2* mutation are shown in Table 4. Infantile spasms were more frequent in patients with a *TSC2* mutation compared to those with a *TSC1* mutation (47.3% vs 23%).

TABLE 2 Baseline patient demographics and clinical characteristics (N = 2216)

Characteristics	Baseline data
Age at diagnosis of TSC[a], years, median (range)	1 (<1-69)
Gender, n (%)	
Male	1062 (47.9)
Female	1154 (52.1)
Patients with molecular testing, n (%)	1000 (45.1%)
Genetic testing, n (%)[b]	
No mutation identified	144 (14.4)
TSC1 mutation[c]	197 (19.7)
TSC2 mutation[c]	644 (64.4)
Variation type, n (%)[d]	
Pathogenic mutation	678 (67.8)
Variant of unknown significance	66 (6.6)
Time from first TSC clinical diagnosis to first molecular testing, months	
Mean (SD)	80.8 (116.5)
Median (range)	23 (<1-721)
Patients with prenatal diagnosis, n (%)	144 (6.5)
Biologic mother/father evaluated for TSC, n	
Mother	936
Father	820
TSC inherited from one parent, n	
Total	51
Mother	30
Father	21
Patients with affected relatives, n (%)[e]	
Total	500 (22.6)
1	275 (12.4)
2	138 (6.2)
3	50 (2.3)
>3	54 (2.4)
Patients with at least one blood relative participating in TOSCA, n (%)	230 (10.4)

SD, standard deviation; TSC, tuberous sclerosis complex; TOSCA, TuberOus SClerosis registry to increase disease Awareness.
[a]Data available for 2179 patients.
[b]Information on the type of mutation was missing for 9 patients.
[c]The count (n) includes 6 patients who had both *TSC1* and *TSC2* mutations.
[d]The count (n) includes 23 patients who had both variation types.
[e]Patients switching from one category to the other during the study visits were counted in each category.

3.3 | Treatment

At baseline, a total of 1226 patients (98.1%) with focal seizures in the overall epilepsy cohort received treatment. The majority received GABAergics as a single agent or in combination with other treatment modalities (803, 65.5%). Additional treatment modalities included mTOR inhibitors (95, 7.7%), surgery (85, 6.9%), ketogenic diet (58, 4.7%), vagus nerve stimulation (47, 3.8%), fructose derivatives (43, 3.5%), and corticotropin (ACTH; 35, 2.9%). Focal seizures were controlled by treatment in 713 patients (58.2%), resolved spontaneously in 9 (0.7%), and were not controlled in 466 patients (38%). Outcome data were not available for 38 patients (3.1%).

A total of 696 patients (96.7%) in the overall epilepsy cohort received treatment for infantile spasms. The most frequent treatment modalities (as single agents or in combination with other treatment modalities) included GABAergics (548, 78.7%) and ACTH (122, 17.5%). Additional treatment modalities included mTOR inhibitors (38, 5.5%), surgery (29, 4.2%), ketogenic diet (27, 3.9%), vagus nerve stimulator (15, 2.2%), and fructose derivatives (9, 1.3%). Infantile spasms were controlled with treatment in 530 (76.3%), resolved spontaneously in 23 (3.3%), and were not controlled in 108 patients (15.5%). Outcome data were not available for 34 patients (4.9%). The type of treatment and overall outcome of focal seizures and infantile spasms in the patients diagnosed with epilepsy before the age of 2 years (early onset seizure group) were similar to those in the overall epilepsy cohort (Table 3). Type of treatment and overall outcome of focal seizures and infantile spasms in relation to mutation type are shown in Table 4.

3.4 | Association between seizure control and intellectual ability

In the overall epilepsy cohort, a total of 563 of 1250 patients with focal seizures reported at baseline (45%) had received an evaluation of their intellectual abilities assessed by the clinician or by standardized tests depending on the local best practice. Of these, 229 patients (40.7%) had normal intellectual ability, whereas the degree of intellectual disability was recorded as mild in 178 (31.6%), moderate in 58 (10.3%), severe in 86 (15.3%), and profound in 12 patients (2.1%). Of 720 patients in the overall epilepsy cohort with a history of infantile spasms, 279 patients (38.8%) had been evaluated by formal tests for IQ. Of these, 61 patients (21.9%) had normal intellectual ability, whereas mild, moderate, severe, and profound degrees of intellectual disability were observed in 82 (29.4%), 73 (26.2%), 46 (16.5%) and 17 patients (6.1%), respectively. The proportion of patients with normal intellectual ability was higher in patients controlled with treatment than in those not controlled with treatment (23.5% vs 7.9%, patients with infantile spasms, and 47.5% vs 26.8%, patients with focal seizures; Figure 1).

In the early onset seizure group, 459 of 984 patients (46.6%) diagnosed with focal seizures had been evaluated for IQ. Of these, 158 patients (34.4%) had normal intellectual ability, whereas mild, moderate, severe, and profound degrees

TABLE 3 Type of epilepsy and treatment outcomes in overall epilepsy cohort and in patients diagnosed at <2 years at baseline

Characteristics	Overall epilepsy cohort (N = 1852), n (%)	Early onset seizure group, (N = 1461), n (%)
Epilepsy type		
Focal seizures[a]	1250 (67.5)	984 (67.4)
Infantile spasms[a]	720 (38.9)	684 (46.8)
Focal seizures only	765 (41.3)	530 (36.3)
Infantile spasms only	246 (13.3)	221 (15.1)
Co-occurrence of infantile spasms and focal seizures	380 (20.5)	375 (25.7)
Treatment[b] for infantile spasm		
No. of patients who received treatment	696 (96.7)	663 (96.9)
Type of treatment		
GABAergics	548 (78.7)	527 (79.5)
ACTH	122 (17.5)	121 (18.3)
mTOR inhibitors	38 (5.5)	-
Surgery	29 (4.2)	-
Ketogenic diet	27 (3.9)	-
Vagus nerve stimulator	15 (2.2)	-
Fructose derivatives	9 (1.3)	-
Treatment outcomes for infantile spasm		
Resolved spontaneously	23 (3.3)	34 (5.0)
Controlled with treatment	530 (76.3)	506 (74.5)
Not controlled with treatment	108 (15.5)	106 (15.6)
Unknown	34 (4 9)	33 (4.9)
Treatment[b] for focal seizures		
No. of patients who received treatment	1226 (98.1)	969 (98.5)
Type of treatment		
GABAergics	803 (65.5)	683 (70.5)
mTOR inhibitors	95 (7.7)	-
Surgery	85 (6.9)	-
Ketogenic diet	58 (4.7)	-
Vagus nerve stimulator	47 (3.8)	-
Fructose derivatives	43 (3.5)	-
ACTH	35 (2.9)	32 (3.3)
Treatment outcomes for focal seizures		
Resolved spontaneously	9 (0.7)	10 (1.0)
Controlled with treatment	713 (58.2)	552 (56.6)
Not controlled with treatment	466 (38.0)	384 (39.3)
Unknown	38 (3.1)	30 (3.1)

ACTH, adrenocorticotropic hormone; mTOR, mammalian target of rapamycin.
[a]Alone or with other seizures.
[b]As single therapy and in combination with other modalities.

of intellectual disability were observed in 151 (32.9%), 81 (17.6%), 57 (12.4%), and 12 (2.6%) patients, respectively. Among the 684 patients diagnosed with infantile spasms in the early onset seizure group, 273 (39.9%) were evaluated for IQ. A total of 59 patients (21.6%) had normal intellectual ability, whereas mild, moderate, severe, and profound degrees of intellectual disability was observed in 82 (30%), 71 (26%), 44 (16.1%), and 17 (6.2%) patients, respectively. Similar to the overall epilepsy cohort, the proportion of patients with normal intellectual ability was higher in the early onset seizure group that had been controlled than the group with uncontrolled focal seizure (41.2% vs 21.4%) and infantile spasms (23.3% vs 7.9%) after treatment.

4 | DISCUSSION

The TOSCA study, which represents the largest cohort of patients with TSC described to date, enrolled patients from 31 countries and from various specialty clinics. The third interim analysis results showed an occurrence at baseline of epilepsy in about 83.6% of the of 2216 enrolled patients, which was in line with both the baseline "core" data of the TOSCA registry[10] and other previous reports,[1,8,13] confirming epilepsy to be the most common clinical presentation of TSC.

A database of 1816 individuals with TSC showed that focal seizures were present in 81.8% of the patients and infantile spasms in 49.2% of the patients included.[2] In agreement with this report, focal seizures were also the most common seizure observed in our cohort, followed by infantile spasms. A history of infantile spasms was reported in about 38.9%

TABLE 4 Characteristics of epilepsy according to mutation type

Characteristics	Overall epilepsy cohort with molecular testing		Early onset seizure group with molecular testing	
	TSC1 mutation (N = 152), n (%)	*TSC2* mutation (N = 569), n (%)	*TSC1* mutation (N = 98), n (%)	*TSC2* mutation (N = 489), n (%)
Epilepsy type				
Focal seizures[a]	113 (74.3)	409 (71.9)	75 (76.5)	350 (71.6)
Infantile spasms[a]	35 (23)	269 (47.3)	34 (34.7)	260 (53.2)
Infantile spasms only	12 (7.9)	67 (11.8)	11 (11.2)	61 (12.5)
Focal seizures only	88 (57.9)	220 (38.7)	52 (53.1)	168 (34.4)
Concomitant infantile spasms and focal seizures	21 (13.8)	163 (28.6)	21 (21.4)	161 (32.9)
Age at diagnosis, years				
Focal seizures				
Mean	3.7	2.2	1.1	0.9
Median	2.0	<1	1	<1
Range	<1-47	<1-59	<1-14	<1-16
Infantile Spasms				
Mean	0.3	0.3	0.3	0.2
Median	<1	<1	<1	<1
Range	<1-6	<1-5	<1-6	<1-4
Treatment[b] for infantile spasm				
No. of patients who received treatment	33 (94.3)	264 (98.1)	32 (94.1)	256 (98.5)
Type of treatment				
GABAergics	22 (66.7)	223 (84.5)	22 (68.8)	216 (84.4)
ACTH	4 (12.1)	43 (16.3)	4 (12.5)	40 (15.6)
Surgery	2 (6.1)	15 (5.7)	-	-
mTOR inhibitors	2 (6.1)	16 (6.1)	-	-
Vagus nerve stimulator	1 (3)	9 (3.4)	-	-
Fructose derivatives	0	2 (0.8)	-	-
Ketogenic diet	0	14 (5.3)	-	-
Treatment outcomes for infantile spasm				
Resolved spontaneously	1 (3)	6 (2.3)	3 (8.8)	9 (3.5)
Controlled with treatment	21 (63.6)	206 (78)	20 (58.8)	199 (76.8)
Not controlled with treatment	10 (30.3)	40 (15.2)	10 (29.4)	40 (15.4)
Unknown	1 (3)	12 (4.5)	1 (2.9)	11 (4.2)
Treatment[b] for focal seizures				
No. of patients who received treatment	113 (100)	402 (98.3)	75 (100)	348 (99.4)
Type of treatment				
GABAergics	67 (59.3)	316 (78.6)	51(68)	280 (80.5)
ACTH	2 (1.8)	7 (1.7)	2 (2.7)	7 (2.0)
Surgery	8 (7.1)	37 (9.2)	-	-
mTOR inhibitors	6 (5.3)	34 (8.5)	-	-

(Continues)

TABLE 4 (Continued)

Characteristics	Overall epilepsy cohort with molecular testing		Early onset seizure group with molecular testing	
	TSC1 mutation (N = 152), n (%)	*TSC2* mutation (N = 569), n (%)	*TSC1* mutation (N = 98), n (%)	*TSC2* mutation (N = 489), n (%)
Vagus nerve stimulator	4 (3.5)	16 (4)	-	-
Fructose derivatives	6 (5.3)	14 (3.5)	-	-
Ketogenic diet	2 (1.8)	33 (8.2)	-	-
Treatment outcomes for focal seizures				
Resolved spontaneously	0	3 (0.7)	0	4 (1.1)
Controlled with treatment	67(59.3)	229 (57.0)	44 (58.7)	195 (55.9)
Not controlled with treatment	42 (37.2)	163 (40.5)	30 (40)	145 (41.5)
Unknown	4 (3.5)	7 (1.7)	1 (1.3)	5 (1.4)

ACTH, adrenocorticotropic hormone; mTOR, mammalian target of rapamycin.
[a]Alone or with other seizures.
[b]As single therapy and in combination.

patients with TSC in the TOSCA study, which was similar to that observed in a single center clinical case series of 291 patients by Chu-Shore et al[1], which reported infantile spasms in approximately 37% of patients with TSC. However, Jeong et al.[2] reported a slightly higher rate of infantile spasms (about 49.2%). This could be because patients with TSC in the series of Jeong et al were self-enrolled by families that might have biased recruitment to more severe cases.

It has been reported that epilepsies associated with TSC most often have their onset during infancy or early childhood, although they may occur at any age, with focal seizures and infantile spasms being the most common seizure types.[5,14] In our study, epilepsy was diagnosed before 2 years of age in about 79% of patients, showing this early onset in the majority of patients. However, seizure onset occurred later in 27% of the cohort, and even in patients older than 40 years of age (about 7 patients [0.6%] diagnosed with focal seizures at age >40 years). This emphasizes that patients with TSC remain at increased risk of epilepsy (mainly focal epilepsies) throughout their lifetime. The occurrence of infantile spasms was higher in the first 2 years (46.8% vs 38.9%), but the occurrence rate of focal seizures was similar (67.4% vs 67.5%) between the whole cohort and the early onset seizure group, respectively. Late-onset epileptic spasms occurred in 2%-6% of patients.[15] This late occurrence of epileptic spasms was reported in structural epilepsies without specifying TSC.[15,16] Our data suggest that TSC can be considered as a cause of late-onset epileptic spasms. These findings mainly highlight that infantile spasms and focal seizures occur at an early age in the majority of patients with TSC. Infantile spasms can precede, co-occur, or follow focal seizures, with this co-occurrence being a characteristic of TSC.[17]

This emphasizes the importance of parental education and the potential role of serial electroencephalography (EEG) recordings to detect possible subclinical seizures. This has been suggested in patients with antenatal diagnosis of TSC or patients diagnosed with TSC before the onset of clinical seizures The question on the usefulness to initiate the treatment for subclinical seizures (seizures on EEG without clinical manifestations), or even paroxysmal EEG activity without subclinical seizures, is still under debate.[18,19] Two projects (EPISTOP[20] and PREVENT[21]) are ongoing with the aim of evaluating the impact of an early presymptomatic treatment to be administered at identification of EEG abnormalities without any clinical seizures reported or recorded in patients with TSC.

With respect to genotype, infantile spasms were more frequently seen in patients with a *TSC2* mutation compared to those with a *TSC1* mutation (47.3% vs 23%). Furthermore, patients with a *TSC2* mutation had an earlier onset of infantile spasms and focal seizures (Table 4). These findings reinforced the observations seen in previous studies evaluating the genotype-phenotype relationships in patients with TSC,[1,22]and could suggest a better efficacy of earlier treatments.

GABAergic drugs were the most frequent therapy used in patients with focal seizures and patients with infantile spasms. The term GABAergic drugs was used for vigabatrin and did not include other GABAergic drugs. This finding is in line with the current recommendations that vigabatrin should be used as a first-line AED treatment for infantile spasms with TSC and for focal seizures occurring before the age of 1 year.[5,23] A better outcome with vigabatrin initiation in association with hormonal therapy was reported in a cohort of patients with infantile spasms.[24] However, patients with TSC were excluded from this study and the potential additional benefit from this inaugural bi-therapy might need additional investigation. The number of patients resistant to

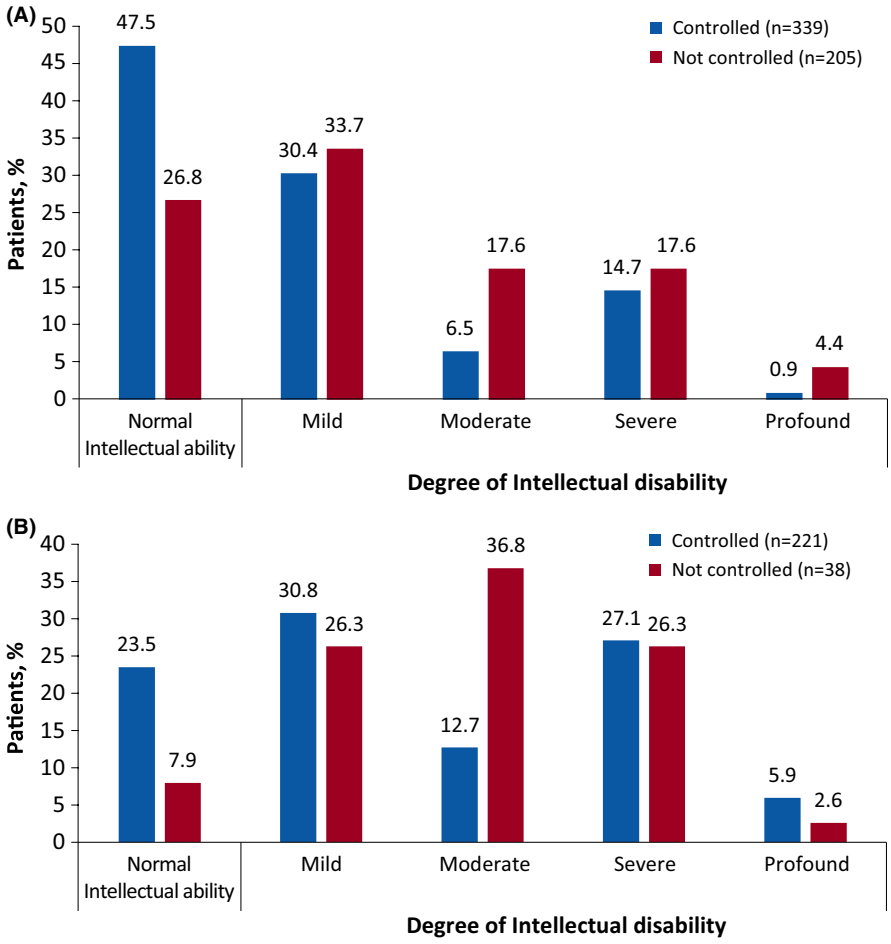

FIGURE 1 Effect of seizure control on intellectual ability. A, Patients with focal seizures. B, Patients with infantile spasms

treatment was higher for focal seizure (38%) than for infantile spasms (15.5%) and was independent of the mutation type. This emphasizes the better control of infantile spasms with a less efficacy on focal seizures control as they might persist or newly occur after the control of spasms. This finding is important for the development of new therapies for epilepsy in TSC, especially for focal seizures that were not controlled as well by available treatments. The recent EXIST 3 study showing higher efficacy of adjunctive everolimus therapy in patients with treatment-refractory seizures associated with TSC compared to placebo, is promising in this regard.[25]

The number of patients not controlled on treatment at baseline was much lower in our study (infantile spasms, 15.5%; focal seizures; 38%) than the 62.5% reported in a previous study by Chu-Shore et al.[1] This lower number of treatment-resistant patients could be explained by the earlier diagnosis age shown by the high number of young patients enrolled in our study and by the fact that patients were diagnosed and managed in specialized reference centers that follow established recommended guidelines.[23] There has been substantial progress in understanding of diagnosis and treatment of epilepsy in patients with TSC during the last decade. The recommendations of serial EEG studies before clinical seizures have allowed the detection of some patients with subtle or infraclinical seizures. In addition, current recommendation on the use of vigabatrin for both infantile spasms and focal seizures for infants might have contributed to this lower rate of pharmacoresistance.[5,23]

Studies exploring the efficacy of the ketogenic diet and vagus nerve stimulation in patients with TSC-associated epilepsy showed that these nonpharmacologic therapies were effective in reducing seizure frequency.[26–29] Surprisingly, the ketogenic diet and vagus nerve stimulation were not commonly used in this cohort with a large proportion of patients with pharmacoresistant epilepsy. This cannot be totally due to lack of availability of such therapies, as many of the TOSCA sites were tertiary centers for TSC and epilepsy treatment. Some explanation can emerge explaining this lower use, as vagus nerve stimulation devices create difficulties for routine use of magnetic resonance imaging (MRI) of the brain or kidney, and the ketogenic diet is more difficult to achieve in patients with psychiatric and behavior disorders that are frequent in the patients with TSC presenting epilepsy. In addition, many of the severely delayed patients can be in specialized institutions where the ketogenic diet is not available. The number of patients that underwent epilepsy surgery was also relatively low in this cohort. Epilepsy surgery for patients with TSC needs specific expertise, as there is often more than a single tuber focus and surgery may need invasive monitoring of seizures to determine

the resection area.[30,31] This might be the main limiting factor for epilepsy surgery but should be a reminder to refer patients with pharmacoresistant epilepsy early on to expert epilepsy surgery centers to define the possibility of such therapy that is showing fair results, even in patients with multiple foci.[32]

The correlation between intellectual ability and seizure control in TSC has been reported.[33–37] Patients with uncontrolled seizures had a higher rate of intellectual disability compared to those with controlled seizures. This association is known primarily for infantile spasms,[33–37]but our cohort showed that pharmacoresistant focal seizures equally impact intellectual development in patients with TSC. Similar to these findings, the present study showed that a smaller proportion of patients who were controlled with treatment had intellectual disability compared to those uncontrolled in both groups of infantile spasms and focal seizures.

We acknowledge selection bias as one of the limitations of our study, given that recruitment was achieved through clinical centers with expertise in TSC. Milder cases, or those without seizure disorders in childhood, may not have been included. This study was recruited mainly from pediatric and adult centers for TSC dedicated to epilepsy care, but with almost 30% of patients (mainly adult) enrolled from other specialties as well. Despite the involvement of several specialty clinics, the rate of epilepsy observed in our study was very high (>80%). Furthermore, due to the observational nature of the registry, only data collected from routine clinical practice were reported. This may in part explain the incomplete genetic and neuropsychological scores data, given the widespread differences in access to clinical evaluation of patients with TSC across the globe even in specialized centers, and the lack of access to neuropsychological evaluation. Nevertheless, participation of a large number of centers (170 sites in 31 countries) with complementary expertise has helped in the inclusion of a significant number of patients with TSC, which is likely to be representative of tertiary hospital clinical practice. The lower number of unknown data for epilepsy in this registry with a very large cohort reflects good quality data collection. Moreover, medical data reported in the registry were collected directly by the patients' physician and not provided by patients and families, ensuring a high level of medical accuracy. Finally, therapies were recorded in groups of AEDs, as the purpose of this registry was to increase disease awareness and to report epilepsy characteristics, and not to evaluate the efficacy of specific therapies.

In conclusion, TOSCA provides valuable insights into the characteristics of epilepsy in patients with TSC. Our aim was to increase awareness on the disease and to present a picture of patients' characteristics and interventions in a very large number of centers dedicated for TSC care around the world. The findings in this study support previous studies highlighting the higher prevalence of epilepsy in patients with TSC with onset during infancy or early childhood, the correlation between seizure control and intellectual outcome, and the ongoing need for therapies for both infantile spasms and focal seizures as well as for even closer developmental observations correlated with these. The findings also emphasize the better seizure control compared to previous studies that might be multifactorial encompassing earlier diagnosis and a widespread use of the international guidelines for therapies. However, standardized neuropsychological and psychiatric assessment is still lacking in many countries, even in reference centers, and this should be better addressed and implemented.[10,38] Finally, the surgery as a rare recourse in the therapy arsenal should be further investigated and a closer interaction with expert centers for epilepsy surgery should be encouraged.

Further analysis of collected data of the TOSCA study will provide more details in understanding the treatment interventions and outcomes and might inform on the development of our knowledge on a rare disease exploring time-related changes through these data regarding different aspects as age of diagnosis, therapies, and therapy responses.

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ORIGINAL ARTICLE

Renal angiomyolipoma in patients with tuberous sclerosis complex: findings from the TuberOus SClerosis registry to increase disease Awareness

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ABSTRACT

Background. Renal angiomyolipoma occurs at a high frequency in patients with tuberous sclerosis complex (TSC) and is associated with potentially life-threatening complications. Despite this frequency and severity, there are no large population-based cohort studies. Here we present baseline and follow-up data of the international TuberOus SClerosis registry to increase disease Awareness (TOSCA) with an aim to provide detailed clinical characteristics of renal angiomyolipoma among patients with TSC.

Methods. Patients of any age with a documented clinic visit for TSC within 12 months or who were newly diagnosed with TSC before participation in the registry were eligible. Data specific to renal angiomyolipoma included physical tumour characteristics (multiple, bilateral, lesion size and growing lesions), clinical signs and symptoms, and management. The effects of age, gender and genotype on the prevalence of renal angiomyolipoma were also evaluated.

Results. Renal angiomyolipoma was reported in 51.8% of patients at baseline, with higher frequency in female patients

(57.8% versus 42.2%). The median age at diagnosis was 12 years. Prevalence of angiomyolipoma was higher in patients with *TSC2* compared with *TSC1* mutations (59.2% versus 33.3%, $P < 0.01$). Of the 1031 patients with angiomyolipoma at baseline, multiple lesions were reported in 88.4% and bilateral in 83.9% of patients, while the size of angiomyolipoma was >3 cm in 34.3% of patients. Most patients were asymptomatic (82%). Frequently reported angiomyolipoma-related symptoms included bleeding, pain, elevated blood pressure and impaired renal function. Embolization and mammalian target of rapamycin inhibitors were the two most common treatment modalities.
Conclusions. The TOSCA registry highlights the burden of renal angiomyolipoma in patients with TSC and shows that renal manifestations are initially asymptomatic and are influenced by gender and genotype. Furthermore, the occurrence of significant problems from angiomyolipoma in a minority of younger patients suggests that surveillance should begin in infancy or at initial diagnosis.

Keywords: mTOR Inhibitor, registry, renal angiomyolipoma, TOSCA, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant genetic disorder resulting from inherited or sporadic germline mutations of *TSC1* or *TSC2* encoding hamartin and tuberin, respectively. It is characterized by hamartomatous lesions in multiple organs, including the brain, kidney, skin, heart, lungs and retina [1].

Renal problems are very frequent in patients with TSC after neurological manifestations and TSC-associated neuropsychiatric disorders and a leading cause of morbidity and mortality in these patients [2–7]. Renal manifestations include angiomyolipoma, epithelial cysts, polycystic kidney disease and renal cell carcinoma [8, 9]. The occurrence rate and clinical characteristics of renal lesions in TSC have been assessed primarily in either single- or two-centre case series [10–12] or in population-based studies with small sample sizes [8, 13, 14] with varied findings. The estimated prevalence of angiomyolipoma varied between studies and ranged from 55% to 80%. Some studies showed a higher proportion of renal angiomyolipoma in females [11, 15], whereas others have shown no gender disparity [10]. Patients with *TSC2* mutations have been reported to exhibit a higher incidence and severity of angiomyolipoma compared with patients with *TSC1* mutations [11, 16]. Patients with TSC-associated renal angiomyolipoma are susceptible to spontaneous life-threatening haemorrhage [4].

Despite considerable progress in the understanding of TSC and associated renal manifestations, there is a need for a large population-based cohort study to better understand clinical characteristics and natural history of renal angiomyolipoma in patients with TSC and its relationship with age, gender and genotype to target surveillance and therapy to those at greatest risk.

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) has been designed to address the knowledge gaps in the natural history of TSC by collecting data from patients across many countries worldwide. The TOSCA registry has provided better insight into the overall TSC manifestations including clinical characteristics of renal angiomyolipoma [17]. In this report, we present baseline and 1-year follow-up data of the TOSCA registry with focus on the clinical characteristics of renal angiomyolipoma.

