TUBEROUS SCLEROSIS COMPLEX - DIAGNOSIS AND MANAGEMENT

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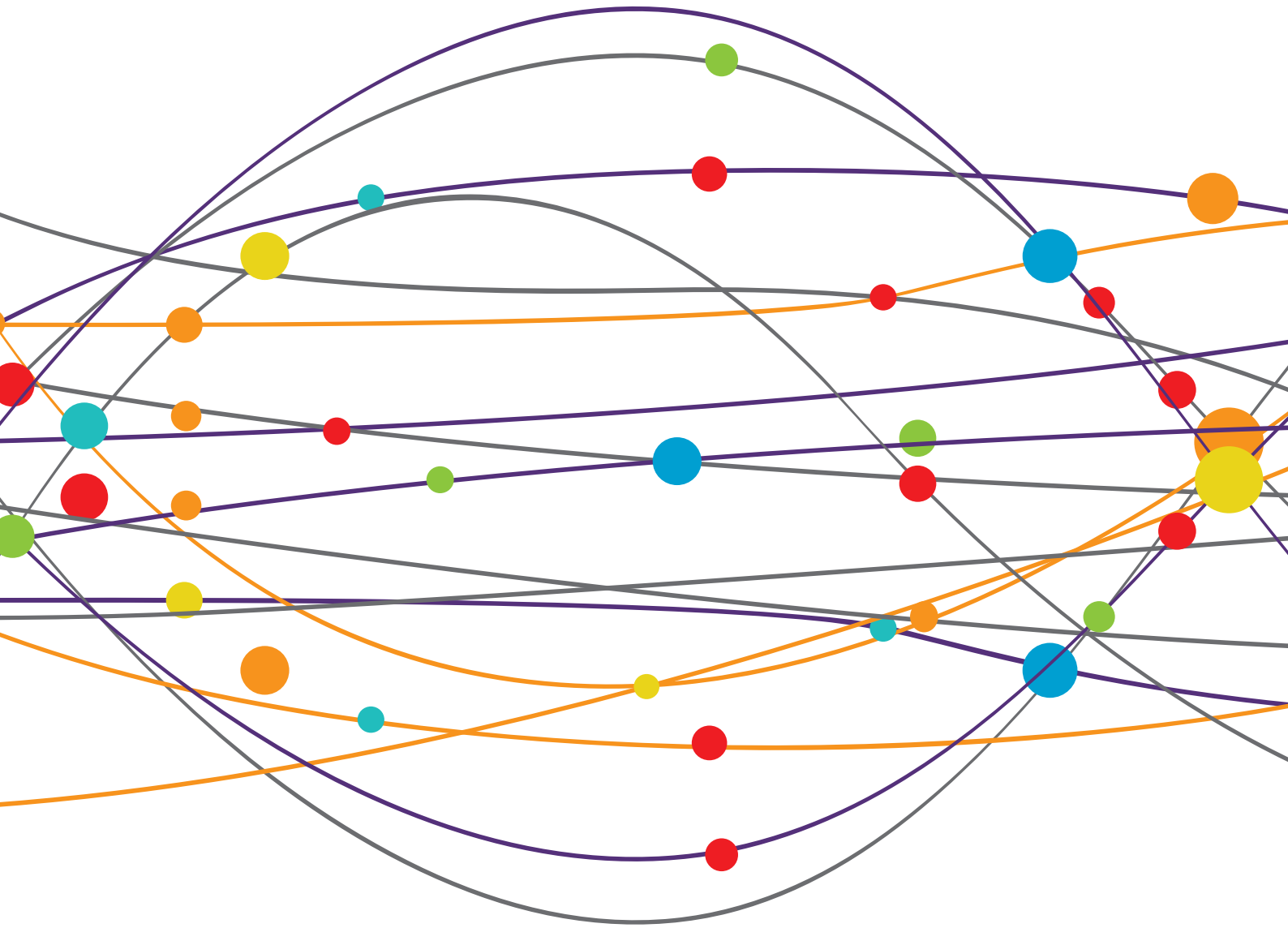

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TUBEROUS SCLEROSIS COMPLEX - DIAGNOSIS AND MANAGEMENT

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EDITORIAL
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Editorial: Tuberous Sclerosis Complex – Diagnosis and Management

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Editorial on the Research Topic

Tuberous Sclerosis Complex – Diagnosis and Management

Tuberous sclerosis complex (TSC) is a multisystem genetic disorder caused by a mutation in either the TSC1 or TSC2 gene and resulting in an overactivation of the mTOR pathway that affects many organs and systems (1).

Though it has been 150 years since the first clinically reported case of tuberous sclerosis, there are still many gaps in our understanding of its pathogenesis, clinical symptomatology, comorbidities, prognostic significance, genetic heterogeneity, and therapeutic possibilities. Moreover, as it is a rare disorder, TSC faces all the difficulties that are characteristic of small cohorts of patients. The creation of a multicentered, multinational TuberOus SClerosis registry to increase disease awareness (TOSCA registry), consisting of 2,221 TSC patients from 31 countries, was intended to address these gaps. Data collected on the natural history of TSC, the incidence of the TSC manifestations, and the age at their onset, are included in several papers published in this Special Issue.

Subependymal giant-cell astrocytomas (SEGAs) are among the most frequent and life-threatening manifestations of TSC. Two papers by Jansen, Belousova, Benedik, Carter, Cottin, Curatolo, Dahlin et al. report the natural course and treatment characteristics in 554 patients from 2,216 TOSCA participants (25%). The median age at diagnosis of SEGAs was 8 years (range, 0–51). SEGAs were symptomatic in 42.1% of patients. Symptoms included increased seizure frequency (15.8%), behavioral disturbance (11.9%), and regression/loss of cognitive skills (9.9%). SEGAs were significantly more frequent in patients with *TSC2* compared to *TSC1* variants (33.7 vs. 13.2%, $p < 0.0001$). Interestingly, mammalian targets of rapamycin inhibitors (mTORi) were almost as frequently used as surgery (49 vs. 59.6%). However, this finding may be biased in the TOSCA study by the high proportion of sites participating in EXIST-1 trial.

Another important finding from the Jansen, Belousova, Benedik, Carter, Cottin, Curatolo, Dahlin, D'Amato et al. paper is the possibility of SEGA development in adult patients. Fourteen adults (2.4%) were newly diagnosed with SEGAs during follow-up, and all had the *TSC2* mutation.

Renal angiomyolipomas (AMLs) are one of the most common renal manifestations in patients with TSC, with potentially life-threatening complications and a poor prognosis. In the study of Kingswood, Belousova, Benedik, Carter, et al., they were significantly more prevalent in female patients ($p < 0.0001$). Although renal AMLs in subjects with *TSC1* mutations develop on average at a later age and are relatively smaller, by age 40 no difference was observed in the percentage of patients with *TSC1* and *TSC2* mutations needing intervention.

Non-functional pancreatic neuroendocrine tumors (PNETs) are rare in TSC, with no specific guidelines outlined for clinical management. Mowrey et al. reported 16 individuals, nine males

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and seven females with an average age of 22.0 years, and characterized the course of the tumors and applied management. The calculated prevalence of non-functional PNETs in their group was 0.65%.

Individuals with TSC are at increased risk of developing both epilepsy and autism. mTOR dysregulation could play a direct role in determining susceptibility to epilepsy, cognitive impairment, and autism spectrum disorder (2). The identification of early signs may therefore become an important prevention tool.

In two papers by Nabbout et al. epilepsy appeared in 85% of patients with a high incidence of drug-resistant epilepsy. *TSC1* mutations were associated with less severe epilepsy phenotypes and more individuals with normal IQ. GABAergics were the first-line treatment in 45% of children with infantile spasms. Prenatal or early infantile diagnosis of TSC provides a unique opportunity to monitor EEG before the onset of clinical seizures. Recently, the EPISTOP trial provided crucial information on the optimal timing for initiating treatment in high-risk infants (3).

A relatively low percentage (12.5–25%) of patients with EEG performed before seizures in the paper by Nabbout et al. may be partly explained by the retrospective character of the study and the large proportion of currently adult patients in the TOSCA registry.

A better understanding of the early biomarkers of developmental outcome could give us a therapeutic window in which early-targeted treatment could obtain greater benefits. De Ridder et al. investigated whether early EEG characteristics in newborns and infants with TSC can be used to predict neurodevelopment. The first recorded EEG of 64 infants with TSC, enrolled in the international prospective EPISTOP trial was correlated with ASD risk based on the ADOS-2 score, and cognitive, language, and motor developmental quotients (Bayley Scales of Infant and Toddler Development III) at the age of 24 months. A dysmature EEG background was associated with lower cognitive, language, and motor developmental quotients at the age of 24 months indicating that early EEG characteristics can be used to predict neurodevelopmental comorbidities in infants with TSC.

The complex relationship between epilepsy and comorbid autism in TSC has been discussed by Specchio et al.. They highlighted the need for early identification and management to optimize favorable outcomes in infants at high risk of developing early seizure onset and autism.

Prenatal or early infantile diagnosis of TSC provides a unique opportunity to monitor EEG before the seizure onset (4). The prevention of epilepsy in infants with TSC is becoming an important challenge. Recently the EPISTOP trial demonstrated that preventive treatment with Vigabatrin reduced the risk and severity of epilepsy (3). Early developmental markers of ASD, such as a social communication deficit, may be identified in the first year of life (5), allowing early intervention in infants at high risk of developing autism, with the potential of optimizing developmental outcomes.

In a *post-hoc* analysis focused on pediatric patients enrolled in the EXIST3 trial, adjunctive everolimus resulted in a sustained reduction in seizure frequency, with particular efficacy in younger children under the age of 6 years (6). Preventive trials with mTOR inhibitors could now be designed in pre-symptomatic infants to evaluate if this strategy could have disease-modifying effects.

Everolimus, a disease-modifying drug that targets the molecular biology of TSC, can address multiple aspects of the disease at the same time. Kingswood, Belousova, Benedik, Budde et al. assessed the long-term safety of everolimus in a non-interventional post-authorization safety study (PASS) in patients with TSC who participated in the TOSCA clinical study and received everolimus for the licensed indications in the European Union. One hundred and eighteen of 179 (66%) patients had an adverse effect (AE) of any grade, with the most common AEs being stomatitis (7.8%) and headache (7.3%). AEs caused dose adjustments in 31.3% and treatment discontinuation in 5% of patients.

To achieve beneficial, suppressing effects of mTORi on growing tumors in TSC, persistent drug treatment is necessary. The aim of EMINENTS prospective, single-center, open-label, single-arm study was to evaluate the cumulative efficacy and safety of reduced doses of everolimus (maintenance therapy) in patients TSC and SEGA. Bobeff et al. included 15 patients who had undergone at least 12 months of treatment with a standard everolimus dose. The dose of everolimus was reduced to three times a week, and patients were followed over a mean duration of 58.37 months. No clinical symptoms of progression were observed in any patients. Regarding AEs, infections and laboratory abnormalities occurred less frequently during maintenance therapy compared to the standard dose regimen.

Marques, Belousova, Benedik, Carter, Cottin, Curatolo, Dahlin, D'Amato, d'Augères, de Vries, Ferreira, Feucht, Fladrowski, Hertzberg, Jansen et al. showed evidence that the TOSCA registry improved the knowledge on the natural history and manifestations of TSC, increased awareness, produced real-world evidence, and helped to identify relevant information for future clinical research.

The authors provided a comprehensive picture of the medical and non-medical health care resources in TSC from information within the TOSCA registry. GABAergic were the most prescribed drugs for epilepsy, and mTOR inhibitors were dramatically replacing surgery in patients with SEGA, despite current recommendations proposing both treatment options (Marques, Belousova, Benedik, Carter, Cottin, Curatolo, Dahlin, D'Amato, d'Augères, de Vries, Ferreira, Feucht, Fladrowski, Hertzberg, Jozwiak et al.).

In another paper, Marques, Thole et al. tested the recommendations from the European Medicine Agency (EMA) on the rare disease registries. They elaborated the compliance and deviations of the TOSCA registry from the EMA guidance on a point-by-point basis, revealing that in most aspects the TOSCA registry met its objective to enhance our understanding of TSC and its manifestations.

Research on TSC to date has focused mainly on the physical manifestations of the disease. One study in this issue examines the psychosocial impact of TSC, which has received until now less attention. Jansen, Vanclooster et al. investigated the quality of life

from the TOSCA study and highlighted the substantial burden of the disease on the personal lives of individuals with TSC. A smooth transition from pediatric to adult care was mentioned by only 36% of caregivers.

TAND poses significant challenges for the diagnosis and management of TSC. Although knowledge is increasing about TAND, little is known about the confounding effects of intellectual ability and the rate of TAND across age, sex, and genotype. de Vries et al. demonstrated in this issue that there is a significant association between levels of intellectual ability and the majority of TAND manifestations. However, no significant age or sex differences were observed from academic difficulties or neuropsychological deficits.

Current recommendations for the delivery of services for TSC patients, including diagnosis, surveillance, treatment, and safe transition from pediatric to adult care, are the focus of Annear et al.. TSC clinics need to offer a range of core services in order to provide comprehensive treatment to TSC patients. Furthermore, TSC clinics should have a multidisciplinary team with a dedicated specialist TSC coordinator, with the aim of ensuring that each TSC patient and their family have a tailored care plan to manage current manifestations and surveillance for future disease manifestations.

In this special issue, several papers were related to TOSCA to expand the current knowledge on diagnosis and management of TSC, allowing the broadening of preventive strategies and stimulating further research in the field. There is now the need to improve TSC management to ensure patients have early access to appropriate treatment and preventive measures.

AUTHOR CONTRIBUTIONS

SJ and PC worked equally on editorships, reviewing the articles, and writing the editorial. Both authors contributed to the article and approved the submitted version.

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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

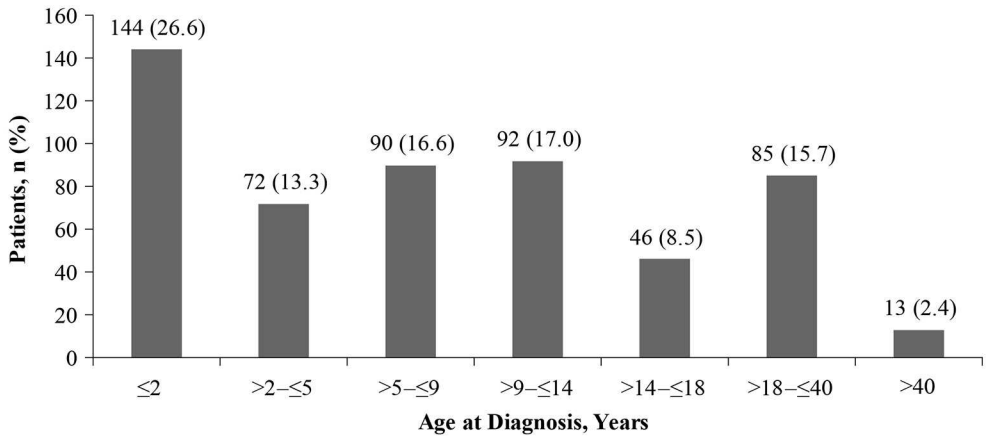

FIGURE 1 | Proportion of patients with SEGAs according to age at SEGA diagnosis ($n = 542$).

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

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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 (± 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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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.

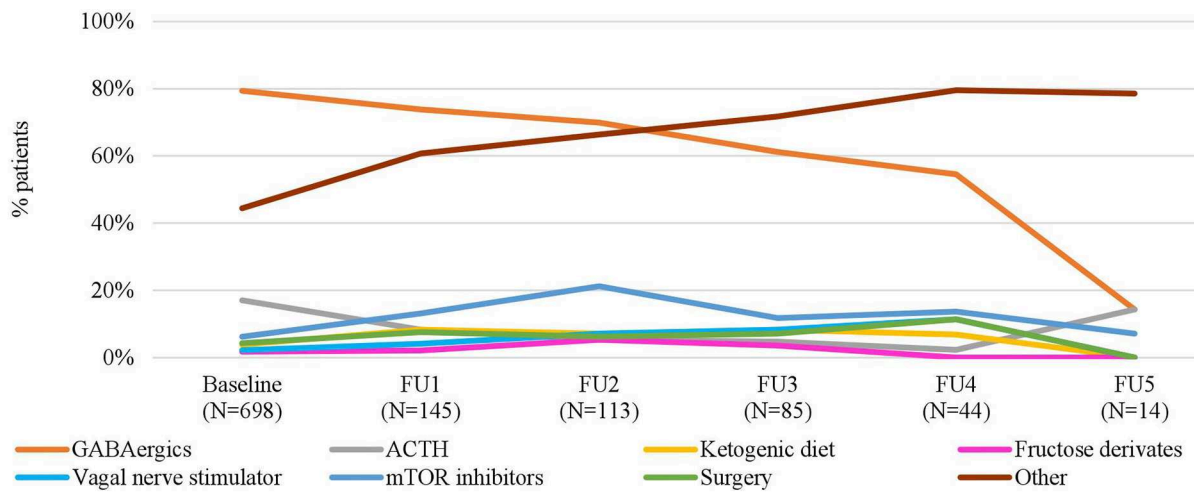
The registry consisted of a "core" part and six associated research projects focusing on: epilepsy, subependymal giant cell astrocytomas (SEGA), renal angiomyolipoma (rAML)/lymphangioleiomyomatosis (LAM), genetics, quality of life (QoL), and TSC-associated neuropsychiatric disorders (TAND); the "core" 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).

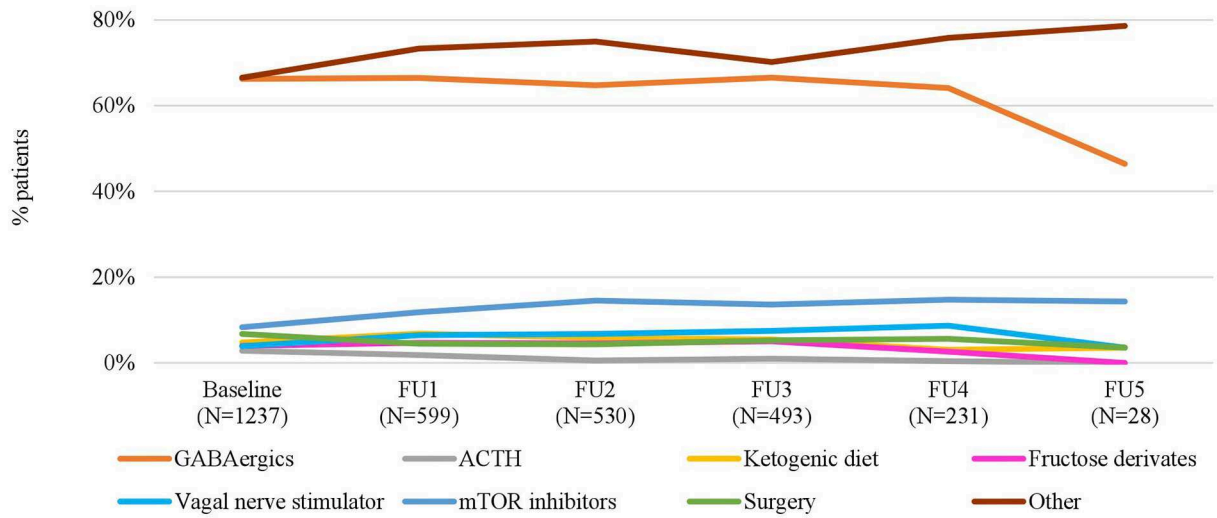
Treatment patterns were analyzed using the core registry data according to 4 clinical manifestations (epilepsy, SEGA, LAM, and rAML), the number of visits [baseline or follow-ups (FU1 to FU5), where FUs were conducted at intervals not longer than 12 months apart], the age group ($\leq$2, >2 to $\leq$5, >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.

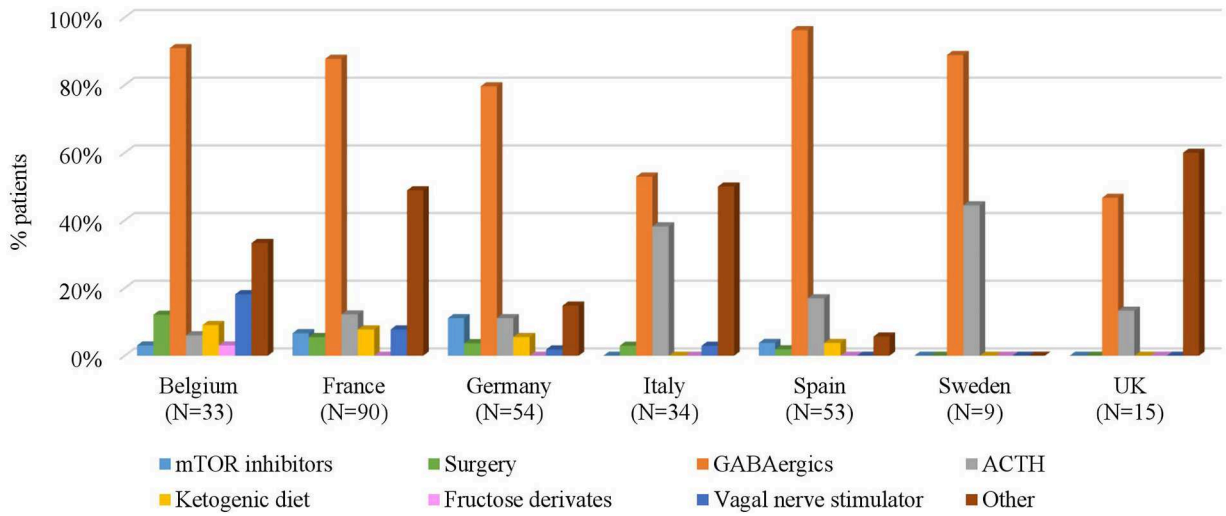
(52.1%) were female. The median age at enrolment was 13 years (range <1–71), and the median age at first TSC diagnosis was 1 year (range <1–69 years). The most common manifestation was epilepsy occurring in 1,879 (85.0%) of patients. Among patients with epilepsy, 1,343 (71.5%) had focal seizures (FS) 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

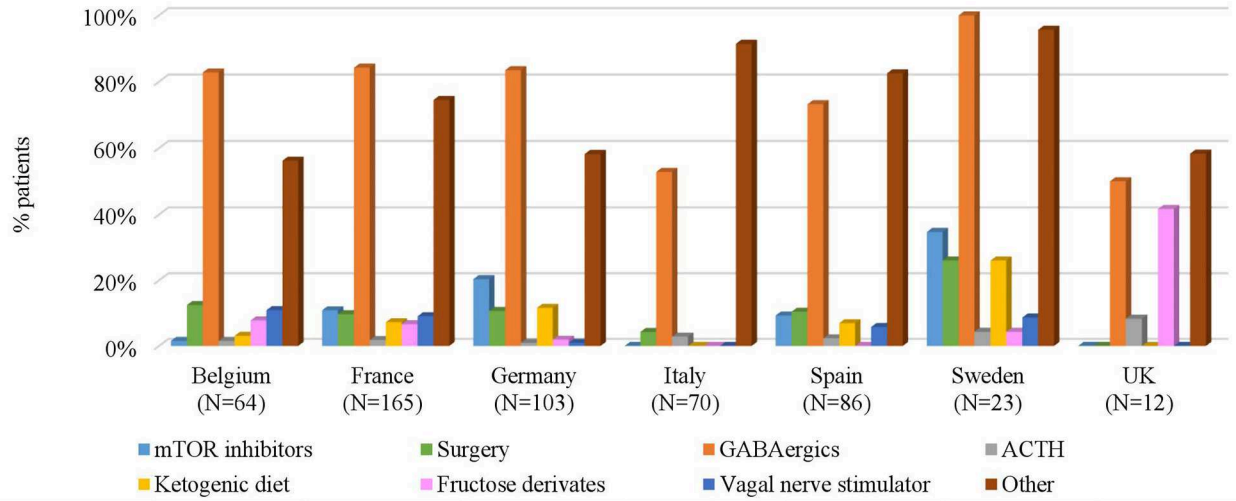
In the TOSCA registry, the proportion of patients who received treatment varied largely depending on the clinical manifestations (**Table 1**), with values at baseline (patients who ever had the manifestation) ranging between 96.8% (698/721) for IS and 32.5% (50/154) for LAM. Almost all patients with 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.

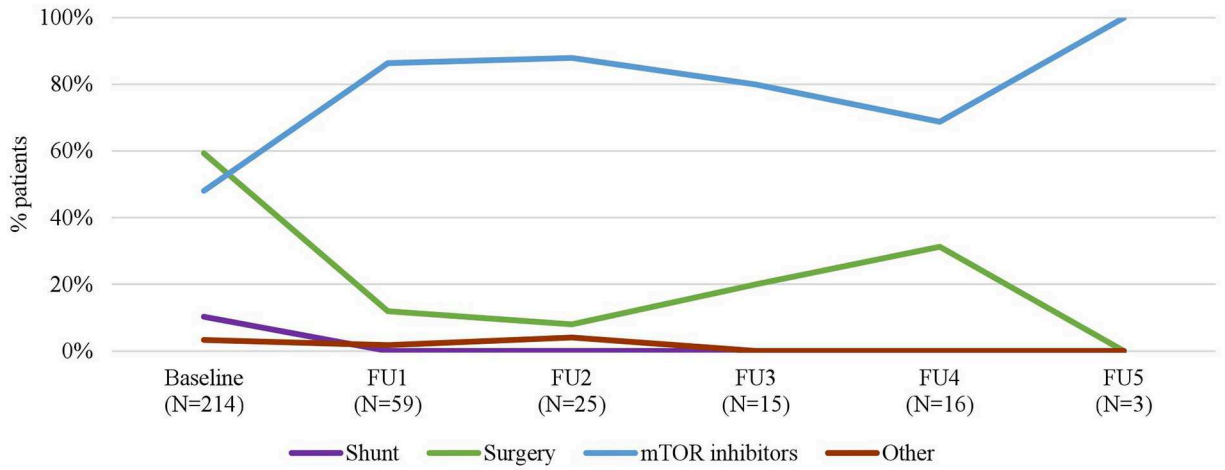
of the patients at baseline, and remained relatively stable over time (**Figures 1**, **2**).

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.

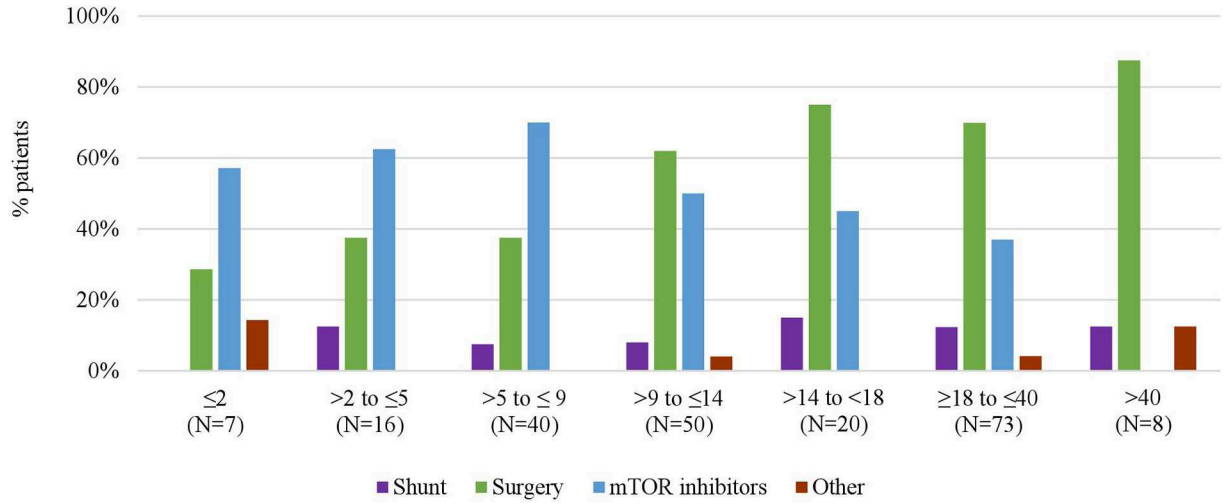
With respect to rAML, the number of patients treated was 315 (29.7%) at baseline, kept at around 30% up to FU 3 and increased in FU 4 (35.0%) and FU 5 (43.8%) (**Table 1**). Similarly to SEGA, mTOR inhibitors and embolization were the most common treatments for rAML patients (**Figure 8**). At baseline, 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**.

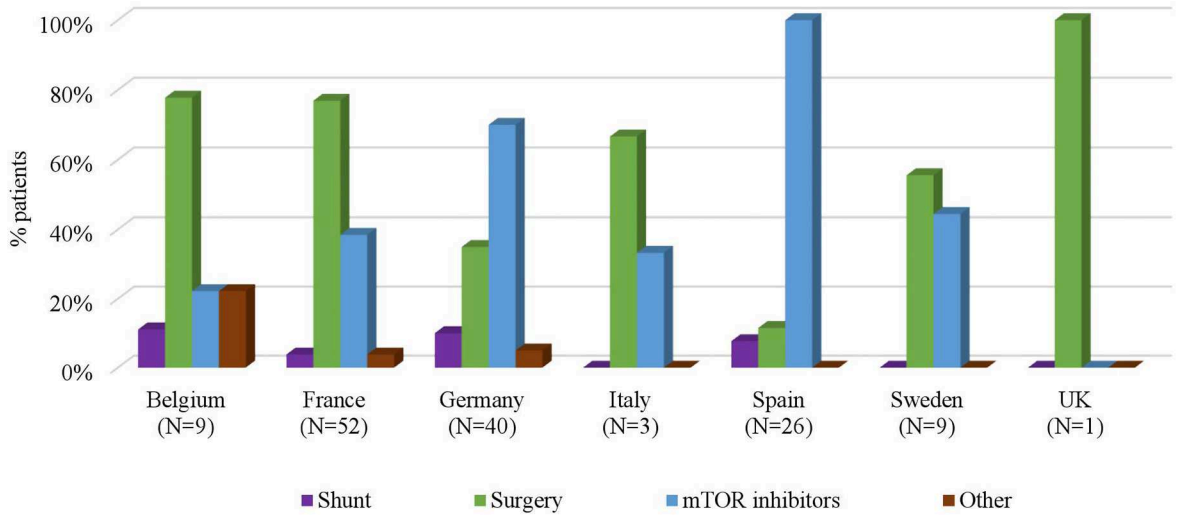
Regarding education, 28 children (31.8%) were not in a mainstream school, and the rest (N = 57; 64.8%) were educated in a mainstream school. Of those who attended a mainstream school, 64.9% received special education within the school, and for 45.6% (26/57) the school offered special programs adequate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

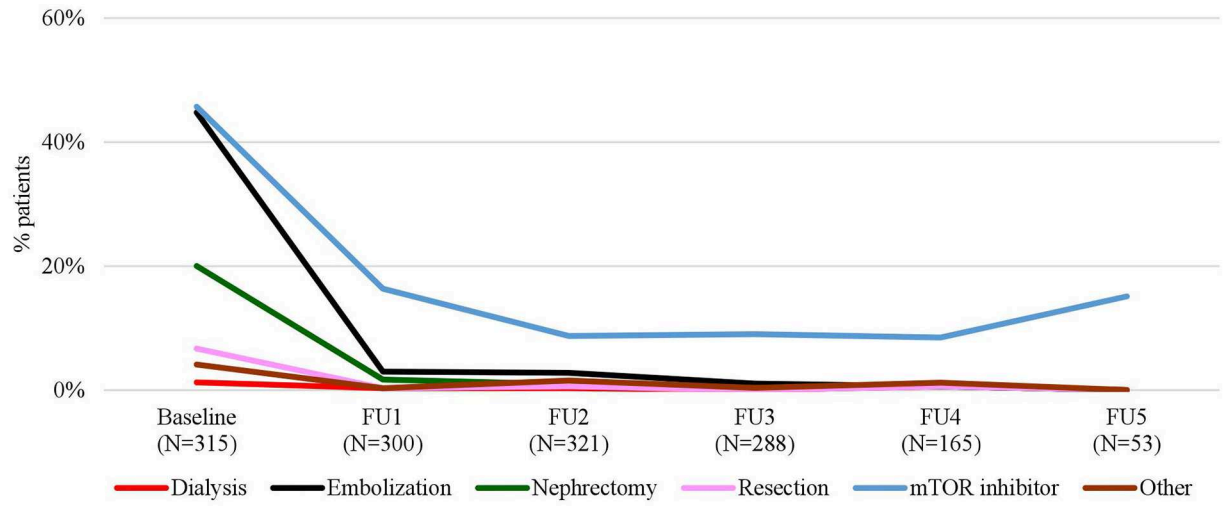
FIGURE 7 | Treatments for SEGA by Country. Patients may receive more than one treatment.

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.

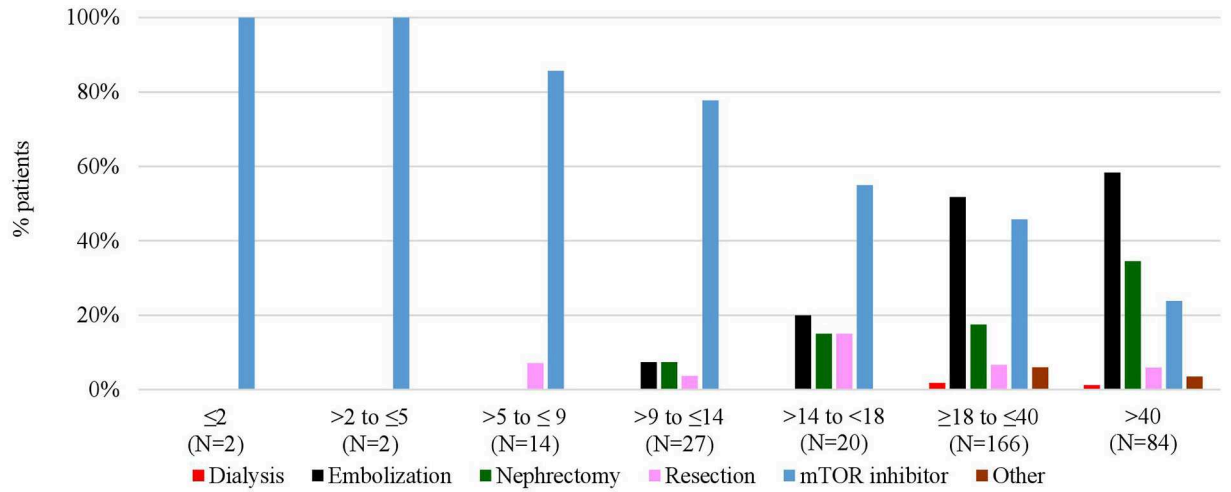
more attention should therefore be paid to the definition of treatment variables.

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.

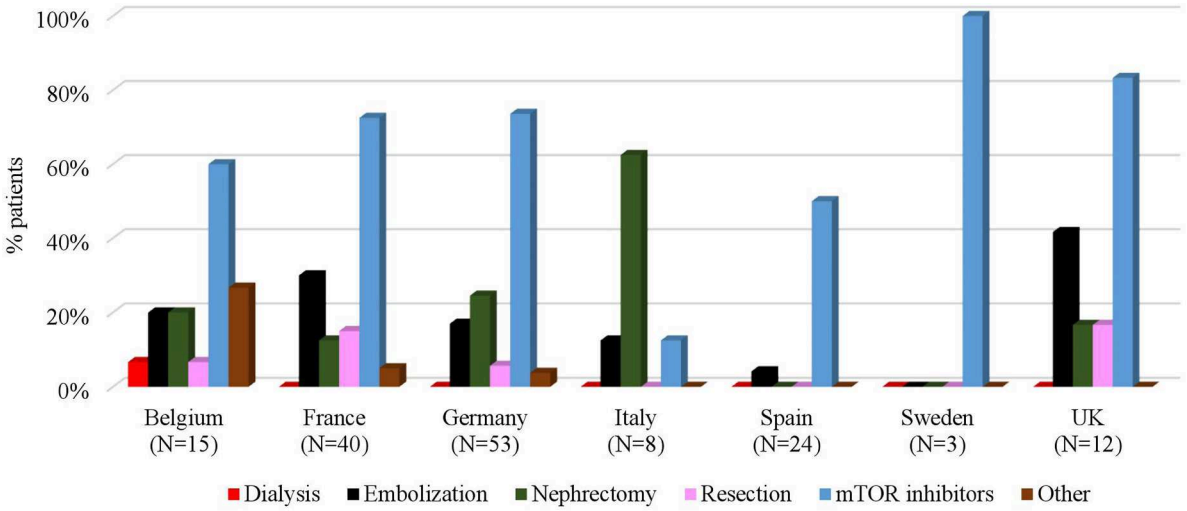
The results observed in rAML and LAM are in line with those observed in patients with SEGA. However, as stated above, since we are considering a population with co-occurring manifestations it is difficult to determine if mTOR inhibitors were used to treat these particular manifestations. It is worth 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%)

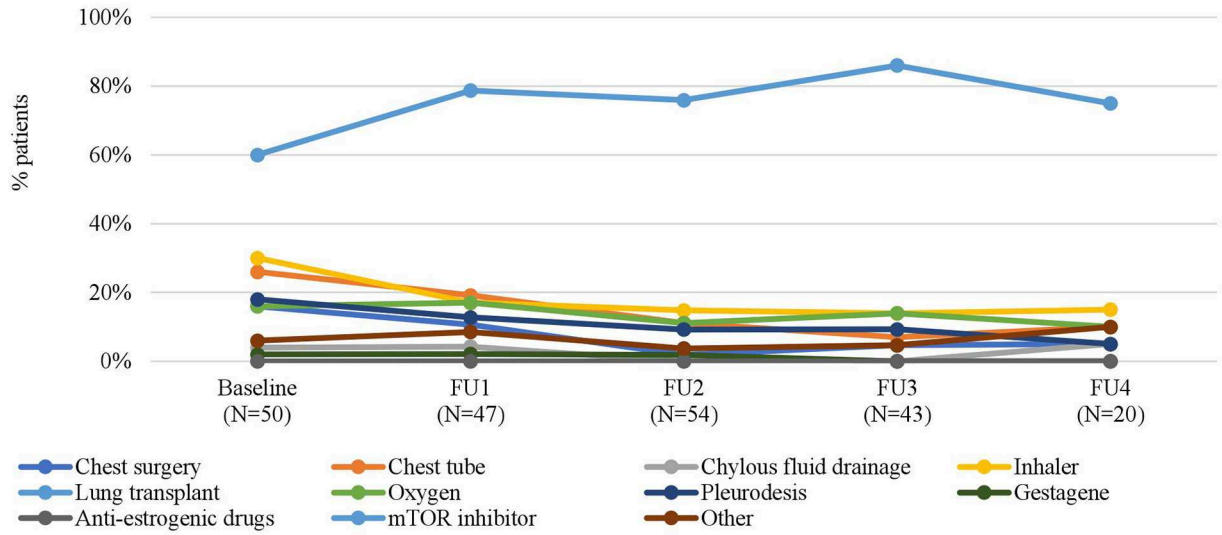
FIGURE 10 | rAML Treatment by Country. Patients may receive more than one treatment.

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.

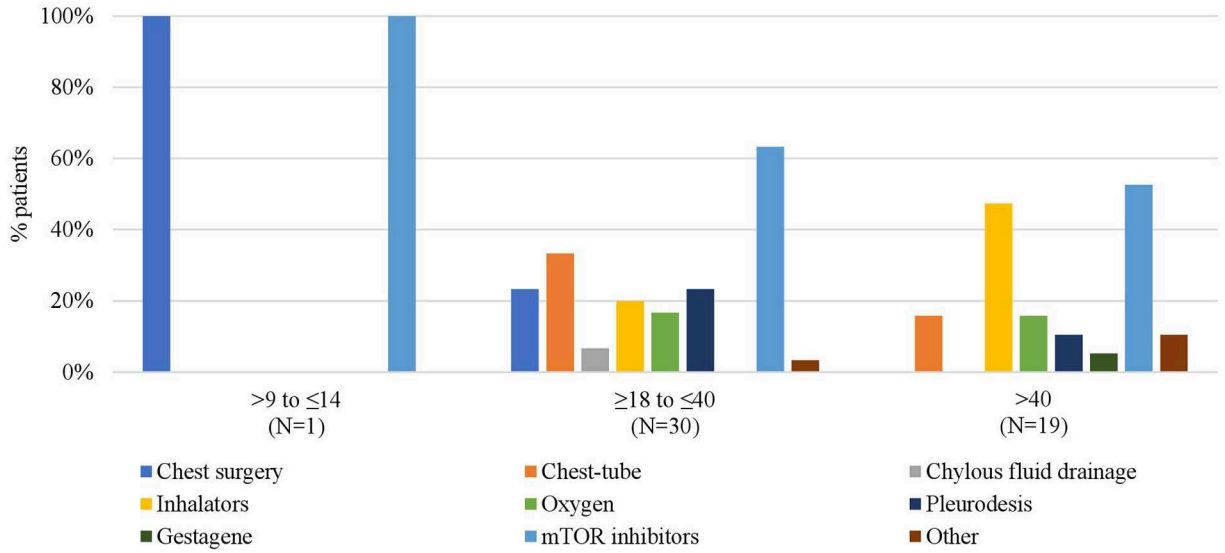
the number of patients in the last follow-up is relatively low compared to the patients for whom data was available at baseline, other studies on use of resources in patients with TSC have been carried out in patient cohorts with a smaller sample size. For instance, a study carried out by Skalicky et 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.

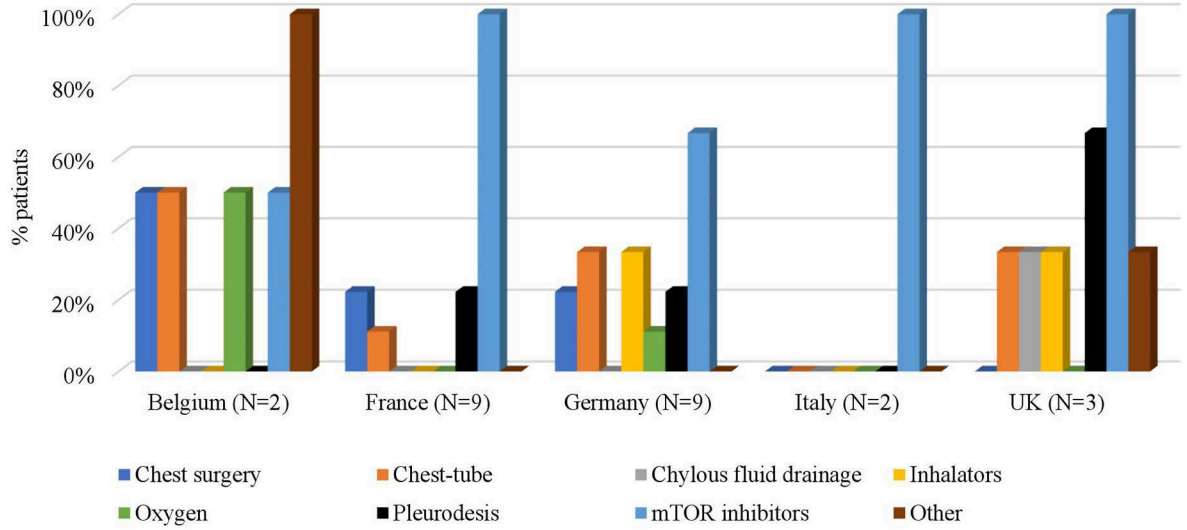
defined by a specific manifestation), while the rest included only patients with epilepsy (10, 12), SEGA (20), LAM (16), or kidney involvement (13).

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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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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OPINION
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Tuberous Sclerosis Complex (TSC): Expert Recommendations for Provision of Coordinated Care

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INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant multisystem genetic disorder characterized by benign tumors in multiple organs, including the skin, brain, kidneys, and lungs and occasional malignant tumors. Hamartomas in the brain, retina, and sometimes other organs also occur (1–3). The estimated prevalence is 1:600–1:10,000 live births in the general population (4–6). Patients present at different ages with different manifestations, and varying degrees of organ involvement (**Figure 1**). CNS manifestations of TSC mainly present in childhood, affect around 85% of patients (8), frequently resulting in epilepsy refractory to treatment, intellectual impairment, autistic spectrum disorder, attention deficit hyperactivity disorder, and behavioral problems (1–3). Renal angiomyolipomas (AMLs) occur in ∼80% of patients (9); kidney disease is the leading cause of death in adults with TSC (10). TSC is complex and highly varied (**Figure 1**) necessitating careful coordination of care, which is lacking for most patients in the UK. Some TSC manifestations are rarer; e.g., subependymal giant cell astrocytoma (SEGA) occurs in around 20–24% of patients (11, 12) (**Figure 2**).

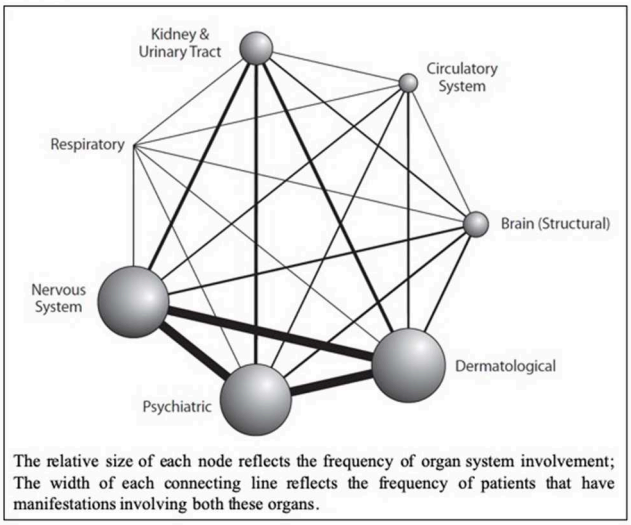

FIGURE 1 | Network Diagram showing primary organ systems affected for each patient from a retrospective cohort analysis of UK TSC patient data ($n = 324$); sourced from the Clinical Practice Research Datalink (CPRD), linked to secondary care data from Hospital Episode Statistics (HES) database, and the Office for National Statistics (ONS) mortality register (Reproduced with permission from Eur J Paediatr Neurol) (7).