MATERIALS AND METHODS

The methods of TOSCA have been described in detail previously [18]. In short, TOSCA is a multicentre, international disease registry conducted at 170 sites across 31 countries worldwide. Between August 2012 and August 2014, patients of any age with a documented clinic visit for TSC in the preceding 12 months or newly diagnosed with TSC were enrolled.

In the TOSCA registry, general information on patient background such as demographic data, family history, genotype, vital signs, prenatal history, clinical features of TSC across all organ systems, comorbidities and rare manifestations were collected at baseline and at regular visits scheduled at a maximum interval of 1 year to ensure an ongoing data stream.

Data specific to renal angiomyolipoma included physical tumour characteristics (multiple, bilateral, lesion size and growing lesions), clinical signs and symptoms and management. The effects of age, gender and genotype on the prevalence of renal angiomyolipoma were also evaluated. Mean age of angiomyolipoma diagnosis at baseline were compared between patients with *TSC1* and *TSC2* mutations using Z test, while Chi-square test was used to analyze association between genotype and renal characteristics (such as history of angiomyolipoma, lesion >3 cm, growing angiomyolipoma, patients with/without signs or symptoms, or treatment received by patients) at baseline. This is an observational study, and therefore no additional clinical or laboratory assessments/interventions were performed other than those required for disease surveillance or management according to the local best practice.

As the registry is observational in nature, results are reported with descriptive statistics only. All eligible patients enrolled in the TOSCA registry were considered in the analysis. Continuous variables were evaluated quantitatively (e.g. frequency, mean, standard deviation, median, range), and categorical variables (e.g. presence/absence of a manifestation) were analysed in terms of frequency distribution at baseline and at follow-up.

This study was designed and conducted according to the Guidelines for Good Clinical Practice and ethical principles outlined in the Declaration of Helsinki [19, 20]. Written informed consents were obtained from all patients, parents or guardians prior to enrolment with prior endorsement by the local human research ethics committee.

RESULTS

Patient demographics and clinical characteristics

As of 30 September 2015 (data cut-off date for the third interim analysis), baseline data were available for 2216 patients and first follow-up visit data were available for 1911 patients. Baseline patient demographics and clinical characteristics are summarized in Table 1. Median age at inclusion was 13 years

(range <1–71 years). Median age at diagnosis of TSC was 1 year (range <1–69 years). There were 144 (6.5%) patients with prenatal diagnoses. Molecular testing for genetic mutations was performed in 1000 (45.1%) patients. Of these, 644 patients (64.4%) had a *TSC2* gene mutation, 197 (19.7%) had a *TSC1* gene mutation, 6 patients had both *TSC1* and *TSC2* gene mutations and 144 (14.4%) had no mutation identified.

Renal angiomyolipoma

A total of 1070/2065 patients (51.8%) had renal imaging and had a history of renal angiomyolipoma. The other 151 patients had not had renal imaging. Of the 1070 patients with documented renal angiomyolipoma, 42.2% were males, and 57.8% females. The frequency at each age distribution is presented in Figure 1. The mean age at the time of diagnosis was 16.9 years [median (range) 12 years (<1–67 years)]. Of 1070 patients with a history of angiomyolipoma, angiomyolipoma was present at baseline in 1031 patients, while renal angiomyolipomas were resolved on treatment in 23 patients (2.1%) and no longer detectable in 16 patients (1.5%). In 1031 patients with renal angiomyolipoma present at baseline, 911 patients (88.4%) had multiple renal angiomyolipoma and 865 patients (83.9%) had bilateral renal angiomyolipoma. Angiomyolipoma size >3 cm was observed in 354 patients (34.3%; 68 patients aged ≤18 years and 286 aged >18 years).

A repeat scan to monitor angiomyolipoma was carried out in 977 patients (44.1%) with known angiomyolipoma. Growth of angiomyolipoma was observed in 218 patients (21.1%; 100 patients aged ≤18 years and 118 aged >18 years). The mean time from the previous scan to the last assessment was 1.3 years. The occurrence rate and physical characteristics of renal angiomyolipoma at first follow-up visit were similar to that observed at baseline (Table 2).

The majority of the patients with renal angiomyolipoma were asymptomatic at baseline (845 patients, 82.0%) and at first follow-up visit (801 patients, 87.4%). Among the symptomatic patients, the most common symptoms reported at baseline and at first follow-up visit were pain (6.1% and 3.7%), elevated blood pressure (5.7% and 5.1%), haemorrhage (5.0% and 1.5%), microscopic haematuria (4.3% and 3.3%) and impaired renal function (3.9% and 3.3%), respectively (Table 2).

Overall, renal angiomyolipomas were treated in 309 patients (28.9%). However, the percentage of patients requiring treatment increased progressively with age to 48.6% by age >40 years (Table 3). The most common treatment modalities (either as monotherapy or in combination with other treatment modalities) were embolization (46.0%) and mammalian target of rapamycin (mTOR) inhibitors (43.4%) (Table 2). The most common treatment modality in patients aged ≤18 years was mTOR inhibitors whereas embolization was most common in patients aged >18 years (Table 3). The most common treatment modality at first follow-up visit was mTOR inhibitors (Table 2).

Table 1. Baseline patient demographics and clinical characteristics (*N* = 2216)

Characteristics	Baseline data
Age at diagnosis of TSC[a], years, median (range)	1 (<1–69)
Gender, *n* (%)	
Male	1062 (47.9)
Female	1154 (52.1)
Patients with molecular testing, *n* (%)	1000 (45.1)
Genetic testing, *n* (%)[b]	
No mutation identified	144 (14.4)
TSC1 mutation[c]	197 (19.7)
TSC2 mutation[c]	644 (64.4)
Both *TSC1* and *TSC2* mutations	6 (0.6)
Variation type, *n* (%)[d]	
Pathogenic mutation	678 (67.8)
Variant of unknown significance	66 (6.6)
Patients with prenatal diagnosis, *n* (%)	144 (6.5)
Patients with at least one blood relative participating in TOSCA, *n* (%)	230 (10.4)

[a]Data available for 2216 patients.
[b]Information on the type of mutation was missing for nine patients.
[c]The count (*n*) includes six patients who had both *TSC1* and *TSC2* mutations.
[d]The count (*n*) includes 23 patients who had both variation types.

Relationship of renal angiomyolipoma with mutation type

Significantly more patients with *TSC2* mutations had renal angiomyolipoma at baseline compared with those with a *TSC1* mutation (59.2% versus 33.3%, P < 0.01). The mean age at diagnosis of renal angiomyolipoma in patients with a *TSC2* mutation

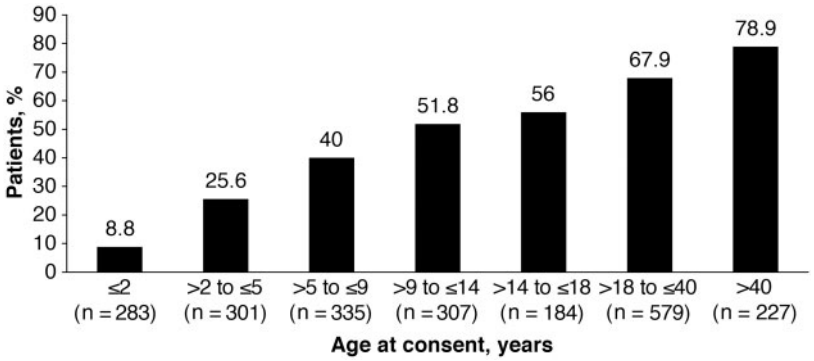

FIGURE 1: Patients with history of renal angiomyolipoma across age groups. Percentage of patients with renal angiomyolipoma in each age group was calculated considering the total number of patients in that age group as the denominator.

Table 2. Renal angiomyolipoma features according to mutation type

Characteristics	Overall ($N = 2216$)		Patients with *TSC1* mutation ($n = 197$)		Patients with *TSC2* mutation ($n = 644$)	
	Baseline	First follow-up visit	Baseline	First follow-up visit	Baseline	First follow-up visit
History of renal angiomyolipoma[a]*	1070 (51.8)	992 (55.8)	63 (33.3)	61 (36.5)	369 (59.2)	344 (62.0)
Mean (range) age at diagnosis, years*	16.9 (<1–67)	–	23.5 (<1–60)	–	13.2 (<1–59)	–
Angiomyolipoma present at the time of assessment	1031 (96.4)	917 (92.4)	58 (92.1)	60 (98.4)	357 (96.7)	309 (89.8)
Multiple	911 (88.4)	808 (81.5)	41 (70.7)	43 (70.5)	330 (92.4)	278 (80.8)
Bilateral	865 (83.9)	770 (77.6)	29 (50.0)	33 (54.1)	308 (86.3)	260 (75.6)
Angiomyolipoma size >3 cm*	354 (34.3)	302 (30.4)	7 (12.0)	10 (16.4)	114 (31.9)	98 (28.5)
Growing angiomyolipoma†	218 (21.1)	173 (17.4)	7 (12.0)	9 (14.8)	86 (24.1)	70 (20.3)
Signs and symptoms						
None‡	845 (82.0)	801 (87.4)	52 (89.7)	55 (91.7)	297 (83.2)	272 (88.0)
Pain not otherwise specified	63 (6.1)	34 (3.7)	2 (3.4)	2 (3.3)	24 (6.7)	11 (3.6)
Elevated blood pressure	59 (5.7)	47 (5.1)	4 (6.9)	3 (5.0)	23 (6.4)	20 (6.5)
Haemorrhage	52 (5.0)	14 (1.5)	0	0	18 (5.0)	5 (1.6)
Haematuria	44 (4.3)	30 (3.3)	0	0	15 (4.2)	10 (3.2)
Impaired renal function	40 (3.9)	30 (3.3)	1 (1.7)	1 (1.7)	10 (2.8)	8 (2.6)
Other	33 (3.2)	11 (1.2)	0	0	10 (2.8)	3 (1.0)
Treatment						
Patients received treatment†	309 (28.9)	272 (27.4)	8 (12.7)	5 (8.2)	99 (26.8)	84 (24.4)
Type of treatment						
Embolization[b]	142 (46.0)	8 (2.9)	2 (25.0)	0	41 (41.4)	2 (2.4)
mTOR inhibitor[b]	134 (43.4)	35 (12.9)	3 (37.5)	0	51 (51.5)	8 (9.5)
Nephrectomy[b]	62 (20.1)	5 (1.8)	3 (37.5)	0	22 (22.2)	1 (1.2)
Resection[b]	20 (6.5)	1 (0.4)	1 (12.5)	0	5 (5.1)	0
Dialysis[c]	3 (1.0)	1 (0.4)	0	0	1 (1.0)	1 (1.2)
Other	13 (4.2)	1 (0.4)	0	0	3 (3.0)	0

Values are presented as n (%), unless otherwise specified.
TSC1 vs *TSC2* at baseline: *$P < 0.01$; †$P < 0.05$; ‡$P = 0.77$.
[a]Percentage were calculated based on number of patients with at least one renal imaging.
[b]Used alone or in combination with other treatment modalities; at baseline in overall population, embolization as single agent was used in 102 of 142 patients; mTOR inhibitors as single agent were used in 87 of 134 patients; nephrectomy as single modality was used in 34 of 62 patients and resection as single modality was used in 11 of 20 patients.
[c]Dialysis was used only in combination with other treatment modalities.

Table 3. Treatment modalities according to age

Treatment modalities	Age at consent for patients with history of renal angiomyolipoma, $n = 1070$						
	≤2 ($n = 25$)	>2 to ≤5 ($n = 77$)	>5 to ≤9 ($n = 134$)	>9 to ≤14 ($n = 159$)	>14 to ≤18 ($n = 103$)	>18 to ≤40 ($n = 393$)	>40 ($n = 179$)
Patients received treatment, *n* (%)	1 (4.0)	1 (1.3)	11 (8.2)	24 (15.1)	27 (26.0)	158 (40.2)	87 (48.6)
Type of treatment[a], *n* (%)							
Dialysis	0	0	0	0	0	2 (1.3)	1 (1.1)
Embolization	0	0	0	2 (8.3)	7 (25.9)	83 (52.5)	50 (57.5)
Nephrectomy	0	0	0	2 (8.3)	3 (11.1)	29 (18.4)	28 (32.2)
Resection	0	0	1 (9.1)	1 (4.2)	3 (11.1)	10 (6.3)	5 (5.7)
mTOR inhibitor	1 (100.0)	1 (100.0)	11 (100.0)	17 (70.8)	16 (59.3)	67 (42.4)	21 (24.1)
Others	0	0	0	0	0	9 (5.7)	4 (4.6)

[a]Used alone or in combination with other treatment modalities.

was 13.2 years (range <1-59 years), which was significantly lower than those with a *TSC1* mutation (23.5 years, range <1-60 years; $P < 0.01$). Furthermore, significantly greater percentage of patients with a *TSC2* mutation compared with those with a *TSC1* mutation had an angiomyolipoma >3 cm in size (31.9% versus 12.0%, $P < 0.01$) or a growing angiomyolipoma (24.1% versus 12.0%, $P < 0.05$; Table 2). The age range of patients with *TSC1* and *TSC2* mutations was similar (Supplementary data, Table S1).

Similar to the overall population, renal angiomyolipoma were asymptomatic in most patients with *TSC1* (89.7%) and *TSC2* (83.2%) mutations, with no differences between the groups ($P = 0.77$). More patients with a *TSC2* mutation required one or more treatment than those with TSC1 mutation (26.8% versus 12.7%, $P < 0.05$). However, most common treatment modalities did not differ based on gene mutation (Table 2).

Other renal manifestations

The other renal features reported at baseline and at first follow-up visit include multiple renal cysts (24.2% and 28.3%), polycystic kidney disease (proven *TSC2/PKD1* mutation; 3.4%

and 4%), renal malignancy (1.1% and 0.7%) and impaired renal function (non-angiomyolipoma-related; 1.9% and 2.2%), respectively. Compared with patients with a *TSC1* mutation, those with *TSC2* mutations had a higher occurrence of multiple renal cysts (33.4% versus 13.7%) and polycystic kidney disease (4.5% versus 0%).

DISCUSSION

The TOSCA study represents the largest cohort of TSC patients, with data accrued from 170 sites across 31 countries worldwide. The study showed several notable findings. Renal angiomyolipoma were reported in 51.8% of patients in the TOSCA cohort, which was lower than that observed in other studies [8, 10–13]. A probable reason for the lower rates of renal angiomyolipoma observed in this study was the younger median age of the TOSCA cohort. As shown in Figure 1, there is markedly increasing prevalence in the adult age range mirroring that of the published series rates of 55–80% [11, 15]. In a retrospective cohort study of 170 patients with TSC, a significant association between advancing age and the incidence of renal angiomyolipoma was reported [11]. Furthermore, the presence of renal angiomyolipoma was unknown for 151 patients (6.8%) suggesting no renal imaging was performed in these patients to confirm or exclude the presence of renal angiomyolipoma. However, it is important to note that the total number of patients enrolled in this study was considerably larger than in previous studies.

A striking finding in our study was the occurrence of angiomyolipoma in very young children and the need for treatment as early as 2 years of age or younger (Table 3 and Figure 1). In the TOSCA cohort the earliest age of diagnosis of angiomyolipoma was <2 years. This compares with other studies, which reported an estimated average age of onset to be between 7.2 and 11.1 years [10–12]. The proportion of patients receiving treatment for angiomyolipoma increased progressively with increasing age. About 15% of patients received treatment by age 14, 27% by age 18, 40% by age 40 and 49% by age >40.

The number of patients with haematuria and hypertension in the TOSCA cohort was low compared with those reported in TSC patients in other studies [21–23]. This low incidence of signs and symptoms could be explained by the high number of young patients enrolled in the study and also by the preemptive treatment of renal angiomyolipoma in patients who were under on-going surveillance (not lapsed from follow-up). On-going surveillance is recommended in patients with asymptomatic tumours per international TSC guidelines [24]. Chopra *et al.* evaluated adherence to surveillance guideline recommendations in an Australian TSC cohort and compared it among adults and pediatric patients [25]. The study showed that there was a significantly lower rate of adherence to surveillance guidelines in adult patients than in pediatric patients. This highlights the need for a focused transition plan for TSC patients transferred to adult care.

Studies have shown a gender disparity among patients with TSC-associated angiomyolipoma, with more occurrences in female patients [11, 21, 26]. A retrospective study by Rakowski *et al.* showed that complications due to angiomyolipoma were more common in women than in men with TSC [11]. Furthermore, about two-thirds of the patients recruited in EXIST-2, a Phase 3 study evaluating the efficacy and safety of everolimus in patients with angiomyolipoma associated with TSC or sporadic lymphangioleiomyomatosis, were women [27]. Both of these findings suggest that women with TSC are more vulnerable to developing renal complications. The gender disparity in angiomyolipoma complications raises a possible role of sex hormones in the pathogenesis of these lesions. Our study showed a similar finding with higher frequency of renal angiomyolipoma in females (57.8%) compared with males, however, this was not statistically significant. A future analysis is planned (after another years data has been collected) to ascertain if the rate of complications or treatment is higher in females.

The effect of mutation type on occurrence rate and severity of renal angiomyolipoma has been reported to be consistently greater among patients with *TSC2* compared with *TSC1* mutations [11, 16]. A study by Dabora *et al.* [16] in 224 unselected patients with TSC showed that frequency and severity of renal angiomyolipoma were significantly higher among patients with *TSC2* mutations than those with *TSC1* mutations (60% versus 31%, $P = 0.03$; mean grade 0.97 versus 0.32, $P = 0.006$). Our study, in a far larger cohort, confirms these findings. In TOSCA, patients with *TSC2* mutations were at higher risk of developing renal angiomyolipoma than those with *TSC1* mutations. The occurrence rate of multiple and bilateral angiomyolipoma lesions were also higher among those with a *TSC2* mutation. In addition, bleeding complications were observed only in patients with a *TSC2* gene mutation (Table 2), suggesting that the risk is higher in this group. These findings suggest that mutational analysis may help predict renal prognosis.

The treatment of renal angiomyolipoma associated with TSC is mainly focused on preventing acute events, preserving renal parenchyma and maintaining kidney function. A surprisingly high rate (34.6%) of patients had lesions >3 cm in diameter, a size where active intervention is recommended [24]. Embolization is the preferred therapy for renal angiomyolipoma presenting with acute haemorrhage, while mTOR inhibitors are the recommended first-line therapy for asymptomatic growing angiomyolipoma lesions ≥3 cm in diameter [9]. Overall, patients with renal angiomyolipoma in our study were most commonly treated with embolization followed by mTOR inhibitors. This balance may change with the expanding use of mTOR inhibitors in younger populations, an important question for this ongoing cohort study to address.

The 2012 International TSC Consensus Group recommends that nephrectomy should be avoided in patients with TSC-associated renal angiomyolipoma [24]. However, there was a high rate of nephrectomy in patients in the TOSCA cohort (20%). When treatment modalities used were stratified by age of patients, mTOR inhibitors were the most common treatment modality in patients aged ≤18 years while embolization and nephrectomy was more common in patients aged >18 years. As stated, mTOR inhibitor therapy is now the recommended

first-line treatment for pre-symptomatic angiomyolipoma and our cohort data included historical treatment in older patients. Renal malignancy has been reported in about 2–4% patients with TSC [28], which is much higher than that reported in a comparable age group in the general population [29]. However, the occurrence rate of renal malignancy observed in this cohort was lower (1.1%) than that reported previously. However, we need to consider that the rate of malignancy was calculated based on patients who survived at the time of analysis.

This study had a number of limitations. First, owing to the observational nature of the registry, only data already available from routine clinical practice was collected. However, participation of a variety of centres with different specialists has helped inclusion of a large number of patients with TSC, which are representative of hospital clinical practice. Furthermore, patients were recruited through clinical centres with expertise in TSC and hence milder cases may not always be seen at these centres.

In conclusion, the TOSCA registry highlights the burden of renal angiomyolipoma in patients with TSC and provides valuable insights in understanding the characteristics, complications and treatment of renal angiomyolipoma in these patients. The data in the TOSCA registry show that renal manifestations are generally initially asymptomatic and are influenced by gender and genotype. Renal angiomyolipoma may occur in patients aged <2 years but the occurrence rate increases markedly with time, with up to 49% of patients requiring treatment interventions over 40 years of age.

SUPPLEMENTARY DATA

Supplementary data are available at ndt online.

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CONFLICT OF INTEREST STATEMENT

J.C.K., P.J.dV., E.B., T.C., V.C., P.C., G.B.d'A., J.C.F., M.F., C.F., C.H., S.J., R.N., F.O'C., J.Q., M.S., R.T., M.D., J.A.L., A.M., S.Y., M.P.B., B.Z. and A.C.J. received honoraria and support for the travels from Novartis. V.C. received personal fees for consulting, lecture fees and travel from Actelion, Bayer, Biogen Idec, Boehringer Ingelheim, Gilead, GSK, MSD, Novartis, Pfizer, Roche and Sanofi; grants from Actelion, Boehringer Ingelheim, GSK, Pfizer and Roche; and personal fees for developing educational material from Boehringer Ingelheim and Roche. P.J.dV. has been on the study steering group of the EXIST-1, -2 and -3 studies sponsored by Novartis, and co-principal investigator on two investigator-initiated studies part-funded by Novartis. R.N. received grant support, paid to her institution, from Eisai and lectures fees from Nutricia, Eisai, Advicenne and GW Pharma. Y.T. received personal fee from Novartis for lecture and for copyright of referential figures from the journals, and received grant from Japanese government for intractable epilepsy research. S.J. was partly financed by the EC Seventh Framework Programme (FP7/2007-2013; EPISTOP, grant agreement no. 602391), the Polish Ministerial funds for science (years 2013-2018) for the implementation of international co-financed project and the grant EPIMARKER of the Polish National Center for Research and Development No STRATEGMED3/306306/4/2016. J.C.K., P.C., C.H., J.A.L. and J.Q. received research grant from Novartis. R.M., L.D'A. and S.S. are employees of Novartis. V.S. reported no conflict of interest. The study participants have not received funds for their participation in the study. The results presented in this manuscript have not been previously published except a poster presentation at TSC International congress on 4 November 2016.

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Tuberous Sclerosis Complex-Associated Neuropsychiatric Disorders (TAND): New Findings on Age, Sex, and Genotype in Relation to Intellectual Phenotype

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Background: Knowledge is increasing about TSC-Associated Neuropsychiatric Disorders (TAND), but little is known about the potentially confounding effects of intellectual ability (IA) on the rates of TAND across age, sex, and genotype. We evaluated TAND in (a) children vs. adults, (b) males vs. females, and (c) *TSC1* vs. *TSC2* mutations, after stratification for levels of IA, in a large, international cohort.

Methods: Individuals of any age with a documented visit for TSC in the 12 months prior to enrolment were included. Frequency and percentages of baseline TAND manifestations were presented by categories of IA (no intellectual disability [ID, intelligence quotient (IQ)>70]; mild ID [IQ 50–70]; moderate-to-profound ID [IQ<50]). Chi-square tests were used to test associations between ID and TAND manifestations.

The association between TAND and age (children vs. adults), sex (male vs. female), and genotype (*TSC1* vs. *TSC2*) stratified by IA levels were examined using the Cochran–Mantel–Haenszel tests.

Results: Eight hundred and ninety four of the 2,211 participants had formal IQ assessments. There was a significant association ($P < 0.05$) between levels of IA and the majority of TAND manifestations, except impulsivity ($P = 0.12$), overactivity ($P = 0.26$), mood swings ($P = 0.08$), hallucinations ($P = 0.20$), psychosis ($P = 0.06$), depressive disorder ($P = 0.23$), and anxiety disorder ($P = 0.65$). Once controlled for IA, children had higher rates of overactivity, but most behavioral difficulties were higher in adults. At the psychiatric level, attention deficit hyperactivity disorder (ADHD) was seen at higher rates in children while anxiety and depressive disorders were observed at higher rates in adults. Compared to females, males showed significantly higher rates of impulsivity and overactivity, as well as autism spectrum disorder (ASD) and ADHD. No significant age or sex differences were observed for academic difficulties or neuropsychological deficits. After controlling for IA no genotype-TAND associations were observed, except for higher rates of self-injury in individuals with *TSC2* mutations.

Conclusions: Findings suggest IA as risk marker for most TAND manifestations. We provide the first evidence of male preponderance of ASD and ADHD in individuals with TSC. The study also confirms the association between *TSC2* and IA but, once controlling for IA, disproves the previously reported *TSC2* association with ASD and with most other TAND manifestations.

Keywords: intelligence quotient, tuberous sclerosis complex, TSC-associated neuropsychiatric disorders, TOSCA, TAND profile

INTRODUCTION

Tuberous sclerosis complex (TSC) is a genetic disorder with prevalence of 1:5,800 live births. It is caused by mutation in either the *TSC1* or *TSC2* gene and characterized by the growth of benign hamartomas in multiple organs including the brain, and is often associated with a high rate of neurological deficits (1). Apart from the range of physical manifestations observed, around 90% of patients with TSC exhibit some neuropsychiatric manifestations and these are associated with the greatest burden of care for families (1–5). Although most people with TSC will have neuropsychiatric disorder, only a small proportion typically ever receive screening, diagnosis, and treatment for these (6). The term TAND (TSC-associated neuropsychiatric disorders) was therefore coined to capture the multi-level manifestations, and a TAND Checklist was developed as a simple screening tool to help in the identification and prioritization of TAND manifestations (7, 8).

TAND manifestations are classified into 6 levels including behavioral, psychiatric, intellectual, academic, neuropsychological, and psychosocial levels (3). Among behavioral difficulties, the reported ranges to date include depressed mood (19–43%), anxiety (41–56%), self-injury (17–69%), aggression (37–66%), temper tantrums (47–70%), overactivity/hyperactivity (22–73%), impulsivity (36–62%), and sleep difficulties (15–74%) (6, 9–11). At the psychiatric level, reported rates include autism spectrum disorder (ASD; 40–50%), attention deficit hyperactivity disorder (ADHD; 30–40%), anxiety and depressive disorder (27–56%) and psychosis (2.3%) (1, 6, 9). At the intellectual level, around 40–50% of individuals with TSC are considered to have normal intellectual ability (IA), and the remaining have some degree of intellectual disability (ID) (2, 12, 13). The majority of individuals with TSC have had difficulties in academic or scholastic skills (2). Individuals with TSC are at high risk of a range of neuropsychological deficits including attention deficits, memory deficits, and executive deficits. At the psychosocial level, family stress and difficulties with self-esteem and self-efficacy are often reported (3, 14).

The etiology of TAND manifestations has received some scientific investigation over the last few decades. It is well-established that epilepsy (infantile spasms and other seizure types) is a clear risk marker for many TAND manifestations, particularly intellectual ability (1, 15, 16). The role of structural brain abnormalities such as cortical tubers or SEGA has been less clear (1, 3, 17). Direct molecular models suggesting that the functional consequences of *TSC1* or *TSC2* mutations may directly lead to TAND, and combinatorial models of the above, have also been suggested (1, 18).

Given the relative rarity of TSC, the evidence-base for TAND manifestations and their patterns have, until recently, been based on relatively small-scale studies that typically examined only some of the levels of TAND, and that were typically from a single country. Very little was known about the differences between children and adults or between those with *TSC1* vs.

TSC2 mutations. In a recent study, we evaluated TAND in a large multicenter international study (TOSCA) and examined profiles of manifestations in children vs. adults, in different age-bands, and in those with *TSC1*, *TSC2*, and no mutation identified (NMI) (2). Findings in the study were based on data from 2,216 participants at the third interim analysis (cut-off 30 September 2015) of the TOSCA natural history study. The study showed significantly higher rates of overactivity and impulsivity in children and higher rates of anxiety, depressed mood, mood swings, obsessions, psychosis, and hallucinations in adults. Individuals with *TSC2* mutations had higher frequency of self-injury, ASD, academic difficulties and neuropsychological deficits, while those with NMI showed a mixed pattern of TAND manifestations. Interestingly, individuals with *TSC1* mutations showed higher rates of impulsivity, anxiety, depressed mood, hallucinations, psychosis, and of ADHD, anxiety and depressive disorders (2).

A key finding from the study was the observation that those with *TSC2* mutations had significantly higher rates of ID. Intellectual ability is known to be a strong correlate or risk marker of behavioral, psychiatric, academic, and neuropsychological deficits both in general population and in individuals with TSC (6, 19). For example, an earlier study in 265 children and adolescents with TSC showed differential rates of many behavioral manifestations, ASD and ADHD, in individuals with and without ID (6). The fundamental role of IA as risk marker for TAND therefore raises concerns about the previous findings of de Vries and colleagues (2) in terms of child vs. adult differences, and about *TSC1* vs. *TSC2* differences in TAND.

It is also well-established that many psychopathologies have been associated with differential rates between male and females. For example, boys and men are typically associated with higher rates of ASD and ADHD, while girls and women are typically associated with higher rates of anxiety and mood disorders (20–24). Studies in TSC to date have shown conflicting findings in relation to sex differences of TAND. In one small study from Wessex, UK a significant male preponderance in the rates of ID was reported (25). In contrast, other studies have shown no difference in the rates of behavioral problems, psychiatric disorders or ID (6, 26). To date no studies have compared academic/scholastic difficulties and neuropsychological deficits between male and female individuals with TSC.

Here, we therefore set out to perform a detailed exploration of the association of TAND manifestations (a) between children and adults, (b) between males and females, and (c) between those with *TSC1* and *TSC2* mutations, in a large international sample of individuals with TSC, stratified for their levels of IA. We hypothesized that, after controlling for levels of IA (a) the significant differences observed between children and adults would be maintained (2), (b) that, as per previous TSC research no sex differences would be observed in TAND (6, 26), and (c) that the *TSC1-TSC2* differences observed in our earlier study would be maintained (2).

PARTICIPANTS AND METHODS

TOSCA, a multicenter, international study in individuals with TSC, was conducted at 170 sites in 31 countries. The study methodology of TOSCA has been detailed previously (27). In brief, the study consisted of a core section and 6 ancillary research projects, focusing each on subependymal giant cell astrocytomas (SEGA), renal angiomyolipoma and lymphangiomyomatosis, genetics, TAND, epilepsy, and quality of life. TAND data were collected from retrospective and prospective information available to study clinicians using a standardized data recording sheet as part of the case report form (CRF). The TAND data recording sheet were a precursor of the TAND Checklist (8). Comprehensive data were collected at baseline and annually thereafter for up to 5 years. Interim analyses of all data collected were done annually. Here we present results of the final analysis (last patient last visit, 10 August 2017).

All TOSCA participants in the final analysis with formal IQ assessment data were included in this study. Frequency and percentages of baseline TAND manifestations were presented by categories of IA [intelligence quotient (IQ) >70 = no ID (noID); IQ = 50–70 = mild ID (MID); IQ <50 = moderate-to-profound ID (M-PID)]. Chi-square test was used to examine the association between ID and TAND manifestations. The association between TAND and age [children [aged ≤18 years] vs. adults [aged >18 years]], sex (male vs. female), and genotype (*TSC1* vs. *TSC2*) stratified by IA (noID, MID, M-PID) was examined using the Cochran–Mantel–Haenszel tests. Statistical significance was set at $p < 0.05$.

The study was designed and conducted in accordance with the Good Clinical Practice principles, the Declaration of Helsinki, and all the local regulations. The Institutional Review Board or Ethics Committee at each participating center approved all the TOSCA related documents. Written informed consent was obtained from all participants, parents, or guardians prior to enrolment.

RESULTS

Overall 2,214 participants with TSC were enrolled into the TOSCA registry from 170 sites across 31 countries. Of these, data of 2,211 eligible participants were analyzed. Data of 3 participants were excluded from the analysis due to major protocol deviations. Of the 2,211 participants, 894 (40.4%) had formal IQ assessments; 395 had normal IQ, 251 had MID and 248 had M-PID. Baseline demographics of this cohort were similar to that of the overall cohort and those without IQ (**Table 1**).

Overall TAND Manifestations and Their Association With Levels of Intellectual Ability (IA)

The overall and stratified frequencies of TAND manifestations in the final TOSCA cohort are depicted in **Table 2**. The majority of behavioral difficulties showed significant association ($P < 0.05$) with the levels of IA, except impulsivity ($P = 0.12$), overactivity ($P = 0.26$), mood swings ($P = 0.08$), hallucinations ($P = 0.20$), and psychosis ($P = 0.06$, **Table 2**). IA showed a significant association with ASD, ADHD, and other psychiatric disorders, but not with depressive disorder ($P = 0.23$) or anxiety disorder ($P = 0.65$). Academic difficulties and neuropsychological deficits were significantly associated with levels of IA (**Table 2**).

TABLE 1 | Demographics of participants in the TOSCA study.

Characteristics	Overall Cohort (*N* = 2,211)	Participants with IQ assessments (*N* = 894)	Participants without IQ assessments (*N* = 1,305)
Age at TSC diagnosis,[a] years, median (range)	1.0 (0–69)	1.0 (0–60)	1 (0–69)
Gender, *n* (%)			
Males	1059 (47.9)	432 (48.3)	621 (47.6)
Females	1152 (52.1)	462 (51.7)	684 (52.4)
Genetic molecular testing performed, *n* (%)	1011 (45.7)	468 (52.3)	543 (41.6)
Genetic testing, *n* (%)			
No mutation identified	148 (14.6)	69 (14.7)	79 (14.5)
TSC1 mutation	191 (18.9)	94 (20.1)	97 (17.9)
TSC2 mutation	649 (64.2)	301 (64.3)	348 (64.1)
Both *TSC1* and *TSC2* mutation	5 (0.5)	0	5 (0.9)
Data not available	18 (1.8)	4 (0.8)	14 (2.6)
Mutation variation type[b], *n* (%)			
Only pathogenic mutation	663 (65.6)	331 (70.7)	332 (61.1)
Only variant of unknown significance	43 (4.3)	18 (3.8)	25 (4.6)
Time from TSC diagnosis to molecular testing, months, mean (SD)	81.8 (116.58)	84 (99.84)	79.8 (129.78)
Participants with prenatal diagnosis, *n* (%)	154 (7.0)	64 (7.2)	90 (6.9)
Participants with biological parent diagnosed with TSC, *n* (%)			
Mother	184 (19.5)	95 (18.3)	98 (21.4)
Father	130 (15.7)	63 (14.9)	67 (16.6)

IQ, intelligence quotient; SD, standard deviation; TSC, tuberous sclerosis complex. [a]Data available for 2,054 participants in the overall cohort. [b]The count (n) also includes 23 participants who had both mutation types.

TAND Manifestations in Children vs. Adults Stratified by Intellectual Ability (IA)

Once controlled for IA, adults showed significantly higher rates of most behavioral difficulties in comparison to children ($P < 0.05$), including severe aggression, self-injury, anxiety, mood swings, hallucination, obsession, and psychosis. Children showed significantly higher rates only of overactivity ($P < 0.05$, **Figure 1A**). No differences were observed between children and adults on sleep difficulties ($P = 0.99$), impulsivity ($P = 0.08$) or severe aggression ($P = 0.10$). At the psychiatric level, the rate of ASD ($P = 0.10$) was not significantly different between children and adults (**Figure 1B**). In contrast, ADHD ($P < 0.05$) were seen at higher rates in children, while anxiety disorders, depressive disorders and other psychiatric disorders were observed at higher rates in adults. No significant differences were seen in the rates of academic difficulties (**Figure 1C**) or neuropsychological deficits (**Figure 1D**) between children and adults in IQ-stratified groups (**Supplementary Table 1**).

TAND Manifestations in Males vs. Females Stratified by Intellectual Ability (IA)

Two behavioral manifestations (impulsivity and overactivity) were seen at significantly higher rates in males than females, while anxiety rates were higher in females (**Figure 2A**, **Supplementary Table 2**). No other behavioral manifestations were statistically significantly different between males and females once controlled for IA. At the psychiatric level, ASD and ADHD were seen at significantly higher rates in males than females, but depressive, anxiety and other psychiatric disorders were not significantly different (**Figure 2B**). No differences were observed between males and females in academic difficulties (**Figure 2C**) or neuropsychological deficits (**Figure 2D**).

TAND Manifestations in *TSC1* vs. *TSC2* Stratified by Intellectual Ability (IA)

After controlling for levels of IA, only one of all the TAND manifestations (self-injury) was observed at significantly higher rates in patients with *TSC2* mutations vs. those with *TSC1* mutations. No genotype-TAND associations were seen on any other behavioral manifestations (**Figure 3A**, **Supplementary Table 3**), psychiatric disorders (**Figure 3B**), academic difficulties (**Figure 3C**) or neuropsychological deficits (**Figure 3D**). In particular, the previously reported association between *TSC2* mutations and ASD was not statistically significant ($P = 0.09$).

DISCUSSION

In this study we set out to examine TAND manifestations in relation to age, sex, and genotype in an IA-stratified sample of individuals from 31 countries. The large-scale cohort allowed us to perform analyses not previously possible. In the overall cohort of 894 participants who had formal IQ evaluations, IA was significantly associated with the majority of behavioral manifestations, apart from impulsivity, overactivity, mood swings, hallucinations, and psychosis. In a similar pattern

TABLE 2 | TAND manifestations in all participants with available IQ data stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]).

TAND manifestation	All participants with IQ data available (*N* = 894) *n* (%)	Level of intellectual ability			*P*-value[a]
		NoID (*n* = 395) *n* (%)	MID (*n* = 251) *n* (%)	M-PID (*n* = 248) *n* (%)	
Behavioral level					
Sleep difficulties	172 (40.3)	46 (31.9)	45 (34.9)	81 (52.6)	0.0004
Severe aggression	100 (23.3)	22 (15.6)	37 (27.2)	41 (26.8)	0.03
Self-injury	63 (14.7)	8 (5.7)	14 (10.6)	41 (26.1)	<0.0001
Impulsivity	201 (47.2)	57 (40.7)	70 (53.0)	74 (48.1)	0.12
Overactivity	191 (44.4)	55 (39.0)	65 (48.5)	71 (45.8)	0.26
Depressed mood	76 (18.3)	37 (26.1)	27 (21.3)	12 (8.2)	0.0003
Anxiety	146 (34.9)	56 (40.0)	54 (40.3)	36 (25.0)	0.009
Mood swings	134 (32.3)	36 (26.3)	50 (39.1)	48 (32.0)	0.08
Obsessions	71 (17.1)	10 (7.2)	26 (20.0)	35 (24.1)	0.0004
Hallucinations	18 (4.3)	5 (3.5)	9 (7.0)	4 (2.8)	0.20
Psychosis	25 (6.0)	3 (2.1)	11 (8.3)	11 (7.6)	0.06
Psychiatric level					
Autism spectrum disorder (ASD)	165 (21.0)	14 (4.0)	31 (14.2)	120 (55.6)	<0.0001
Attention deficit hyperactivity disorder (ADHD)	167 (22.2)	56 (16.0)	55 (25.5)	56 (29.9)	0.0004
Depressive disorder	42 (5.7)	23 (6.7)	13 (6.3)	6 (3.2)	0.23
Anxiety disorder	87 (11.7)	38 (11.0)	28 (13.5)	21 (11.1)	0.65
Other psychiatric disorder	61 (8.2)	17 (4.9)	20 (9.6)	24 (12.6)	0.005
Academic level					
Participants with academic/scholastic difficulties	450 (68.0)	143 (47.2)	156 (82.5)	151 (88.8)	<0.0001
Participants assessed for difficulties	290 (76.9)	96 (75.0)	103 (79.8)	91 (75.8)	0.62
Neuropsychological level					
Participants assessed for neuropsychological skills	408 (58.1)	183 (56.5)	123 (60.9)	102 (58.0)	0.61
Participants with any deficit (Performance<5th percentile)	250 (69.6)	69 (41.3)	92 (90.2)	89 (98.9)	<0.0001

Values are expresses as number (%). Percentages are calculated excluding missing/unknown data.

IQ, intelligence quotient; noID, no intellectual disability; MID, mild intellectual disability; M-PID, moderate-to-profound intellectual disability; TAND, tuberous sclerosis complex-associated neuropsychiatric disorders.

[a] *P-value calculated from chi-square to test the association between categories of intellectual disability (NoID, MID and M-PID) and presence of respective TAND manifestation.*

at the psychiatric level, IA was associated with ASD, ADHD, and other psychiatric disorders, but not with depressive disorders or anxiety disorders. Academic difficulties and neuropsychological deficits showed a clear association with the levels of IA.

In terms of differences between children and adults, we predicted that all age-related TAND manifestations previously observed (2) would be maintained in stratified groups. In the earlier study overactivity, impulsivity and ADHD were more prominent in children, while anxiety, mood swings, depressed mood, psychosis, hallucinations, depressive disorder, and anxiety disorder were more prominent in adults. After controlling for IA, only overactivity was observed at significantly a higher rate in children, while most other behavioral manifestations had higher rates in adults. These observations challenge previous data that suggested an improvement or reduction in behavioral difficulties in individuals with TSC over time. In keeping with general population patterns, even after IA stratification, ADHD was observed at higher rates in children, and depressive and anxiety disorders at higher rates in adults. No academic difficulties or neuropsychological deficits showed age-based patterns after stratification. Mindful of the fact that these findings are based on cross-sectional rather than longitudinal data, our results suggest the need for careful longitudinal examination of behavioral change and emergence of psychopathology over time in TSC.

We predicted that, based on previous TSC research (6, 26), no sex differences would be observed. Contrary to the hypothesis, impulsivity, overactivity, anxiety, and obsessions, as well as ASD and ADHD were significantly more common in males. These observations are therefore the first clear evidence of a sex-related preponderance of ASD, ADHD and related behavioral manifestations in TSC. Anxiety symptoms were observed at higher rates in females, but, interestingly, no sex differences were observed in rates of anxiety disorders. Findings suggest that, at least for some psychopathologies in TSC, sex may play a contributory role. Future research should therefore consider the potential role of sex alongside genetic and other environmental factors in the pathway to psychopathology in TSC. Our results

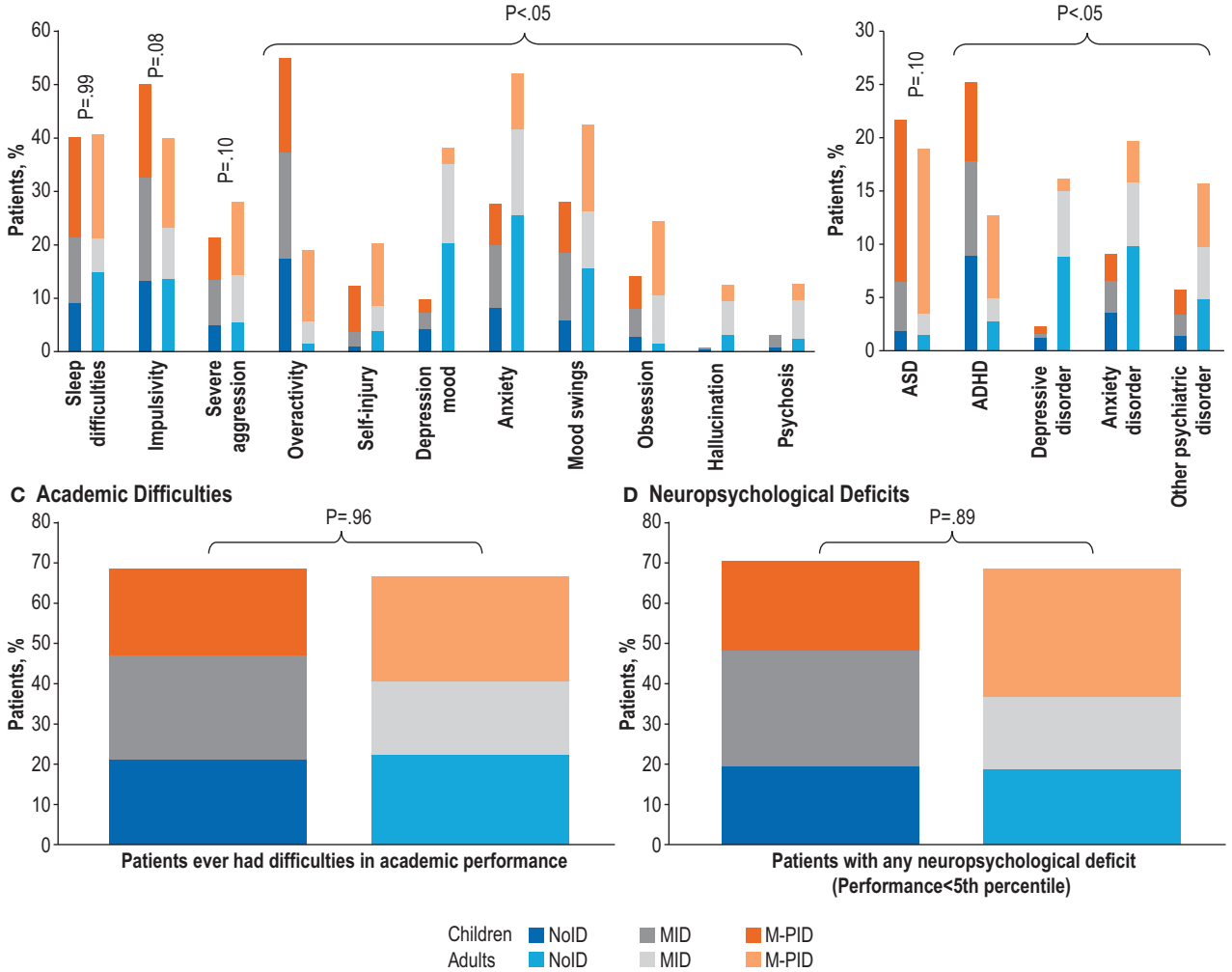

FIGURE 1 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in children vs. adults. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated excluding missing/unknown data.

certainly highlight the need to control for sex in any comparative studies involving individuals with TSC.

Given previous reports of an association between *TSC2* and more severe TSC manifestations, we predicted the same pattern for TAND. We observed a clear correlation between levels of IA and genotype, with *TSC2* more likely to be associated with ID. However, after controlling for levels of IA, only one of all the genotype-TAND correlations was statistically significant (self-injury, $P = 0.0496$). We are cautious not to over-interpret what might have been a spurious finding. Importantly, the previously suggested association between *TSC2* mutations and ASD was not replicated in our data. These results support the previous evidence of the strong association between levels of intellectual ability and psychopathologies in the general population (28, 29), and provide the first clear evidence of the association between IA and all levels of TAND investigated here. However, our findings did not suggest a specific association between *TSC1* or *TSC2* and TAND once levels of IA had been controlled for. Our findings therefore underline the importance of controlling for the levels of IA in any future study that may wish to compare or contrast TAND in individuals with *TSC1* and *TSC2* mutations.

Overall our findings underline the prominent role of IA as a risk marker for TAND manifestations, illustrated the differences in TAND profiles between children and adults over and above IA, and, for the first time, identified male sex as an additional risk marker for TAND. Together, these highlight the need always to consider intellectual ability, age, and sex in any TAND-related research investigation.

Implications for Clinical Practice

The findings reported here support the value of an intellectual ability evaluation of all individuals with TSC. Even though we reported the largest cohort with formal IQ assessments to date ($n = 894$), this represented only 40.4% of the overall TOSCA cohort. Even in expert TSC centers, IQ was therefore not routinely evaluated. With regards to age-related

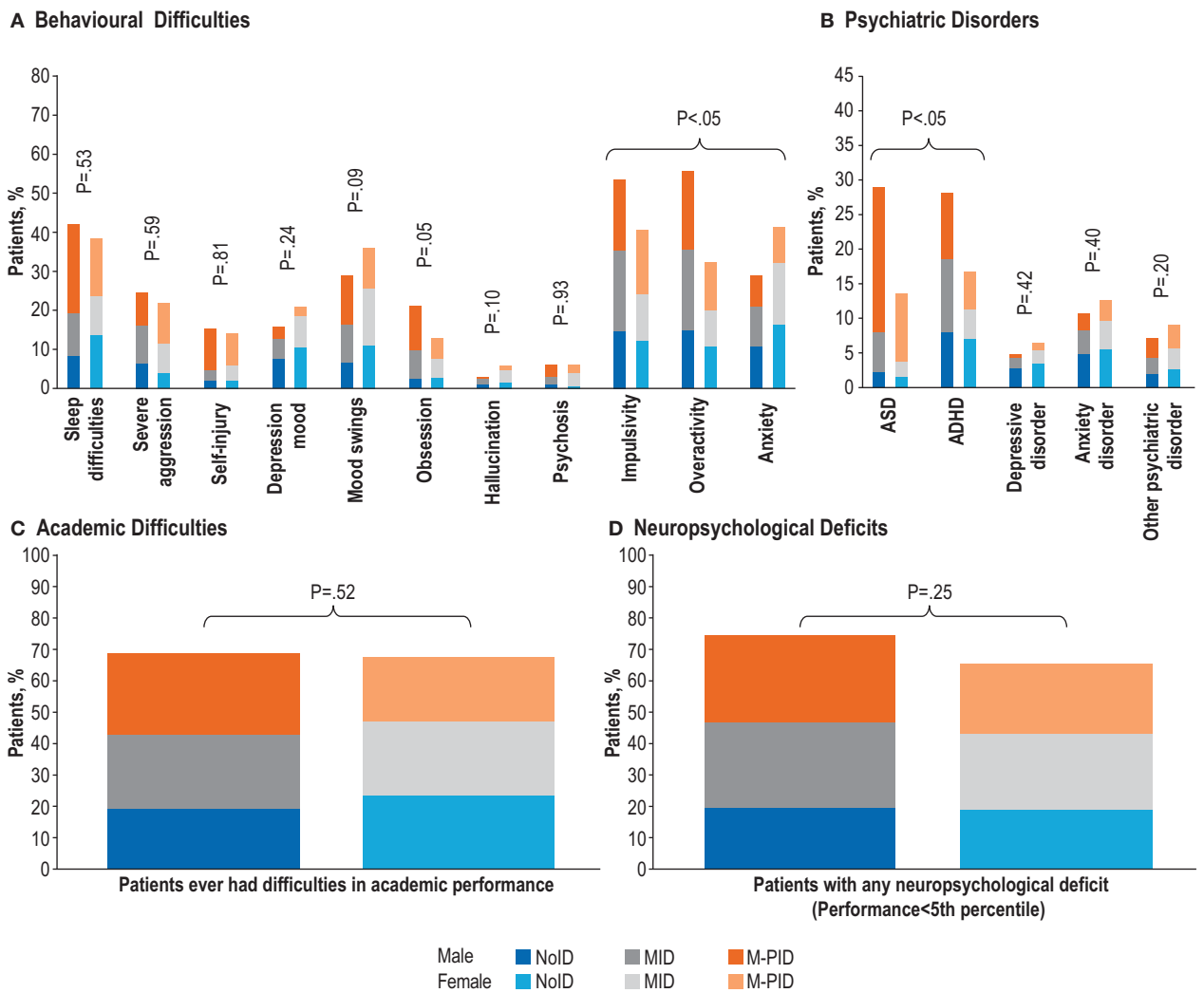

FIGURE 2 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in male vs. female. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated excluding missing/unknown data.

changes, overactivity showed lower rates in adults, but the majority showed higher rates in adults stratified by IA. It will be important not to interpret this as "worsening" of behaviors in adults with TSC given that our dataset was cross-sectional. Longitudinal studies will be important to examine this aspect, but, for clinical practice, results suggest that not all behavioral manifestations may always improve. The clear increase in mood and anxiety symptoms and disorders into adulthood emphasizes the dynamic nature of TAND, and underlines the importance of annual screening for TAND using tools such as the TAND Checklist, as recommended in the International Consensus Guidelines (8, 30). The sex differences observed with higher rates of ASD and ADHD in males with TSC are in keeping with general population observations, and raise interesting scientific questions. From a clinical perspective, even though some sex differences were observed, it is also clear that all males and females should be monitored for all TAND manifestations. At a clinical level the absence of genotype-TAND correlations suggests that, apart from the greater likelihood of ID in association with *TSC2*, clinicians should not suggest to families to expect significantly different TAND profiles in an individual with *TSC1* vs. *TSC2*. All individuals with TSC should therefore be screened and monitored for all TAND manifestations throughout their lifespan.

Limitations

We acknowledge the limitations intrinsic to a large-scale, international, non-interventional/observational study. These included the fact that participants were recruited from expert TSC centers around the world, included evaluation in a range of languages, and the fact that evaluations were performed based on standard clinical practice in each center, rather than on a pre-specified set of evaluation instruments. However, these

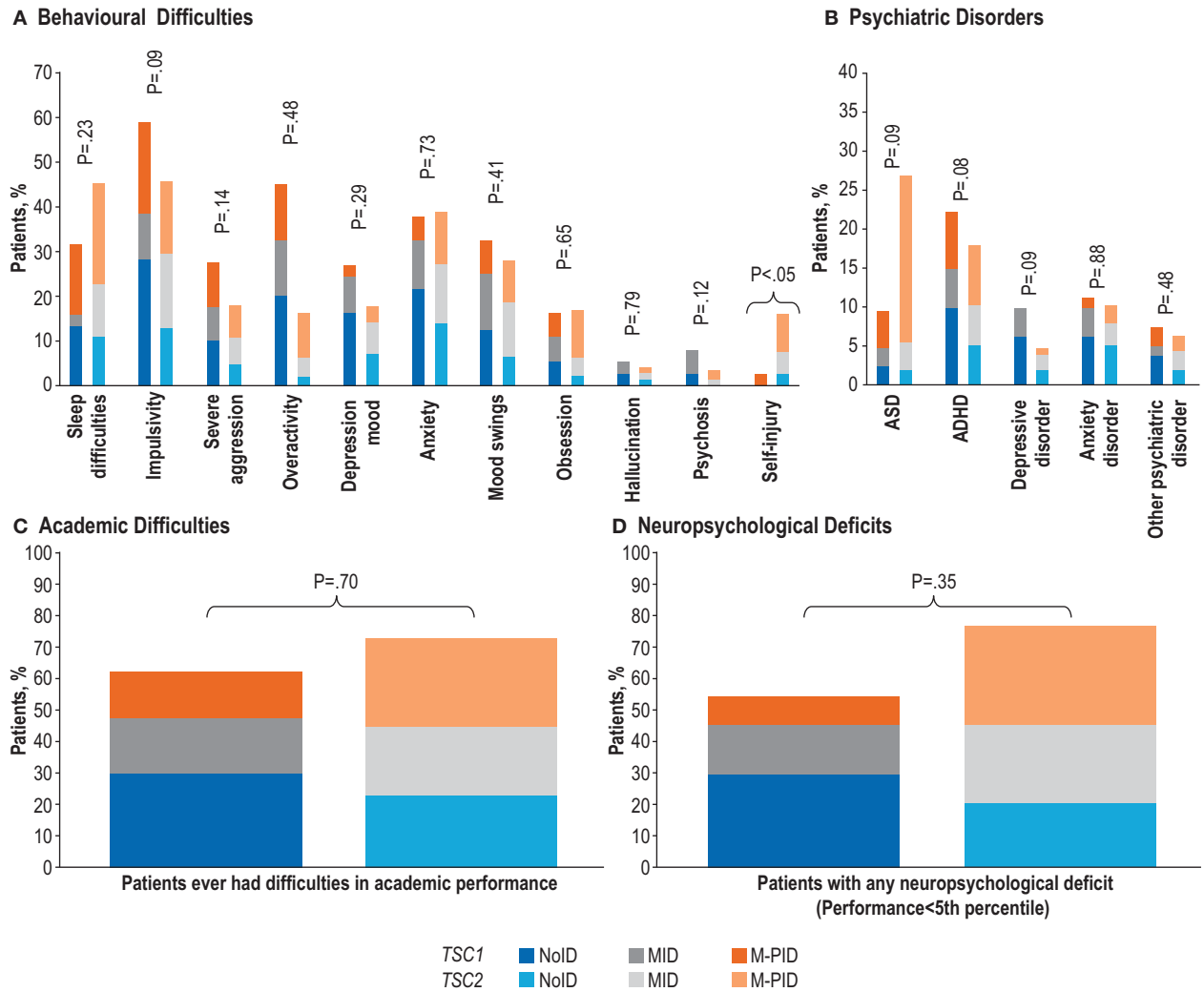

FIGURE 3 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in *TSC1* vs. *TSC2*. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated excluding missing/unknown data.

limitations are, at least in part, off-set by the large-scale and "real-world" nature of the cohort across multiple centers and countries. We acknowledge the high proportion of non-reported (missing) data by sites, including IA evaluation on only 40.4% of the cohort. This finding emphasizes that, even in expert TSC centers, TAND manifestations are often not examined and therefore not treated. We also acknowledge that we focused here on the association between intellectual ability, age, sex, and genotype and that we did not include the potential contributions of physical risk markers (e.g., seizures, SEGA or other TSC manifestations) into our modeling of associations.