The major unsolved problem in TSC is refractory epilepsy and TSC-associated neuropsychiatric disorders (TAND); of which preliminary evidence suggests refractory epilepsy is a major cause (13–15).

TSC, like other complex rare diseases, is a major burden to patients, families and healthcare systems. Optimizing care will alleviate some of this while waiting for medical research to deliver a cure.

Classically, a clinical diagnosis of TSC is made by identifying major and minor features (**Table 1**) (1, 16). With wider availability of genetic testing, identification of pathogenic mutations in *TSC1* or *TSC2* is now sufficient to establish a diagnosis, regardless of the presence of clinical features (1, 16), and is particularly useful in confirming a suspected diagnosis, as many clinical TSC manifestations are infrequent in young patients (1, 16).

The approval of the mTORC1 inhibitor—everolimus—for the treatment of AMLs, SEGA, and refractory epilepsy represents a significant advance in the potential management of the disease (17–19). Whilst not licensed in Europe, the Federal Drugs Agency (FDA) have also approved sirolimus for use in pulmonary lymphangioleiomyomatosis (LAM) (18). Refractory seizures adversely affect early development (20). Furthermore, appropriate early treatment of infantile spasms with vigabatrin has been shown to reduce the long-term impact of the neurological and neuropsychiatric aspects of TSC on patients (13, 14).

A retrospective UK cohort study linking Clinical Practice Research Datalink (CPRD) to Hospital Episode Statistics (HES) data identified 334 patients with TSC revealed a much lower frequency of complications than would be expected from previous research; the disparity possibly reflecting under-recognition, and hence suggestive of inadequate medical care (7).

It is clear from these findings, and the observation that many new patients referred to TSC clinics have never had holistic systematic monitoring, that many patients receive inadequate care. In the UK, about 1000 TSC families are known to the UK Tuberous Sclerosis Association, known as the TSA (Patient organization), and a similar number (usually the same families) attend UK specialist TSC clinics. Therefore, in most other cases, the quality of care delivered is unknown.

Given the range of organ systems affected by TSC, its treatment requires coordination across a number of medical specialties over a patient's lifetime (**Table 2**). Currently in the UK, 16 centers host specialist TSC clinics—but most UK TSC patients are not currently managed within them. These specialist clinics have often been founded by enthusiastic clinicians but are frequently inadequately funded.

The transition from pediatric to adult services can be particularly challenging in the absence of a systematic service. In Wales, a specialist TSC clinic that has been established through a partnership, between a pharmaceutical company and the NHS, awaits the development of a fully sustainable commissioning model. In Northern Ireland, a TSC clinic has been running since 1995, and directly reviews the majority of TSC patients in the region.

In the UK, specialized service specifications are in place for adults and children with genetic disorders such as cystic fibrosis and inherited metabolic disorders. These are funded by NHS England, the Department of Health, Social Services and Public Safety in Northern Ireland, and Welsh Health Specialized Services Committee in Wales. However, no similar service or service specification is yet available for TSC patients.

We propose a comprehensive, holistic model of care—to manage patients that present with a range of manifestations, requiring specialist management from a wide range of specialties (**Figures 1**, **2**).

REVIEW OF INTERNATIONAL GUIDELINES ON TSC SURVEILLANCE AND MANAGEMENT

The 2012 consensus statement on TSC surveillance and management, together with UK guidelines published this year make a number of recommendations for patient screening (1, 16, 21), with additional recommendations specific to AML, SEGA, LAM, and TSC-related epilepsy reported in disease-specific guidelines (22–24). A summary of the UK clinical guidelines, targeted at patients and general physicians has been made available by the UK TSA (25).

Additional baseline investigations to assess the extent of disease and organ involvement (**Table 3**), play an important role in guiding later treatment decisions.

The treatment and long-term surveillance needs (**Table 4**) (16) should be determined, based on the extent of disease at baseline, and tailored to the patient.

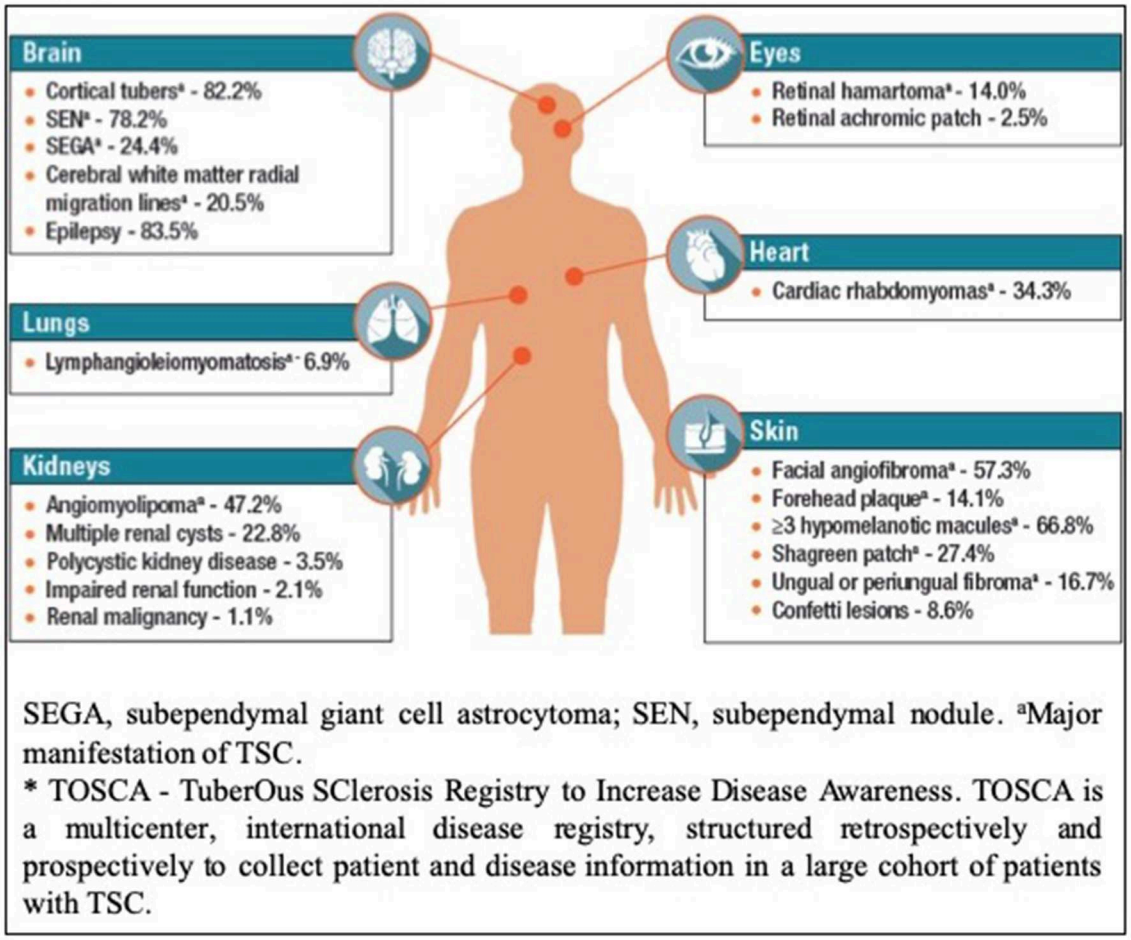

FIGURE 2 | Disease Manifestations of TSC Reported at Baseline in TOSCA* Participants (n = 2,093) (Reproduced with permission of TOSCA consortium, presented at The International TSC research Conference Tokyo 2018).

RECOMMENDATIONS FOR THE DELIVERY OF SERVICES FOR TSC PATIENTS

A "hub and spoke" model of care is proposed, with a central network of TSC-centers, co-ordinated by specialists, and supported by a regional network of clinicians, that offer access to a comprehensive set of TSC-related specialist services. Hospital specialists should work collaboratively with patients, their families and their community doctors (General Practitioners or general physician) to provide support and advice and a pathway for dealing with problems that need specialist care. Since holistic care of TSC patients requires input from many different specialties, treatment of TSC patients should be discussed within the regional network by a multidisciplinary team (MDT), with the aim of ensuring that each TSC patient and their family have a tailored care plan to manage current disease manifestations, and surveillance for future TSC manifestations.

To achieve this, Specialist TSC services should ensure:

- **Diagnosis:** Patients with TSC are identified by clinical evaluation and/or genetic testing.
- **Surveillance:** Provision of multi-disciplinary evaluation–through alignment with regional genetic services (for genetic counseling to patients and their families), and with other clinical specialties to ensure access to appropriate care for all patients.
- **Treatment:** The appropriate access and use of TSC therapies.
- **Safe transition from pediatric to adult care.**
- **Information and Support:** Collaboration with patients/family and other organizations to provide access to TSC-specific information.
- **Research:** Facilitate patients and their families to become involved in relevant research projects.

Regional TSC clinics should be responsible for the diagnosis of patients with TSC, and the provision of routine care and support for patients and their families. Regional clinics should be supported by a dedicated TSC specialist coordinator, who has responsibility for coordinating the service, ensuring timely surveillance, and coordinating care between different specialist services, developing individualized plans for patient follow-up, and ensuring continuity of care for TSC patients transitioning

TABLE 1 | Major and minor clinical manifestations of tuberous sclerosis complex (1).

MAJOR FEATURES
Hypomelanotic macule (≥3, at least 5 mm in diameter)
Angiofibroma (≥3) or fibrous cephalic plaque
Ungual fibroma (≥2)
Shagreen patch
Multiple retinal hamartomas
Cortical dysplasias*
Subependymal nodules
Subependymal giant cell astrocytoma
Cardiac rhabdomyoma
Lymphangioleiomyomatosis
Angiomyolipoma (≥2)
MINOR FEATURES
"Confetti" skin lesions
Dental enamel pits (>3)
Intraoral fibromas (≥2)
Retinal achromic patch
Multiple renal cysts
Non-renal hamartomas

**Cortical dysplasias includes tubers and cerebral white matter radial migration lines.*

TABLE 2 | A wide range of healthcare services are involved in the diagnosis, management, and treatment of the various manifestations of TSC. These include:

- Primary care
- Pediatrics/Community pediatrics
- Genetics
- Diagnostic radiology
- Interventional radiology
- Surgery
- Cardiology
- Respiratory medicine
- Nephrology
- Dermatology
- Neurology/Pediatric neurology
- Neurosurgery
- Oncology
- Fetal medicine
- Urology
- Ophthalmology
- Psychiatry
- Psychology
- Child, adolescent, and adult learning disability psychiatry

to adulthood. Alongside this, linking regional clinics with TSC patient support groups (e.g., in the UK, the Tuberous Sclerosis Association (TSA), is vital to ensure that patients and their families receive comprehensive support). Regional clinics are in an ideal position to gather clinical and prevalence data to monitor needs locally and facilitate future research.

To allow regional TSC clinics to fulfill this pluripotent role, they need to offer or have access to a range of core services, including:

- Genetic Testing and Genetic Counseling.
- Neurology and Neuroimaging.
- Nephrology, Urology, General and Interventional Radiology services.
- Clinical Psychology, Psychiatry, and Developmental Pediatrics.
- Collaboration with patient/family organizations.
- Collaboration with patient's community physician (General Practitioner).

TABLE 3 | Surveillance and management recommendations for newly diagnosed or suspected TSC (16, 21).

Organ system or specialty area	Recommendation
Genetics	• Obtain three-generation family history to assess for additional family members at risk of TSC. • Offer genetic testing for family counseling or when TSC diagnosis is in question but cannot be clinically confirmed.
Brain	• Perform magnetic resonance imaging (MRI) of the brain to assess for the presence of tubers, subependymal nodules (SEN), migrational defects, and subependymal giant cell astrocytoma (SEGA). • Evaluate for TSC-associated neuropsychiatric disorder (TAND), using the TAND checklist (26). • During infancy, educate parents to recognize infantile spasms, even if none have occurred at time of first diagnosis • Obtain baseline routine electroencephalogram (EEG). If abnormal, especially if features of TAND are also present, follow-up with a 24-h video EEG to assess for subclinical seizure activity.
Kidney	• Obtain MRI of the abdomen to assess for the presence of angiomyolipoma and renal cysts. • Screen for hypertension by obtaining an accurate blood pressure. • Evaluate renal function by determination of glomerular filtration rate (GFR).
Lung	• Perform baseline pulmonary function testing (pulmonary function testing and 6-min walk test) and high-resolution chest computed tomography (HRCT), even if asymptomatic, in patients at risk of developing lymphangioleiomyomatosis, typically females 18 years, or older. Adult males, if symptomatic, should also undergo testing. • Provide counsel on smoking risks and estrogen use in adolescent and adult females.
Skin	• Perform a detailed clinical dermatologic inspection/exam.
Teeth	• Perform a detailed clinical dental inspection/exam.
Heart	• Consider fetal echocardiography to detect individuals with high risk of heart failure after delivery when rhabdomyomas are identified. • via prenatal ultrasound. • Obtain an echocardiogram in pediatric patients, especially if younger than 3 years of age. • Obtain an electrocardiogram (ECG) in all ages to assess for underlying conduction defects.
Eye	• Perform a complete ophthalmologic evaluation, including dilated fundoscopy, to assess for retinal lesions, and visual field deficits.

The roles of each of these core services is summarized in **Table 5**. Where regional centers are unable to provide a core service, there should be a clear pathway through which that service can be accessed. Furthermore, regional centers should also have access to the necessary facilities to cater for the specific needs of TSC patients. For example, TSC-related intellectual impairment and autistic spectrum disorder may necessitate that surveillance brain and renal imaging be performed under general anesthetic. This requires co-ordination of such procedures in an appropriate day-unit, or via a formal inpatient admission, with the support of specialized pediatric and adult anesthetists.

In addition to the "core" services, in order to provide comprehensive treatment to TSC patients, regional TSC centers

TABLE 4 | Surveillance and management recommendations for patients already diagnosed with definite or possible TSC (16, 21).

Organ system or specialty area	Recommendation
Genetics	• Offer genetic testing for family counseling or when TSC diagnosis is in question but cannot be clinically confirmed.
Brain	• Obtain brain MRI 1–3 yearly in asymptomatic TSC patients aged under 25 years to monitor for new occurrence of SEGA. • Patients with asymptomatic large/growing SEGA, with or without ventricular enlargement should undergo MRI scans more frequently and the patients and their families should be educated regarding the potential of new symptoms. Patients with asymptomatic SEGA in childhood should continue to be imaged periodically as adults to ensure there is no growth. • For acutely symptomatic SEGA, neurosurgical resection, with or without cerebral spinal fluid diversion (shunt) is advocated. • For asymptomatic but growing SEGA, either surgical resection or medical treatment with mTOR inhibitors may be used. In determining the best treatment option, discussion should include the risks of complication and adverse outcomes, cost, length of treatment, and potential impact on TSC-associated comorbidities. • At least annual screening for TAND features at each clinical visit, using the TAND checklist (26). Comprehensive formal evaluation for TAND at key developmental time points: infancy (0–3 years), preschool (3–6 years), primary school (6–9 years), adolescence (12–16 years), early adulthood (18–25 years), and as needed thereafter. Management strategies should be based on the TAND profile of each patient and should be based on evidence-based good practice guidelines/practice parameters for individual disorders (e.g., autism spectrum disorder, attention deficit hyperactivity disorder, anxiety disorder). Always consider the need for an individual educational program (IEP). Sudden change in behavior should prompt medical/clinical evaluation to look at potential medical causes (e.g., SEGA, seizures, renal disease). • Routine electroencephalograph (EEG) should be performed in individuals with known or suspected seizure activity. The frequency of routine EEG should be determined by clinical need rather than a specific defined interval. Prolonged video EEG, 24 h or longer, is appropriate when seizure occurrence is unclear or when unexplained sleep, behavioral changes, or other alteration in cognitive or neurological function is present. • Vigabatrin is the recommended first-line therapy for infantile spasms. ACTH can be used if treatment with vigabatrin is unsuccessful. Anticonvulsant therapy of other seizure types in TSC should generally follow that of other epilepsies. • Epilepsy surgery should be considered for medically refractory TSC patients, but special consideration should be given to children at younger ages experiencing neurological regression and is best if performed at epilepsy centers with experience and expertise in TSC.
Kidney	• Obtain MRI of the abdomen to assess for the progression of angiomyolipoma and renal cystic disease every 1–3 years throughout the lifetime of the patient. • Assess renal function (including determination of glomerular filtration rate) and blood pressure at least annually. • First-line therapy for renal AMLs presenting with acute hemorrhage is embolization followed by corticosteroids, with nephrectomy to be avoided if possible. • First-line therapy for asymptomatic, growing AMLs measuring larger than 3 cm in diameter is treatment with an mTOR inhibitor. Selective embolization or kidney-sparing resection are acceptable second-line therapy for asymptomatic AMLs.
Lung	• Perform clinical screening for lymphangioleiomyomatosis (LAM) symptoms, including exertional dyspnoea and shortness of breath, at each clinic visit. Counseling regarding smoking risk and estrogen use should be reviewed at each clinic visit for individuals at risk of LAM. • Obtain HRCT every 5–10 years in asymptomatic individuals at risk of LAM if there is no evidence of lung cysts on their baseline HRCT. Individuals with lung cysts detected on HRCT should have annual pulmonary function testing (pulmonary function testing and 6-min walk) and HRCT interval reduced to every 2–3 years. • mTOR inhibitors may be used to treat LAM patients with moderate to severe lung disease or rapid progression. TSC patients with LAM are candidates for lung transplantation but TSC comorbidities may impact transplant suitability.
Skin	• Perform a detailed clinical dermatologic inspection/exam annually. • Rapidly changing, disfiguring, or symptomatic TSC-associated skin lesions should be treated as appropriate for the lesion and clinical context, using approaches such as surgical excision, laser(s), or possibly topical mTOR inhibitor. • Facial angiofibromas (And some other skin lesions) respond to systemic or topical mTOR inhibitor; which can prevent more severe disease later if started early (27, 28).
Teeth	• Perform a detailed clinical dental inspection/exam at minimum every 6 months and panoramic radiographs by age 7, if not performed previously. • Symptomatic or deforming dental lesions, oral fibromas, and bony jaw lesions should be treated with surgical excision or curettage when present.
Heart	• Obtain an echocardiogram every 1–3 years in asymptomatic pediatric patients until regression of cardiac rhabdomyomas is documented. More frequent or advanced diagnostic assessment may be required for symptomatic patients. • Obtain electrocardiogram (ECG) every 3–5 years in asymptomatic patients of all ages to monitor for conduction defects. More frequent or advanced diagnostic assessment such as ambulatory and event monitoring may be required for symptomatic patients.
Eye	• Annual ophthalmologic evaluation in patients with previously identified ophthalmologic lesions or vision symptoms at the baseline evaluation. • NB this frequency may not be necessary for most patients and the recommendation may be changed in the forthcoming 2019 revision of the International TSC Clinical Guidelines. • More frequent assessment, including those treated with vigabatrin, is of limited benefit and not recommended unless new clinical concerns arise.

TABLE 5 | TSC clinic—core services.

Core Services	Role
1. Genetic testing and counseling	• Diagnostic opinion and management advice, including perinatally. • Arrange genetic testing, when indicated, and aid with interpretation of results. • Cascade genetic testing to identify asymptomatic disease in parents and relatives & stratify risk of developing TSC manifestations. • Discuss options for prenatal & pre-implantation genetic diagnosis.
2. Neurology and neuroradiology	• Access to pediatric and adult neurology services with specific epilepsy expertise, including epilepsy, and learning disability nurses. • Access to Neurophysiological tests including routine electroencephalogram (EEG) for patients with suspected or known seizure activity, and video-telemetry. • Access to Neuroradiological investigations: Baseline brain MRI (including MRI under general anesthesia where required): children and young adults with TSC should have a surveillance MRI every 2–3 years.
3. Nephrology, Urology, General, and Interventional Radiology	• Access to pediatric and adult nephrology, urology and interventional radiology services. • Radiological monitoring should include baseline and 1–3 yearly surveillance MRI (including under general anesthesia where required), depending on the presence and size of lesions. • MRI is the optimal renal imaging modality; CT or ultrasound may be acceptable alternatives in some circumstances. Where possible, 3D Volumetric analysis for AML to monitor change in lesions.
4. Clinical Psychology, Psychiatry and Developmental Pediatrics	• Assess and diagnose intellectual, behavioral, and psychiatric conditions associated with TSC. • Monitoring should include baseline evaluation of cognition, regular screening for TAND (or more frequently if required), and comprehensive formal evaluation of TAND at key developmental milestones (21).

would also need to have access to additional specialist support services, including Dermatology, Respiratory, Cardiology, Neuropsychiatry, and Obstetrics/Gynecology. The role of each of these additional services in relation to TSC patients is summarized in **Table 6**.

TSC Clinics need access to highly specialized services, of which there are four in the UK, including for Pulmonary LAM, Pediatric epilepsy surgery, Neurosurgery & Neuro-oncology and Neuropsychiatric services (summarized in **Table 7**).

In addition to ensuring access to appropriate services, there are key responsibilities for the regional centers in ensuring holistic care for TSC patients and their families.

Regional services need to ensure provision of the supportive care needed by patients and their families, including referral for individualized education plans for patients, genetic counseling for family members, and ongoing support for both the patient and their family from a patient association.

There is a need to monitor patient movement through the service to ensure that all patients are offered appropriate, regular surveillance and timely follow-up. Patients should be offered the most up-to-date, evidence-based surveillance, and those patients with multiple complications of TSC should attend joint clinics, or have the monitoring of different manifestations performed in a single session (e.g., combined surveillance/monitoring of SEGA and renal AML through a coordinated MRI scan of both brain & renal tract—particularly where a general anesthetic is required to achieve the imaging), in order to minimize individual patients' time in hospital. Such efforts would not only help to reduce the costs of patient monitoring but help to improve patients' and careers' experience of care, and their quality of life.

TSC regional centers should ensure that the service is aligned with National guidelines such as those published by NICE or the Renal Association on how to manage transitional care for patients moving from pediatric to adult services, with bespoke plans drawn up for individual patients where necessary.

TSC centers and networks should collaborate with the current available networks of local/community services (e.g., Community pediatricians and mental health services) to optimize care and minimize cost.

Pediatric and adult TSC centers, if not co-located, need to collaborate proactively to ensure safe transition of care from children's' to adult services. This is a time when patients are often lost to follow up.