CONCLUSION

The TOSCA study confirmed the association between levels of IA and TAND manifestations, suggesting IA as risk marker for most TAND manifestations and provided the first evidence of a male preponderance of ASD and ADHD in individuals with TSC. The study also confirmed the association between *TSC2* and IA but disproved the previously reported *TSC2* association with ASD and most other TAND manifestations once controlled for IA. Overall, the study reinforces the high frequency of TAND manifestations in all individuals with TSC across age, sex, and genotype, and strengthens the evidence-base for regular screening, comprehensive evaluation and intervention for the dynamic and variable range of neuropsychiatric manifestations associated with TSC.

DATA AVAILABILITY STATEMENT

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The

protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The study was designed and conducted in accordance with the Good Clinical Practice principles, the Declaration of Helsinki, and all the local regulations. The Institutional Review Board or Ethics Committee at each participating center approved all the TOSCA related documents. Written informed consent was obtained from all participants, parents, or guardians prior to enrolment.

List of Ethics Committees

The study protocol and all amendments were reviewed and approved (if applicable) by independent Ethics Committee/Institutional Review Board for each centre: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); Comité Etico Investigación Clínica de Euskadi (CEIC-E); Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; Research Ethics Committee of the University of Tartu (UT REC); Ethikkommission der Medizinischen Universität Graz; North Wales REC – West; Regionala Etikprövningsnämnden i Göteborg; REK – Regionale komiteer for medisinsk og helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie "Pomnik Centrum Zdrowia Dziecka"; Ethikkommission bei der Ludwig-Maximilians-Universitat München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Deveropment of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Childrens Hospital of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yan-sen University; The First Affiliated Hospital of Guangzhou Medical University; Shenzhen Children's Hospital; West China Hospital, Sichuan University; Xijing Hospital; Children's Hospital of Chongqing Medical University; Wuhan Children's Hospital; The Second Affiliated Hospital of Xi'an Jiaotong University; Guangdong 999 Brain Hospital; Seoul National University Hospital Institutional Review Board; National Taiwan University Hospital (NTUH) Research Ethics Committee (REC); Institutional Review Board of the Taichung Veterans General Hospital; Institutional Review Board of Chung Shan Medical University Hospital; Institutional Review Board, Tungs' Taichung MetroHarbor Hospital; Institutional Review Board of National Cheng Kung University Hospital; Metro South Human Research Ethics Committee; Sydney Children's Hospital Network Human Research Ethics Committee; St Vincents Hospital Human Research Ethics Committee; Royal Melbourne Hospital Human Research Ethics Committee; Siriraj Institutional Review Board; The Institutional Review Board, Faculty of Medicine, Chulalongkorn University, King Chulalongkorn Memorial Hospital; The Committee on Human Rights Related to Research Involving Human Subjects; Institutional Review board, Royal Thai Army Medical Department IRB RTA, Phramongkutklao College of Medicine; Research Ethics Committee, Faculty of Medicine, Chiang Mai University; Research and Development, Queen Sirikit National Institute of Child Health; Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town; Shaare Zedek Meidcla Center Helsinki Committee; Sheba Medical Center Helsinki Committee; Tel Aviv Sourasly Medical Center Helsinki Committee; General University Hospital of Patras Ethics Committee; Pendeli Children's Hospital Ethics Committee; General University Hospital of Athens "G. 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PV, TC, VC, GB, CF, FO'C, JQ, YT, and SY designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. EB, MB, PC, MD, JF, MF, CH, SJ, JK, JL, AM, RN, VS, MS, RT, BZ, and AJ designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. LD'A designing the study, trial management, data collection, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. RM designing the study, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. SS designing the study, trial statistician, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00603/full#supplementary-material

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Renal Manifestations of Tuberous Sclerosis Complex: Key Findings From the Final Analysis of the TOSCA Study Focussing Mainly on Renal Angiomyolipomas

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Renal angiomyolipomas are one of the most common renal manifestations in patients with tuberous sclerosis complex (TSC), with potentially life-threatening complications and a poor prognosis. Despite the considerable progress in understanding TSC-associated renal angiomyolipomas, there are no large scale real-world data. The aim of our present study was to describe in detail the prevalence and outcome of renal angiomyolipomas in patients with TSC, enrolled into the TuberOus SClerosis registry to increase disease Awareness (TOSCA) from 170 sites across 31 countries worldwide. We also sought to evaluate the relationship of TSC-associated renal angiomyolipomas with age, gender

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and genotype. The potential risk factors for renal angiomyolipoma-related bleeding and chronic kidney disease (CKD) were studied in patients who participated in the TOSCA renal angiomyolipoma substudy. Of the 2,211 eligible patients, 1,062 (48%) reported a history of renal angiomyolipomas. The median age of TSC diagnosis for the all subjects ($n = 2,211$) was 1 year. The median age of diagnosis of renal angiomyolipoma in the 1,062 patients was 13 years. Renal angiomyolipomas were significantly more prevalent in female patients ($p < 0.0001$). Rates of angiomyolipomas >3 cm ($p = 0.0119$), growing lesions ($p = 0.0439$), and interventions for angiomyolipomas ($p = 0.0058$) were also higher in females than males. Pre-emptive intervention for renal angiomyolipomas with embolisation, surgery, or mammalian target of rapamycin (mTOR) inhibitor may have abolished the gender difference in impaired renal function, hypertension, and other complications. The rate of interventions for angiomyolipomas was less common in children than in adults, but interventions were reported in all age groups. In the substudy of 76 patients the complication rate was too low to be useful in predicting risk for more severe CKD. In addition, in this substudy no patient had a renal hemorrhage after commencing on an mTOR inhibitor. Our findings confirmed that renal angiomyolipomas in subjects with *TSC1* mutations develop on average at the later age, are relatively smaller in size and less likely to be growing; however, by age 40 years, no difference was observed in the percentage of patients with *TSC1* and *TSC2* mutations needing intervention. The peak of appearance of new renal angiomyolipomas was observed in patients aged between 18 and 40 years, but, given that angiomyolipomas can occur later, lifelong surveillance is necessary. We found that pre-emptive intervention was dramatically successful in altering the outcome compared to historical controls; with high pre-emptive intervention rates but low rates of bleeding and other complications. This validates the policy of surveillance and pre-emptive intervention recommended by clinical guidelines.

Keywords: mTOR, registry, renal angiomyolipoma, TOSCA, tuberous sclerosis complex

INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare, autosomal dominant genetic disorder characterized by hamartomatous lesions in multiple organs such as brain, kidneys, skin, lungs, eyes, and heart (1, 2). Renal manifestations are one of the most common causes of morbidity and were historically reported as the primary cause of death in adult TSC patients (3–5). The relative importance of mechanisms postulated to lead to impaired renal function are unknown (6) but a major risk factor may be intervention for renal angiomyolipomas (7).

Renal angiomyolipomas are the most common renal manifestations in patients with TSC, with an estimated prevalence ranging from 55 to 80% (8–11). They are usually multiple and bilateral, progress with age and cause more problems in females (12, 13). Angiomyolipomas >3 cm in diameter have an increased risk of bleeding or invade adjacent normal renal parenchyma, potentially leading to kidney failure (10, 14). A retrospective cohort study showed that modifiable factors such as hypertension, proteinuria, and hyperfiltration occur frequently and early in patients with TSC and could play an important role in the development of chronic kidney disease (CKD) in these patients (15). Renal cysts, although asymptomatic in most patients, may be aggressive due to associated polycystic disease in a minority of patients and can even result in development of end stage renal disease in childhood or early adulthood (10, 16). Mutation studies have shown the occurrence and severity of TSC-associated renal angiomyolipomas and cysts to be higher among patients with *TSC2* mutation than those with *TSC1* mutation (8, 17).

Previously we have reported interim analysis data of the TOSCA (TuberOus SClerosis registry to increase disease Awareness) study, highlighting the burden of TSC-associated renal angiomyolipoma and showed that renal angiomyolipomas are initially asymptomatic, influenced by gender and genotype and can occur in younger patients (13). Here we present the final analysis data of the TOSCA registry with detailed overall characteristics of TSC-associated renal angiomyolipoma and its association with age, gender, and genotype. We have also analyzed possible risk factors for bleeding from renal angiomyolipomas and for CKD in patients with TSC from the TOSCA renal angiomyolipoma substudy.

MATERIALS AND METHODS

The study methodology has been published previously (18). In brief, TOSCA was a large-scale non-interventional study in patients with TSC. The study was designed with a core section and six ancillary substudies (research projects with more detailed focus on subependymal giant cell astrocytomas, renal angiomyolipoma, and lymphangioleiomyomatosis, genetics, TSC-associated neuropsychiatric disorder, epilepsy, and patient's quality of life). Here we present findings from the core study and renal angiomyolipoma substudy.

The TOSCA study was designed and conducted according to the Guidelines for Good Clinical Practice and ethical principles outlined in the Declaration of Helsinki. Written informed consent was obtained from all patients, parents, or guardians prior to enrolment with prior endorsement by the local human research ethics committee.

TABLE 1 | Baseline patient demographics and clinical characteristics.

Characteristic	All patients (*N* = 2,211)	Patients with renal angiomyolipoma (*N* = 1062)
Patients by age at consent		
≤2 years	282 (12.8)	25 (2.4)
>2 to ≤5 years	301 (13.6)	76 (7.2)
>5 to ≤9 years	334 (15.1)	133 (12.5)
>9 to ≤14 years	307 (13.9)	164 (15.4)
>14 to <18 years	138 (6.2)	79 (7.4)
≥18 to≤40 years	625 (28.3)	411 (38.7)
>40 years	224 (10.1)	174 (16.4)
Median (range) age at diagnosis of TSC,[a] years	1.0 (<1–69)	1.0 (<1–67)
Gender		
Male	1,059 (47.9)	447 (42.1)
Female	1,152 (52.1)	615 (57.9)
Genetic molecular testing performed	1,011 (45.7)	525 (49.4)
Genetic testing results[b,c]		
No mutation identified	148 (14.6)	80 (15.2)
TSC1 mutation	191 (18.9)	63 (12.0)
TSC2 mutation	649 (64.2)	373 (71.0)
Both *TSC1* and *TSC2* mutations	5 (0.5)	2 (0.4)
Mutation variation type[c]		
Only pathogenic mutation	663 (65.6)	343 (65.3)
Only variant of unknown significance	43 (4.3)	23 (4.4)
Both	23 (2.3)	5 (1.0)
Time from TSC clinical diagnosis to molecular testing, months, mean (SD)	81.8 (116.58)	118.3 (133.4)
Patients with prenatal TSC diagnosis	154 (7.0)	53 (5.0)

SD, standard deviation; TSC, tuberous sclerosis complex.
Values are expressed as n (%) unless otherwise specified. [a]Data available for 2,174 patients (all patients) and 1050 patients (cohort with renal angiomyolipoma at baseline). [b]Genetic testing results were not available for 18 patients (all patients) and 7 patients (cohort with renal angiomyolipoma at baseline). [c]Percentages were calculated from number of patients with genetic molecular testing performed.

Participants and Procedure

In the core study, patients of any age with TSC were enrolled from 170 sites across 31 countries and were followed for up to 5 years. Investigators from 18 sites across eight countries also agreed to participate in this renal angiomyolipoma substudy and enrolled a total of 76 patients, after receiving separate informed consent from the patients.

In the core study, patient data including demographics and clinical features of TSC across all organ systems, comorbidities, and rare manifestations, were collected at baseline and at regular visits scheduled at a maximum interval of 1 year. For the purpose of this manuscript, we presented data specific to renal

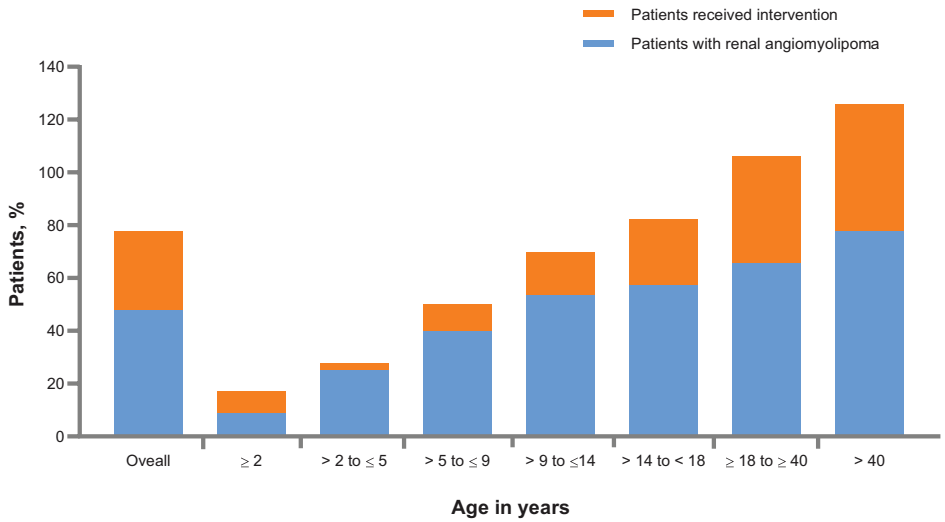
TABLE 2 | Clinical characteristics of renal angiomyolipoma in overall population.

Characteristic	Baseline *N* = 2,211	Follow-up 1 *N* = 2,099	Follow-up 2 *N* = 1,935	Follow-up 3 *N* = 1,664
Past history of renal angiomyolipoma	1,062 (48.0)	–	–	–
Median (range) age at angiomyolipoma diagnosis, years	13 (<1–67)	–	–	–
Renal angiomyolipoma ongoing during the study[a]	1,024 (96.4)	1,024 (96.0)	1,002 (96.3)	909 (96.2)
Multiple	901 (88.0)	896 (87.5)	880 (87.8)	822 (90.4)
Bilateral	859 (83.9)	854 (83.4)	834 (83.2)	784 (86.2)
Lesion >3 cm	342 (33.4)	327 (31.9)	320 (31.9)	282 (31.0)
Growing	216 (21.1)	193 (18.8)	205 (20.5)	168 (18.5)
Renal angiomyolipoma symptoms and complications[b]				
None	840 (82.0)	894 (87.3)	885 (88.3)	816 (89.8)
Elevated blood pressure	58 (5.7)	48 (4.7)	42 (4.2)	38 (4.2)
Hematuria (blood in urine)	43 (4.2)	31 (3.0)	22 (2.2)	20 (2.2)
Hemorrhage	55 (5.4)	16 (1.6)	15 (1.5)	13 (1.4)
Impaired renal function	39 (3.8)	35 (3.4)	36 (3.6)	34 (3.7)
Pain	63 (6.2)	37 (3.6)	27 (2.7)	17 (1.9)
Other	30 (2.9)	13 (1.3)	16 (1.6)	12 (1.3)
Patients received treatment for angiomyolipoma[c]	315 (29.7)	300 (28.1)	321 (30.8)	288 (30.5)
mTOR inhibitor	144 (45.7)	49 (16.3)	28 (8.7)	26 (9.0)
Embolization	141 (44.8)	9 (3.0)	9 (2.8)	3 (1.0)
Nephrectomy	63 (20.0)	5 (1.7)	3 (0.9)	1 (0.3)
Resection	21 (6.7)	1 (0.3)	2 (0.6)	0
Dialysis	4 (1.3)	1 (0.3)	1 (0.3)	0
Other	13 (4.1)	1 (0.3)	5 (1.6)	1 (0.3)

mTOR, mammalian target of rapamycin.
Values are expressed as n (%) unless otherwise specified. [a]Percentages calculated based on denominator of patients with history of renal angiomyolipoma. [b]Percentages calculated from number of patients with renal angiomyolipoma ongoing during the study. [b]The numbers include patients who experienced more than one symptoms simultaneously. [c]Treatment received as monotherapy or polytherapy.

angiomyolipoma including occurrence rate, annual incidence of newly diagnosed angiomyolipoma, maximum diameter on ultrasound or magnetic resonance imaging, clinical symptoms and complications, and management at baseline and during follow-up. The number of patients who completed follow-up 4 and follow-up 5 visits were low due to their late enrolment in the study, and hence follow-up data of only the first 3 years of the core study are reported here.

In the 76 patients in the renal substudy data was collected on; prevalence and size of renal angiomyolipomas and complication rates (including bleeding, hypertension, and CKD). We also present the effects of treatment with embolization or mammalian



Patients received intervention, n	315	2	2	14	27	20	166	84
Patients with a history of renal angiomyolipoma, n	1062	25	76	133	164	79	411	174

FIGURE 1 | Patients with history of renal angiomyolipoma and intervention received across age groups at baseline.

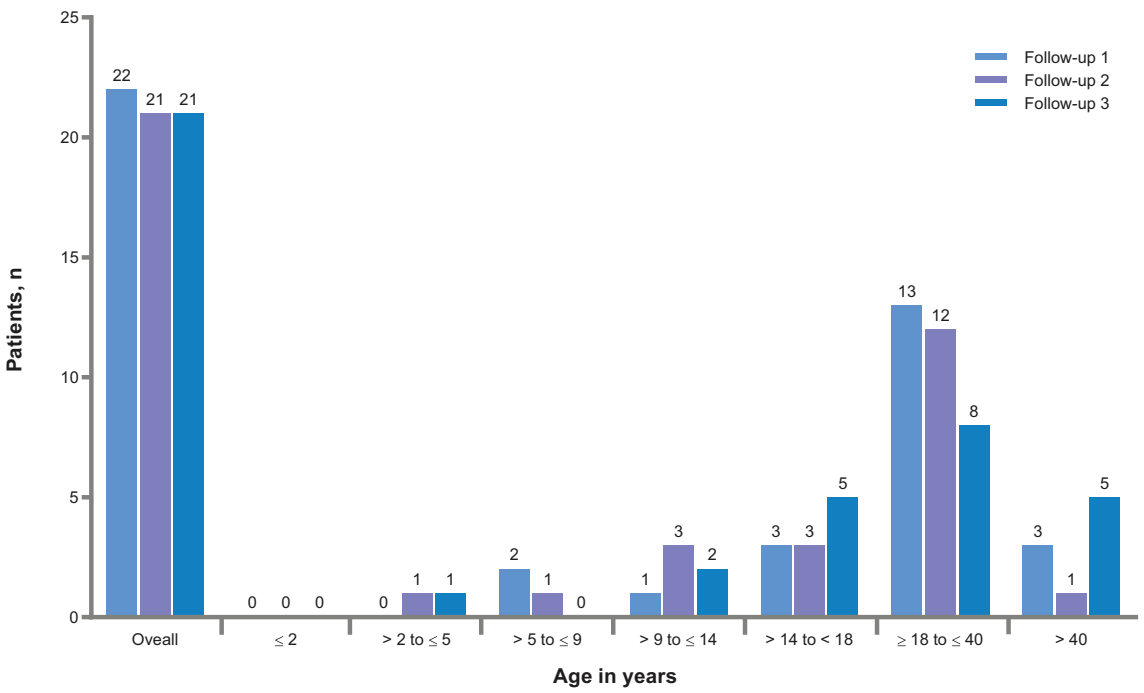


FIGURE 2 | Newly diagnosed renal angiomyolipoma after baseline visit.

target of rapamycin (mTOR) inhibitors on the risk of renal impairment. For the substudy, only the baseline data are reported here, as very few patients had follow-up visits due to their late enrolment in the study.

Data Analyses

All eligible patients enrolled in the TOSCA registry and renal angiomyolipoma substudy, without any major protocol deviations, were included in the analysis. Given that the study was observational in nature, results reported in this manuscript are primarily descriptive statistics. Continuous variables were evaluated quantitatively (e.g., frequency, mean, standard deviation, median, range), and categorical variables (e.g., presence/absence of a manifestation) were analysed in terms of frequency distribution at baseline and at follow-ups.

The Cochran–Mantel–Haenszel test was performed to evaluate the rates of renal angiomyolipomas stratified by age groups (<18 and ≥18 years), gender (male and female) and mutation (*TSC1* and *TSC2*). The exact binomial test was used to evaluate the difference between proportion of patients with renal angiomyolipomas and those received treatment among both genders, regardless of age, and genetic mutation. Furthermore, we evaluated reported association of angiomyolipoma-related variables at baseline visit (rates of angiomyolipomas, angiomyolipomas with lesion >3 cm, growing angiomyolipomas, treatment of angiomyolipomas and symptoms) by age (<18 vs. ≥18 years), gender (male vs. female) and mutation (*TSC1* vs. *TSC2*) using Chi-square test. Statistical significance was set at $p < 0.05$.

RESULTS

Findings From the Core Study

A total of 2,214 patients were enrolled from 170 sites across 31 countries. Of these, data of 2,211 eligible patients were analysed. Data of three patients were excluded due to major protocol deviations. Most patients were enrolled at sites where the principal investigators were pediatric neurologists (53%) or neurologists (17%).

Baseline demographics and clinical characteristics are summarized in **Table 1**. There were more females (52.1%) than males (47.9%), the majority of patients were under the age of 18 years (61.6%) and the median age at consent for the study was 13 years. The median age at first TSC diagnosis was 1 year (mean 6.9 years, range: <1–69 years). Molecular genetic testing was performed in 1,011 patients (45.7%). Of these, 64.2% had a *TSC2* mutation and 18.9% *TSC1* mutation. In 14.6% of patients, no mutation was identified. Of the 1,011 tested patients, 663 (65.6%) had pathogenic mutation, 43 (4.3%) had a variant of unknown significance and 23 patients (2.3%) had both a pathogenic mutation and variant of unknown significance. In 282 patients, the pathogenicity of the mutation was not recorded. Prenatal diagnosis of TSC was reported in 154 patients (7%). Parents of 1,036 of 2,211 patients (56.3%) were evaluated for TSC. Of these, 180 (17.4%) had mother, 126 (12.2) had fathers and 4 (0.4%) had both parents diagnosed with TSC. A considerable proportion of patients (23.6%) had relatives affected with TSC and patients with relatives also enrolled in TOSCA (10.6%).

Clinical Characteristics of Renal Angiomyolipomas

A history of renal angiomyolipomas was reported in 1,062 (48%) patients (**Table 2**, **Figure 1**). Baseline demographics of cohort with renal angiomyolipomas were similar to the overall cohort (**Table 1**). Of 1,024 patients (96.4%) with ongoing renal angiomyolipoma, 901 (88%) had multiple lesions, 859 (83.9%) had bilateral lesions, 342 (33.4%) had lesions >3 cm in size and 216 (21.1%) had growing lesions. The median age at diagnosis

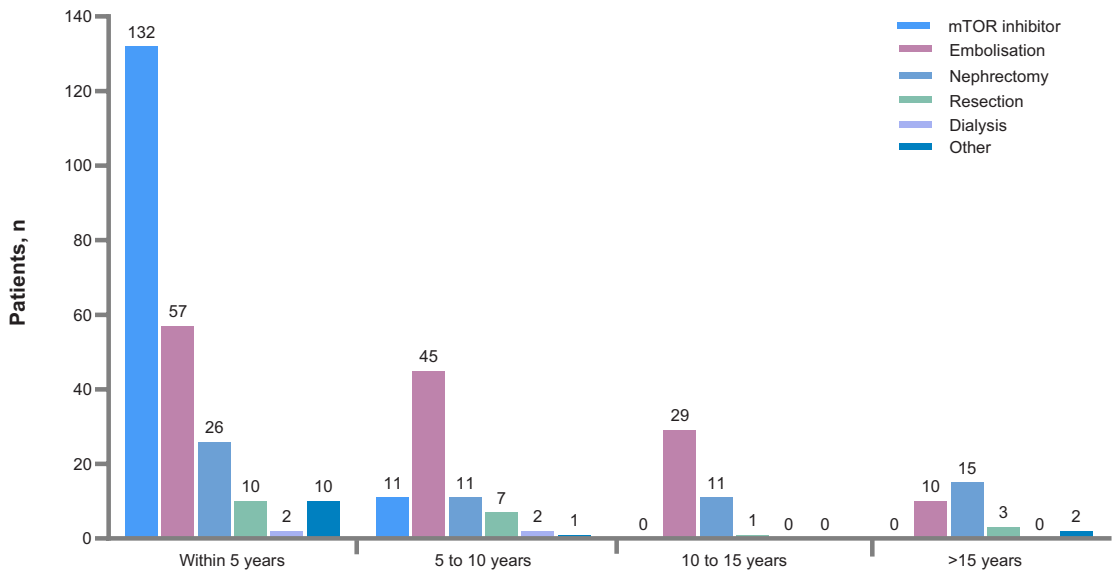


FIGURE 3 | Time since interventions prior to baseline. mTOR, mammalian target or rapamycin.

was 13 years (mean 17 years, range <1–67 years). Median time from the previous scan to last assessment was 1 year (range, <1–21).

Renal angiomyolipomas were asymptomatic in most patients (840 of 1,024 patients, 82%). Very few patients experienced renal angiomyolipoma-related symptoms or complications (**Table 2**). After baseline visit, newly diagnosed renal angiomyolipomas were reported in 22 (2.1%), 21 (2.0%), and 21 (2.2%) patients at follow-up 1, follow-up 2, and follow-up 3, respectively (**Figure 2**). A total of 315 patients (29.7%) had received treatment for renal angiomyolipomas at baseline. In these patients, mTOR inhibitors (45.7%), embolization (44.8%), and nephrectomies (20%) were the common treatment modalities. During the follow-ups, more patients received treatment with mTOR inhibitors than embolization (**Table 2**), and mTOR inhibitors appear to become a predominant treatment in recent years (**Figure 3**). However, the rate of nephrectomy was similar in each period prior to baseline.

Relationship of Renal Angiomyolipoma With Age

The proportion of patients with angiomyolipomas increased with age (from 8.9% in patients aged ≤2 years to 77.7% in patients aged >40 years. Similarly, use of pre-emptive treatment increased with age (**Figure 1**). Newly diagnosed renal angiomyolipomas were more common in adults (**Figure 2**). There was an increased rate of symptoms and complications with age (**Table 3**). Embolization

TABLE 3 | Renal angiomyolipoma symptoms and complications stratified by age.

Complication and symptom	Overall (*N* = 2,211)	Age at consent, years						
		≤2 (*n* = 282)	>2 to ≤5 (*n* = 301)	>5 to ≤9 (*n* = 334)	>9 to ≤14 (*n* = 307)	>14 to <18 (*n* = 138)	≥18 to ≤40 (*n* = 625)	>40 (*n* = 224)
None	840 (82.0)	23 (100.0)	74 (100.0)	122 (96.1)	147 (93.0)	71 (92.2)	298 (74.7)	105 (63.3)
Elevated blood pressure[a]	58 (5.7)	0 (0)	0 (0)	0 (0)	5 (3.2)	5 (6.5)	25 (6.3)	23 (13.9)
Hemorrhage[a]	43 (4.2)	0 (0)	0 (0)	0 (0)	2 (1.3)	1 (1.3)	23 (5.8)	17 (10.2)
Haematuria[a]	55 (5.4)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	37 (9.3)	18 (10.8)
Impaired renal function[a]	39 (3.8)	0 (0)	0 (0)	1 (0.8)	2 (1.3)	0 (0)	16 (4.0)	20 (12.0)
Pain[a]	63 (6.2)	0 (0)	0 (0)	1 (0.8)	1 (0.6)	1 (1.3)	38 (9.5)	22 (13.3)
Other	30 (2.9)	0 (0)	0 (0)	3 (2.4)	2 (1.3)	1 (1.3)	17 (4.3)	7 (4.2)

All the values are expressed as n (%). [a] *The numbers include patients who experienced more than one symptom simultaneously.*

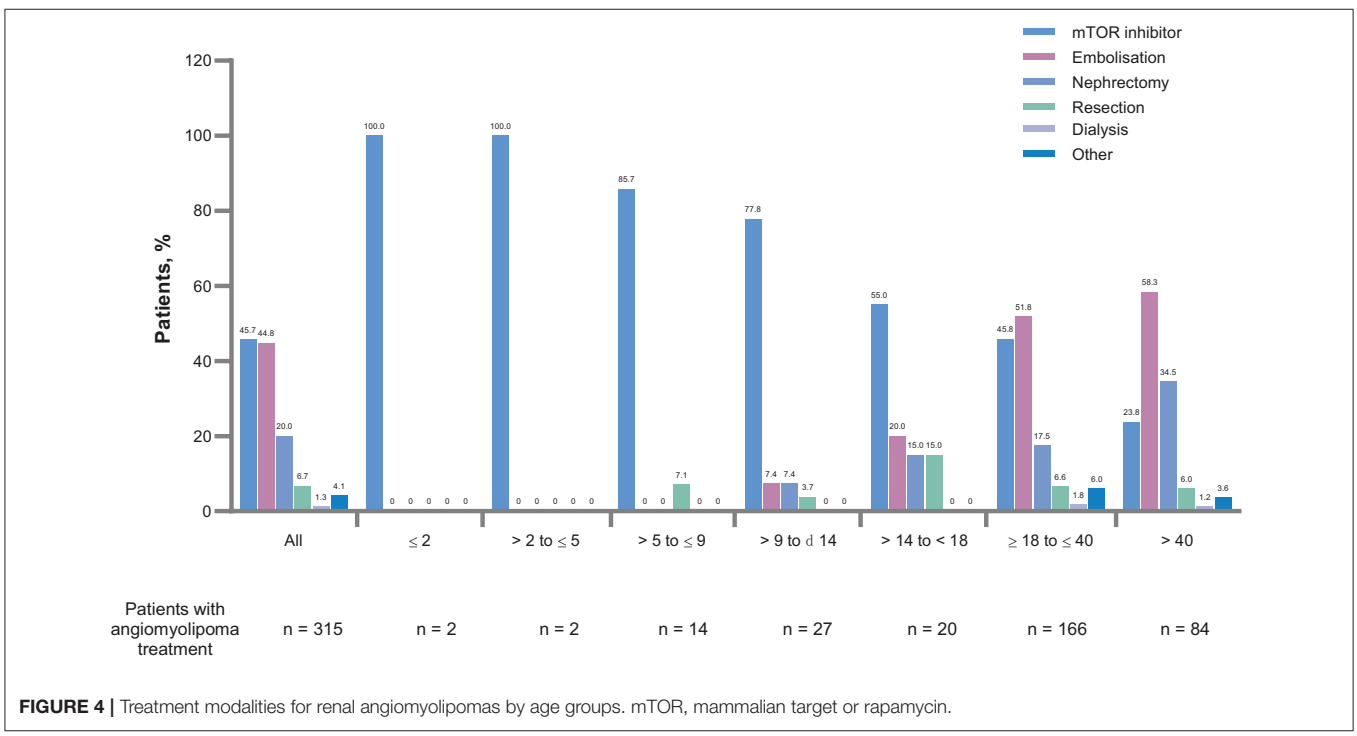


FIGURE 4 | Treatment modalities for renal angiomyolipomas by age groups. mTOR, mammalian target or rapamycin.

TABLE 4 | Clinical characteristics of renal angiomyolipoma by gender.

Characteristics	Female $N = 1,152$	Male $N = 1,059$	Odds ratio (95% CI)	*P*-value
Past history of renal angiomyolipoma	615 (53.4)	447 (42.2)	1.6 (1.3, 1.9)	<0.0001
Median (range) age at angiomyolipoma diagnosis, years	14 (<1–63)	11 (<1–67)	–	0.9891
Renal angiomyolipoma ongoing during the study[a]	590 (95.9)	434 (97.1)		
Multiple	524 (88.8)	377 (86.9)	1.2 (0.8, 1.8)	0.3436
Bilateral	502 (85.1)	357 (82.3)	1.3 (0.9, 1.8)	0.1585
Lesion >3 cm	212 (35.9)	130 (30.0)	1.4 (1.1, 1.9)	0.0119
Growing	135 (22.9)	81 (18.7)	1.4 (1.0, 1.9)	0.0439
Renal angiomyolipoma signs and symptoms[b,c]				
None	466 (79.0)	374 (86.2)	0.6 (0.4, 0.8)	0.0031
Elevated blood pressure	31 (5.3)	27 (6.2)	0.8 (0.5, 1.4)	0.5083
Haematuria (blood in urine)	29 (4.9)	14 (3.2)	1.6 (0.8, 3.0)	0.1829
Hemorrhage	41 (6.9)	14 (3.2)	2.2 (1.2, 4.2)	0.0090
Impaired renal function	27 (4.6)	12 (2.8)	1.7 (0.8, 3.4)	0.1345
Pain	50 (8.5)	13 (3.0)	3.0 (1.6, 5.6)	0.0003
Others	22 (3.7)	8 (1.8)	2.1 (0.9, 4.7)	0.0771
Treatment received for renal angiomyolipoma[d]	203 (33.0)	112 (25.1)		
mTOR inhibitor	95 (46.8)	49 (43.8)	1.1 (0.7, 1.78)	0.6395
Embolization	84 (41.4)	57 (50.9)	0.7 (0.4, 1.1)	0.0894
Nephrectomy	47 (23.2)	16 (14.3)	1.8 (1.0, 3.4)	0.0629
Resection	16 (7.9)	5 (4.5)	1.8 (0.6, 5.1)	0.2503
Dialysis	3 (1.5)	1 (0.9)	1.7 (0.2, 16.1)	0.6618
Other	10 (4.9)	3 (2.7)	1.9 (0.5, 6.9)	0.3428

CI, confidence interval; mTOR, mammalian target of rapamycin.
Values are expressed as n (%) unless otherwise specified. [a]Percentages calculated based on denominator of patients with history of renal angiomyolipoma. [b]Percentages calculated from number of patients with renal angiomyolipoma ongoing during the study. [c]The numbers include patients who experienced more than one symptom simultaneously. [d]Treatment received as monotherapy or polytherapy.

was more common in adults (54% vs. 9.2%), whereas children were mostly treated with mTOR inhibitors (73.8 vs. 38.4%), **Figure 4**).

Relationship of Renal Angiomyolipoma With Gender

Of the 2,211 enrolled patients, 1,152 (52.1%) were female and 1,059 (47.9%) were male. A history of renal angiomyolipomas was reported at a significantly higher frequency in female than male patients (53.4 vs. 42.2%, $p < 0.0001$, **Table 4**). Newly diagnosed renal angiomyolipomas were also more common in female patients (2.3 vs. 1.8%). The gender difference (female vs. male) in the rates of renal angiomyolipomas remained statistically significant when stratified by age [<18 years [38.97 vs. 31.54%]; $p < 0.0001$ and ≥18 years [71.35 vs. 65.18%]; $p < 0.0001$].

The median age at diagnosis of renal angiomyolipomas in female patients was 14 years (mean 18.4 years, range <1–63 years), while it was 11 years (mean 15.1 years, range <1–67 years) in male patients. The difference in the age at diagnosis between male and female patients were not significant ($p = 0.9891$). Five hundred and ninety females and 434 males had renal angiomyolipomas ongoing during the study. There was no significant differences between females and males in the occurrence of multiple lesions (88.8 vs. 86.9%, $p = 0.3436$) and bilateral angiomyolipomas (85.1 vs. 82.3%, $p = 0.1585$). Compared to males, females had significantly higher rates of lesions >3 cm in size (35.9 vs. 30.0%, $p = 0.0119$) and growing lesions (22.9 vs. 18.7%, $p = 0.0439$) at baseline. In both male and female patients, renal angiomyolipomas were asymptomatic in most patients at baseline (male: 86.2 vs. female: 79%). Most angiomyolipoma-related symptoms occurred equally in females and males. These include elevated blood pressure (5.3 vs. 6.2%, $p = 0.5083$), haematuria (4.9 vs. 3.2%, $p = 0.1829$) and impaired renal function (4.6 vs. 2.8%, $p = 0.1345$). However, compared to males, females had significantly higher rates of hemorrhage (6.9 vs. 3.2%, $p = 0.0090$) and pain (8.5 vs. 3%, $p = 0.0003$). Overall, the rate of intervention at baseline were significantly higher among females than males (33 vs. 25.1%, $p = 0.0058$). However, there was no significant gender difference (male vs. female) observed in the rates of specific interventions: embolization (50.9 vs. 41.4%; $p = 0.0894$), mTOR inhibitors (46.8 vs. 43.8%; $p = 0.6395$), nephrectomy (23.2 vs. 14.3%; $p = 0.0629$), resection (7.9 vs. 4.5%; $p = 0.2503$), and dialysis (1.5 vs. 0.9%; $p = 0.6618$).

Relationship of Renal Angiomyolipoma With Mutation Type

The prevalence of angiomyolipomas was significantly higher in patients with *TSC2* vs. *TSC1* mutations (57.5 vs. 33%, $p < 0.0001$; **Table 5**). The mean age at diagnosis of renal angiomyolipomas was 13.3 years (median, 9 years, range <1–59 years) in patients with a *TSC2* mutations, while it was 22.5 years (median 21 years, range <1–60 years) in those with a *TSC1* mutations. Patients with *TSC2* mutations also had significantly higher rates of multiple angiomyolipomas (92.3 vs. 67.2, $p < 0.0001$), bilateral angiomyolipomas (87 vs. 47.5%, $p < 0.0001$) angiomyolipoma lesions >3 cm (31.2 vs. 11.5%, $p = 0.0013$) and growing angiomyolipomas (23.2 vs. 9.8%, $p = 0.0150$).

Similar to the overall sample, renal angiomyolipomas were asymptomatic in most patients with *TSC1* (90.2%) and *TSC2* (83.1%) mutations. However, bleeding events were observed only in patients with *TSC2* mutations (haematuria, 3.9% and hemorrhage, 5.2%). No significant difference in the rates of intervention of any sort was observed between those with *TSC1* mutations and *TSC2* mutations ($p < 0.0801$, **Table 5**).

Other Renal Manifestations

The other renal features reported at baseline were multiple renal cysts (24.6%), polycystic kidney disease (proven TSC2/PKD1 mutation; 3.4%), renal malignancy (1.4%), and impaired renal function (non-angiomyolipoma-related; 1.9%) (**Table 6**). Compared with patients with a *TSC1* mutation, those with *TSC2* mutations had a higher occurrence of multiple

TABLE 5 | Clinical characteristics of renal angiomyolipoma by mutational status.

Characteristics	Patients with *TSC1* mutation *N* = 196	Patients with *TSC2* mutation *N* = 654	Odds ratio (95% CI)	*p*-value
Past history of renal angiomyolipoma	63 (33.0)	373 (57.5)	2.8 (2.0, 3.9)	<0.0001
Male	28 (44.4)	169 (45.3)	–	–
Female	35 (55.6)	204 (54.7)	–	–
Median (range) age at angiomyolipoma diagnosis, years	21 (<1–60)	9 (<1–59)	–	0.0035
Renal angiomyolipoma ongoing during the study[a]	61 (93.8)	362 (96.5)		
Multiple	41 (67.2)	334 (92.3)	6.1 (3.1, 11.8)	<0.0001
Bilateral	29 (47.5)	315 (87.0)	8.1 (4.4, 14.7)	<0.0001
Lesion >3 cm	7 (11.5)	113 (31.2)	3.6 (1.6, 8.2)	0.0013
Growing	7 (11.5)	85 (23.5)	2.9 (1.2, 7.2)	0.0150
Renal angiomyolipoma signs and symptoms[b]				
None	55 (90.2)	301 (83.1)	0.6 (0.2, 1.3)	0.1881
Elevated blood pressure	4 (6.6)	23 (6.4)	0.9 (0.3, 2.8)	0.9098
Haematuria (blood in urine)	0	14 (3.9)	NE	0.1234
Hemorrhage	0	19 (5.2)	NE	0.0709
Impaired renal function	1 (1.6)	10 (2.8)	1.7 (0.2, 13.2)	0.6297
Pain	2 (3.3)	24 (6.6)	2.0 (0.5, 8.8)	0.3335
Other	0	9 (2.5)	NE	0.2195
Treatment received for renal angiomyolipoma[a,c]	9 (13.8)	103 (27.5)	–	p<0.0801
mTOR inhibitor	4 (44.4)	56 (54.4)	1.5 (0.4, 5.9)	0.5670
Embolization	2 (22.2)	41 (39.8)	2.3 (0.5, 11.7)	0.2983
Nephrectomy	3 (33.3)	23 (22.3)	0.6 (0.1, 2.5)	0.4534
Resection	1 (11.1)	6 (5.8)	0.5 (0.1, 4.6)	0.5299
Dialysis	0	1 (1.0)	NE (NE)	0.7665
Other	0	3 (2.9)	NE (NE)	0.6038

CI, confidence interval; mTOR, mammalian target of rapamycin; TSC, tuberous sclerosis complex.
Values are expressed as n (%) unless otherwise specified. [a]Percentages calculated based on denominator of patients with history of renal angiomyolipoma. [b]Percentages calculated from number of patients with renal angiomyolipoma ongoing during the study. [c]Treatment received as monotherapy or polytherapy.

renal cysts (33.6 vs. 13.3%) and polycystic kidney disease (4.7 vs. 0%).

Findings From the Angiomyolipoma Substudy

A total of 76 patients [24 (31.6%) male and 52 (68.4%) female] were enrolled into the substudy from eight countries [France (n = 25), United Kingdom (n= 15), Belgium and Japan (n = 11, each), Turkey (n = 6), Poland (n = 4), and Germany and Spain (n = 2, each)]. Most patients were Caucasians (57 patients, 75%). Hypertension was reported in 19 patients (25%). Pre-existing antihypertensive medication was reported in 12 patients (63.2%).

TABLE 6 | Rates of other renal manifestations at baseline in overall population and by mutational status.

	Overall *N* = 2,211	Patients with *TSC1* mutation *N* = 196	Patients with *TSC2* mutation *N* = 654
Renal manifestations in patients with angiomyolipomas			
Multiple renal cysts	544 (24.6)	26 (13.3)	220 (33.6)
Polycystic kidneys	Not applicable*	0	31 (4.7)
Renal malignancy	31 (1.4)	4 (2.0)	8 (1.2)
Renal manifestations in patients without angiomyolipoma			
Impaired renal function	43 (1.9)	6 (3.1)	18 (2.8)

CI, confidence interval; mTOR, mammalian target of rapamycin; N/A, not applicable; TSC, tuberous sclerosis complex.
*Values are expressed as n (%). *PKD was observed only in those with TSC2 mutations.*

Risk Factors of Bleeding From Renal Angiomyolipomas

Of the 76 patients with renal angiomyolipomas, hemorrhage was reported in three patients at baseline, who were not taking mTOR inhibitors (patients aged 31, 34, and 43 years). All three of them were female and had *TSC2* mutations, with largest angiomyolipoma diameter between 66 and 96 mm.

Risk Factors of Chronic Kidney Disease

A total of 42 patients reported CKD at baseline. Of these, seven (16.7%) had grade 3a/3b CKD (GFR 30–59), and four (9.5%) had grade 4 CKD (GFR 15–29). Thirty-six of 42 CKD patients had typical renal angiomyolipomas, eight had atypical renal angiomyolipomas and two had other renal angiomyolipomas. There was no correlation between CKD stage and type of angiomyolipoma. Mean age at diagnosis of renal angiomyolipoma was 14.5 years for patients with grade 1 CKD, 26.4 years for patients with grade 2 CKD, 35 years for patients with grade 3a CKD, 22 years for patients with grade 3b CKD and 34 years for patients with grade 4 CKD. Size of renal angiomyolipomas were between 3 and 180 mm. Simple cysts were reported in 16 patients (38.1%) and polycystic kidney disease in two patients (4.8%). Of the three patients with CKD and cysts, but without renal angiomyolipoma at baseline, two had grade 1 CKD and one had grade 2 CKD.

Effect of Embolization or mTOR Inhibitor Treatment on CKD and Bleeding

Out of 76 patients enrolled, 47 patients received treatment; 20 were treated with mTOR inhibitors alone, four with embolization alone and five with both mTOR inhibitors and embolization at baseline. Among the 20 patients who were treated with mTOR inhibitors alone, eight (40%) had grade 2 CKD, four (20%) had grade 3a/3b CKD, and two had grade 4 CKD. No patient had unselected proteinuria while 7 patients (35%) had

albuminuria grade 1. No patient on mTOR inhibitors alone had renal hemorrhage.

Among the four patients treated with embolization alone, one (25%) had grade 1 CKD, one (25%) had grade 2 CKD, and one (25%) had grade 4 CKD. Data was missing for one patient. One (25%) patient had proteinuria, while two (50%) had grade 1 albuminuria. No patient had renal hemorrhage.

DISCUSSION

The results from this final analysis have several novel observations. The prevalence of angiomyolipoma as well as rates of angiomyolipoma-related complications were higher in females than in male patients. This effect might be attributed to the presence of estrogen and progesterone receptors on the tumors (19). However, the mechanism of hormonal modulation on angiomyolipoma growth is not yet known. Female patients were also more likely to have bilateral, multiple and growing renal angiomyolipoma than male patients. This was in line with the other studies suggesting a higher propensity of angiomyolipoma growth in female patients (9, 20). Angiomyolipomas were dignosed at a later age in females (median age 14 years) than in male patients (median age 11 years), but this difference was not statistcally significant.

In our previous publication from the TOSCA core section interim analysis (13), we reported that the occurrence rate of renal angiomyolipomas was lower in the TOSCA cohort compared to other published literature (8, 9). Rates of haematuria and hypertension were also lower compared with those reported in TSC patients in other studies (6, 7, 21, 22), this may be a reflection of the age relatively young age of our subjects and possibly under-ascertainment. These lower rates of occurrence of renal angiomyolipomas and angiomyolipoma-related complications could be explained by a different (younger) age range of our population; however the current analysis shows that angiomyolipoma prevalence rose progressively with age, to 77.7% in those over 40 years of age, whereas complication rates remained much lower than in other studies. This suggests that active surveillance and a policy of pre-emptive treatment may have been successful in altering the natural history of renal TSC.

Patients with *TSC2* mutations were reported to exhibit a higher incidence and severity of both renal angiomyolipoma and cysts than those with *TSC1* mutations (8). In our study, the prevalence of angiomyolipoma was significantly higher in those with *TSC2* mutations. This was in line with the previous other reports (7, 8, 17, 23). We also observed that patients with *TSC2* mutations had angiomyolipoma at early age and experienced higher rates of bleeding complications (haematuria and hemorrhage). Rates of multiple angiomyolipomas, bilateral angiomyolipoma, renal angiomyolipoma lesions of >3 cm were significantly higher in those with *TSC2* mutations than those with *TSC1* mutations. Furthermore, more patients with *TSC2* mutations received intervention for renal angiomyolipoma than those with *TSC1* mutations.

As expected polycystic kidney disease was only found in those with *TSC2* mutations because it is the result of a deletion stretching across the TSC2 and PKD1 genes on chromosme 16 (The "contiguous gene syndrome") (24).

The study showed that pre-emptive treatment was used increasingly commonly with age (**Figure 1**) and this was associated with a very low rate of bleeding and significant renal impairment. **Figures 3**, **4** show that mTOR inhibitors are now the most commonly used treatment.

Despite the fact that overall prevalence of hemorrhage and CKD was too low to accurately define risk factors, in our sub-study we observed that all the three patients who had hemorrhage had *TSC2* mutation. Majority of the patients had grade 1/2 CKD (31 patients, 73.8%). Patients with CKD grade 2 or more were older but there was a clear trend for more advanced CKD stages.

Renal malignancy has been reported in about 2–4% of patients with TSC (25), which is much higher than that reported in a comparable age group in the general population (26). The occurrence rate of renal malignancy observed in this cohort was lower (1.4%) than that reported previously, in TSC (8, 25).

CONCLUSION

Renal angiomyolipomas are the major kidney risk for those with TSC; other renal complications are less common. We have shown a marked increase in the prevalence of intervention for renal angiomyolipomas, from $<10\%$ in those under 2 years of age to 48% in those over 40. The risk of needing an intervention was higher and begins earlier in those with a *TSC2* mutation, but the difference disappears by age 40 years. Gender differences were much smaller, but in females the occurrence of angiomyolipomas was significantly greater, as were angiomyolipomas >3 cm and the need for intervention. However, there was no absolute cut-off between the differences in any of these categories which means lifelong surveillance is important in all patients. In the substudy of 76 subjects none had a renal hemorrhage after commencing on an mTOR inhibitor. The most encouraging finding was that pre-emptive intervention was dramatically successful in altering the outcome compared to historical controls; with high pre-emptive intervention rates but low rates of bleeding and other complications. This validates the policy of surveillance and pre-emptive intervention recommended by clinical guidelines.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

LIST OF ETHICS COMMITTEES

The study protocol and all amendments were reviewed and approved (if applicable) by independent ethics committee/institutional review board for each centre: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent

Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); Comité Etico Investigación Clínica de Euskadi (CEIC-E); Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; Research Ethics Committee of the University of Tartu (UT REC); Ethikkommission der Medizinischen Universität Graz; North Wales REC—West; Regionala Etikprövningsnämnden i Göteborg; REK—Regionale komiteer for medisinsk og helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie Pomnik Centrum Zdrowia Dziecka; Ethikkommission bei der Ludwig-Maximilians-Universitat München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Deveropment of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Children's Hospital of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yan-sen University; The First Affiliated Hospital of Guangzhou Medical University; Shenzhen Children's Hospital; West China Hospital, Sichuan University; Xijing Hospital; Children's Hospital of Chongqing Medical University; Wuhan Children's Hospital; The Second Affiliated Hospital of Xi'an Jiaotong university; Guangdong 999 Brain Hospital; Seoul National University Hospital Institutional Review Board; National Taiwan University Hospital (NTUH) Research Ethics Committee (REC); Institutional Review Board of the Taichung Veterans General Hospital; Institutional Review Board of Chung Shan Medical University Hospital; Institutional Review Board, Tungs' Taichung MetroHarbor Hospital; Institutional Review Board of National Cheng Kung University Hospital; Metro South Human Research Ethics Committee; Sydney Children's Hospital Network Human Research Ethics Committee; St Vincent's Hospital Human Research Ethics Committee; Royal Melbourne Hospital Human Research Ethics Committee; Siriraj Institutional Review Board; The Institutional Review board, Faculty of Medicine, Chulalongkorn University, 3rd Floor, Ananthamahidol Building, King Chulalongkorn Memorial Hospital; The Committee on Human Rights Related to Research Involving Human Subjects; Institutional Review Board, Royal Thai Army Medical Department IRB RTA, 5th Floor, Phramongkutklaowejvitya Building, Phramongkutklao College of Medicine; Research Ethics Committee, Faculty of Medicine, Chiang Mai University; Research and Development, Queen Sirikit National Institute of Child Health; Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town; Shaare Zedek Medical Center Helsinki Committee; Sheba Medical Center Helsinki Committee; Tel Aviv Sourasly Medical Center Helsinki Committee; General University Hospital of Patras Ethics Committee; Pendeli Children's Hospital Ethics Committee; General University Hospital of Athens G. 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ETHICS STATEMENT

The studies involving human participants were reviewed and approved by all ethics committees involved in the TOSCA study (see list of ethics committees in article). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JK, EB, MB, PC, MD, JF, MF, CH, SJ, JL, AM, RN, VS, RT, BZ, AJ, and MS designed the study, patient accrual, clinical care, data interpretation, drafted, revised, final review, and approval of the manuscript. TC, VC, GB, PV, CF, FO'C, JQ, YT, and SY designed the study, data interpretation, drafted, revised, final review, and approval of the manuscript. LD'A designed the study, trial management, data collection, data analysis, data interpretation, drafted, revised, final review, and approval of the manuscript. RM designed the study, data analysis, data interpretation, drafting, revised, final review, and approval of the manuscript. SS designed the study, trial statistician, data analysis, data interpretation, drafted, revising, final review, and approval of the manuscript. All authors contributed to the article and approved the submitted version.

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Burden of Illness and Quality of Life in Tuberous Sclerosis Complex: Findings From the TOSCA Study

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Research on tuberous sclerosis complex (TSC) to date has focused mainly on the physical manifestations of the disease. In contrast, the psychosocial impact of TSC has received far less attention. The aim of this study was therefore to examine the impact of TSC on health, quality of life (QoL), and psychosocial well-being of individuals with TSC and their families. Questionnaires with disease-specific questions on burden of illness (BOI) and validated QoL questionnaires were used. After completion of additional informed consent, we included 143 individuals who participated in the TOSCA (TuberOus SClerosis registry to increase disease Awareness) study. Our results highlighted the substantial burden of TSC on the personal lives of individuals with TSC and their families. Nearly half of the patients experienced negative progress in their education or career due to TSC (42.1%), as well as many of their caregivers (17.6% employed; 58.8% unemployed). Most caregivers (76.5%) indicated that TSC affected family life, and social and working relationships. Further, well-coordinated care was lacking: a smooth

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transition from pediatric to adult care was mentioned by only 36.8% of adult patients, and financial, social, and psychological support in 21.1, 0, and 7.9%, respectively. In addition, the moderate rates of pain/discomfort (35%) and anxiety/depression (43.4%) reported across all ages and levels of disease demonstrate the high BOI and low QoL in this vulnerable population.