Finally, there is a need to audit the services offered to and used by patients with TSC, so as to ensure that patients are treated appropriately. A very helpful way to ensure that clinic services develop into exactly what is needed by patients and families is to audit services using PREMS (Patient reported experience measures) (29) and PROMS (Patient reported outcome measures) (30).

Regular review of services will help to identify any potential opportunities for improved efficiency, as well as ensure that patients are consistently screened and treated according to best practice. With this aim, a national database should be established to facilitate the coordination of care between centers, auditing of services, planning of resource allocation, and TSC-related research.

DISCUSSION

The rarity and heterogeneity of TSC presentations offers a number of challenges to the implementation of best practice care; treatment and follow-up is consequently frequently fragmented, disjointed, and suboptimal.

There is a need to improve TSC management to ensure patients have early access to appropriate treatment and

TABLE 6 | TSC clinic—additional services.

Additional services	Role
Dermatology	• All patients with TSC should have an annual review of their skin, carried out in the regional TSC clinic. • Patients should be referred for specialist dermatological advice when required.
Respiratory	• A high-resolution computed tomography (HRCT) of the chest should be performed at 18–21 years, particularly in post-pubertal females, who are at higher risk of developing pulmonary LAM. • In asymptomatic patients with no sign of LAM on the HRCT chest, scanning should be repeated to screen for new onset disease every 5–10 years. • Patients with pulmonary LAM should undergo regular pulmonary function tests, assuming the patient is able to cooperate. HRCT should be repeated at 2–3-years intervals to monitor for changes in known lesions. Patients with progressive or complex disease, should be referred to, or discussed with, the LAM highly specialized service based in Nottingham (**Table 6**).
Cardiology	• Affected infants and children should receive a baseline echocardiogram, and electrocardiogram (ECG) if any new-onset TSC-related symptoms are identified.
Neuropsychiatry	• Patients with TSC-related psychiatric comorbidities frequently require treatment with psychotropic medications. Regional centers should have input into identifying the most appropriate treatment for these patients, as their care may be complicated by a high rate of comorbid illness, poor response and a high risk of adverse side effects, and potential drug interactions due to polypharmacy.
Pregnancy	• All women of reproductive age should be offered contraceptive advice. • Women with a pregnancy where the fetus is at risk of/known to have TSC should be referred to specialized fetal medicine services to consider invasive testing. In the absence of an identifiable mutation, monitoring for cardiac rhabdomyomas and/or other genetic testing can occur. • All women should be offered pre-pregnancy counseling, including genetic counseling. • During pregnancy women should be sign-posted to antenatal care in a high-risk combined maternal medicine service.

TABLE 7 | Highly specialized centers for TSC in the UK.

Highly specialized centers for TSC	Role
Pulmonary LAM	• Patients with TSC and symptomatic pulmonary LAM should in the first instance be assessed in their local respiratory center. If appropriate, they may be referred to a specialist center (e.g., in England, this is the LAM center at Nottingham University Hospital Trust, as described in the NHS England service specification).
Pediatric epilepsy surgery	• Children with TSC-related drug-resistant epilepsy should be referred to an NHS England commissioned Children's Epilepsy Surgery Service (CESS) center for consideration of intervention (Great Ormond Street Hospital or King's College Hospital in London, services are also located in Bristol, Birmingham, and Manchester/Liverpool).
Neurosurgery and neuro-oncology	• Patients with SEGA should have their overall management overseen by the specialist neurosurgical and neuro-oncological service.
Neuropsychiatry	• Complex neuropsychiatric presentations should be considered for referral to NHS England-commissioned centers (in Manchester, Newcastle, or London) to access diagnostic assessments, and management advice for Autism Spectrum Disorder and associated neuropsychiatric conditions.

preventive measures—both to minimize long-term effects of TSC where possible, and to support a frequently vulnerable patient group and their families. In particular, there are three elements that are both essential for the success of a TSC clinic, yet frequently missing. These include dedicated neuropsychiatric input, access to CT/MRI imaging under general anesthetic, and perhaps most importantly, a dedicated specialist TSC co-ordinator. A mechanism to deliver optimal care is essential if patients are to gain the best outcomes; including monitoring and intervention for SEGA, renal AMLs, LAM, and TAND, and early improvement in refractory epilepsy.

Hepatic lesions are common in TSC but very rarely cause any clinical problems (31). They do not need to be regularly monitored.

The primary physician of a patient is usually their general pediatrician or, in adults, their general practitioner or general physician. They may delegate responsibility for holistic management of TSC care to a hospital specialist but remain responsible for other aspects of their patient's care, so that collaboration and good communication is essential.

Finally, it should be acknowledged that optimal management of TSC is a field of active research and new recommendations will continue to be made. For example, It is now recognized that regular surveillance EEGs in infants can identify infants who are about to begin having seizures (32, 33). Emerging evidence from the Epistop trial and historic case series suggest starting therapy promptly or before the onset of clinical seizures, may markedly improve outcomes (13, 14). Similarly, genetic testing cannot yet be used to accurately predict an individual's prognosis, only the average risk in a group, but this is likely to change in the near future (9, 34).

We advocate that specialist expertise be provided by centralized TSC "hubs," with routine patient management coordinated centrally and undertaken in regional TSC networks to facilitate optimal resource use and improve the comprehensive care of TSC patients. The TSC hub-and-spoke model will form a coordinated care network, that will also provide a structure to

facilitate the education of health care professionals and affected families, and to facilitate TSC research. This model for TSC care may also serve as a blueprint for improving the quality of care for patients with other rare diseases in evolving, ever more efficient, healthcare services.

AUTHOR CONTRIBUTIONS

NA and FE participated in the development of the service specification and the drafting of the manuscript. JK participated in the development of the service specification, the drafting, and reviewing of the manuscript. PC advised on adult neurological aspects of TSC in particular relating to epilepsy. PM helped to draft the manuscript and has read and approved the final manuscript. RA, ZB, RB, PB, AC, MT, EF, DG, AH, EJ, SJ, SRJ, LK, GL, FO'C, JC, AO, JS, and CS read and approved the final manuscript.

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The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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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

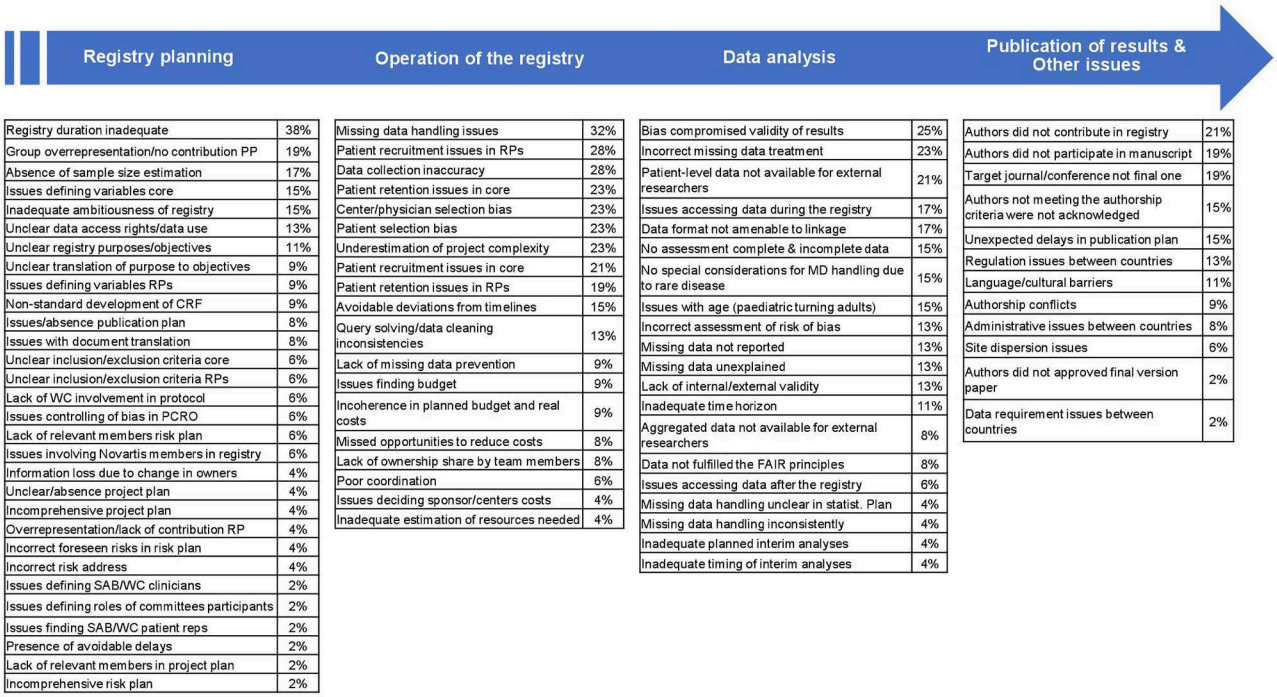
The limited duration of the registry (up to 5 years) was considered the most common issue amongst the survey respondents (38%). There was a consensus amongst those answering the questionnaire on the appropriateness of having a long-term registry and some respondents stated that a longer follow-up would 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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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

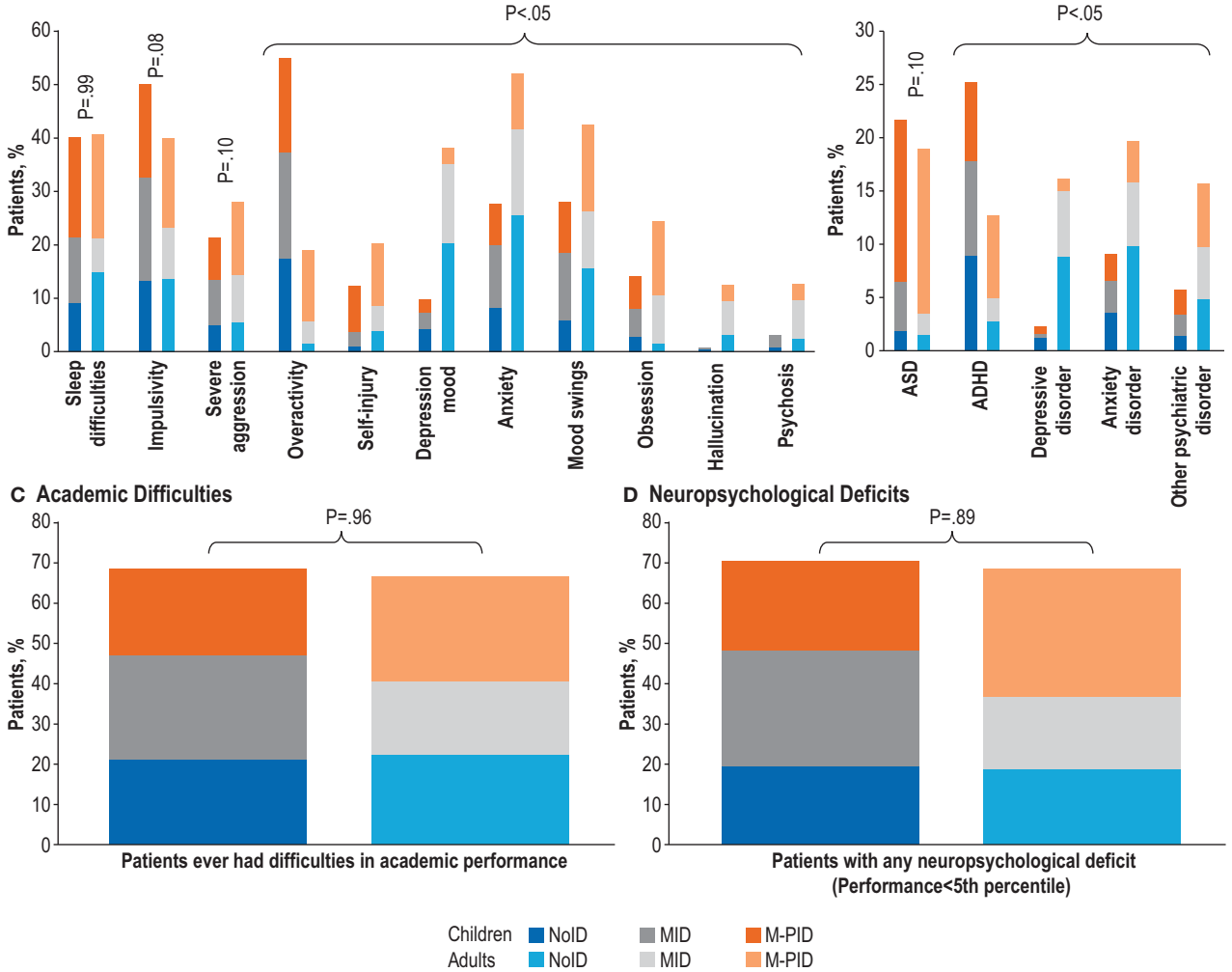

FIGURE 1 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in children vs. adults. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated 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

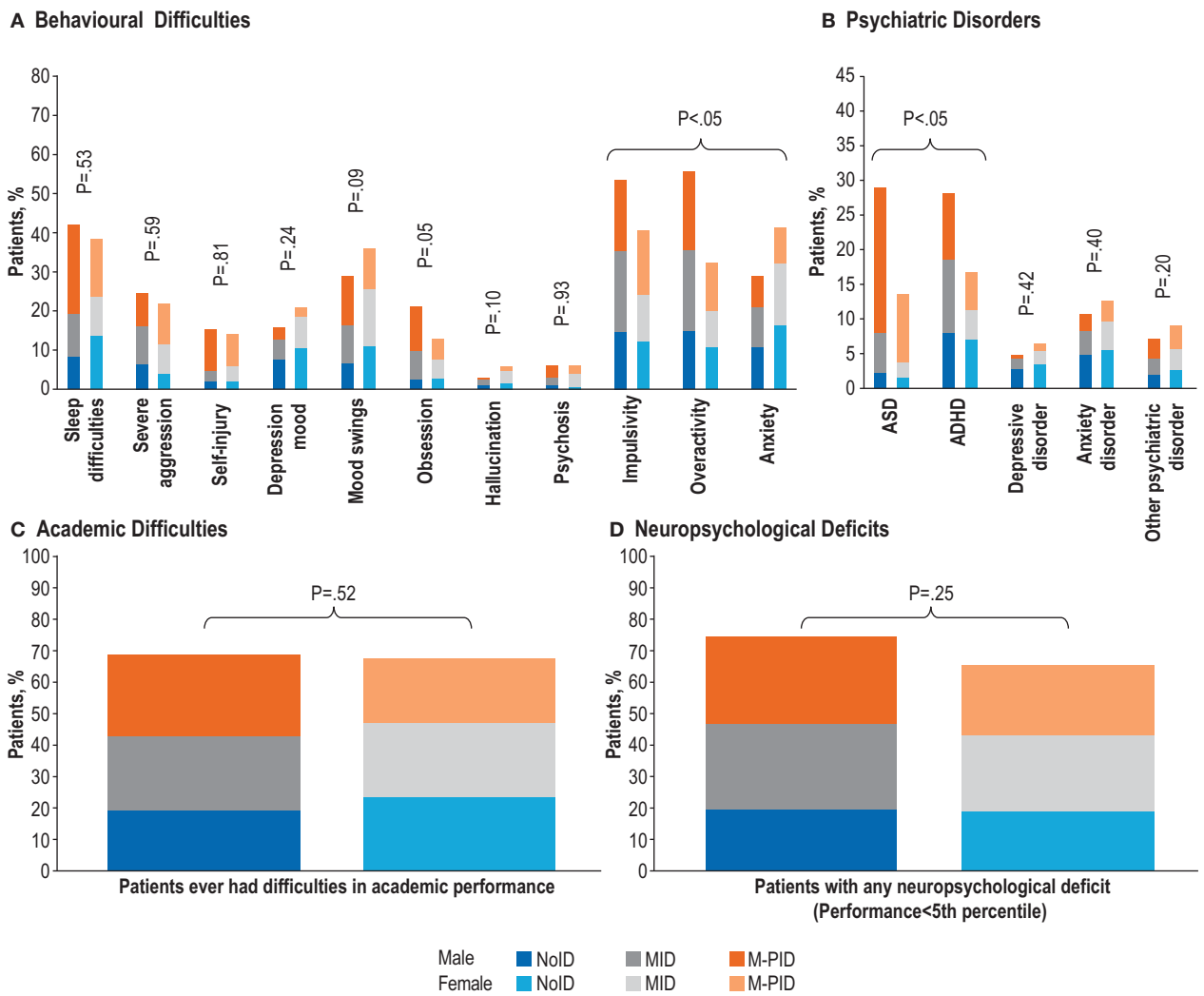

FIGURE 2 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in male vs. female. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated 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

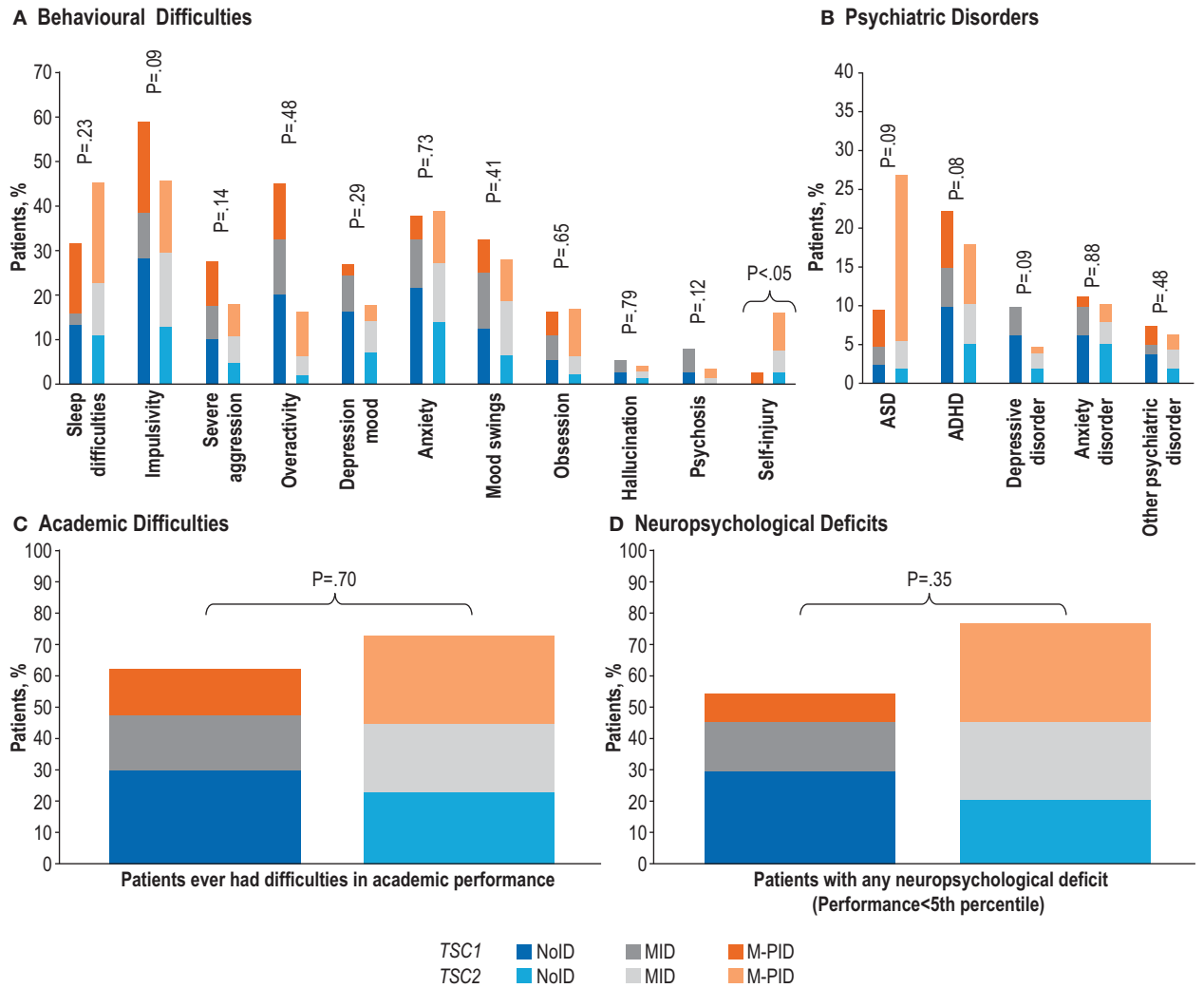

FIGURE 3 | Frequency of TAND features stratified by levels of intellectual ability (noID [IQ>70], MID [IQ 50–70] and M-PID [IQ<50]) in *TSC1* vs. *TSC2*. **(A)** Behavioral difficulties. **(B)** Psychiatric disorders. **(C)** Academic difficulties. **(D)** Neuropsychological deficits. Percentages calculated 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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AUTHOR CONTRIBUTIONS

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

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Autism and Epilepsy in Patients With Tuberous Sclerosis Complex

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Introduction: Individuals with Tuberous Sclerosis Complex (TSC) are at increased risk of developing both epilepsy and autism spectrum disorder (ASD), but the relationship between these conditions is little understood. We reviewed published reports to elucidate the relationship between ASD, epilepsy, and TSC, and to define the genetic and neurological risk factors.

Methods: Articles (January 2004–May 2019) were identified via PubMed, EMBASE, and CENTRAL databases. Article inclusion required report on individuals with TSC-associated ASD and epilepsy with prevalence, odds ratio, or rate report on the comorbidity of ASD in epileptic patients due to TSC.

Results: A total of 841 abstracts were identified in the original search. Thirty-six articles were included, which identified study populations, ASD measures used, and study confounders as bias factors. This review included 2,666 TSC patients, with a mean age of 15.9 years (range 1.94–30.3 years). The percentage of TSC patients with epilepsy *and* autism was 33.7%. Patients with TSC *and* autism showed more frequent seizures and earlier epilepsy onset than TSC patients without autism. ASD and intractable epilepsy were both predicted by a higher number of areas with dysplastic features revealed in brain MR scans. ASD, the onset of seizures in children <2 years of age, and >3 tubers have all been associated with an increased risk of refractory epilepsy in TSC patients. However, the direction of the relationship is not clear because a history of epilepsy, or infantile spasms in patients with TSC is also associated with an increased likelihood of ASD. Overall, 73.2% of patients carried *TSC2* genetic variant and, among patients with TSC and autism, the percentage of *TSC2* individuals was 85.6%.

Conclusions: The complex interrelationship between TSC, autism, and epilepsy, coupled with limited knowledge on the neurobiological basis for the interrelationship, limits overall understanding and opportunities for management. The results of this review highlight the need for early identification and management to optimize favorable outcomes in the most vulnerable individuals with TSC. Regardless of whether studies are considered individually or collectively, interpretation is made difficult due to the differences between the studies, most notably between methods and diagnostic criteria used to assess intellectual ability.