Keywords: tuberous sclerosis complex, quality of life, burden of illness, epilepsy, TOSCA

INTRODUCTION

Tuberous sclerosis complex (TSC) is a multi-system genetic disorder with a global incidence of 1 per 6,000–10,000 live births. Over a million people are estimated to be affected worldwide (1). It is characterised by growth of benign tumours in various organs throughout the body, including the brain, kidney, lungs, and skin (2). It is also associated with behavioural, psychiatric, intellectual, academic, neuropsychological, and psychosocial difficulties, grouped under the umbrella term TAND (TSC-Associated Neuropsychiatric Disorders) (3, 4). The clinical presentation of TSC manifestations is complex (5–8). Its natural course varies between individuals, with symptoms occurring at variable ages and severity ranging from very mild to severe, which may even lead to death. Furthermore, individuals with TSC are expected to have lifelong follow-up care to ensure the early detection of potentially life-threatening complications. The diverse clinical presentation represents significant disease, healthcare, and treatment burden (9).

To date, the majority of TSC research has concentrated on the pathophysiology, epidemiology, diagnosis, and treatment of the condition (10). Relatively little has been done to evaluate the impact of TSC on the quality of life (QoL) and social well-being of individuals with TSC and their families. A number of researchers have focused on the burden of specific aspects of TSC, such as epilepsy, subependymal giant cell astrocytoma (SEGA), facial angiofibroma, and renal angiomyolipoma (8, 9, 11–14). Others have evaluated the impact of specific treatments on QoL such as following epilepsy surgery (15), or have studied specific groups such as the impact on adult caregivers (10, 16). A retrospective study that evaluated parents of 99 children with TSC showed that about 50% reported clinically significant parental stress. The stress was related to the presence of current seizures, a history of psychiatric diagnosis, intellectual disability, and/or behavioural problems in the children (17). A web-based United Kingdom (UK) survey of individuals with TSC and their caregivers showed significantly lower health state utility values (HSUVs) compared with the general population reference value for the UK value set of the three-level version of the EuroQol-5D (EQ-5D-3L). This indicates substantial impairment in individuals with TSC (18). Zöllner et al. performed a systematic review on the burden of illness (BOI) in TSC and included 33 articles published up to October 2019, only 14 of which addressed QoL (19). We sought to assess the impact of TSC on the lives of individuals or their caregivers in terms of BOI and QoL, using a combination of ancillary disease-specific questions on BOI and validated QoL questionnaires in seven European countries.

METHODS

TOSCA, a natural history registry in TSC, was conducted in 170 sites across 31 countries worldwide. A detailed description of the methods of the TOSCA study has been provided previously (20). The registry consists of a "core" section and six "petals" or "research projects". Here, we present findings from one of the research projects focusing on BOI and QoL in individuals with TSC.

Participants

Selection of countries participating in this research project was based on the availability of the validated QoL questionnaires in the primary language used in that country. Based on this criterion, TSC individuals of any age from seven European countries were eligible for this specific research project, after signing an additional consent form.

Measuring Burden of Illness

All enrolled individuals were asked to complete a set of ancillary questions addressing social care needs (circumstances of living arrangements, financial, social, and psychological support, and information sources), healthcare needs (health insurance, medical care and level of satisfaction, genetic testing, and genetic counselling), impact on education and employment, impact on family, and transition from paediatric to adult care (**Supplementary Material**). These ancillary questions were developed by patient representatives, who were part of the TOSCA Working Committee in collaboration with the TSC patient associations. Draft questionnaires were reviewed by two caregivers for clarity and comprehensiveness. When individuals were unable to complete the questionnaires by themselves, caregivers were asked to complete the proxy version of the questionnaires (caregiver report).

Measuring Quality of Life

For evaluating QoL, validated questionnaires in local languages were administered to individuals with TSC/caregivers who participated in this research project. These included the following: (1) EuroQol-5D (EQ-5D), a self-complete questionnaire for adults (age, ≥18 years); the EQ-5D proxy version 1 was completed by the caregiver for children or adolescents for adults who were unable to complete the report by themselves; (2) QoL in Epilepsy Inventory-31-Problems (QOLIE-31)-P for adults (age, ≥18 years) with epilepsy, completed by the individuals themselves; (3) QoL in Childhood Epilepsy (QOLCE) for children <10 years old with epilepsy (completed by caregivers); (4) QoL in Epilepsy Inventory for

Adolescents-48 (QOLIE-AD-48) for children aged 11–17 years with epilepsy, completed by the subjects themselves.

Data Analyses

Data on QoL and BOI were recorded once (i.e., no follow up requested) before the data cut-off date (10 August, 2017). A copy of the collected paper questionnaires was sent from each clinical site to the clinical research organization (CRO) for data entry in the TOSCA study. Data were then extracted and analysed by the CRO. Responses to the BOI questions and QOL scales were summarised by descriptive statistics (number of responders, mean, standard deviation, median, range, frequency), considering age-based subgroup as children (<11 years), adolescents (age 11 to <18 years) and adults (age ≥18 years).

Individuals with TSC or their caregivers, rated their level of impairment across five dimensions (mobility, self-care, usual activities, pain/discomfort, and anxiety/depression). Each dimension has three levels: no problems, some problem and confined to bed. The mean thermometer score for EQ-5D and mean health state score for QOLIE-31-P questionnaire were recorded on a scale from 0 to 100, with 0 being the worst health state imaginable and 100 the best. Furthermore, each patient rated the importance of the seven QOLIE 31-P sub scales (energy, mood, daily activities, cognition, medication effects, seizure worry, and overall quality of life) from one to seven, with one being the most important topic and seven the least important one. The sub-scale scores of QOLIE-31-P questionnaire were the means of the converted item scores multiplied by the distress score. The total QOLIE-31-P score was calculated by dividing the sum of the sub scales by the sum of the distress scores

TABLE 1 | Demographic characteristics.

	Overall (*N* = 143)
Sex #	
Male	54 (37.8)
Female	88 (61.5)
Age at consent (years)	
n	142
Mean (SD)	19.8 (15.24)
Median (range)	14 (3–72)
Duration of TSC (years)	
n	141
Mean (SD)	13.5 (9.44)
Median (range)	11.2 (1.6–43.5)
Country	
Belgium	24 (16.8)
France	30 (21.0)
Germany	11 (7.7)
Italy	58 (40.6)
Spain	11 (7.7)
Sweden	6 (4.2)
UK	3 (2.1)
Individuals with epilepsy	
n(%)	67 (46.9)
Duration of epilepsy (years) at start of research project	
n	66
Mean (SD)	16.6 (12.53)
Median (range)	12.8 (2.7–55.4)

#Information on sex was not available for 1 patient. Values are expressed as n (%) unless otherwise stated.

TABLE 2 | Social care needs: self- and caregiver-reported outcomes.

	Self-reported individuals with TSC		Individuals with TSC reported by caregivers	
	Adolescents *N* = 17	Adults *N* = 38	Children/ Adolescents *N* = 71	Adults *N* = 17
Circumstances of living arrangements				
Lives alone	NA	5 (13.2)	NA	1 (5.9)
Lives with spouse/partner	NA	21 (55.3)	NA	2 (11.8)
Lives with other family	NA	10 (26.3)	NA	13 (76.5)
Information missing	NA	2 (5.3)	NA	1 (5.9)
Help with daily activities needed				
Yes	NA	3 (7.9)	NA	8 (47.1)
No	NA	35 (92.1)	NA	9 (52.9)
Assistance at home				
Nurse	0	0	1 (1.4)	1 (5.9)
Daily assistance by professional carer (paid)	1 (5.9)	0	5 (7.0)	1 (5.9)
Caregiver assistance from friend/family/relative (not paid)	0	5 (13.2)	16 (22.5)	7 (41.2)
Individuals felt that assistance and support at home was not sufficient	5 (29.4)	16 (42.1)	31 (43.7)	6 (35.3)
Financial, social, and psychological support				
Disability allowance	6 (35.3)	8 (21.1)	39 (54.9)	13 (76.5)
Caregiver allowance	1 (5.9)	0	9 (12.7)	0
Social worker assistance	1 (5.9)	0	6 (8.5)	1 (5.9)
Social services support	1 (5.9)	0	3 (4.2)	2 (11.8)
Psychological counselling	2 (11.8)	3 (7.9)	10 (14.1)	0
Used sources for information about rights and benefits				
Physician	7 (41.2)	25 (65.8)	46 (64.8)	9 (52.9)
Internet/Websites	9 (52.9)	14 (36.8)	49 (69.0)	7 (41.2)
Patient group	2 (11.8)	5 (13.2)	20 (28.2)	6 (35.3)
Social worker	1 (5.9)	1 (2.6)	21 (29.6)	3 (17.6)
Local government	1 (5.9)	4 (10.5)	6 (8.5)	2 (11.8)
Nurse	0	2 (5.3)	3 (4.2)	3 (17.6)
Most useful source				
Physician	9 (52.9)	25 (65.8)	34 (47.9)	8 (47.1)
Internet/Websites	4 (23.5)	4 (10.5)	19 (26.8)	3 (17.6)
Patient group	2 (11.8)	2 (5.3)	12 (16.9)	4 (23.5)
Social worker	1 (5.9)	0	13 (18.3)	3 (17.6)
Local government	1 (5.9)	2 (5.3)	3 (4.2)	0
Nurse	0	0	1 (1.4)	0

NA not applicable. Values are expressed as n (%).

multiplied by 100. If more than half the items in a sub-scale had not answered, the sub-scale was not included in the total score. For each sub scale of QOLCE, the answer for each item was converted to a 0 to 100 point score, where high scores reflect the highest level of functioning.

RESULTS

Hundred fouty three individuals (88 children and adolescents, and 55 adults) from seven European countries were enrolled in this research project as part of the TOSCA study (**Table 1**). The mean time since initial diagnosis of TSC was 13.5 years (median, 11.2 years; range, 1.6–43.5). Of the 143 individuals enrolled, 67 (28 adults) had epilepsy (46.9%). The mean duration of epilepsy was 16.6 years (median, 12.8 years; range, 2.7–55.4).

Burden of Illness: Self-Reported Outcomes

17 adolescents (19.3%; aged between 11 and <18 years) and 38 adults (69.1%) completed the questionnaire independently. Of these, one (5.9%) adolescent and five adults (13.2%) needed extra assistance at home. In most cases, assistance was provided by unpaid caregivers (a family member or friend). 29.4% adolescents and 42.1% of adults felt that assistance and support at home was not sufficient (**Table 2**). Financial, social, and psychological support was received by 8 (21.1%), 0 (0%), and 3 (7.9%) of adult respondent, respectively.

Nine adolescents (52.9%) and 16 adults (42.1%) had access to public and/or private insurance (**Table 3**). Although none of the individuals reported that they had to pay extra for private insurance due to TSC, two adults (5.3%) reported that health or any kind of insurance was denied due to TSC. TSC was managed by TSC specialists in 12 adolescents (70.6%) and 28 adults (73.7%). Twenty-nine adults (76.3%) reported that they had access to a TSC clinic when required, while no access to TSC clinics were reported by six adults (15.8%). TSC was managed by more than three physicians in 15 adults (39.5%). Smooth transition from paediatric to adult care was reported by only 14 adults (36.8%). Nearly one fifth of patients were dissatisfied with various aspects of their medical care and nearly 50% were not able to report if their care followed clinical guidelines (**Figure 1**).

TSC was reported to have impacted the career/education progress in three adolescents (17.6%; **Table 4**). Fourteen adolescents (82.4%) were in mainstream education. Six adolescents (35.3%) received additional support in class; no adolescents were home-schooled. Of the 38 adults, 20 (52.6%) were employed and seven were not able to work (4 due to TSC; 3 due to other reasons). Sixteen adults (42.1%) expressed that TSC had affected their career or education in different ways: impact on career progression/promotions (25%), choice of career (25%), loss of employment (31.3%), part-time rather than full-time work (31.3%), or attainment of education level (37.5%).

Burden of Illness: Caregiver-Reported Outcomes

Parents/Caregivers completed the questionnaires for 71 children and adolescents (80.7%; 38 girls and 32 boys) and 17 adults (30.9%; 11 female and 6 male)

TABLE 3 | Health care needs: self- and caregiver-reported outcomes.

	Self-reported individuals with TSC		Caregivers-reported individuals with TSC	
	Adolescents *N* = 17	Adults *N* = 38	Children/ adolescents *N* = 71	Adults *N* = 17
Individuals with health insurance				
Private insurance	2 (11.8)	7 (18.4)	31 (43.7)	6 (35.3)
Public insurance	6 (35.3)	14 (36.8)	37 (52.1)	4 (23.5)
No insurance	7 (41.2)	15 (39.5)	11 (15.5)	8 (47.1)
Individuals thought to have paid extra for private insurance due to TSC condition	0	0	1 (1.4)	0
Public insurance was denied due to TSC	0	2 (5.3)	9 (12.7)	1 (5.9)
Genetic testing				
Patient had genetic testing for TSC	13 (76.5)	31 (81.6)	57 (80.3)	16 (94.1)
Patient was offered genetic testing but did not do it	1 (5.9)	1 (2.6)	3 (4.2)	0
Patient had not been offered genetic testing for TSC	0	3 (7.9)	7 (9.9)	1 (5.9)
Genetic counselling				
Patient had genetic counselling	9 (52.9)	26 (68.4)	43 (60.6)	10 (58.8)
Patient was offered genetic counselling but decided not to have it	0	0	3 (4.2)	0
Patient had not been offered genetic counselling for TSC	4 (23.5)	6 (15.8)	19 (26.8)	4 (23.5)
Number of doctors managing TSC				
1	8 (47.1)	12 (31.6)	17 (23.9)	6 (35.3)
2	3 (17.6)	5 (13.2)	11 (15.5)	1 (5.9)
3	0	3 (7.9)	11 (15.5)	2 (11.8)
>3	6 (35.3)	15 (39.5)	31 (43.7)	7 (41.2)
Data not provided	0	3 (7.9)	1 (1.4)	1 (5.9)
TSC is managed by*				
General practitioner/family doctor	1 (5.9)	9 (23.7)	17 (23.9)	6 (35.3)
TSC specialist	12 (70.6)	28 (73.7)	39 (54.9)	16 (94.1)
Other specialist	7 (41.2)	19 (50.0)	49 (69.0)	7 (41.2)
Access to TSC clinic				
Individuals had access to clinic when required	13 (76.5)	29 (76.3)	43 (60.6)	16 (94.1)
Distance to TSC clinic from home				
<50 km	10 (58.8)	14 (36.8)	18 (25.4)	4 (23.5)
>50 km	3 (17.6)	15 (39.5)	30 (42.3)	12 (70.6)
Individuals in contact with national TSC association				
Yes	9 (52.9)	14 (36.8)	36 (50.7)	9 (52.9)
No	7 (41.2)	22 (57.9)	33 (46.5)	7 (41.2)
Data not available	1 (5.9)	2 (5.3)	2 (2.8)	1 (5.9)

**Participants may have provided more than one answer. Values are expressed as n (%).*

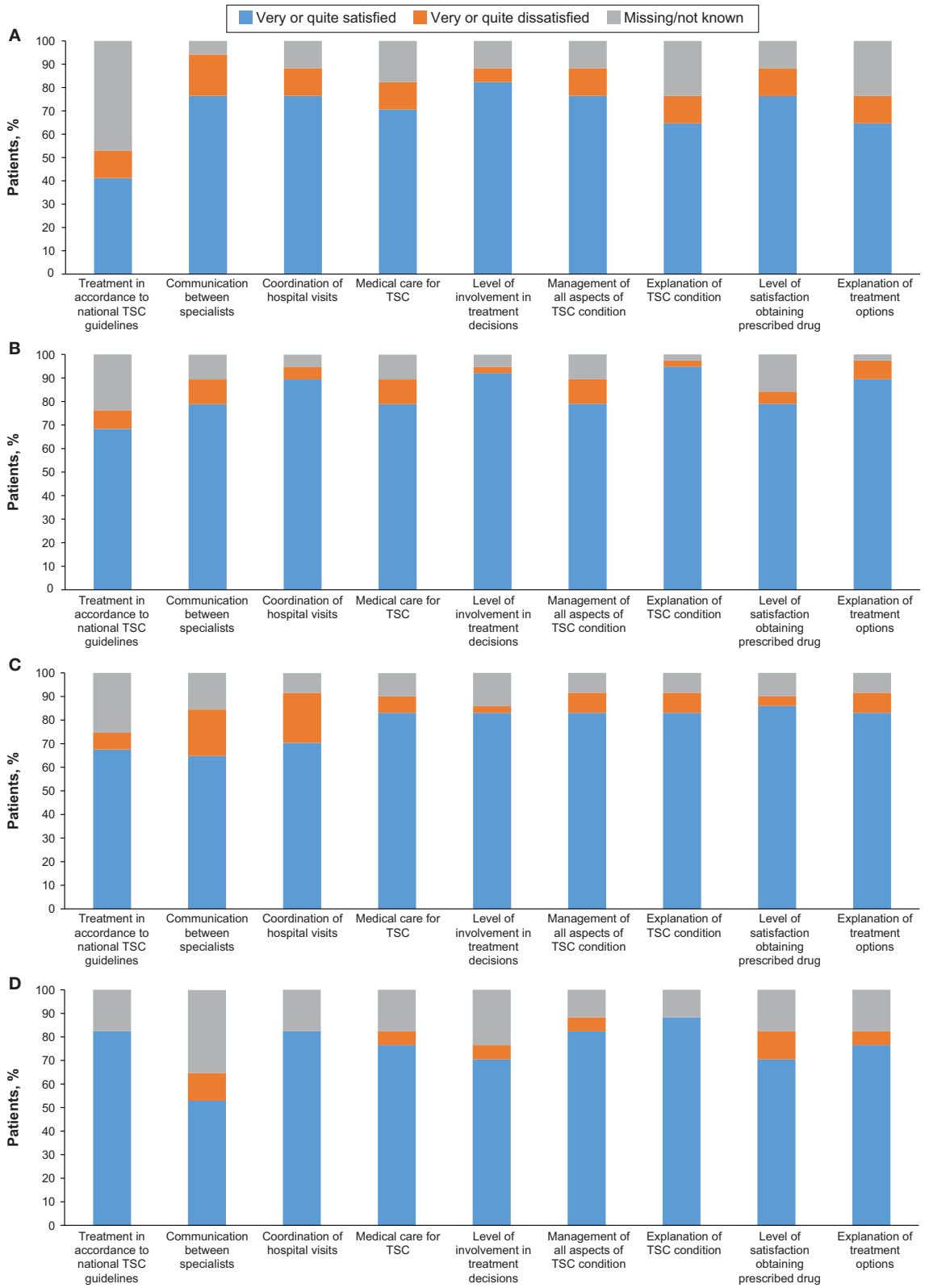

FIGURE 1 | Satisfaction with treatment aspects in **(A)** Self-reported children, **(B)** Self-reported adults, **(C)** Caregiver-reported children, and **(D)** Caregiver-reported adults.

TABLE 4 | Impact of TSC on education, employment and relationships.

	Self-reported individuals with TSC		Caregivers-reported individuals with TSC	
	Adolescents *N* = 17	Adults *N* = 38	Children/ adolescents *N* = 71	Adults *N* = 17
Impact on education				
Impact of TSC on career/education of self or caregivers (in case of children)[a]	3 (17.6)	16 (42.1)	47 (66.2)	12 (70.6)
Career progression/promotions	0	4 (25.0)	17 (36.2)	1 (8.3)
Choice of career	0	4 (25.0)	16 (34.0)	1 (8.3)
Loss of employment	2 (66.7)	5 (31.3)	10 (21.2)	1 (8.3)
Part-time work rather than full time	0	5 (31.3)	25 (53.2)	1 (8.3)
Education level attained	1 (33.3)	6 (37.5)	3 (6.4)	10 (83.3)
Current employment status of self or caregivers (in case of caregiver-reported children)				
Employed (either full or part-time)	11 (64.7)	20 (52.6)	47 (66.2)	3 (17.6)
Unable to work due to condition	0	4 (10.5)	8 (11.3)	10 (58.8)
Unable to work but not due to condition	0	3 (7.9)	8 (11.3)	1 (5.9)
Student	2 (11.8)	2 (5.3)	0	1 (5.9)
Homemaker	4 (23.5)	7 (18.4)	10 (14.1)	1 (5.9)
Impact of TSC on relationships of self or caregivers (in case of caregiver-reported children)				
Family relationships	3 (17.6)	8 (21.1)	29 (40.8)	4 (23.5)
Social relationships	2 (11.8)	14 (36.8)	36 (50.7)	11 (64.7)
Working colleague relationships	0	4 (10.5)	17 (23.9)	1 (5.9)
Child is in mainstream education	14 (82.4)	NA	43 (60.6)	NA
Child receives additional support in class	6 (35.3)	NA	31 (43.7)	NA
Additional support causes child additional problems	2 (11.8)	NA	13 (18.3)	NA

[a] *Individuals may have reported one or more ways of impact of career/education. Values are expressed as n (%).*

who were unable to complete the questionnaires by themselves.

Of the 71 caregiver-reported children and adolescents, 20 (28.2%) needed help at home, provided mainly by unpaid caregivers in 80% of cases (**Table 2**). Of the 17 caregiver-reported adults, one (5.9%) was living alone, two (11.8%) with a partner, and 13 (76.5%) with other family members. Eight (47.1%) individuals needed help with daily activities. About half of the caregiver-reported individuals (50.7% children and adolescents, and 52.9% adults) were in contact with their local TSC associations.

TSC was managed by TSC specialists in 39 (54.9%) caregiver-reported children and adolescents, and 16 (94.1%) caregiver-reported adults (**Table 3**). Twenty-three caregivers (32.4%) reported that their children and adolescents did not have access to TSC specialist clinics but most caregiver-reported adults (94.1%) did. Most caregiver-reported children and adolescents (80.3%) and caregiver-reported adults (94.1%) received genetic testing for TSC, but genetic counselling was received only by 60.6% of children and adolescents, and 58.8% of adults. None of the six (35.3%) caregiver-reported adults who received private insurance felt that they had to pay extra due to TSC and only one patient (5.9%) reported that health or any kind of insurance was denied due to TSC.

Caregivers have reported that TSC had affected the career or education of their children and adolescents in different ways. These include part-time work rather than full time (53.2%), impact on career progression/promotions (36.2%), choice of career (34.0%), loss of employment (21.2%), impact on educational attainment (6.4%). Of the 17 caregiver-reported adults, only three (17.6%) were employed while 10 (58.8%) were unable to work due to TSC. Ten (83.3%) carer-reported adults reported impact of educational attainment. Relationships of caregivers had been impacted due to child's TSC in 53.5% of cases with impact on the family, social, and working colleague relationships were reported in 29 (40.8%), 36 (50.7%), and 17 (23.9%) cases, respectively. Impact on the family, social and working relationships by TSC condition have been noted in 76.5% of caregiver-reported adults.

Quality of Life (QoL) in TSC

EQ-5D Questionnaire

Overall, EQ-5D (or Q-5D proxy version 1) questionnaires were completed for all 143 participants. Difficulty in mobility was reported by 34 individuals (23.8%) and 32 (22.4%) experienced difficulty in self-care. Twenty-six individuals (18.2%) were unable to perform usual activities, fifty individuals (35%) had moderate pain or discomfort and four individuals (2.8%) had extreme pain or discomfort. Sixty-two individuals (43.4%) reported moderate anxiety/depression, while six individuals (4.2%) reported extreme anxiety/depression. Anxiety/depression and pain/discomfort were reported in both self-reported as well as caregiver-reported groups and present in both children and adolescents, and adults (**Figure 2**). On the thermometer scale of 0–100 (100 being the best state of health imaginable and 0 as worst state imaginable) the mean score was 70.6.

QOLIE-31-P Questionnaire

The QOLIE-31-P questionnaire was completed by 24 individuals. The total score of the QOLIE-31-P questionnaire was 71.6 (standard deviation [SD]: ±16.7, **Table 5**). The mean (±SD) score for different sub-scales were: energy (47.0 ± 27.6), mood (53.4 ± 29.8), daily activities (67.0 ± 33.3), cognition (63.6 ± 37.5), medication effects (56.9 ± 31.5), seizure worry (49.8 ± 31.4), and overall quality of life (53.8 ± 29.1).

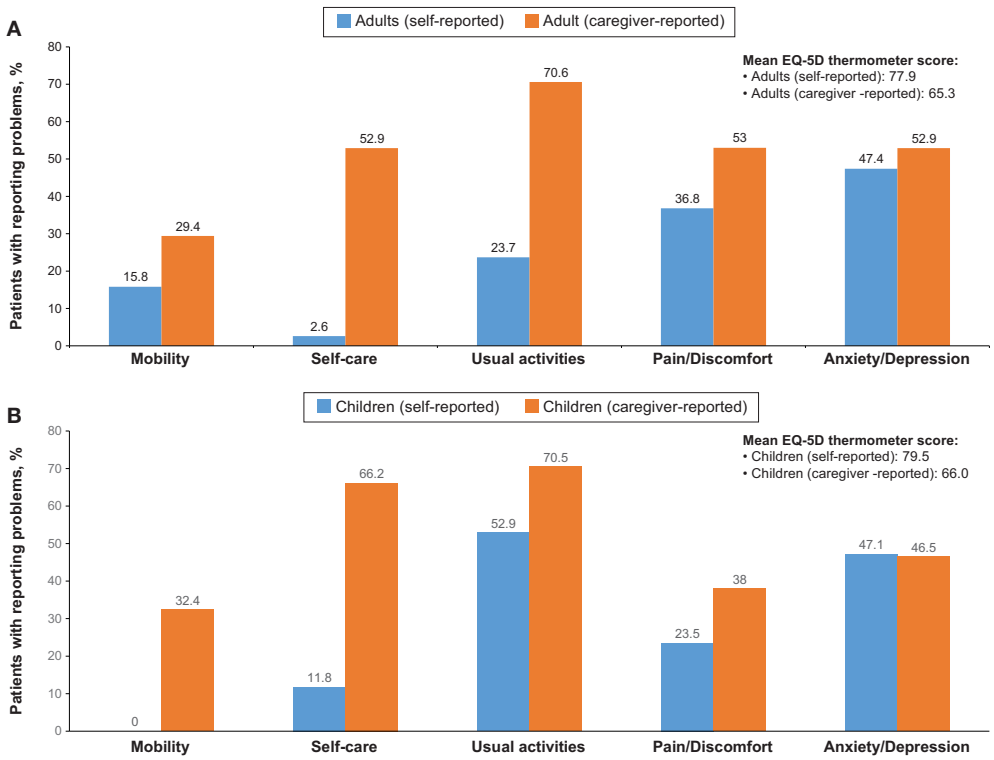

FIGURE 2 | Summary of EQ-5D Questionnaire in **(A)** adults and **(B)** children.

QOLCE Questionnaire

The QOLCE questionnaire was completed by 70 caregivers. The mean QOLCE score was 52.3 (SD: ±18.9, **Table 5**). The mean (±SD) scores of different sub-scales were: QoL (51.5 ± 27.5), physical restrictions (44.6 ± 24.4), energy/fatigue (54.5 ± 22.7), depression (70.7 ± 17.6), anxiety (58.8 ± 20.5), control/helplessness (56.1 ± 20.1), self-esteem (63.9 ± 19.6), attention/concentration (37.5 ± 28.7), memory (54.2 ± 23.8), language (42.1 ± 28.7), other cognitive functions (31.7 ± 29.0), social interactions (53.6 ± 21.7), social activities (63.8 ± 35.5), stigma (66.1 ± 36.4), behaviour (50.5 ± 20.6), and general health (48.5 ± 27.3). The highest score was reported for depression and the lowest for other cognitive functions.

QOLIE-AD-48 Questionnaire

Eight adolescents aged 11–17 years with epilepsy completed the questionnaire. The mean total QOLIE-AD-48 questionnaire score was 74.2 (SD: ±13.9, **Table 5**). The score of the sub-scales were epilepsy impact (82.7 ± 20.2), memory/concentration (74.1 ± 22.3), physical functioning (83.1 ± 18.1), stigma (81.9 ± 22.2), social support (69.5 ± 22.5), school behaviour (97.7 ± 3.2), attitudes toward epilepsy (30.4 ± 7.6), and health perceptions (61.5 ± 9.9).

DISCUSSION

This study aimed to evaluate BOI and QoL in children and adolescents, and adults with TSC and their families. BOI focused on social care needs, health (care) needs, and impact of TSC on education, employment, and family life. Individuals' QoL was assessed by means of standardized measures of QoL. To our knowledge, this study represented the most comprehensive and multinational evaluation of BOI and QoL in TSC to date.

Four main findings were highlighted by this study. BOI in families with TSC patients was high, as shown by their experiences of insufficient assistance at home and from social services. Individuals with TSC reported significant use of healthcare services but considered the support from TSC associations and patient organizations as inadequate. Also, the impact of TSC on individuals' education, employment, and social and family life was profound. Regarding quality of life, both children and adolescents, and adults reported moderate-to-severe levels of pain or discomfort and anxiety or depression, which was also indicated by their caregivers.

Individuals with TSC and their families have unmet needs with respect to support from social workers who provide various services, corresponding to previous findings (8). Most services were not available, or not offered or performed properly. Possibly, these professionals were insufficiently aware of the specific needs of individuals with or lack the experience to provide appropriate support. Another explanation for this unmet need might be difficulty in reaching out to families of individuals with TSC by social workers due to practical reasons, or families of individuals with TSC had personal barriers to seek help. Clearly, our findings underline the urgent need for increased awareness among social services about the importance of early and systematic follow-up

TABLE 5 | Summary of QOLIE-31-P, QOLCE and QOLIE-AD-48 questionnaire scores.

	n	Mean	SD	Median	Range
QOLIE-31-P					
Energy	24	47.0	27.6	45.0	2.5–90
Mood	24	53.4	29.8	63.5	3.6–92
Daily activities	24	67.0	33.3	73.1	3.8–100
Cognition	24	63.6	37.5	71.1	0.3–100
Medication effects	24	56.9	31.5	57.5	1.3–100
Seizure worry	24	49.8	31.4	45.8	0.4–100
Overall QoL	24	53.8	29.1	58.1	3.3–95
Final Score	24	71.6	16.7	75.8	27.3–93.4
QOLCE					
QoL	67	51.5	27.5	50.0	0–100
Physical restrictions	69	44.6	24.4	45.8	0–100
Energy/fatigue	67	54.5	22.7	62.5	0–100
Depression	68	70.7	17.6	75.0	8.3–100
Anxiety	68	58.8	20.5	50.0	25–100
Control/helplessness	64	56.1	20.1	50.0	18.8–100
Self-esteem	65	63.9	19.6	70.0	15–95
Attention/concentration	66	37.5	28.7	32.3	0–100
Memory	58	54.2	23.8	56.3	0–100
Language	60	42.1	28.7	44.4	0–100
Other cognitive functions	64	31.7	29.0	25.0	0–100
Social interactions	56	53.6	21.7	60.0	0–100
Social activities	67	63.8	35.5	66.7	0–100
Stigma	56	66.1	36.4	75.0	0–100
Behaviour	69	50.5	20.6	48.4	0–93.8
General health	68	48.5	27.3	50	0–100
Final score	70	52.3	18.9	51.5	12.2–91.7
QOLIE-AD-48					
Epilepsy impact	8	82.7	20.2	91.7	39.6–95.8
Memory/concentration	8	74.1	22.3	82.5	45–100
Physical functioning	8	83.1	18.1	87.5	55–100
Stigma	8	81.9	22.2	83.3	33.3–100
Social support	8	69.5	22.5	59.4	43.8–100
School behaviour	8	97.7	3.2	100.0	93.8–100
Attitudes toward epilepsy	7	30.4	7.6	31.3	18.8–37.5
Health perceptions	8	61.5	9.9	58.3	50.0–75
Final score	7	74.2	13.9	81.2	46.1–85.7

of individuals with TSC and their environment (21). When such needs remain unrecognised, family members feel urged to take on various responsibilities and failed to introduce further professional care in a timely manner, preventing optimal guidance with attention to individual goals or preferences.

Individuals with TSC showed various clinical manifestations for which they visited health specialists. Throughout their lives, they made significant use of healthcare services as a result of the regular multidisciplinary medical care indicated for the management of TSC (22). However, the present study showed that high healthcare utilization and followed-up by a TSC specialist or clinic were unrelated to involvement of TSC associations and patient organizations in the individual's care trajectory. Reasons could be that patients were not familiar with them, not convinced of their significance for their own situation or experience sufficient support from their own private network. It was also plausible that these societal partners failed to reach families with TSC in the right way or did not meet their expectations regarding types of support.

The observed lack of appropriate care services was also reflected in differences between individuals in terms of health insurance, and genetic testing, and counselling. These findings indicate a need for revision and standardization of insurance policies for people with TSC or chronic conditions in general, as well as clinical care characterized by a personalized and transparent approach. Despite this imbalance between care need and care provision, individuals in this study reported satisfaction with how their disease was treated and monitored. Furthermore, the transition from paediatric to adult TSC care was an important area of concern (23). Although this phase is generally considered challenging or difficult (24), our results showed a smooth process in almost half of the cases. Transition-enhancing practices such as use of an individual action plan, implementation of a transition protocol and setting up a mixed paediatric-adult team with a transition coordinator might be useful in TSC care (25, 26).

TSC had a strong influence on the education and professional career of affected individuals. Especially in adults, their level of education, choice of career, career progression and promotion, and employment rate were impacted by the disease. Apart from the presence of TSC, other influences, directly or indirectly, related to the illness should be taken into account. Having few professional expectations for the future, being confronted with negative attitudes of colleagues and lacking arrangements to improve working conditions, might all further reduce the patient's opportunities at work (27, 28). The impact of TSC on education was relatively minor in the group of self-reporting adolescents, a finding that is likely biased by their assumed milder phenotype since they were able to fill-out the BOI and QOL questionnaires independently. Previous research showed a higher degree of absenteeism, impaired performance, and lower productivity at school in paediatric patients (28). It seems therefore advisable to guide young patients on study choice and keep track of adults' working life, while listening to expressed questions, concerns, and problems.

TSC has significant effects on the social well-being and family life of both young and adult individuals with TSC. The patients' high dependence on their environment can lead to feelings of disorientation, loneliness, and clinically significant stress levels in patients, but also in family members (10, 17). Our data show the marked effect of TSC on the income, career, and psychological well-being of the individual's family. Therefore, it is essential to identify and approach the sources of such familial distress, which vary according to the patient's personal characteristics, health status, and living environment. Problems in children and adults with TSC such as severe epilepsy and other persistent health problems, neuropsychiatric disorders (TAND) and a lack of support from the family's network can put a heavy burden on the family of individuals with TSC (2, 29, 30). As a result, the family may become isolated as friendships and professional relationships receive less attention (16). However, it has been shown that external support might help building the family's resources, as they can cope better with the multifaceted problems

of TSC and regularly shift their attention from the disease to pleasant events and moments in life (10).

With regard to QoL, moderate to severe levels of pain or discomfort and anxiety or depression were reported by individuals with TSC of all ages as well as by their caregivers. In order to achieve a comprehensive view of health-related QoL in individuals with TSC, research suggested to investigate other indicators such as fatigue, emotional stress, and participation (31). In particular, participation is important, as this multidimensional concept captures how the patient's health determines his or her participation in daily life, taking into account functional and intellectual disabilities. Assessing the individual's participation rate in terms of education, social activities, and leisure time is required for the development of interventions, which enable a long life with a good QoL (32). In future studies on BOI and QoL, standardized instruments to measure participation such as questionnaires for patients and carers could be used (33, 34).

When interpreting the results of this study, certain limitations need to be taken into account. Not all patients completed all questionnaires in the study, and only a small subsample of patients from the TOSCA registry enrolled in the present study. Although the information was collected from both individuals who were able to self-report as well as from caregivers of individuals who were unable to self-report, the overall disease severity of the cohort is likely to be milder compared to that of the global TOSCA registry cohort. Only 46.85% of patients in the current study was reported to have epilepsy in contrast to 83.5% in the overall TOSCA cohort (35). Since epilepsy is known to have a major impact on QoL (36), the burden of illness reported here might reflect the impact at the milder end of the spectrum. Furthermore, these subjects were all recruited from clinicians specialized in TSC care. Therefore, the level of care and satisfaction in the general TSC population is likely to be lower.

Although no data on intellectual ability were collected, 65% of children were following mainstream education. Although school systems differ across countries and attending mainstream education does not imply that children have normal intellectual ability, it seems likely that this reflects again a potential bias towards the milder end of the spectrum. The lack of a personal perspective is another limitation of the study. The questionnaire used to measure BOI contained questions that were developed together with families, which ensures a large patient-oriented input. Although no qualitative research was conducted, a short analysis of the questionnaire's open data fields did confirm the quantified BOI (data not shown).

CONCLUSION

Our study confirms the impact of TSC on education, career and social life of patients, and their families. This disease-specific impact is also reflected in patients' quality of life, including moderate-to-high levels of pain or discomfort and anxiety or depression. Unfortunately, despite families' frequent use of healthcare services, provision of well-organized TSC care is not evident as shown by their experiences of insufficient social support and discontinuous pediatric to adult care trajectories. These difficulties further increase the impact on the different life domains of families living with TSC, who would benefit from better coordinated educational, psychosocial, and medical support.

DATA AVAILABILITY STATEMENT

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by independent ethics committee/institutional review board for each centre involved in the study (see **Supplementary Materials** for full list). Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

AJ, EB, MB, PC, MD, JF, MF, CH, SJ, JK, JL, AM, RN, VS, MS, RT, and BZ designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. SV data interpretation, drafting, revising, final review, and approval of the manuscript. PV designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. CF designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. CF, GB, TC, VC, FO'C, JQ, YT, and SY designing the study, data interpretation, drafting, revising, final review, and approval of the manuscript. LD'A designing the study, trial management, data collection, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. RM designing the study, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. SS designing the study, trial statistician, data analysis, data interpretation, drafting, revising, final review, and approval of the manuscript. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00904/full#supplementary-material

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RESEARCH Open Access

Natural clusters of tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND): new findings from the TOSCA TAND research project

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Abstract

Background: Tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) have unique, individual patterns that pose significant challenges for diagnosis, psycho-education, and intervention planning. A recent study suggested that it may be feasible to use TAND Checklist data and data-driven methods to generate natural TAND clusters. However, the study had a small sample size and data from only two countries. Here, we investigated the replicability of identifying natural TAND clusters from a larger and more diverse sample from the TOSCA study.

Methods: As part of the TOSCA international TSC registry study, this embedded research project collected TAND Checklist data from individuals with TSC. Correlation coefficients were calculated for TAND variables to generate a correlation matrix. Hierarchical cluster and factor analysis methods were used for data reduction and identification of natural TAND clusters.

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Results: A total of 85 individuals with TSC (female:male, 40:45) from 7 countries were enrolled. Cluster analysis grouped the TAND variables into 6 clusters: a scholastic cluster (reading, writing, spelling, mathematics, visuo-spatial difficulties, disorientation), a hyperactive/impulsive cluster (hyperactivity, impulsivity, self-injurious behavior), a mood/anxiety cluster (anxiety, depressed mood, sleep difficulties, shyness), a neuropsychological cluster (attention/concentration difficulties, memory, attention, dual/multi-tasking, executive skills deficits), a dysregulated behavior cluster (mood swings, aggressive outbursts, temper tantrums), and an autism spectrum disorder (ASD)-like cluster (delayed language, poor eye contact, repetitive behaviors, unusual use of language, inflexibility, difficulties associated with eating). The natural clusters mapped reasonably well onto the six-factor solution generated. Comparison between cluster and factor solutions from this study and the earlier feasibility study showed significant similarity, particularly in cluster solutions.

Conclusions: Results from this TOSCA research project in an independent international data set showed that the combination of cluster analysis and factor analysis may be able to identify clinically meaningful natural TAND clusters. Findings were remarkably similar to those identified in the earlier feasibility study, supporting the potential robustness of these natural TAND clusters. Further steps should include examination of larger samples, investigation of internal consistency, and evaluation of the robustness of the proposed natural clusters.

Keywords: ASD, Cluster analysis, Factor analysis, Natural TAND clusters, TAND, Tuberous sclerosis complex, TOSCA, Registry, Neuropsychiatric

Background

Tuberous sclerosis complex (TSC) is a complex multi-system genetic disorder with a vast and variable age-related presentation of physical and neuropsychiatric manifestations [1–3]. It is associated with a substantial economic and psychosocial burden on the affected individuals and their families [1, 4–7].

In spite of the high rates and burden of neuropsychiatric manifestations in individuals with TSC, a 2010 study from the UK reported that only 18% of all families had ever received any of the recommended evaluations or treatments for the range of neuropsychiatric manifestations [8]. These findings suggested a large assessment and treatment gap in TSC. In order to reduce this gap, the Neuropsychiatry Panel of the International Consensus Guidelines Group coined the term TAND (TSC-associated neuropsychiatric disorders) in 2012 [9] and presented a standardized nomenclature to describe the range of neuropsychiatric manifestations observed in TSC across six levels—behavioral, psychiatric, intellectual, academic, neuropsychological, and psychosocial. The Neuropsychiatry Panel also recommended that all individuals with TSC should be screened for TAND on an annual basis [9]. In order to support screening for TAND, a TAND Checklist was developed through a participatory research strategy and pilot validated [10, 11].

Individuals with TSC have unique and highly variable TAND profiles. This uniqueness and multi-dimensionality of TAND often lead to 'treatment paralysis' where most clinical teams feel overwhelmed by the complexity of the neuropsychiatric presentations of their patients with TSC, thus posing a significant challenge to clinicians for diagnosis, psycho-education, and intervention planning [12, 13]. To reduce the assessment gap and treatment paralysis seen in the TSC community, the possibility of identifying "natural clusters" of the TAND phenomena was hypothesized by Leclezio and de Vries [12]. They proposed that, if data-driven strategies could identify a manageable number of clusters, this could reduce the assessment and treatment gap by providing clinical next steps [13]. The researchers proposed this to be an essential first step towards personalisation of clinical concerns, guiding the generation of evidence-based treatments for TAND and adding precision to training and fundamental neuroscience research [13].

In a feasibility study, Leclezio and colleagues explored methods that may identify natural clusters [14]. Findings identified WARD's cluster analysis and exploratory factor analysis as potential methods and produced six natural clusters with good face validity. However, the study had a small sample size (n = 56) and included patients from only two countries (South Africa and Australia). Given the highly heterogeneous nature of TAND manifestations, it was therefore not clear to what extent the six identified clusters would be replicable.

In this study, we set out to examine a new sample of individuals with TSC across ages and abilities from seven countries to determine whether data reduction methods would be able to replicate and extend the findings from the feasibility study performed by Leclezio et al. [14].

Methods

Design

The detailed methodology of the overall TOSCA clinical study has been published previously [15]. In brief, TOSCA was a non-interventional, multicenter, natural history registry of individuals with TSC. The study was designed with a "core" section and six research projects,

each focusing on a specific area of TSC—subependymal giant cell astrocytoma, renal angiomyolipoma, genetics, epilepsy, quality of life, and TAND. Here, we present data on the research project focusing on TAND.

Subjects and procedures for this research project

All centers participating in the TOSCA clinical study were invited to participate in the TAND research project. Centers from seven countries opted to participate. All TOSCA participants from these countries were therefore invited to participate in this study. Upon provision of a dedicated informed consent for the TAND research project, the TAND Checklist was administered to individuals with TSC or their caregivers by a study physician [10]. The TAND Checklist follows the neuropsychiatric levels of investigation outlined previously [10, 11] and consists of the following 12 sections: (1) basic developmental milestones; (2) current level of functioning; (3) behavioral difficulties; (4) psychiatric disorders diagnosed; (5) intellectual ability; (6) academic difficulties; (7) neuropsychological deficits; (8) psychosocial functioning; (9) parent, caregiver, or self-rating of the impact of TAND; (10) prioritization list; (11) additional concerns; and (12) health care professional rating of the impact of TAND. The questions require simple yes or no responses in most sections.

Data analysis

In contrast to "hypothesis-testing" statistical approaches where data are analyzed in relation to an a priori prediction, unsupervised learning or data-driven methods searches for previously undetected patterns or groupings in a dataset without any a priori rules, predictions, or labels to data. In this study, we used cluster analysis and factor analysis, two unsupervised learning/data-driven statistical methods, to help understand the complex TAND data. The objectives of cluster and factor analysis methods are, however, different. Cluster analysis aims to group observations (e.g., a sample of subjects or variables) into distinct groups in a way that objects in that group are more similar to each other than to those in other clusters or groups. Many different methods are used for cluster analysis. In the proof-of-principle study by Leclezio et al. [14], a wide range of cluster analysis methods were explored and the WARD method was identified as the most suitable method for the TAND Checklist data used. WARD is a hierarchical cluster analysis method. The method starts with each object as a separate cluster. At each sequential step, the two closest clusters are merged. The WARD method bases the closeness of clusters on within cluster variance. The sequential merging is typically visualized in a dendrogram (or hierarchical tree).

In contrast to the intuitive stepwise WARD clustering algorithm, factor analysis is based on fitting a model to the data. Factor analysis is typically used as a data reduction method to reduce a larger set of variables into a much smaller number of factors. The model assumes a few unobservable "latent (or underlying) factors" in the data. Factor analysis uses the correlations between variables (e.g., TAND checklist items) to identify latent factors representing a group of highly correlated variables. (A group of highly correlated variables will tend to vary jointly, thus reducing the within group variance). Factor analysis data are typically visualized as correlation matrices showing the factor loadings of items included in each factor. Factor score plots represent a different visualization method and show how factor scores contribute to each factor. In the Leclezio et al. study [14], a range of exploratory factor analysis methods were used for extraction and rotation of data to find a factor solution that best matched the cluster analysis method. Ultimately both methods (cluster and factor analysis) group similar items, but follow very different approaches. In general, where the two methods converge on the same findings, this allows one to place increased confidence in those findings.

In order to replicate the proof-of-concept work by Leclezio et al. [14], we included exactly the same variables for analysis. The following sections of the TAND Checklist were included: Section 3, behavioral challenges (19 questions/variables); Section 6, academic skills (four variables); and Section 7, neuropsychological skills (six variables). In the original study, variables were included that were (a) descriptive of observed phenomena, e.g., the behavioral, scholastic or neuropsychological levels, and (b) that could have been answered without access to specialist care (e.g., no need for diagnosis or formal testing). Given that all the variables had binary (yes/no), a scoring coefficient was used to compute a correlation matrix for the variables of interest. In case of missing values, variables were omitted pairwise in correlation computations. Hierarchical cluster analysis was used to identify natural clusters and to generate a clustering tree (dendrogram) visually representing the merging of TAND variables and suggesting a suitable number of clusters. Factor analysis was performed for data reduction based on correlation between the variables. The number of factors in the model was matched to the number of natural clusters identified. Cluster and factor solutions were compared to examine overlap between the two data reduction methods. In the absence of access to data to perform a direct statistical comparison, a narrative comparison was made of the cluster and factor solutions between this study and the feasibility study [14].

Results

Eighty-five individuals (31 adults and 54 children) from 7 countries were enrolled in this research project. The

demographic characteristics of the participants are shown in Table 1. Median age at consent was 14 years (mean, 17.8 years; range, 2–72 years).

Cluster analysis and exploratory factor analysis

Hierarchical clustering identified six natural clusters of TAND variables as the most parsimonious solution. A dendrogram detailing these six natural clusters is shown in Fig. 1. The first cluster included difficulties with reading, writing, spelling, mathematics, visuo-spatial tasks, restlessness, and disorientation, suggesting a natural "scholastic" cluster. The second cluster included mood swings, aggressive outbursts, and temper tantrums, suggesting a natural "dysregulated behavior" cluster. The third cluster included difficulties in attention/concentration, deficits in memory, neuropsychological attention deficits, dual/multi-tasking, and executive skills. These characteristics suggested a natural "neuropsychological" cluster. The fourth cluster included anxiety, depressed mood, sleep difficulties, and extreme shyness, suggesting a natural "mood/anxiety" cluster. The fifth cluster included self-injurious behavior, hyperactivity, and impulsivity, suggesting a natural "hyperactive/impulsive" cluster. The sixth cluster included delayed language, poor eye contact, repetitive behaviors, unusual use of language, rigidity or inflexibility, and difficulties associated with eating. These characteristics suggested a natural "autism spectrum disorder (ASD)-like" cluster. The exploratory factor analysis findings are shown in Figs. 2 and 3.

Table 1 Demographic characteristics

	Overall participants (N = 85)
Sex, *n* (%)	
Male	40 (47.1)
Female	45 (52.9)
Age strata (years), *n* (%)	
≤ 2	1 (1.2)
> 2 to ≤ 5	11 (12.9)
> 5 to ≤ 9	16 (18.8)
> 9 to ≤14	17 (20.0)
> 14 to < 18	9 (10.6)
≥ 18 to ≤ 40	22 (25.9)
> 40	9 (10.6)
Age at consent, years	
Mean (SD)	17.8 (14.57)
Median (range)	14 (2–72)
Country of residence, *n* (%)	
Belgium	18 (21.2)
France	33 (38.8)
Germany	7 (8.2)
Spain	7 (8.2)
UK	4 (4.7)
Japan	15 (17.6)
Turkey	1 (1.2)

SD standard deviation, *UK* United Kingdom

Comparison of cluster analysis and factor analysis

The similarities and differences between cluster analysis and exploratory factor analysis are shown in Fig. 4. The six factors mapped reasonably well onto the natural clusters identified as linked to scholastic skills, ASD, dysregulated behavior, neuropsychological deficits, hyperactive/impulsive behaviors, and mood/anxiety. With the exception of poor eye contact, there was a 100% overlap between the "ASD-like" natural TAND cluster and the ASD-related factor solution (delayed language, repetitive behaviors, unusual use of language, rigidity or inflexibility, and difficulties associated with eating). In the hyperactive/impulsive natural TAND cluster, factor analysis included one additional characteristic (restlessness), but the other items were identical. In the dysregulated behavior natural TAND cluster, factor analysis included one additional characteristic (extreme shyness), and grouped mood swings with neuropsychological attention deficits and behavioral attention deficits. Aggressive outbursts and temper tantrums were both present in the dysregulated behavior cluster and factor. With regard to the mood/anxiety natural TAND cluster, factor analysis had grouped extreme shyness with other items in the dysregulated behavior cluster. Other mood/anxiety items were the same in the cluster and factor solutions. In the scholastic natural TAND cluster, factor analysis included three neuropsychological variables (dual/multi-tasking, memory, and executive skills), but the other items were identical. A separate "neuropsychological attentional factor" with high cross-loading onto the other neuropsychological variables and the neuropsychological cluster was identified.

Narrative comparison of findings between the feasibility study (Leclezio et al. 2018) and the present study

Cluster solutions

The majority of items from the TAND Checklist were grouped similarly between the two studies. Both the feasibility study and this study showed six natural clusters, with identical findings for the dysregulated behavior and mood/anxiety clusters between the studies (Table 2). In the ASD-like cluster, five variables (language, unusual language, repetitive behavior, poor eye contact, and eating difficulties) were identical between the studies. However, this study also included peer difficulties and inflexibility with the ASD-like cluster. This grouping has good face validity in relation to the clinical characteristics of ASD. In terms of the scholastic cluster, all core scholastic items (difficulties with reading, writing,

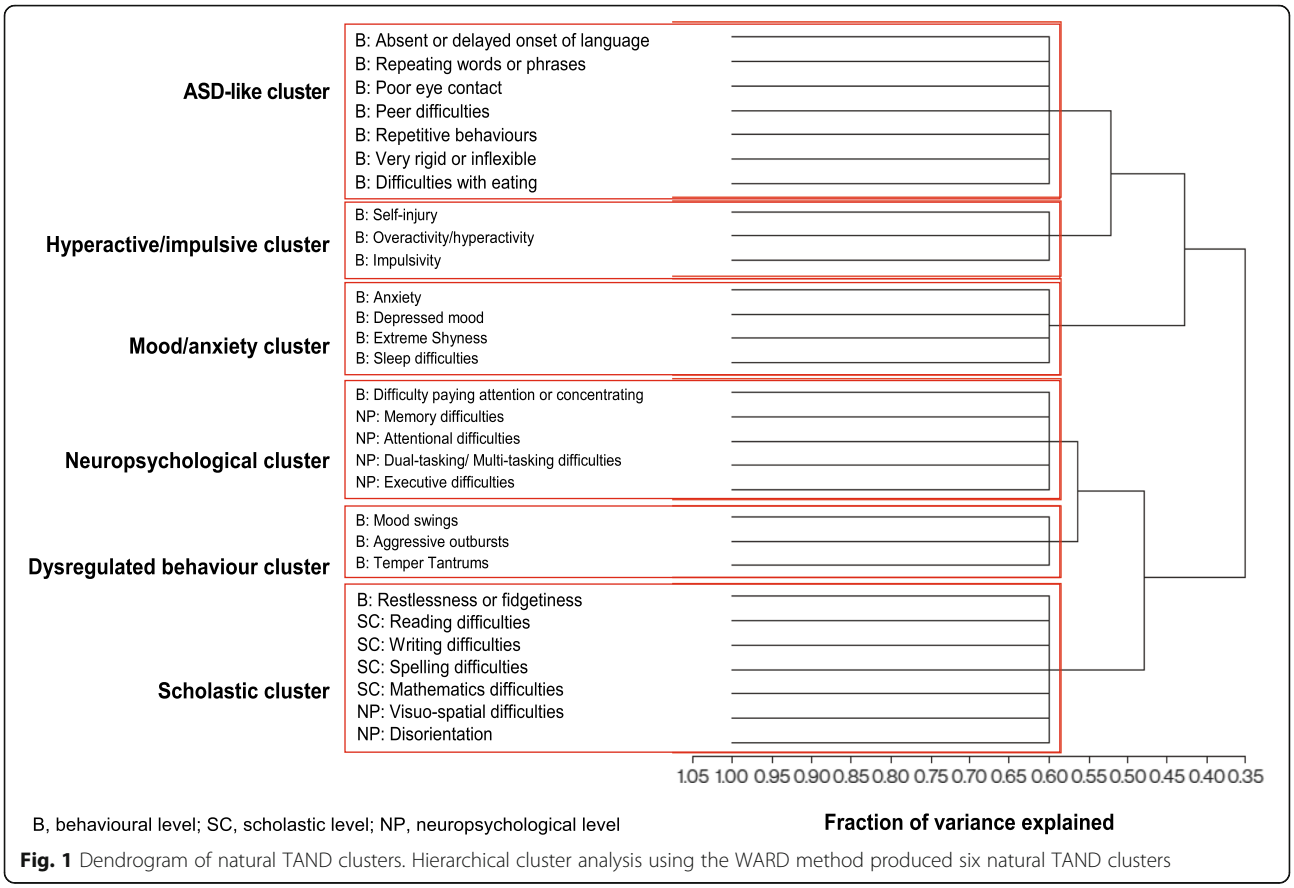


Fig. 1 Dendrogram of natural TAND clusters. Hierarchical cluster analysis using the WARD method produced six natural TAND clusters

spelling, mathematical problems) were grouped together in the feasibility study and in this study. However, two items that appeared more neuropsychological in construct (disorientation and visuo-spatial deficits) were also grouped in the scholastic cluster in the present study. In the hyperactive/impulsive cluster, overactivity, and impulsivity were grouped together in the feasibility study and in this study, but restlessness (grouped with hyperactive/impulsive behaviors in the feasibility study) was clustered in the scholastic cluster in this study. In both studies, attention deficits (behavioral level and neuropsychological attention deficits) clustered separately from the overactive/impulsive items.