Keywords: tuberous sclerosis complex, epilepsy, autism spectrum disorder, prognostic factors, age at onset, genetic, TSC1, TSC2

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INTRODUCTION

Tuberous Sclerosis Complex (TSC) is a rare genetic multisystem disorder characterized by hamartoma formation in several organs and systems (1, 2), with an estimated birth incidence of 1 in 5,800 (3). TSC is caused by mutation in either *TSC1* (chromosome 9q34) or *TSC2* (16p13.3) gene, encoding for hamartin and tuberin, respectively (4). These two proteins, along with TBC1D7, form a heterotrimeric complex regulating the activity of mTOR complex 1 (mTORC1), which is a key regulator of cell metabolism and proliferation. mTORC1 dysregulation is the main reason for aberrant growth and differentiation underlying the formation of TSC-related lesions, either in the brain or other organs (1).

Neurologic and developmental issues such as epilepsy, autism spectrum disorder (ASD), and developmental delay (DD), are major sources of morbidity in people of all ages with TSC and typically present in infancy or early childhood (1). Epilepsy is estimated to occur in about 80% of TSC patients, typically within the first 3 years of life, and considered to be a result of the genetic mutation leading to an imbalance between excitation and inhibition of gamma-amino-butyric acid (GABA) receptors. Dysregulation of the neurotransmission of GABA has also been proposed as a neurobiological link between epilepsy and ASD in TSC patients (5).

ASD is an early onset, lifelong, neurobiological disorder characterized by impairments in communication and social interaction along with the presence of restricted and repetitive patterns of behavior, interests or activities, and is prevalent in 1.85% of children aged 8 years (6–8). In the last 5 years, some longitudinal studies have explored the early emerging symptoms and prompt intervention in infants with high familial risk of ASD (9, 10). In contrast, very few studies have addressed this topic in ASD associated with specific syndromes or genetic conditions (11–13).

TSC is one of the major syndromes associated with ASD. The prevalence of ASD in TSC ranges from 26 to 45%, depending on the sample, ASD criteria, and the testing methodologies employed (14, 15). Some autistic features are present in about half of patients with TSC. A number of factors have been identified as being associated with ASD in TSC, including brain lesion load, prominent lesion type, the size and location of the tubers, cyst-like tubers, *TSC2* mutation, early onset and refractory seizures, and the presence and severity of cognitive impairment (1, 16). Prompt cessation of early seizures can, in at least some cases, improve neuropsychiatric outcome (17, 18).

To our knowledge, no review has yet examined the relationship between ASD and epilepsy in patients with TSC. We performed a review of the literature to assess the prevalence and risk factors for ASD in patients with TSC and epilepsy, and to investigate the relationship and comorbidity between these conditions. The main aims of this review were: to identify the frequency of both ASD and epilepsy within the TSC population, and to elucidate the relationship between ASD and epilepsy in individuals with TSC.

Abbreviations: ADC, apparent diffusion coefficient; ASM, antiseizure medication; ASD, Autism Spectrum Disorder; EEG, electroencephalogram; DD, developmental delay; FA, fractional anisotropy; GABA, gamma-amino-butyric acid; ID, intellectual disability; MRI, magnetic resonance imaging; PDD, pervasive developmental disorder; QUIPS, Quality in Prognosis Strategy; TSC, Tuberous Sclerosis Complex.

METHODS

The results of the present review were reported according to the preferred reporting items for reviews and meta-analyses (PRISMA) and adheres to a structured review protocol (19).

Search Strategy and Article Selection

Two authors (NP and NS) performed a search of PubMed, EMBASE, and CENTRAL databases using the following search strategy: "autism" OR "autistic" OR "asperger" OR "autism spectrum disorder" OR "pervasive" OR "pervasive developmental disorder" OR "PDD" OR "ASD" AND "epilepsy" OR "seizure" OR "epileptic" OR "convulsion" AND "tuberous sclerosis complex" OR "tuberous sclerosis" OR "TSC."

Studies were initially included if they:

1) Involved individuals with ASD and epilepsy symptomatic of TSC.
2) Reported prevalence, odds ratio, or numerical report of the comorbidity of ASD in patients with epilepsy due to TSC.
3) Were written in English.
4) Were based on human research.
5) Were published within 15 years of the search date (January 2004–May 2019), which was considered a sufficient period to capture publications with the most reliable and appropriate diagnostic and management procedures.

Two authors independently screened all titles and abstracts of studies identified by the initial search. The full text of an article was obtained when either reviewer thought that it might fulfill the inclusion criteria. Upon uncertainty for inclusion of a publication, an additional author was consulted (LDP).

Full articles were reviewed for relevance and articles were excluded if they did not include data relating to the prevalence of epilepsy/seizures in the TSC population. Articles also had to contain a reported or calculable prevalence for ASD in the text (if not provided in the abstract).

Based on the Quality in Prognosis Strategy (QUIPS) tool, the most commonly found risk factors for bias in the studies reviewed included study participation, ASD measure, and study confounders. Many [14] of the reviewed articles included participants drawn from one clinic or hospital (18, 20–32); others [5] had a specific age range (12, 13, 33–35) or a particular subset of the TSC population (18, 35–47, 49, 50). Only 18 of the included articles reported the diagnostic criteria for ASD (**Table 1**). Large variations were noted in the measures and criteria used to define ASD and many of the articles relied on reports of ASD by parents and caregivers. Comparisons between various studies were subject to a number of potential confounders, including a failure to report seizure onset, type, and frequency for epilepsy, antiseizure medication (ASM), genetic susceptibility, or other relevant baseline measures. Only articles that unequivocally reported the above-mentioned information were included in **Tables 2**–**4**. From **Table 2**, eight articles were

TABLE 1 | Demographic information and prevalence rates of autism and epilepsy/seizures in Tuberous Sclerosis Complex patients reported within each of the articles included in this review.

Article	Study type	TSC patients, *n*	Male, *n* (%)	Mean age (unless median reported)	TSC patients with epilepsy/seizures, *n* (%)	TSC patients with autism, *n* (%)	Autism assessment	TSC patients with epilepsy and autism, *n* (%)
Baumer et al. (36)	Retrospective cohort	17	10 (59%)	7.2 y	10 (59%)	5 (29%)	n/r	n/r
Baumer et al. (37)	Retrospective clinical records (MRI)	51	31 (61%)	9.25 y	36 (71%)	19 (37%)	DSM IV/V and ADOS	18 (35%)
Benova et al. (38)	Prospective imaging	22	13 (59%)	6.3 y	20 (91%)	9 (41%)	ADI-R	9 (41%)
Capal et al. (51)	TACERN Prospective longitudinal study	130	68 (52%)	23.3 mo	95 (73%)	Symptoms only studied	AOSI and ADOS-2	n/r
Caylor et al. (39)	Exome sequencing in 3 families	3	2 (67%)	16.3 y	3 (100%)	1 (33%)	n/r	1 (33%)
Chopra et al. (40)	Cohort	45	22 (49%)	14.8 y	35 (78%)	15 (33%)	n/r	n/r
Chou et al. (20)	Cohort MRI	25	14 (56%)	11 y	23 (92%)	5 (20%)	n/r	n/r
Cusmai et al. (41)	Retrospective cohort	44	19 (43%)	13.8 y	44 (100%)	13 (30%)	n/r	13 (30%)
de Vries et al. (33)	Postal survey	265	106 (40%)	Reported age in groups (<5 and >18 were excluded)	238 (90%)	119 (45%)	n/r	n/r
Doherty et al. (42)	Retrospective study	44	21 (48%)	n/r	44 (100%)	9 (20%)	PDD	9 (20%)
Eluvathingal et al. (43)	MRI and PET scans of consecutive patients	78	44 (56%)	8 y	78 (100%)	Symptoms only studied	Gilliam Asperger's Disorder Scale (GADS)* and VABS	n/r
Gül Mert et al. (21)	Case study of clinical records	83	43 (53%)	33.5 mo	83 (100%)	28 (34%)	n/r	28 (34%)
Huang et al. (22)	Medical records	32	16 (50%)	n/r	26 (81%)	6 (19%)	n/r	n/r
Iscan et al. (23)	Brain imaging	17	10 (59%)	9.5 y	15 (88%)	1 (6%)	n/r	0
Jeste et al. (13)	Longitudinal study	36	22 (62%)	32.1 mo	34 (94%)	18 (50%)	ADOS	18 (50%)
Kilincaslan et al. (44)	Case study of patients with refractory epilepsy	6	4 (67%)	16.25 y[a]	6 (100%)	3 (50%)	CARS and AuBC	3 (50%)
Kingswood et al. (46)	Retrospective longitudinal cohort	334	157 (47%)	30.3 y	257 (77%)	41 (13%)	n/r	n/r
Kopp et al. (28)	Clinical records	99	45 (45%)	7.7 y	87 (88%)	31 (31%)	n/r	n/r
Kosac and Jovic (25)	Retrospective cohort (clinical records)	44	18 (41%)	19.4y	39 (89%)	6 (14%)	n/r	5 (11%)
Metwellay et al. (32)	Cross sectional observational study	24	18 (75%)	6.2 y	21 (88%)	11 (46%)	ADI-R and ADOS	n/r
Mizuguchi et al. (45)	Randomized trial	29	17 (59%)	8.76 y[a]	29 (100%)	20 (69%)	PARS	20 (69%)
Moavero et al. (34)	Epistop prospective study	82	45 (55%)	n/r evaluated at 6, 12, and 18 mo	51 (62%)	25 (30%)	ADOS and BSID	19 (23%)
Muzykewicz et al. (52)	Retrospective chart review	241	118 (49%)	20 y	208 (86%)	86 (36%)	Neuropsychological exam or clinical opinion	n/r

(Continued)

TABLE 1 | Continued

Article	Study type	TSC patients, *n*	Male, *n* (%)	Mean age (unless median reported)	TSC patients with epilepsy/seizures, *n* (%)	TSC patients with autism, *n* (%)	Autism assessment	TSC patients with epilepsy and autism, *n* (%)
Numis et al. (22)	Retrospective cohort (clinical records)	103	47 (46%)	13.05 y	91 (88%)	41 (40%)	DSM-IV, Child symptom inventory-4, BASC-2 and Gilliam Asperger's Disorder Scale (GADS)*	40 (39%)
Overwater et al. (47)	RCT	32	16 (50%)	12 y[a]	25 (78%)	17 (53%)	ADOS and CANTAB	n/r
Pascual-Castroviejo (26)	Retrospective review of MRI data	45	23 (51%)	n/r	45 (100%)	16 (36%)	n/r	16 (36%)
Saltik et al. (27)	Retrospective study of clinical records	21	11 (52%)	7.5 y	21 (100%)	2 (10%)	DSM-IV	2 (10%)
Samir et al. (35)	Prospective EEG and MRI	30	16 (53%)	4.66 y	30 (100%)	12 (40%)	ADIR and ADOS	12 (40%)
Spurling Jeste et al. (12)	Prospective study as part of a multisite longitudinal study	40	n/r	data reported at 6 mo intervals	36 (90%)	22 (55%)	AOSI and ADOS	22 (55%)
Staley et al. (49)	Retrospective review of clinical records	257	n/r	19 y	210 (82%)	23 (9%)	Gilliam Asperger's Disorder Scale (GADS)*	n/r
Toldo et al. (28)	Retrospective and prospective cohort study	32	16 (50%)	9.75 y	24 (75%)	22 (69%)	n/r	n/r
Vignoli et al. (29)	Cohort Study	42	18 (43%)	19.3 y[a]	42 (100%)	17 (40%)	SCQ	17 (40%)
Wataya-Kaneada et al. (30)	Comparison study of current vs. historical data from patients with TSC	166	70 (42%)	26.6 y	138 (83%)	35 (21%)	Pediatric and pychiatric departments (no diagnostic criteria) in Japan	n/r
Wilbur et al. (31)	Retrospective review of clinical records	81	41 (51%)	10 y[a]	74 (91%)	20 (25%)	n/r	20 (25%)
Wong and Khong (53)	MRI records	22	10 (45%)	15.25 y	21 (95%)	7 (32%)	DSM-IV/ADIR	7 (32%)
Yang et al. (50)	Systematic analysis of genotypic and clinical data of Chinese patients	117	60 (51%)	5.17 y	113 (97%)	27 (23%)	n/r	n/r

ADI-R, The Autism Diagnostic Interview-Revised; ADOS, The Autism Diagnostic Observation Schedule; AOSI, Autism Observation Scale for Infants; AuBC, Autism Behavior Checklist; BASC, Behavioral Assessment System for Children; BSID, Bayley Scales of Infant Development; CARS, Childhood Autism Rating Scale; DSM, Diagnostic Statistics Manual; MRI, magnetic resonance imaging; n/r, not reported; PARS, Pervasive Developmental Disorders Autism Society Japan Rating Scale; PET, Positron emission tomography; SCQ, Social Communication Questionnaire; TSC, Tuberous Sclerosis Complex; VABS, Vineland Adaptive Behavior Scales.

[a]*Median age reported.*

**(54)*.

TABLE 2 | Summary of history of epilepsy in patients with Tuberous Sclerosis Complex.

Article	Epilepsy/seizures present in TSC	Age at onset, mean	Epileptic spasms, *n*	Epilepsy/seizure type	Refractory epilepsy (%)	Seizure frequency
Benova et al. (38)	20	8.1 mo	5	n/r	n/r	Daily ($n = 14$); weekly ($n = 2$); monthly ($n = 4$)
Capal et al. (51)	95	5.6 mo	39	Focal szs ($n = 21$); mixed ($n = 42$) Generalized szs ($n = 4$); unclassified ($n = 6$)	n/r	n/r
Caylor et al. (39)	3	n/r	1	Frontal lobe epilepsy ($n = 1$); Focal szs ($n = 1$);	1 (33%)	n/r
Chou et al. (20)	23	<1 y ($n = 13$); <2 y ($n = 19$)	10	n/r	11 (48%)	n/r
Cusmai et al. (41)	44	<1 y	29	Focal motor szs ($n = 19$); generalized szs ($n = 1$)	14 (32%)	n/r
Doherty et al. (42)	44	n/r	23	n/r	n/r	n/r
Gul Mert et al. (21)	83	25.46 mo	21	Focal ($n = 23$); multifocal ($n = 12$); generalized ($n = 26$)	15 (18%)	n/r
Huang et al. (22)	26	≤6 mo ($n = 11$); 7–12 mo ($n = 8$); ≥12 mo ($n = 4$)	7	Complex partial ($n = 4$); simple partial ($n = 4$); generalized ($n = 7$); clonic ($n = 1$); tonic ($n = 1$; myoclonic ($n = 1$)	n/r	n/r
Iscan et al. (23)	15	24.7 mo	4	Generalized ($n = 3$); mixed ($n = 4$); Complex partial ($n = 2$) myoclonic ($n = 1$); febrile ($n = 1$)	n/r	n/r
Jeste et al. (13)	34	5.75 mo	n/r	n/r	6 (18%)	Monthly (26%); weekly (7%); daily (27%)
Kilincaslan et al. (44)	6	<6 mo ($n = 3$); <2 y ($n = 2$); 7 y ($n = 1$)	4	Complex partial ($n = 2$); simple partial ($n = 2$); atonic/atypical absence ($n = 1$)	6 (100%)	>1 a day ($n = 4$); >1 a week ($n = 2$)
Kopp et al. (24)	87	0.9 y	51	Complex partial history ($n = 78$); mixed seizures history ($n = 18$)	n/r	Mean per month 39.9 ($n = 66$)
Kosac and Jovic (25)	39	2.8 y	10	Focal szs (84.6%); Secondary generalized szs (39.3%)	n/r	n/r
Metwellay et al. (32)	21	<6 mo ($n = 12$); >6 mo ($n = 9$)	13	Generalized ($n = 3$); Focal ($n = 4$); Partial with secondary generalization ($n = 1$)	16 (76%)	n/r
Mizuguchi et al. (45)	29	n/r	n/r	n/r	n/r	n/r
Moavero et al. (34)	51	<1 y ($n = 38$); <2 y ($n = 13$)	10	n/r	32 (63%)	n/r
Muzykewicz et al. (52)	208	n/r	92	n/r	141 (68%)	n/r
Numis et al. (18)	91	1.9 y	44	n/r	60 (66%)	1.75 per week
Overwater et al. (47)	25	n/r	7	n/r	14 (56%)	n/r
Pascual-Castroviejo et al. (26)	45	n/r	23	n/r	n/r	n/r
Saltik et al. (27)	21	<1 y (76.1%)	5	Focal szs ($n = 20$); diffuse tonic-clonic ($n = 3$); atonic ($n = 3$); absence ($n = 1$)	13 (62%)	n/r
Samir et al. (35)	30	<6 mo ($n = 16$); ≥6 mo ($n = 14$)	17	Focal szs ($n = 5$); secondary generalization ($n = 8$)	19 (63%)	n/r
Spurling Jeste et al. (12)	36	5.8 mo	26	n/r	n/r	n/r
Vignoli et al. (29)	42	7.9 mo	11	n/r	11 (26%)	Monthy ($n = 7$); Weekly ($n = 10$)
Wataya-Kaneada et al. (30)	143	n/r	n/r	n/r	20 (14%)	n/r
Wilbur et al. (31)	74	12 mo median	26	Focal (66%); epileptic spasms (26%); generalized (5%)	n/r	n/r
Wong et al. (55)	21	33 mo	8	n/r	3 (14%)	n/r
Yang et al. (50)	113	n/r	55	n/r	n/r	n/r

n/r, not reported; Szs, seizures; TCS, Tuberous Sclerosis Complex.

TABLE 3 | Summary of family history of Tuberous Sclerosis Complex (TSC) and genetic mutations in patients with TSC.

Article	TSC patients, *n*	TSC patients with epilepsy and autism, *n* (%)	Seizure/epilepsy in patients with ASD
Baumer et al. (37)	51	18 (35%)	n/r
Benova et al. (38)	22	9 (41%)	2/9 ES
Caylor et al. (39)	3	1 (33%)	1/1 focal to bilateral seizure
Cusmai et al. (41)	44	13 (30%)	8/13 ES, 5/13 focal motor
Doherty et al. (42)	44	9 (20%)	n/r
Gül Mert et al. (21)	83	28 (34%)	n/r
Iscan et al. (23)	17	0	n/r
Jeste et al. (13)	36	18 (50%)	13/18 ES
Kilincaslan et al. (44)	6	3 (50%)	2/3 ES, 3/3 focal seizure, 2/3 tonic seizure
Kosac and Jovic (25)	44	5 (11%)	n/r
Mizuguchi et al. (45)	29	20 (69%)	n/r
Moavero et al. (34)	82	19 (23%)	2/15 ES
Numis et al. (18)	103	40 (39%)	24/40 ES
Pascual-Castroviejo et al. (26)	45	16 (36%)	n/r
Saltik et al. (27)	21	2 (10%)	n/r
Samir et al. (35)	30	12 (40%)	11/12 ES
Spurling Jeste et al. (12)	40	22 (55%)	14/22 ES
Vignoli et al. (29)	42	17 (40%)	n/r
Wilbur et al. (31)	81	20 (25%)	n/r
Wong and Khong (53)	22	7 (32%)	n/r

n/r, not reported; TSC, Tuberous Sclerosis Complex; ASD, Autism spectrum disorder; ES, epileptic spasms.

excluded because of no mention of onset, type, or frequency of epileptic seizures; 16 articles were excluded from **Table 3** because of no mention of number of patients with epilepsy, TSC and autism; 14 articles were excluded from **Table 4** because of no mention of genetic mutation in *TSC1* and *TSC2*. In this review we have used the terminology "infantile spasms" for infants with ES (with or without hypsarrhythmia), who may or may not have had cognitive regression. This operational definition was chosen because it was not always possible to determine whether the infants had hypsarrhythmia or cognitive regression. In the tables and figures, however, the term "epileptic spasms" has been used because this refers to that specific type of seizure.

RESULTS

A total of 841 abstracts were identified in the original search. Of these, 673 were duplicates or congress abstracts only. The remaining abstracts and articles were reviewed for inclusion/exclusion criteria, and a total of 36 articles were considered suitable for inclusion (**Figure 1**). Included articles are presented in **Table 1**. In total, 2,666 patients with TSC were included in this review, with a mean age of 15.9 years (range 1.94–30.3 years). TSC populations included within the selected articles were predominantly male; males represented 52.5% of overall participants, ranging from 41 to 75% of patients in articles (**Table 1**).

Prevalence of Autism and Epilepsy in Patients With TSC

Of the patients with TSC included with available data in this review, the overall percentage of patients with autism was 29.8% (732 of 2,458 patients with available data), ranging from 6% (23) to 69% (28, 45), and those with epilepsy/seizures was 88.2% (2,352 of 2,666 patients), ranging from 59 to 100% (21, 26, 27, 29, 35, 39, 41–45) (**Table 1**). Patients with epilepsy *and* autism are also reported where available (**Table 1**), with the overall percentage being 33.7% (279 of 828 patients with available data) and ranging from 10% (27) to 69% (45).

Epilepsy

The mean age for onset of epilepsy was below 33 months; however, data were available for 859 patients only. Infantile spasms were reported in 42.8% of TSC populations studied (**Table 2**), ranging from 20% (34) to 67% (44). Other epilepsy types were less frequently reported within the articles reviewed, but Huang et al. (22) suggested that focal seizures were also frequent in infants with TSC under 1 year of age. Reports of refractory epilepsy in patients with TSC ranged from 14% to 100% (44), although the latter specifically focused on 6 TSC patients with refractory epilepsy.

The relationship between epilepsy, ASD, and TSC is complex. Autism, the onset of seizures in children <2 years of age and with >3 tubers (21, 31) have all been associated with an increased risk of refractory epilepsy in TSC patients. However, the direction of the relationship is unclear because a history of epilepsy (33) or infantile spasms (31, 35) in patients with TSC is also associated with an increased likelihood of ASD. Patients with TSC *and* autism showed more frequent seizures than TSC patients without autism (18) and an earlier age of onset of epilepsy has been associated with ASD (18, 28, 31), delayed language, intellectual disability (ID), and poor cognitive flexibility (28). In **Table 3** are reported the epilepsy features in patients with TSC and autism.

Phenotype/Behavior

Clinically significant behavioral problems and social withdrawal are common in young children with TSC (28). Conditions including mood disorder, anxiety, ADHD, and aggressive behavior were reported in 66% of a pediatric population with TSC ($n = 241$) (52). Aggressive behavior was associated with both increased severity of epilepsy and features of autism/pervasive developmental disorder (PDD) (52).

Early identifiers of autism or autistic-like features in patients with TSC include early DD or a slowing in nonverbal cognition (13, 38). Studies of very young infants with TSC suggest early delay in visual reception (12) and under-developed fine-motor skills to be markers of the development of autism traits (12, 34).

TABLE 4 | Summary of family history of Tuberous Sclerosis Complex (TSC) and genetic mutations in patients with TSC.