Factor solutions

We observed less consistency in factor solutions between the two studies. In the ASD-like factor of this study, almost all the variables were identical to those in the feasibility study, except that our factor analysis excluded self-injury, disorientation, poor eye contact, and difficulty in visuo-spatial tasks, and included inflexibility in the factor (Table 2). In the overactive/impulsive factor, three variables (overactive, impulsive, and restlessness) were identical, but inflexibility and self-injury grouped with different factors. Both dysregulated behavior and mood/anxiety factors had almost identical variables, apart from anxiety and extreme shyness that switched factors between the studies. The mood/anxiety factor in the present study excluded memory. In this study, we observed a combined "scholastic and neuropsychological" factor and a new "attentional" factor that included behavioral attention deficits, neuropsychological attention deficits, and mood swings.

Discussion

Identification of natural TAND clusters through data-driven methods has been proposed as a potential solution for the "treatment paralysis" seen in TSC, given the highly variable and apparently unique nature of TAND profiles in individuals. In a proof-of-principle study, Leclezio, Gardner, and de Vries showed the feasibility of using data reduction methods in TAND and identified six putative natural clusters [14]. However, the sample size of the Leclezio study was very small, and individuals were recruited from only two countries. Given these limitations and the highly heterogeneous nature of TSC, we set out to replicate the feasibility findings in a larger sample of 85 individuals, including children, from seven countries. We observed six natural TAND clusters (scholastic, ASD-like, dysregulated behavior, neuropsychological, overactive/

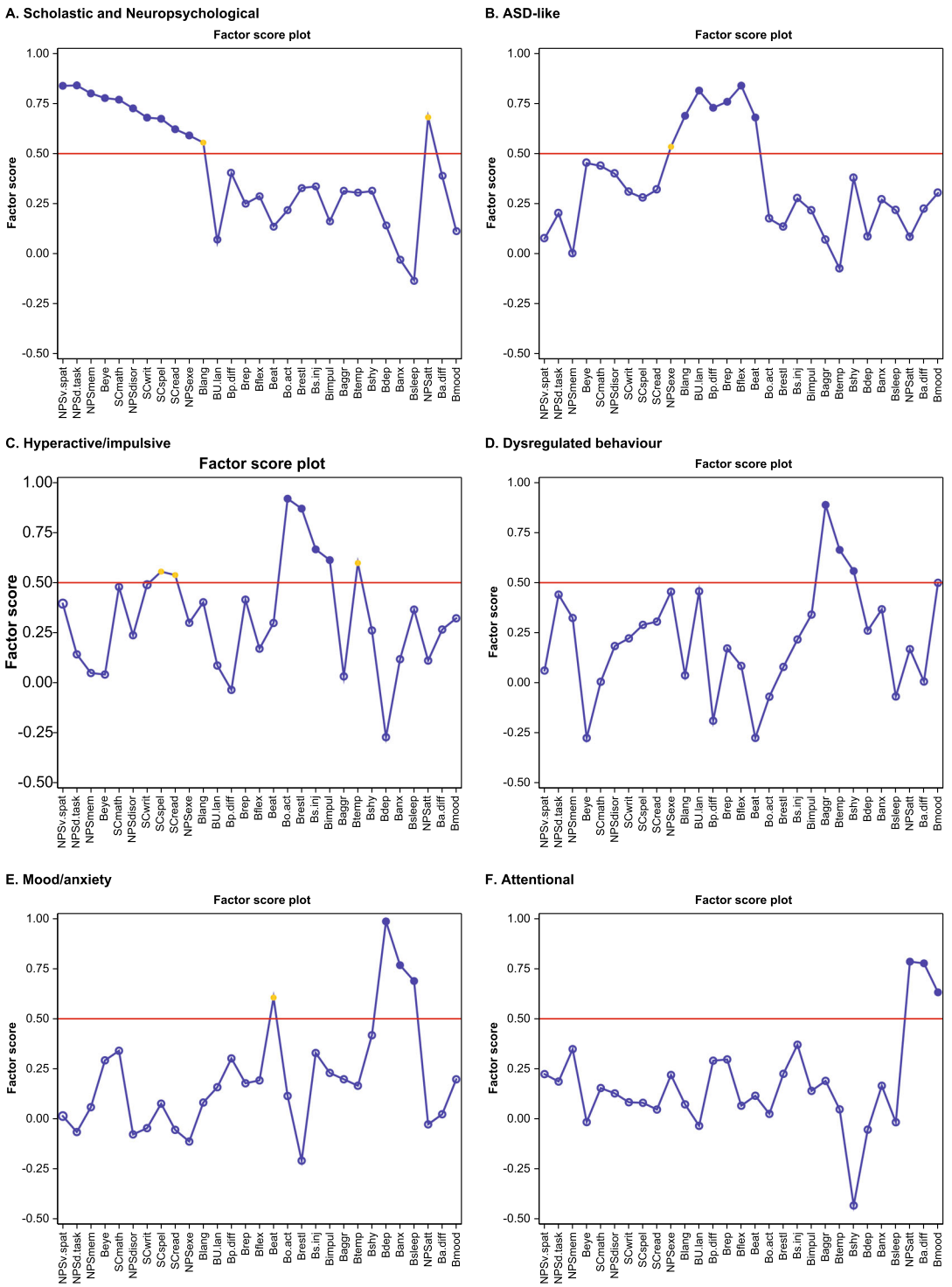
		Factor 1	Factor 2	Factor 3	Factor 4	Factor 5	Factor 6
Scholastic and Neuropsychological	NP: Visuo-spatial difficulties	0.83583	0.07226	0.39345	0.05909	0.01320	0.22280
	NP: Dual-tasking/ Multi-tasking difficulties	0.83785	0.19796	0.14001	0.43847	-0.06660	0.18583
	NP: Memory difficulties	0.79784	-0.00295	0.04675	0.32173	0.05763	0.34768
	B: Poor eye contact	0.77454	0.44973	0.03914	-0.27795	0.29152	-0.01694
	SC: Mathematics difficulties	0.76654	0.43490	0.47697	0.00308	0.33951	0.15322
	NP: Disorientation	0.72319	0.39631	0.23567	0.18159	-0.07848	0.12646
	SC: Writing difficulties	0.67688	0.30495	0.48804	0.22007	-0.04714	0.08217
	SC: Spelling difficulties	0.67113	0.27549	0.55357	0.28732	0.07523	0.07947
	SC: Reading difficulties	0.61890	0.31630	0.53624	0.30368	-0.05575	0.04592
	NP: Executive difficulties	0.58782	0.53123	0.29794	0.45316	-0.11409	0.21852
ASD-like	B: Absent or delayed onset of language	0.55197	0.68604	0.40020	0.03528	0.08076	0.07167
	B: Repeating words or phrases	0.05721	0.81218	0.08408	0.45502	0.15750	-0.03560
	B: Peer difficulties	0.39793	0.72617	-0.03757	-0.19178	0.30124	0.28987
	B: Repetitive behaviours	0.24647	0.75627	0.41315	0.16955	0.17720	0.29627
	B: Very rigid or inflexible	0.28407	0.83678	0.16840	0.08205	0.19145	0.06498
	B: Difficulties with eating	0.13308	0.67763	0.29688	-0.27745	0.60532	0.11476
Hyperactive/impulsive	B: Overactivity/hyperactivity	0.21398	0.17140	0.91800	-0.07167	0.11355	0.02413
	B: Restlessness or fidgetiness	0.32541	0.13027	0.86841	0.07716	-0.20976	0.22436
	B: Self-injury	0.33251	0.27280	0.66463	0.21447	0.32840	0.36963
	B: Impulsivity	0.15839	0.21173	0.61116	0.33871	0.22882	0.13916
Dysregulated behaviour	B: Aggressive outbursts	0.30989	0.06515	0.03004	0.88699	0.19715	0.18904
	B: Temper Tantrums	0.30154	-0.07842	0.59652	0.66214	0.16468	0.04668
	B: Extreme Shyness	0.30903	0.37446	0.25949	0.55643	0.41720	-0.43373
Mood/anxiety	B: Depressed mood	0.13794	0.08105	-0.27402	0.25867	0.98596	-0.05463
	B: Anxiety	-0.03202	0.26662	0.11574	0.36518	0.76733	0.16451
	B: Sleep difficulties	-0.13616	0.21330	0.36346	-0.07046	0.68812	-0.01763
Attentional	NP: Attentional difficulties	0.67846	0.07946	0.10889	0.16585	-0.02831	0.78501
	B: Difficulty paying attention or concentrating	0.38441	0.21981	0.26405	0.00420	0.02226	0.77660
	B: Mood swings	0.11144	0.29992	0.32030	0.49751	0.19683	0.63167

B, behavioural level; SC, scholastic level; NP, neuropsychological level

Fig. 2 Exploratory factor analysis results of a six-factor solution to identify the latent constructs underlying the TAND variables. The figure shows the rotated factor pattern using the Varimax method. Coefficients in blue represent the largest coefficient values for each variable across all 6 factors. All other coefficients with values > 0.5 are shown in yellow

impulsive, and mood/anxiety). These were remarkably similar to those identified by Leclezio et al. in the feasibility study [14], but had more mixed results in factor solutions, thus providing partial replication of the finding of potential natural TAND clusters. However, while some items were clearly differently grouped using data-driven strategies between the feasibility study and this study, many similarities were seen, suggesting that, in spite of the vast heterogeneity of TAND, there may be robust natural clusters of TAND manifestations that should be explored further in larger-scale studies [16–18].

Currently, many families and clinical teams are unaware of which of all the possible TAND manifestations to look out for and how to provide appropriate evidence-based, next-step interventions. If a limited number of natural clusters are confirmed, clinical monitoring, and next steps of psycho-education and intervention for six or so clusters of difficulties would be much more feasible. For instance, it may be possible then to develop modular training based on specific clusters, such as specific programs for dysregulated behavior in TSC or for mood/anxiety cluster features.



NPSv.spat, visuo-spatial difficulties; NPSd.task, dual-tasking/multi-tasking; NPSmem, memory difficulties; Beye, poor eye contact; SCmath, mathematics difficulties; NPSdisor, disorientation; SCwrit; writing difficulties; SCspel, spelling difficulties; SCread, reading difficulties; NPSexe, executive difficulties; Blang, absent or delayed language; BU.lan, repeating words or phrases; Bp.diff, peer difficulties; Brep, repetitive behaviours; Bflex, rigidity or inflexibility; Beat, eating difficulties; Bo.act, overactivity/hyperactivity; Brestl, restlessness or fidgetiness; Bs.inj, self-injury; Bimpul, impulsivity; Baggr, aggressive outbursts; Btemp, temper tantrums; Bshy, extreme shyness; Bdep, depressed mood; Banx, anxiety; Bsleep, sleep difficulties; NPSatt, attentional difficulties; Ba.diff, difficulty paying attention or concentrating; Bmood, mood swings

Fig. 3 Visualization of the factor score graph showing factor scores of individual TAND variables in relation to the six-factor solution derived from exploratory factor analysis. The closer a factor score is to + 1 the stronger the influence of the factor is on that variable. Solid blue dots represent the largest coefficient values for each variable across all 6 factors and solid yellow dots represent all other coefficients with values > 0.5. Blue circles represent coefficients with values < 0.5

It was of interest that some of the natural clustering was in groups that make intuitive diagnostic sense from clinical criteria, such as the ASD-like cluster. TSC is known to be one of the medical conditions most strongly associated with ASD [6]. However, it was also interesting to observe that the hyperactive/impulsive features did not cluster with the inattention features, in contrast with the typical clinical grouping of manifestations associated with attention deficit/hyperactivity disorder (ADHD). In both the feasibility and this study, behavioral attention deficits were more likely to cluster with neuropsychological attention-executive skill deficits. All these proposals will require further evaluation in larger-scale studies.

For the purposes of this early-phase replication study, we wanted to see if, first, we were able to identify robust methodologies and whether they would replicate in an independent sample, and second, whether natural clusters could be identified even in the absence of age and intellectual ability data. The association between age and intellectual ability on TAND clusters, however, raises interesting conceptual and empirical questions. It is likely that TAND cluster profiles may emerge or change over time. For instance, the scholastic cluster is likely not to be relevant in the first few years of life. Similarly, intellectual ability may be a very strong marker of the likelihood of TAND clusters. These important questions will require larger-scale and longitudinal datasets.

In comparison to the feasibility study [14] where only English-speaking participants were used, we deliberately aimed to include a more culturally and linguistically diverse sample to examine the robustness of the putative TAND clusters identified. The sample therefore included French, Dutch, English, German, Spanish, Turkish, and Japanese participants. The TAND Checklist has been translated and authorized in 17 languages to date, and where available, those language versions were used. Larger-scale studies may allow for a comparison of TAND cluster profiles in different cultural and language groups. However, to date, there are no clinical suggestions that TAND manifestations have differential cultural expression.

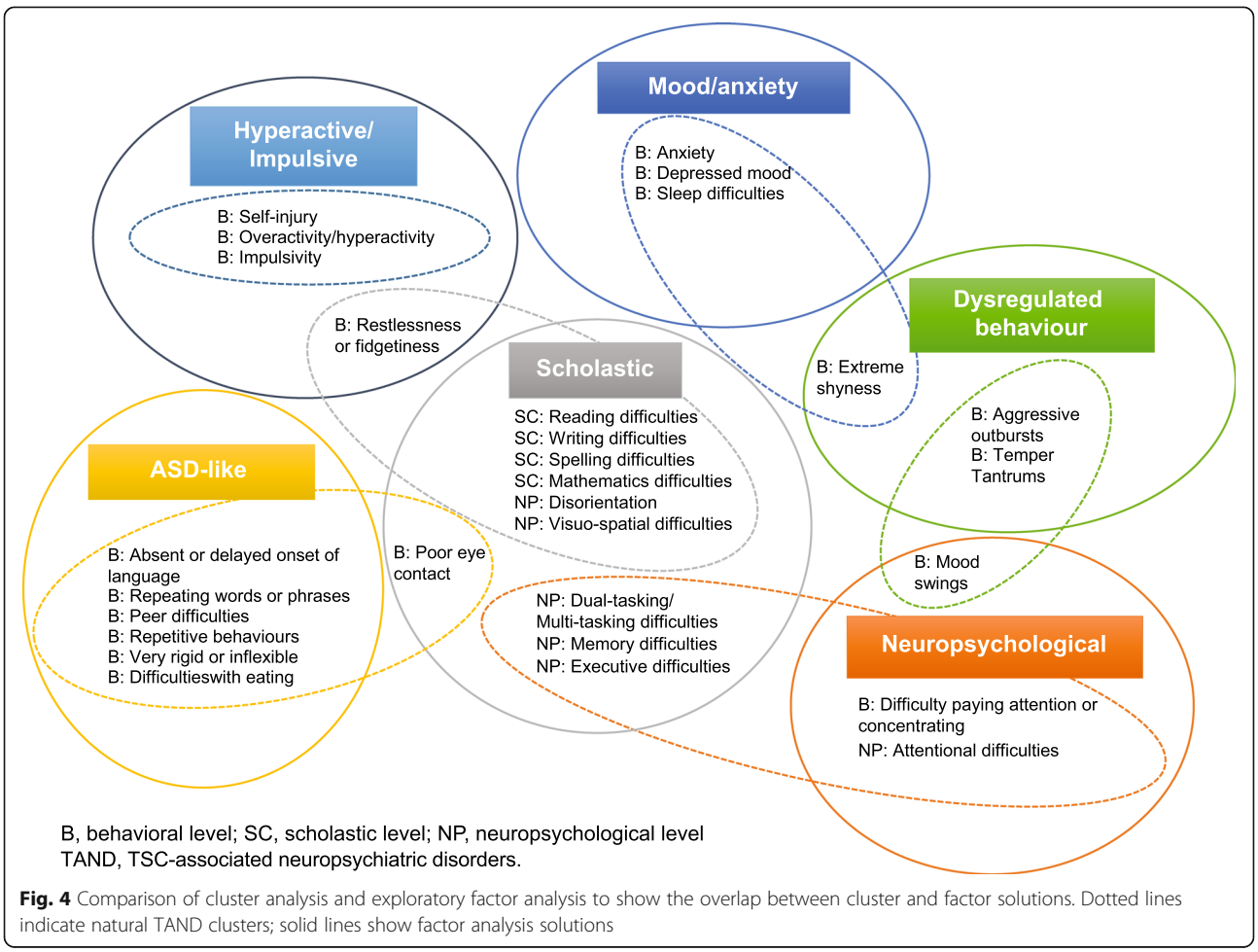


Fig. 4 Comparison of cluster analysis and exploratory factor analysis to show the overlap between cluster and factor solutions. Dotted lines indicate natural TAND clusters; solid lines show factor analysis solutions

Table 2 Comparison of clusters and factors between the feasibility study (Leclezio et al. 2018) and this study (the replication study)

Clusters and factors	Variables		
	Both feasibility and replication studies	Replication study (current study)	Feasibility study (Leclezio et al. 2018)
TAND clusters			
1. Mood/anxiety	Anxiety (Banx) Depressed mood (Bdep) Extreme shyness (Bshy) Sleep difficulties (Bsleep)	-	-
2. Dysregulated behavior	Mood swings (Bmood) Aggressive outbursts (Baggr) Temper tantrums (Btemp)	-	-
3. ASD-like	Absent or delayed language (Blang) Repeating words or phrases (BU.lan) Poor eye contact (Beye) Repetitive behaviors (Brep) Difficulties with eating (Beat)	Peer difficulties (Bp.diff) Rigidity/inflexibility (Bflex)	Self-injury (Bs.inj) Visuo-spatial difficulties (NPSv.spat)
4. Hyperactive/impulsive	Overactivity (Bo.act) Impulsivity (Bimpul)	Self-injury (Bs.inj)	Rigidity/inflexibility (Bflex) Restlessness (Brestl)
5. Neuropsychological	Difficulty paying attention (Ba.diff) Memory difficulties (NPSmem) Attention difficulties (NPSatt) Dual-tasking difficulties (NPSd.task) Executive difficulties (NPSexe)	-	Visuo-spatial difficulties (NPSdisor) Peer difficulties (Bp.diff)
6. Scholastic	Reading difficulties (SCread) Writing difficulties (SCwrit) Spelling difficulties (SCspel) Mathematics difficulties (SCmath)	Restlessness (Brestl) Visuo-spatial difficulties (NPSv.spat) Disorientation (NPSdisor)	-
TAND factors			
1. Scholastic and Neuropsychological	Dueal-task difficulties (NPSd.task) Executive difficulties (NPSexe) Mathematics difficulties (SCmath) Reading difficulties (SCread) Writing difficulties (SCwrit) Spelling difficulties (SCspel)	Visuo-spatial difficulties (NPSv.spat) Memory difficulties (NPSmem) Disorientation (NPSdisor) Poor eye contact (Beye)	Attention difficulties (Ba.diff) Neuropsychological attention difficulties (NPSatt)
2. ASD-like	Absent or delayed language (Blang) Repeating words or phrases (BU.lan) Peer difficulties (Bp.diff) Repetitive behaviors (Brep) Eating difficulties (Beat)	Rigidity/inflexibility (Bflex)	Visuo-spatial difficulties (NPSv.spat) Disorientation (NPSdisor) Self-injury (Bs.inj) Poor eye contact (Beye)
3. Hyperactive/impulsive	Restlessness (Brestl) Overactivity (Bo.act) Impulsivity (Bimpul)	Self-injury (Bs.inj)	Rigidity/inflexibility (Bflex)
4. Dysregulated behavior	Aggressive outbursts (Baggr) Temper tantrums (Btemp)	Extreme shyness (Bshy)	Anxiety (Banx) Mood swings (Bmood)
5. Mood/anxiety	Depressed mood (Bdep) Sleep difficulties (Bsleep)	Anxiety (Banx)	Memory difficulties (NPSmem) Extreme shyness (Bshy)
6. Attentional	-NA-	Neuropsychological attention difficulties (NPSatt) Attention difficulties (Ba.diff) Mood swings (Bmood)	-NA-

ASD autism spectrum disorder

The columns show all TAND Checklist items included in the study and the abbreviation for each variable in parenthesis e.g. Anxiety (Banx)

Limitations and next steps

There are several potential limitations to this study. We acknowledge that, even though this study sample was larger and more diverse than that of the feasibility study, the sample size was still small, even for a rare disease. We were aiming to recruit from a large natural history study (TOSCA study) and were therefore hopeful to include a much larger sample for this study. However, given that it was embedded in an industry-funded observational trial, a formal procedure for opting in at a country level was required. Where countries opted in, all participants at centers were included. While we therefore acknowledge an "administrative" bias in recruitment, we have no reason to suspect a clinical ascertainment bias, given that all subjects from participating centers had a TAND Checklist completed.

Interestingly, there is no consensus in the literature about the required sample size for cluster analysis, and a number of small-scale studies such as ours have identified meaningful natural clusters [19]. Some authors have suggested a minimum sample size of $n = 100$, while others emphasized the importance of an optimal variable/subject ratio with a 1:10 ratio (1 variable to 10 subjects) as most stringent suggestion [20]. Given the differences observed between the feasibility and replication data sets, we propose that it would be important to proceed to examination of larger-scale samples, ideally in excess of the 1/10 (variable/subject) ratio. Secondly, apart from cluster and factor analysis, it would be important to evaluate the internal consistency of putative natural clusters and to examine the robustness of these clusters using bootstrapping methodologies. These extra steps will extend the investigation of the psychometric properties and robustness of the putative natural TAND clusters. We also acknowledge that the natural clusters were generated using only the TAND Checklist data. There may therefore be other natural clusters that could be identified using different kinds of fine-grain data. However, the purpose of the TAND Checklist was to provide a simple and easy-to-use tool for clinical practice. For this reason, we set out to examine the potential of the TAND Checklist data to generate natural TAND Clusters, given that such a strategy has a far greater potential for larger-scale implementation.

Conclusion

In spite of the highly heterogeneous nature of TAND manifestations, the data-driven strategies used here in search of natural TAND clusters were able to replicate the findings from the feasibility study in a larger sample of children and adults with the pen-and-paper TAND Checklist data collected across seven countries. The study not only identified several similarities between the findings from the two data sets but also identified key aspects and next steps that will require larger-scale data, replication, and expansion. If these steps could replicate and extend the natural TAND clusters suggested in these preliminary studies, the natural TAND clusters may have the potential to help develop novel approaches to identification and treatment of TAND and may suggest novel data-driven strategies to subgroup individuals with TSC for clinical and research purposes.

Abbreviations

ADHD: Attention deficit/hyperactivity disorder; ASD: Autism spectrum disorder; TAND: TSC-associated neuropsychiatric disorders; TSC: Tuberous sclerosis complex

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Availability of data and materials

Novartis supports the publication of scientifically rigorous analysis that is relevant to patient care, regardless of a positive or negative outcome. Qualified external researchers can request access to anonymized patient-level data, respecting patient informed consent, contacting study sponsor authors. The protocol can be accessed through EnCePP portal http://www.encepp.eu/ (EU PAS Register Number EUPAS3247).

Ethics approval and consent to participate

The study protocol and all amendments were reviewed and approved (if applicable) by an independent ethics committee/institutional review board for each center: National Hospital Organization Central Ethics Committee; Gazi University Clinical Research Ethics Committee; Independent Multidisciplinary Committee on Ethical Review of Clinical Trials; Peking Union Medical College Hospital; Commissie Medische Ethiek UZ Brussel; CNIL (Commission National de l'Informatique et des Libertés), CCTIRS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); Comité Etico Investigación Clínica de Euskadi (CEIC-E); Consejeria de Salud y Bienestar Social, Dirección General de Calidad, Investigación, Desarrollo e Innovación, Comité Coordinador de Ética de la Investigación Biomédica de Andalucía; Research Ethics Committee of the University of Tartu (UT REC); Ethikkommission der Medizinischen Universität Graz; North Wales REC–West; Regionala Etikprövningsnämnden i Göteborg; REK–Regionale komiteer for medisinsk og helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie "Pomnik Centrum Zdrowia Dziecka"; Ethikkommission bei der Ludwig-Maximilians-Universitat München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for Chile Health and Development of IRB; Osaka University Hospital of IRB; Ethics Committee at Moscow Institute of Pediatrics and Pediatric Surgery; Peking University First Hospital; Sanbo Brain Hospital Capital Medical University; Tianjin Children's Hospital; Childrens Hospital Of Fudan University; Zhongshan Hospital Fudan University; Fudan University Shanghai Cancer Center; The Second Affiliated Hospital of Guangzhou Medical University; The First Affiliated Hospital, Sun Yat-Sen University; The First Affiliated Hospital Of Guangzhou Medical University; Shenzhen Children's Hospital; West China Hospital, Sichuan University; Xijing Hospital; Children's Hospital of Chongqing Medical University; Wuhan Children's Hospital; The Second Affiliated Hospital of Xi'an Jiaotong University; Guangdong 999 Brain Hospital; Seoul National University Hospital Institutional Review Board; National Taiwan University Hospital (NTUH) Research Ethics Committee (REC); Institutional Review Board of the Taichung Veterans General Hospital; Institutional Review Board of Chung Shan Medical University Hospital; Institutional Review Board, Tungs' Taichung MetroHarbor Hospital; Institutional Review Board of National Cheng Kung University Hospital; Metro South Human Research Ethics Committee; Sydney Children's Hospital Network Human Research Ethics Committee; St Vincent's Hospital Human Research Ethics Committee; Royal Melbourne Hospital Human Research Ethics Committee; Siriraj Institutional Review Board; The Institutional Review board, Faculty of Medicine, Chulalongkorn University, 3rd Floor, Ananthamahidol Building, King Chulalongkorn Memorial Hospital; The committee on Human Rights Related to Research Involving Human Subjects; Institutional Review board, Royal Thai Army Medical Department IRB RTA, 5th Floor, Phramongkutklaowejvitya Building, Phramongkutklao College of Medicine; Research Ethics Committee, Faculty of Medicine, Chiang Mai University; Research and Development, Queen Sirikit National Institute of Child Health; Human Research Ethics Committee, Faculty of Health Sciences, University of Cape Town; Shaare Zedek Meidcla Center Helsinki committee; Sheba Medical Center Helsinki committee; Tel Aviv Sourasly Medical Center Helsinki committee; General University Hospital of Patras Ethics Committee; Pendeli Children's Hospital Ethics Committee; General University Hospital of Athens 'G. 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Consent for publication
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Competing interests
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