Article	TSC pts, *n*	*TSC1* (all patients), *n* (%)	*TSC1* (patients with autism), *n* (%)	*TSC2* (all patients), *n* (%)	*TSC2* (patients with autism), *n* (%)	No mutation identified (all patients), *n* (%)	No mutation identified (patients with autism), *n* (%)	Family history of TSC, *n* (%)
Benova et al. (38)	22	7 (32%)	2 (22%) ($n = 9$)	12 (55%)	5 (56%) ($n = 9$)	–	–	n/r
Caylor et al. (39)	3	2 (67%)	1 (100%) ($n = 1$)	1 (33%)	0 ($n = 1$)	–	–	n/r
Chopra et al. (40)	45	9 (20%)	1 (7%) ($n = 15$)	24 (53%)	12 (80%) ($n = 15$)	11 (24%)	n/r	5 (11%)
Chou et al. (20)	25	n/r	n/r	n/r	n/r	n/r	n/r	2 (8%)
Cusmai et al. (41)	44	2 (9%) ($n = 23$)	n/r	20 (87%) ($n = 23$)	n/r	1 (4%) ($n = 23$)	n/r	n/r
Doherty et al. (42)	44	10 (23%)	n/r	26 (59%)	n/r	n/r	n/r	n/r
Huang et al. (22)	32	6 (19%)	1 (17%) ($n = 6$)	26 (81%)	5 (83%) ($n = 6$)	–	–	n/r
Iscan et al. (23)	17	n/r	n/r	n/r	n/r	n/r	n/r	4
Jeste et al. (13)	34	5 (16%) ($n = 31$)	4 (27%) ($n = 15$)	26 (84%) ($n = 31$)	11 (73%) ($n = 15$)	–	–	n/r
Kopp et al. (24)	99	15 (16%)	n/r	58 (62%)	n/r	21(22%)	n/r	20 (20%)
Kosac and Jovic (25)	44	3 (30%) ($n = 10$)	n/r	5 (50%) ($n = 10$)	n/r	n/r	n/r	11 (25%)
Moavero et al. (34)	82	20 (24%)	n/r	59 (72%)	n/r	3 (4%)	n/r	n/r
Muzykewicz et al. (52)	241	50[a] (27%) ($n = 191$)	n/r	106[a] (55%) ($n = 191$)	n/r	34 (18%) ($n = 191$)	n/r	n/r
Numis et al. (18)	103	24 (23%)	3 (7%) ($n = 41$)	58 (56%)	27 (66%)	10 (10%)	6 (22%) ($n = 41$)	n/r
Overwater et al. (47)	32	7 (22%)	n/r	21 (66%)	n/r	4 (13%)	n/r	n/r
Saltik et al. (27)	21	n/r	n/r	n/r	n/r	n/r	n/r	7 (33%)
Samir et al. (35)	30	n/r	n/r	n/r	n/r	n/r	n/r	4 (13%)
Staley et al. (49)	257	51 (27%) ($n = 192$)	n/r	109 (57%) ($n = 192$)	n/r	n/r	n/r	n/r
Vignoli et al. (29)	42	10 (24%)	n/r	30 (71%)	n/r	2 (5%)	n/r	n/r
Wataya-Kaneada et al. (30)	166	21 (28%) ($n = 75$)	n/r	24 (32%) ($n = 75$)	n/r	30 (39%) ($n = 75$)	n/r	17 (23%) ($n = 75$)
Wilbur et al. (31)	81	2 (33%) ($n = 6$)	n/r	4 (67%) ($n = 6$)	n/r	–	n/r	6 (7%)
Yang et al. (50)	117	16 (14%)	2 (9) ($n = 27\%$)	101 (86)	25 (93%) ($n = 27$)	–	–	14 (12%)

n/r, not reported; TSC, Tuberous Sclerosis Complex; pts, patients.

[a]*One patient had both TSC1 and TSC2 mutations.*

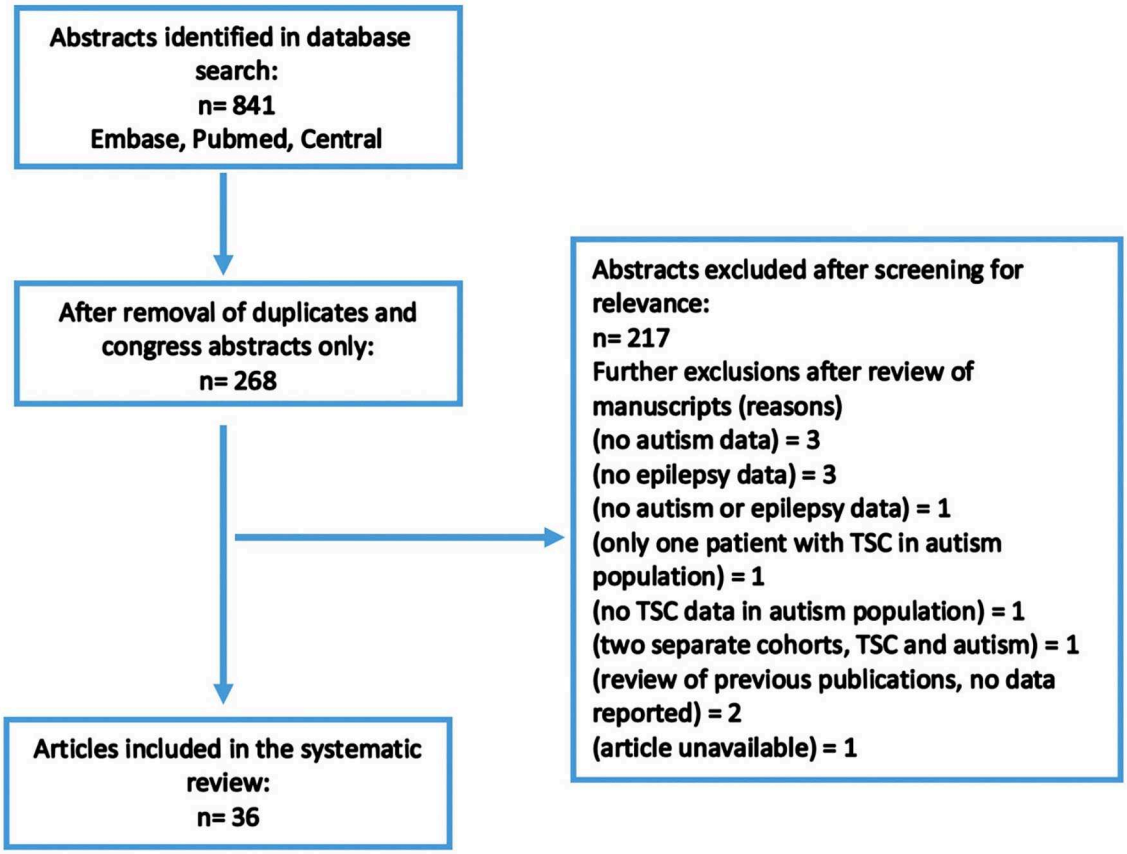

FIGURE 1 | Search strategy.

Deficits across all domains of the Bayley Scales of Infant Development (BSDI) at 1 year of age were predictive of higher autism traits on the Autism Diagnostic Observation Schedule (ADOS) at 2 years within a prospective study of infants with TSC ($n = 82$) in 10 sites across Europe and Australia (34). ID is often more common in TSC patients with ASD than those with TSC alone (31). Behavioral problems have been reported to be exacerbated by seizure frequency and a mixed seizure profile (24). Results of a study exploring the relationship between cognitive delay and clinical features of TSC in Egypt reported that the age of seizure onset ($p = 0.044$) and number of brain tubers ($p = 0.06$) increased the odds for cognitive delay in 24 children with TSC (32). Similarly, ID has been associated with early onset of seizures, infantile spasms (35), and intractable epilepsy (38). Early seizure onset was the most significant predictor of DD at 2 years of age in a longitudinal prospective analysis of developmental outcomes in infants (0–3 years) with TSC (51). Since the data in most of the reported studies did not specify infantile spasms, many of the early onset seizures could have been infantile spasms. The neurologic symptoms of TSC, refractory epilepsy, ASD, and ID have all shown an interrelationship (30), making specific relationships between ASD, ID, and epilepsy difficult to discern.

Self-injurious behavior in patients with TSC was associated with a history of infantile spasms and seizures, ID, ASD, and *TSC2* mutations (49). Aggressive behavior was also associated with ID and *TSC2* mutations (52), suggesting a potential genetic link.

Data on severity of autism and developmental delay were sparse and therefore not reported.

Genotype

Refractory epilepsy (38), ID (24), and autism in TSC patients have all been associated with the *TSC2* genotype (29, 40, 50). The *TSC2* genotype was more common than *TSC1* genotype among TSC patients overall (**Table 4**), with the exception of one study that focused on individuals from three families, in which three individuals had a diagnosis of TSC: two with the *TSC1* genotype and one with the *TSC2* genotype (39). Overall, 73.2% of TSC individuals had the *TSC2* genotype—ranging from 32% (30) to 89% (41)—and 26.8% had the *TSC1* genotype—ranging from 9% (41) to 67% (39) (**Table 4**). Among patients with TSC and autism, 85.6% had the *TSC2* genotype. Autistic behavior correlated with nonsense mutations in the *TSC2* gene group in a retrospective review of medical records from patients with TSC in Taiwan ($n = 32$) (22).

Neuroimaging

A magnetic resonance imaging (MRI) study including 25 children (aged >2 years) reported that lesion load within the left temporal lobe was positively correlated with the neurological severity score ($r = 0.609$; $p = 0.001$). This finding was supported in an electroencephalogram (EEG) study that found greater interictal epileptiform features in the left temporal lobe only (18).

Two studies exploring potential impact of TSC proteins on white-matter tract pathways have identified abnormal diffusion characteristics, which are believed to arise from abnormal neuronal and axonal organization and hypomyelination (36, 37). Furthermore, these effects were each associated with TSC, epilepsy, and autism (36, 37). In a diffusion MRI study exploring the directionality of water movement [fractional anisotropy (FA)], TSC alone was related to lower callosal FA values than controls—and this difference was greater in the TSC patients with autism than without—when comparing study groups of TSC patients with either epilepsy (with and without comorbid autism; $n = 19$ and $n = 32$, respectively) or autism alone ($n = 46$) with a healthy control group ($n = 89$) (37). A positron emission tomography (PET) study comparing TSC patients with and without a cerebellar lesion ($n = 20$ vs. $n = 57$, respectively) reported that the group with cerebellar lesions had higher overall autistic symptomology (i.e., social isolation and communicative/developmental disturbance) and that these deficits were associated with right-sided cerebellar lesions (43).

The size, number, and anatomical location of tubers have all independently been linked to autism and/or epilepsy in TSC (22, 26, 35, 38, 42), although this relationship has not always been established (55). The number of tubers is strongly associated with infantile spasms (42) and ASD (35). Tubers of larger size were associated with increased likelihood of seizures and autism (26), and higher prevalence of cyst-like tubers was associated with ASD (22). ASD and intractable epilepsy were both predicted by a higher number of areas with dysplastic features (38). ASD or PDD have been linked with tubers in the frontal areas of the brain (35), increased tuber count in the occipital lobe (42), cystic-like tubers, and tubers in insular and temporal areas (22). Infantile spasms are more likely to occur in children with cortical tubers in the parietal lobes (55).

Pharmacological Treatment

Data relating to ASM use was not commonly provided in the studies included in this review. Where reported, the mean number of ASMs per patient with TSC ranged from 1.46 to 3.95 (12, 13, 18, 38). Combination treatment with two ASMs or more was common and, where reported, the number of TSC patients using polytherapy ranged from 52 to 100% (20, 21, 25, 29, 38). Common ASMs included valproic acid, carbamazepine, topiramate, lamotrigine, and vigabatrin (25, 38, 41). Only two of the reviewed studies reported individual use of ASMs among TSC patients, and these data are summarized in **Figure 2**.

Early treatment with ASMs may be of importance, since better long-term epileptic encephalopathy outcomes were reported in those treated early in a randomized trial of early vs. later treatment with vigabatrin (41). In general, studies should distinguish between early and later treatment of epilepsy in TSC, considering that later treatment of seizures in TSC is often disappointing and research reports that the development of ID is predicted by the number of ASMs used (potentially related to delay in effective treatment) to treat epilepsy in children with TSC (38).

The studies in our review with data on individuals with uncontrolled epilepsy reported these to represent 14–100% of patients with a history of epilepsy, with the majority of studies reporting >40% of the epilepsy population still having seizures (**Table 2**). Although these data suggest a greater proportion of TSC patients with difficult-to-treat epilepsy than is typical of a general population, the bias in study participation remains a caveat to such speculation.

Three studies evaluated the effects of an mTOR kinase inhibitor, everolimus, which can be used to reduce tumor size (44, 45, 47, 48). The first, a three-armed randomized trial in Japan ($n = 29$), reported adjunctive everolimus treatment to significantly reduce seizure frequency in TSC patients with refractory epilepsy, with a trend for improvements in ASD symptoms (45). A similar finding was reported in a small case study evaluating everolimus for refractory epilepsy in six TSC patients with refractory epilepsy (44). This second study also reported improvement in ASD symptoms, such as social contact, language, and repetitive behavior (44). However, the third study—a recent randomized controlled trial conducted in the Netherlands including 32 children with TSC—found no benefit of everolimus on cognitive or neuropsychological functioning, or autism traits, in comparison with placebo (47). In this third study, age at enrollment was high—the median age was 11.5 years for patients on placebo and 12.2 years for patients on everolimus—therefore, firm conclusions cannot be drawn (47). However, early treatment with everolimus might be required for improvement in features such as social contact, language and repetitive behavior; there is a need for formal studies to determine whether this is the case.

DISCUSSION

Based on the 36 articles included in this review, our findings were consistent with previous reports of high rates of epilepsy in patients with TSC (5). Interestingly, epilepsy was reported in 83.6% of patients with TSC in an international TuberOus SClerosis registry to increase disease Awareness (TOSCA); however, data on the prevalence of ASD in this population were not reported (56). The prevalence of autism in patients with TSC in the subjects included in this review is high, but is consistent with previous estimates of syndromic ASD in TSC (14, 15, 57).

The risk of autism is increased by early onset seizures (18, 28, 31, 35) and by DD and ID (28), which in turn have been associated with early onset epilepsy and infantile spasms (28, 31, 32, 35, 38). Existence of phenotypic variability should be acknowledged: TSC is also associated with high-functioning autism, normal intelligence, hypercalculia, and drug-resistant epilepsy with an EEG pattern characterized by hypsarrhythmia and electrical status epilepticus during sleep (58).

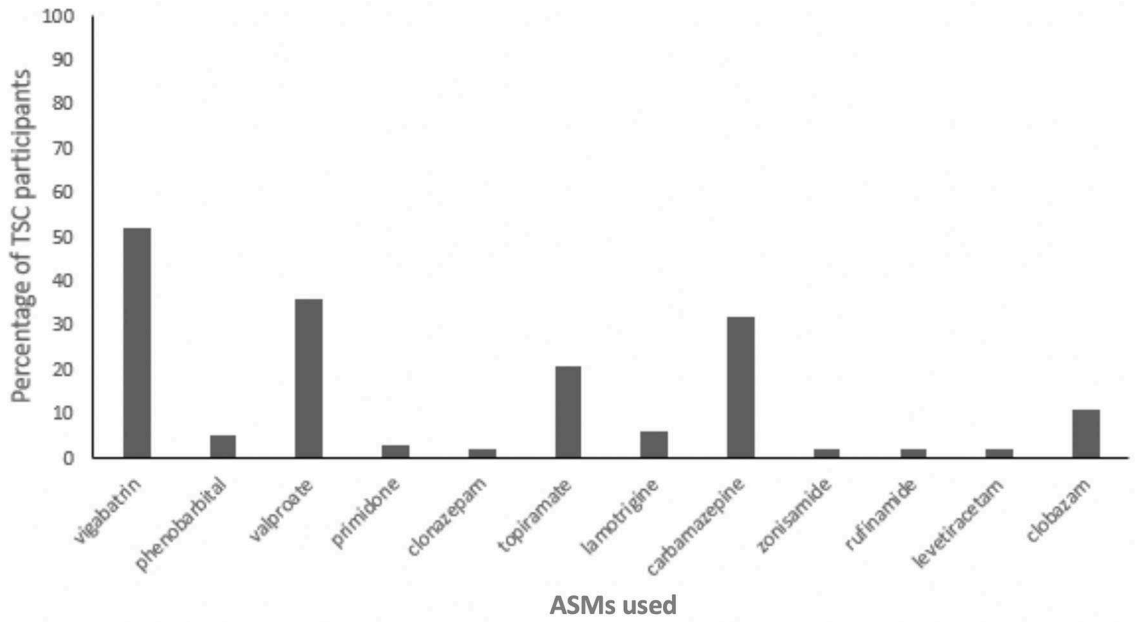

FIGURE 2 | Proportion of Tuberous Sclerosis Complex (TSC) participants using antiseizure medications (ASMs) ($n = 66$) (38, 41).

The relationship between TSC, epilepsy, and ASD is highly complex. A poor prognosis of epilepsy outcomes is largely reported to be exacerbated by ASD (18, 21, 31). An additive neuroanatomical impact of TSC, epilepsy, and autism has been proposed that is predominantly evident in white-matter pathways (36, 37), supporting the association between autism, epilepsy, and DD/ID in patients with TSC.

Evidence suggests both epilepsy and autism are linked with mutations on the *TSC1* and *TSC2* genes. Mutations in the *TSC2* gene are more prevalent in association with epilepsy and autism (18, 22, 29, 38, 40, 50). Early genotyping may, therefore, help identify TSC patients at increased risk of poorer long-term outcomes.

In terms of autism, neuroimaging studies report that tuber features, such as larger size or increased number of cyst-like tubers, are associated with increased risk (22, 26). It is also demonstrated that diffusion imaging abnormalities correlate with reduced myelination in TSC patients (59) and the effect of mTOR overactivation on white matter might be modified by pharmacological inhibition (60). Moreover, TSC patients with autism have been documented to have a reduction of fractional anisotropy in different white-matter regions, and this happens over the first 2 years of life (61). Since size, type, and location of tubers influence the longer-term risk of autism and epilepsy in TSC, early characterization of such features could assist in determining the focus of early intervention.

Cells in the central nervous system express TSC1 and TSC2 proteins throughout childhood and into adulthood. These proteins help regulate myelination, axon guidance, and dendritic arborization, promoting normal synaptic formation and function (37). Dysregulation of the neurotransmission of GABA, resulting from genetic mutations of TSC, has previously been argued to underlie development of epilepsy and autism in this population (5). Limited evidence suggests that treatment with everolimus, particularly if commenced early, may improve epilepsy outcomes and reduce the risk of autism in TSC patients (44, 45). Data coming from the EXIST-3 trial confirm that adjunctive everolimus might reduce seizure frequency in pediatric patients with treatment-refractory seizures associated with tuberous sclerosis complex also in patients younger than 6 years (62). However, these findings were based on few trials and contradictory evidence also exists (47). Additional research into alternative treatment strategies and an increased focus on the longer-term outcomes would help elucidate whether size, type, and location of tubers influence the longer-term risk of autism and epilepsy in TSC.

Early treatment with ASMs to control epilepsy is reported to improve longer-term epilepsy outcomes (41), and controlled epilepsy is associated with reduced symptoms of autism (44, 45). ID and DD are in turn associated with increased presence of autism (31, 34), so the number and choice of ASMs in infants with TSC needs to be managed with care. **Figure 3** is a diagrammatic overview of the complex relationship between the phenotypic features of TSC and polytherapy treatment with ASMs based on the evidence reviewed here.

LIMITATIONS

Although we identified 36 articles reporting autism and epilepsy in TSC, only approximately half of these articles indicated which patients were experiencing either of these comorbid conditions. Very few of the included studies summarized the potential prognostic features of patients with all three conditions (TSC,

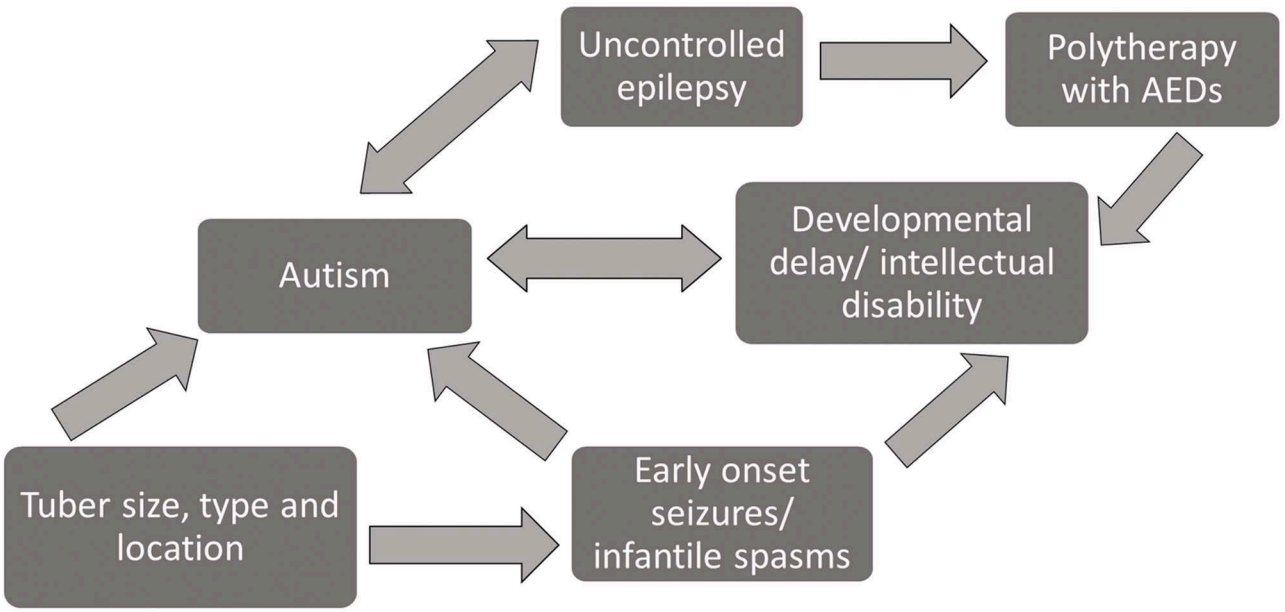

FIGURE 3 | Diagram of interrelationship between phenotypic profiles and prognostic risk factors among patients with TSC.

epilepsy, and autism). This review has therefore identified a need for future studies to focus on common associative factors.

A second limitation was that, because the studies were conducted in different settings across different countries, practices were not standardized with respect to identification of TSC, epilepsy, and—above all—autism. Different diagnostic criteria were used to identify patients with TSC according to the clinical practice of the country or region. Likewise, the tools used to define the presence of autism varied considerably. In some cases, the diagnosis of autism was not confirmed, but relied on reports from parents and caregivers. In populations that only focused on very young infants, in whom a clinical diagnosis of autism was not possible, the conclusions regarding risk of autism were based on autistic features, which do not necessarily indicate a later clinical outcome.

The methodological approaches of the included articles also varied widely and ranged from small clinical series to large retrospective studies, each with differing strengths and limitations. One of the challenges of establishing a representative sample of individuals with TSC is the rarity of the disease. The TSC populations within the included articles ranged from infants to adults, sometimes within the same study. Consequently, the core features of TSC and age of onset of the conditions may not have been reliable.

Lastly, the quality of the available data does not allow a meaningful review to be performed.

CONCLUSIONS

Early onset epilepsy, frequently represented by epileptic encephalopathy, can be considered one of the risk factors for ID in TSC patients. However, the role of genetic variations should be highlighted as the major player in determining both epilepsy and intellectual disability due to mTOR overactivation (63).

In terms of further defining the prognostic features of epilepsy and autism within TSC, large prospective studies, such as TACERN or those conducted by the EPISTOP group (34, 51, 64), are helping to identify early biomarkers for treatment.

The prevalence of autism and epilepsy in TSC is much higher than that in the general population, both alone and as comorbid features. We summarized the phenotypic, genetic, and neurological risk factors for the association of autism and epilepsy in TSC patients from available data, but the inherent limitations of the source studies should be noted.

The relationship between these three conditions is complex. Early identification of the risk factors, together with early use of m-TOR inhibitors might be a priority to optimize favorable outcomes in this vulnerable population.

AUTHOR CONTRIBUTIONS

NS conceptualized and designed the study, drafted the initial manuscript, supervised data collection, and reviewed and revised the manuscript. NP, MT, and LD designed the data collection instruments, collected data, carried out the initial analyses, and reviewed and revised the manuscript. AF and RM collected data, carried out the initial analyses, and reviewed and revised the manuscript. FV and PC conceptualized and designed the study and critically reviewed the manuscript for important intellectual content. 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

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 (%).*

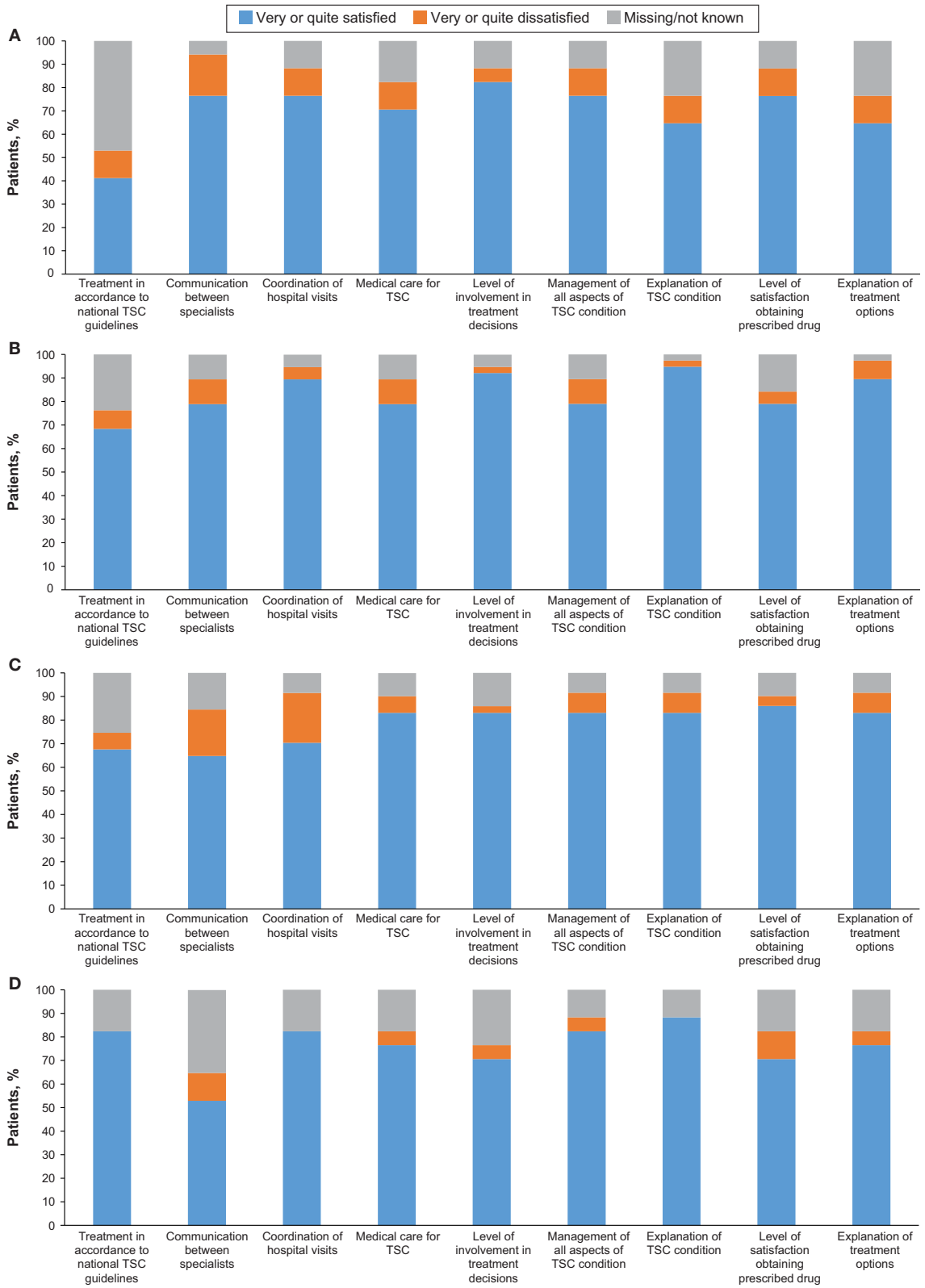

FIGURE 1 | Satisfaction with treatment aspects in **(A)** Self-reported children, **(B)** Self-reported adults, **(C)** Caregiver-reported children, and **(D)** Caregiver-reported adults.

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).

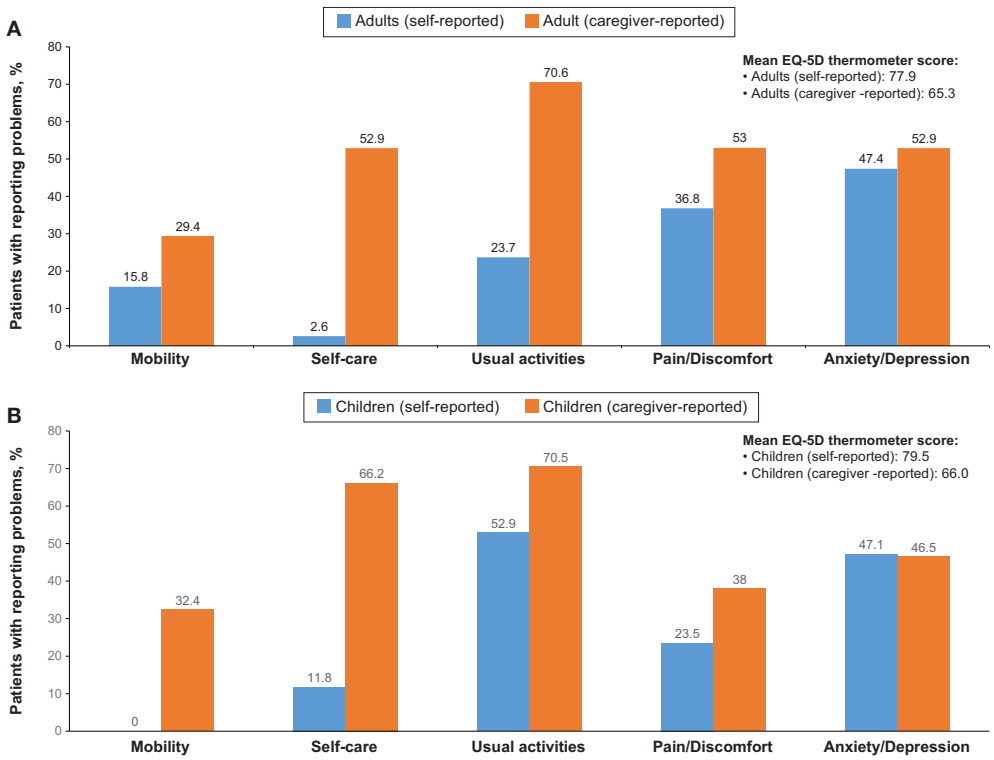

FIGURE 2 | Summary of EQ-5D Questionnaire in **(A)** adults and **(B)** children.

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

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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

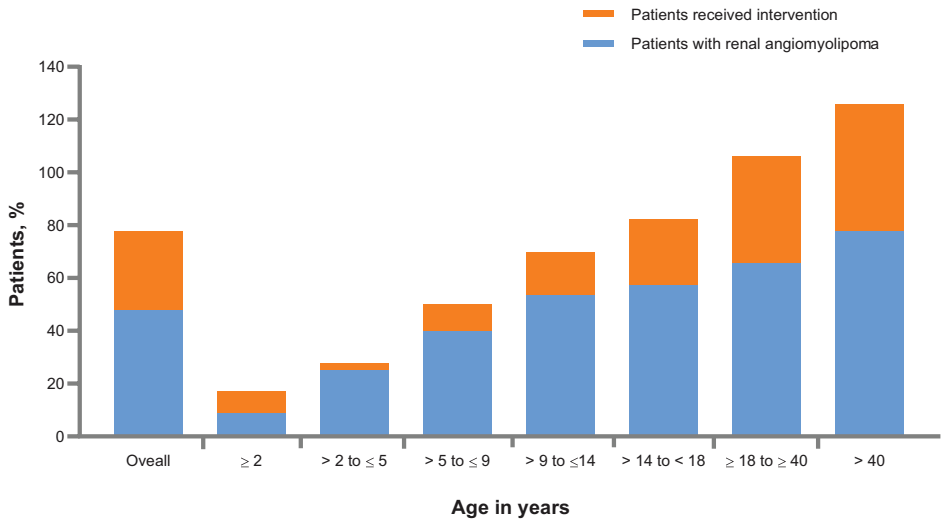
TABLE 2 | Clinical characteristics of renal angiomyolipoma in overall population.

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.

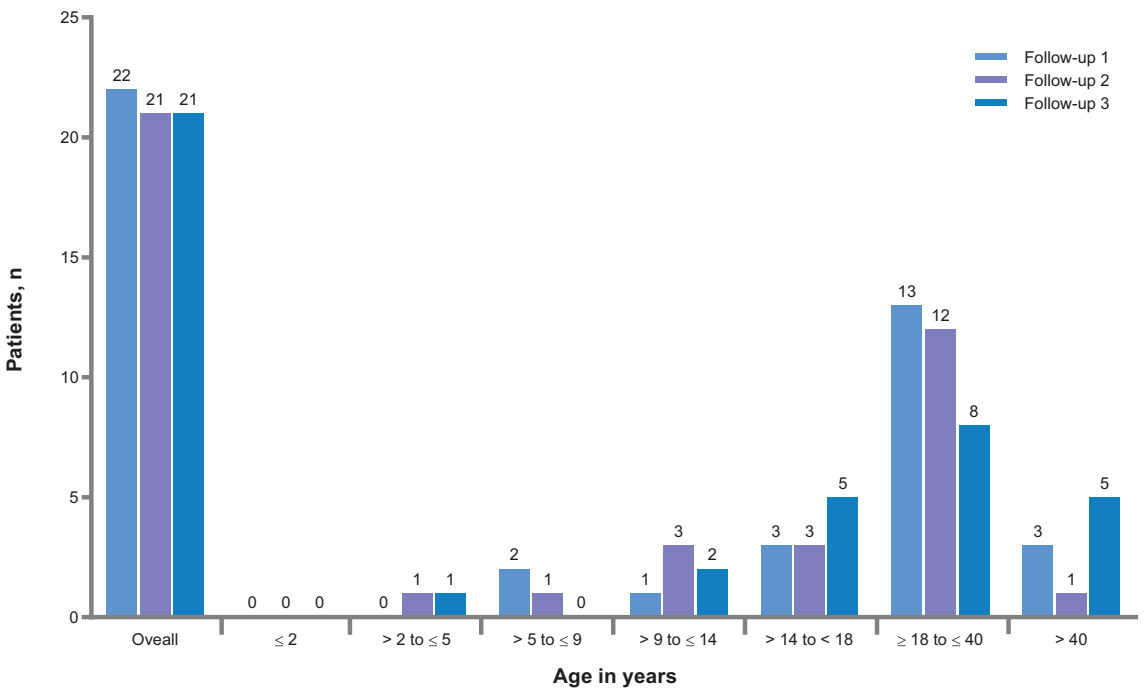


FIGURE 2 | Newly diagnosed renal angiomyolipoma after baseline visit.

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

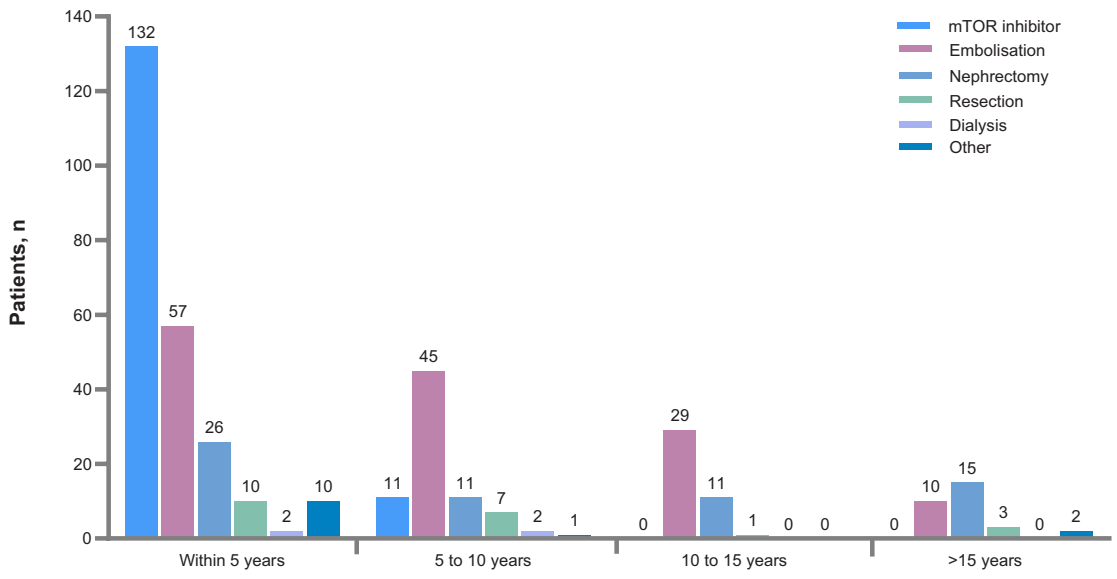


FIGURE 3 | Time since interventions prior to baseline. mTOR, mammalian target or rapamycin.

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.*

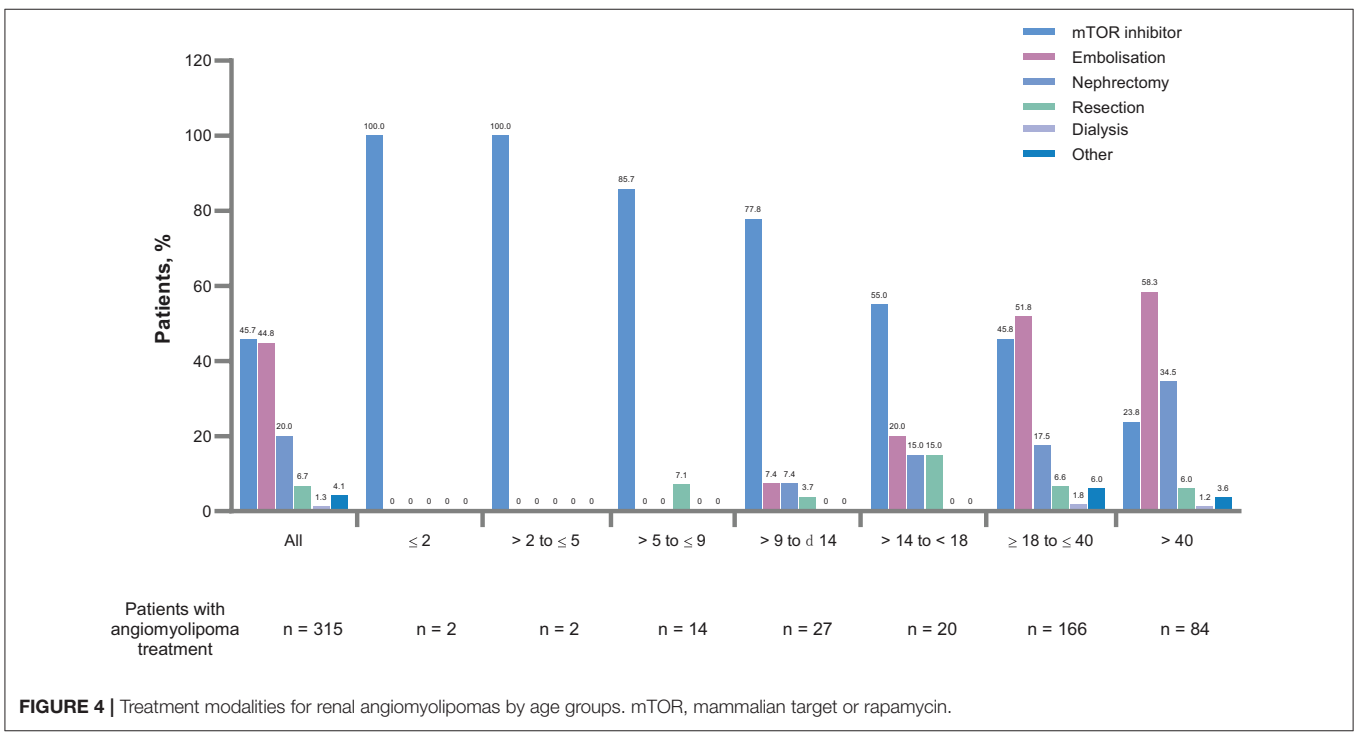


FIGURE 4 | Treatment modalities for renal angiomyolipomas by age groups. mTOR, mammalian target or rapamycin.

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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Prediction of Neurodevelopment in Infants With Tuberous Sclerosis Complex Using Early EEG Characteristics

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Tuberous Sclerosis Complex (TSC) is a multisystem genetic disorder with a high risk of early-onset epilepsy and a high prevalence of neurodevelopmental comorbidities, including intellectual disability and autism spectrum disorder (ASD). Therefore, TSC is an interesting disease model to investigate early biomarkers of neurodevelopmental comorbidities when interventions are favourable. We investigated whether early EEG characteristics can be used to predict neurodevelopment in infants with TSC. The first recorded EEG of 64 infants with TSC, enrolled in the international prospective EPISTOP trial (recorded at a median gestational age 42 4/7 weeks) was first visually assessed. EEG characteristics were correlated with ASD risk based on the ADOS-2 score, and cognitive, language, and motor developmental quotients (Bayley Scales of Infant and Toddler Development III) at the age of 24 months. Quantitative EEG analysis was used to validate the relationship between EEG background abnormalities and ASD risk. An abnormal first EEG (OR = 4.1, p-value = 0.027) and more specifically a dysmature EEG background (OR = 4.6, p-value = 0.017) was associated with a higher probability of ASD traits at the age of 24 months. This association between an early abnormal EEG and ASD risk remained significant in a multivariable model, adjusting for mutation and treatment (adjusted OR = 4.2, p-value = 0.029).

A dysmature EEG background was also associated with lower cognitive (p-value = 0.029), language (p-value = 0.001), and motor (p-value = 0.017) developmental quotients at the age of 24 months. Our findings suggest that early EEG characteristics in newborns and infants with TSC can be used to predict neurodevelopmental comorbidities.

Keywords: tuberous sclerosis complex (TSC), EEG, biomarker, neurodevelopment, autism (ASD), TAND profile

INTRODUCTION

Tuberous sclerosis complex (TSC) is an autosomal dominant disorder affecting ∼1 in 5,800 individuals (1). This disorder is caused by loss of function mutations in the tumour-suppressor genes *TSC1* or *TSC2*, encoding the proteins hamartin and tuberin. Both proteins are key components of a TSC protein complex that regulates the state of activation of the Ras homolog enriched in brain GTPase and hence the mammalian target of rapamycin complex 1 (mTORC1) (2, 3). Overactivation of mTORC1 results in disorganized cellular growth, differentiation, metabolism, and impaired autophagy, leading to the formation of hamartomatous lesions in various organs, causing a multisystem disorder (2). However, the clinical features of TSC are both age-dependent and highly variable (2).

In patients with TSC, both epilepsy and neurodevelopmental comorbidities including intellectual disability and autism spectrum disorder (ASD) are common. Epilepsy affects 80–90%, 6% of TSC infants develop seizures during the first month of life, and 60–70% develop epilepsy within the first year of life (4–7). About 60% of TSC patients develop drug-resistant epilepsy (4, 8). In addition, 40–50% of TSC patients have intellectual disability. ASD is diagnosed in 21–50% of patients with TSC (9–12). In TSC several characteristics are associated with intellectual disability and ASD, including having a *TSC2* mutation, young age at seizure onset, a high seizure burden, epileptic spasms, and drug-resistant epilepsy (6, 13–16). Similarly, TSC patients with intellectual disability have a higher prevalence of epilepsy (88%) and a higher rate of drug-resistant epilepsy (65%) (1, 2).

Recent studies have shown that early interventions can improve seizure control and can preserve neurodevelopment to some extent (13, 17). A small non-randomized pilot clinical trial of early intervention with vigabatrin at the time of multifocal interictal epileptiform discharges (IED) detection, led to a significant reduction in drug-resistant epilepsy, more seizure-free patients, and fewer patients with intellectual disability at 24 months in comparison to an historical control group in which vigabatrin was started only after seizure onset (18). More recent follow-up of these TSC children showed persistent improvement at school age (19).

Prenatal or early postnatal diagnosis of TSC is currently possible, due to the visualisation of cardiac TSC related-lesions or cortical tubers (2). Since early diagnosis is feasible, TSC is an interesting disease model to study both epileptogenesis and investigate potential biomarkers of neurodevelopmental comorbidities. Early abnormal EEG activity had been shown to be predictive for later epilepsy. Abnormal EEG activity can be observed before the development of clinical seizures in TSC (20). In a prospective study of 32 infants with TSC, 20 patients developed seizures. In 17/20 IED preceded the onset of seizures, by an average interval of 3·6 months (21) A recent quantitative EEG study showed that increased EEG connectivity in infants with TSC preceded the onset of epileptic spasms (22).

In this exploratory EEG study we investigated whether the first EEG, recorded in infants with TSC, and taken below the age of 4 months could be used to predict neurodevelopment and especially ASD risk at the age of 24 months. Our hypothesis was that early EEG characteristics, such as IED and background abnormalities can predict neurodevelopmental outcome. In addition to the visual analysis of the EEG, we used quantitative EEG methodology for assessment and quantification of EEG background abnormalities.

PATIENTS AND METHODS

Patients

This EEG study was part of the EPISTOP project. The EPISTOP project was a multicentre long-term, prospective study evaluating clinical and molecular biomarkers of epileptogenesis in TSC (NCT02098759). As part of this biomarker study, serial EEGs were collected during this project. A second aim of the EPISTOP study was to investigate the potential benefit of preventive treatment with vigabatrin after the appearance of IED on the EEG (focal IED for more than 10% of the recording time, multifocal IED, generalized IED or hypsarrhythmia, assessed by the local electroencephalographer) and before seizure onset vs. conventional follow-up and treatment only after the onset of clinical or electrographic seizures.

Patients were enrolled from November 2013 to August 2016 at 10 sites. Male or female infants of age ≤4 months with a definite diagnosis of TSC (23), but without previous seizures, or antiepileptic treatment were enrolled after informed consent of their caregivers, which was obtained in accordance with the Declaration of Helsinki. The EPISTOP study was approved by local ethical committees at all study sites.

EEG

The EEG was recorded for at least 1 h, including wake and sleep at least until stage two. The EEG was performed using a minimum of 19 electrodes according to the 10–20 system. A reduced array with nine electrodes was allowed in infants under 3 months of corrected age (Fp1, Fp2, C3, C4, T4, T3, O1, O2, Cz). The video-EEGs were anonymized, coded and uploaded to a secure central server at the University of Leuven in Belgium. EEG assessment

was performed blinded to TSC gene mutation, clinical (with exception of the age of the patient) and outcome data and was done using BrainRT^{TM} software version 3·5 (OSG BVBA, Rumst, Belgium). For this EEG study, the first EEG after enrolment was analysed. All EEGs were analysed separately by three experienced clinical EEG readers. When there was disconcordance, consensus was reached after discussion between the three readers.

For each EEG the presence or absence of IED and electrographic seizures was assessed. An electrographic seizure was defined as an ictal EEG pattern, without clinical correlate and with a duration of at least 10 s (24). Background was scored as follows: normal, focal slowing, and/or a dysmature EEG background. Focal slow wave activity indicates focal cerebral pathology and dysfunction of the underlying brain region (25). A dysmature EEG background was defined as a background inappropriate for the age of the child. Defining characteristics of a dysmature EEG background are: an abnormal discontinuity, persistence of extremely slow delta waves (<2 Hz at term age), asynchrony, or several transient waveforms inappropriate for the gestational age (GA), or poor development of sleep stages according to GA (26–28).

These dysmaturity features can also be analysed by a variety of quantitative approaches (26, 29–31). In this study, four sets of features were derived from the EEGs to describe dysmaturity in young infants (see **Supplementary Material**):

1. The power in the common EEG frequency bands (δ_1, δ_2, θ, α β) to assess the persistence of slow waves.
2. Quantitative EEG features derived from the range EEG (rEEG) [an estimation of amplitude-integrated EEG (aEEG)]. Specifically, the difference in distance from the rEEG median from the upper or the lower margin, known as rEEG asymmetry, was used to measure the discontinuity of the signal.
3. With maturation, the EEG background evolves from a discontinuous to a more continuous pattern, showing less regularity and more complexity. This complexity of the EEG signal was measured by means of entropy: the higher the complexity, the higher the entropy (29).
4. The regularity of the EEG signal was assessed by means of the Hurst Exponent: the higher the regularity, the higher the Hurst exponent (30).

Outcome Measures

Neurodevelopmental outcomes were studied at the age of 24 months. ASD risk was based on the Autism Diagnostic Observation Scale 2 (ADOS-2) score (Toddler Module), which is the gold standard for assessing and diagnosing ASD. If the ADOS-2 test could not be performed due to the child being non-verbal, having a non-verbal age equivalent below 12 months, or was not able to walk independently, Diagnostic and Statistical Manual of Mental Disorders-5 (DSM-5) clinical criteria were applied. Non-verbal children with a total score of 0–9, 10–13, and >13 were classified as "no risk," "mild/moderate risk," and "high risk," respectively. For verbal children the cut-off scores for the three risk categories were 0–7, 8–11, and >11. Cognitive, language and motor developmental quotients (DQ) were based on the Bayley Scales of Infant and Toddler Development III (BSID-III) results at the age of 24 months. Tests were performed by neuropsychologists certified through formal reliability training at each centre and sent to a central research team in Rome for analysis and classification.

Statistical Analysis

Continuous non-EEG characteristics between groups were assessed by Mann-Whitney *U*-test, because of deviation from the normal distribution demonstrated by Q-Q plots and Kolmogorov-Smirnov test. Both univariable and multivariable linear models were used to assess the relation between EEG characteristics and the DQs on the cognitive, language and motor test of the BSID-III at 24 months. Since the DQs on the cognitive and language test were not normally distributed, the DQs were logarithmic and reciprocal transformed, respectively. Associations between discrete variables and ASD risk were analysed using univariable, and multivariable logistic regression analyses. The selection of non-EEG variables in multivariable models was based on literature search on predicting variables of neurodevelopmental outcome in infants with TSC and based on our univariable models including non-EEG variables (**Supplementary Tables 1, 2, 4**) (14, 18, 19). The most important non-EEG characteristic considered in multivariable analyses were the mutation and the treatment strategy (preventive or conventional treatment). Due to the low sample size, no interaction terms were added to the model. In multivariable logistic regression models all variables were entered in block. Omnibus tests of model coefficients and Nagelkerke R^2 are reported below each multivariable logistic regression model. Multicollinearity was assessed for each multivariable logistic regression model. Tolerance values were >0.1 and VIF values were <10, indicating no collinearity between the variables. A two-sided p-value < 0.05 was considered statistically significant. Analyses were performed using the Statistical Package for the Social Sciences (SPSS version 26·0; Armonk, NY: IBM Corp.).

Quantitative EEG Analysis

For each set of the quantitative EEG features, the most discriminant features were extracted to classify ASD outcome. A binary classification model was developed with linear discriminant analysis (LDA) using three-fold testing, which means that two thirds of patients were used to develop the model and one third to test its performance. The results were reported in terms of misclassification error [percentage of misdiagnosis E(%)] and area under the receiver operating curve (AUC).

RESULTS

For this EEG study, 64 first EEGs of EPISTOP patients were available for in dept analysis (**Table 1**). The median GA and chronological age at first EEG were 42 4/7 weeks (IQR [40 2/7–45 2/7 weeks]) and 25 days (IQR [15.25–50.75 days]), respectively. In eighty-four percent of the patients the first recorded EEG was done before a GA of 48 weeks. In 63/64 patients, complete neurodevelopmental follow up was available. In one patient only

the cognitive DQ at 24 months was known as the language and motor DQs and ASD assessment at 24 months were missing.

Nineteen infants (30%) were diagnosed with ASD traits at the age of 24 months (**Table 1**). When we assessed the relation between early EEG abnormalities and ASD risk, we found that 15/36 (42%) children with an abnormal first EEG were diagnosed with ASD symptoms at the age of 24 months, compared to 4/27 (14%) of the children with a normal first EEG. In a univariable logistic regression analysis, an abnormal first EEG was significantly associated with a higher probability of ASD at the age of 24 months (p-value = 0.027). The odds ratio (OR) was 4.1 (95% CI = [1.2 – 14.4]). In a multivariable logistic regression analysis including the treatment strategy (preventive treatment or conventional follow-up and only treatment after seizure onset) and the pathogenic TSC variant (*TSC1* or *TSC2*) as covariables, an abnormal first EEG remained significantly associated with a higher probability of ASD at the age of 24 months [p-value = 0.029, adjusted OR = 4.2 (95% CI = [1.2 − 15.5])] (**Table 2**, **Figure 1**). The positive predictive value (PPV), or how often an abnormal first EEG correctly predicted ASD symptoms at the age of 24 months, was 42%. The negative predictive value (NPV), or how often a normal EEG was associated with no ASD symptoms at 24 months of age, was 85% (Sensitivity: 79%, specificity: 52%). Additional analysis of EEG characteristics showed that a dysmature EEG was significantly associated with a higher probability of ASD symptoms at 24 months [8/14 (57%) of patients with a dysmature EEG vs. 22% (11/49) of patients with a mature EEG, univariable p-value = 0.017, unadjusted OR = 4.6 (95% CI = [1.3 − 16.1])] (**Figure 1**). In multivariable logistic regression analysis, the strong association was no longer significant [p-value = 0.092, adjusted OR = 6.3 (95% CI = [0.7 − 52.9])] (**Table 2**). The PPV of a dysmature EEG and ASD traits at the age of 24 months was 57%. The NPV of a mature EEG and no ASD symptoms was 78% (Sensitivity: 42%, specificity: 86%). Other characteristics of the first EEG, such as the presence of IED or focal slowing were not associated with ASD risk at 24 months (**Supplementary Table 1**).

TABLE 1 | Baseline and EEG characteristics of the study cohort and neurodevelopmental outcome at 24 months.

	Overall cohort (*N* = 64)
BASELINE	
GA at birth	38 1/7 weeks (37–40)
Sex	
Male	35 (55%)
Female	29 (45%)
Mutation	
Pathogenic *TSC1* variant	17 (27%)
Pathogenic *TSC2* variant	46 (72%)
No identified variant	1 (1%)
Preventive treatment	19 (30%)
EEG CHARACTERISTICS	
Age at first EEG	
GA (weeks)	42 4/7 (40 2/7–45 2/7)
Chronological age (days)	25 (15.25–50.75)
Abnormal first EEG*	37 (58%)
Presence of IED	28 (44%)
Focal IED	7 (11%)
Multifocal IED	21 (33%)
Multifocal IED: 1 hemisphere	2 (3%)
Multifocal IED: 2 hemispheres	19 (30%)
Electrographic seizures	6 (9%)
Background abnormalities	23 (36%)
Dysmature EEG background**	14 (22%)
Focal EEG slowing	15 (23%)
NEURODEVELOPMENTAL OUTCOME	
ASD symptoms (Data available 63/64)	19 (30%)
DQ cognitive BSID-III (Data available 64/64)	75 (65–90.75)
DQ language BSID-III (Data available 63/64)	68 (59–77)
DQ motor BSID-III (Data available 63/64)	73 (67–85)

*Data are n (%) or median (IQR). *Abnormal first EEG: IED, background abnormalities, or electrographic seizures. **Dysmature EEG characteristics: abnormal discontinuity for the GA (10/14), persistence of high levels of interhemispheric asynchrony inappropriate for the GA (3/14), and extremely slow delta waves (1/14). GA, gestational age; IED, interictal epileptiform discharges; ASD, autism spectrum disorder; DQ, developmental quotient; BSID-III, Bayley Scales of Infant and Toddler Development III.*

TABLE 2 | Multivariable logistic regression models predicting ASD symptoms at the age of 24 months.

	B	**S.E**	***p*-value**	**OR (adjusted)**	**95% CI**
PREDICTOR VARIABLES OF THE FIRST MULTIVARIABLE MODEL					
Abnormal EEG	1.446	0.661	0.029	4.2	1.2–15.5
Conventional follow-up and treatment	−0.338	0.655	0.606	0.7	0.2–2.6
Pathogenic *TSC2* variant	−0.210	0.682	0.759	0.8	0.2–3.1
Overall model χ^2 (df 3, N = 63) = 5.485 p-value 0.140–R^2 Nagelkerke = 0.120					
PREDICTOR VARIABLES OF THE SECOND MULTIVARIABLE MODEL					
Abnormal EEG	1.348	0.820	0.100	3.9	0.7–19.2
Abnormal EEG background	−0.842	1.052	0.423	0.4	0.06–3.4
Dysmature EEG background	1.836	1.088	0.092	6.3	0.7–52.9
Conventional follow-up and treatment	−0.758	0.732	0.300	0.5	0.1–2.0
Pathogenic *TSC2* variant	−0.558	0.738	0.449	0.6	0.1–2.4
Overall model χ^2 (df 5, N = 63) = 9.014 p-value = 0.108–R^2 Nagelkerke = 0.191					

The first multivariable model includes abnormal EEG, conventional follow-up and treatment (infants not receiving preventive treatment) and a pathogenic variant in TSC2 as predictor variables. The second multivariable model includes the EEG background abnormalities and maturation, conventional follow-up and treatment (infants not receiving preventive treatment) and a pathogenic variant in TSC2 as predictor variables. Below the included variables the goodness-of-fit statistic and Nagelkerke R^2 for the multivariable models are reported in italic. B = regression coefficient, S.E., standard error of the regression coefficient, OR, odds ratio, 95% CI, 95% confidence interval of the odds ratio.

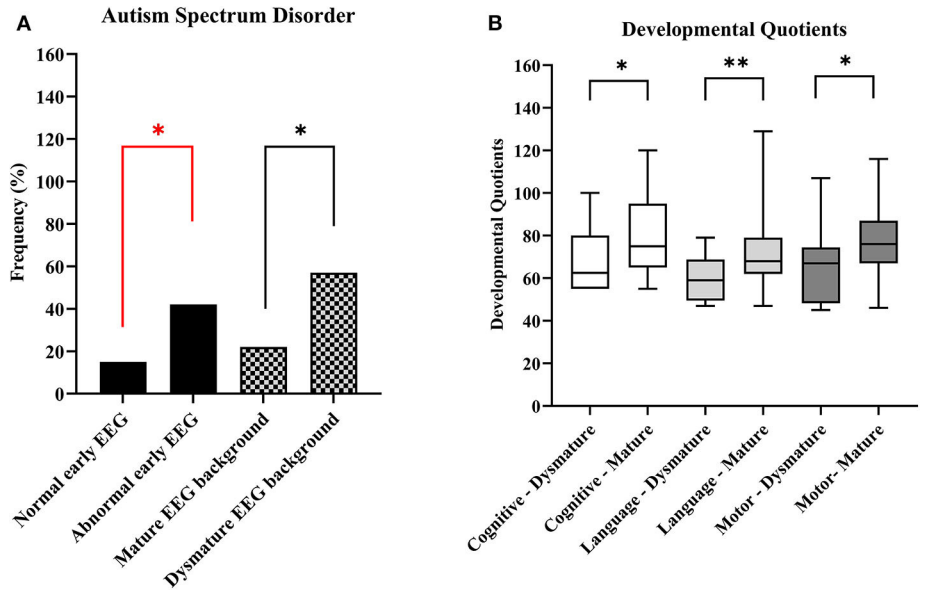

FIGURE 1 | Overview of EEG characteristics and neurodevelopmental outcome at the age of 24 months. **(A)** Autism spectrum disorder by EEG abnormalities. **(B)** Cognitive, language and motor developmental quotients based on Bayley Scales of Infant and Toddler Development -III test results. *p-value < 0.05, **p-value < 0.01. A red line indicates significance in a multivariable model.

The quantitative analysis of EEG background confirmed the association of an early abnormal/dysmature EEG, and ASD traits at the age of 24 months. A dysmature EEG is characterised by less complexity, with less entropy and higher regularity. The boxplots in **Figure 2** show in TSC patients with ASD traits significantly less entropy [MSE(20)] and higher regularity (Hurst Exponent) compared to children with no ASD risk. Also more asymmetry in the rEEG in the lower frequency bands was found in TSC patients with ASD traits as result of the persistence of slow-waves and discontinuity in the lower frequencies. The discriminatory power of this quantitative analysis to predict ASD traits in infants with TSC was further confirmed by the results of the binary classification models developed with LDA (see Appendix and **Supplementary Table 4**). The AUC (a measure of classification accuracy) of the different LDA models was in the range of 66–79% (**Table 3**).

The median cognitive, language, and motor DQs based on BSID-III results at 24 months were: 75 (IQR [65 – 90.75]), 68 (IQR [59 – 77]), and 73 (IQR [67 – 85]), respectively (**Table 1**). Children with an early abnormal EEG background had significantly lower cognitive [median 70 (IQR [55 – 80]) vs. 80 (IQR [67.50 – 95]), p-value = 0.029], language [median 59 (IQR [53 – 71]) vs. 71 (IQR [62 – 81.75]), p-value = 0.006], and motor (median 70 (IQR [55 – 79]) vs. 76 (IQR [67.50 – 89]), p-value = 0.042] DQs at 24 months compared to those with a normal EEG background. The cognitive [median 62·50 (IQR [55 – 80]) vs. 75 (IQR [65 – 95], p-value = 0.029], language [median 59 (IQR [49.50 – 68.75]) vs. 68 (IQR [62 – 79]), p-value = 0.001], and motor [median 67 (IQR [48.25 – 74.50]) vs. 76 (IQR [67 – 87]), p-value = 0.017] DQs were significantly different between the children with a dysmature and those with a mature EEG background at 24 months of age (**Figure 1**). Using a multivariable linear model, also including other EEG characteristics, as well as the pathogenic TSC variant (*TSC1* or *TSC2*) and the treatment strategy, the results were no longer significant. In these multivariable linear models, a *TSC2* mutation was significantly associated with a lower cognitive and motor DQs at 24 months (**Supplementary Tables 3, 5, 6**). Other EEG characteristics, such as the presence of IED and focal slowing, were not significantly associated with the cognitive, language, and motor DQs at 24 months of age (**Supplementary Tables 2, 4**).

DISCUSSION

The main finding of our study is that an abnormal first EEG in neonates and infants with TSC, and more specifically a dysmature EEG background was associated with a higher probability of ASD traits at the age of 24 months. The sensitivity of this finding was low (42%), but the specificity was high (86%). Hence, an infant with TSC and an EEG in the first weeks of life showing a mature background was less frequently diagnosed with ASD symptoms at 24 months. A dysmature EEG background was also associated with lower DQs on cognitive, language, and motor BSID-III test results. It is important to stress that the predictive value was not influenced by mutation status, or by preventive or standard anti-epileptic treatment during follow up. We were also able to confirm our qualitative findings with a more quantitative approach, which ultimately might become a more reliable biomarker of EEG background development and abnormalities. Our findings are of major clinical importance as an early abnormal EEG might trigger early diagnosis and management of ASD in TSC children.

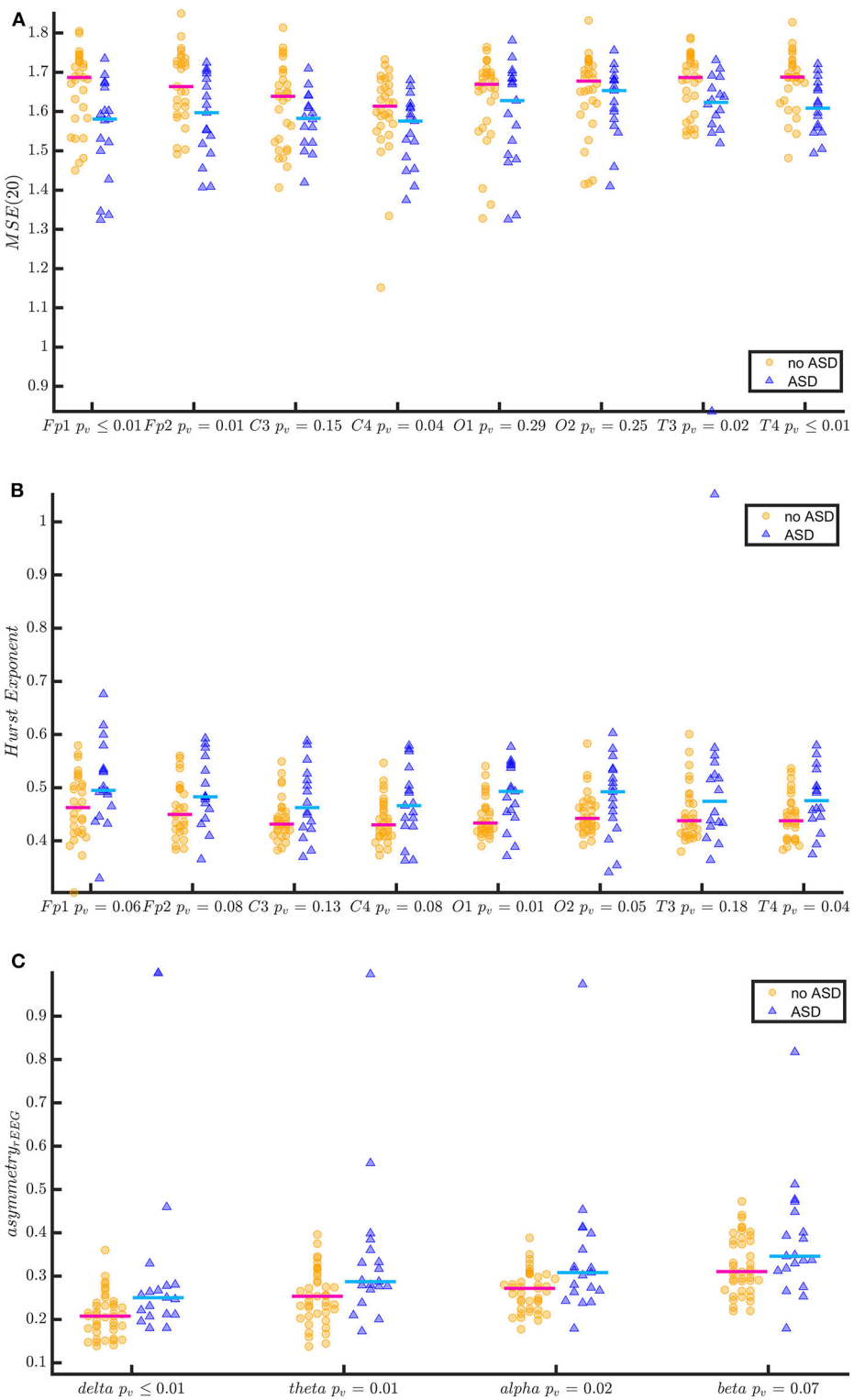

FIGURE 2 | Quantitative EEG features of TSC patients with and without ASD at the age of 24 months. The figure shows the entropy at scale 20 [MSE(20)] **(A)** Hurst Exponent **(B)** and the asymmetry of the range EEG **(C)** (estimate of amplitude integrated EEG) in two groups (no ASD vs. ASD at 24 months). The EEGs of patients with ASD at 24 months show a more asymmetric range EEG, a higher Hurst Exponent (more regularity) and lower entropy at lower frequencies [MSE(20)] (less complexity). In **(A,B)**, the comparisons are reported for each EEG channel. In **(C)**, the comparisons are reported for the frequency bands. *P*-values have been derived by Kruskal-Wallis test.

TABLE 3 | Results of the binary classification models developed with linear discriminant analysis.

	E (%)	AUC (%)
Power	32.59	66
Entropy	33.01	79
Asymmetric rEEG	30.26	69
Fractality	22.12	74

Results of the binary classification models developed with linear discriminant analysis (LDA) for the prediction of ASD symptoms at the age of 24 months. The results are reported in terms of misclassification error [percentage of misdiagnosis, E(%)], and area under the receiving operating curve (measure of classification accuracy, AUC). The different features sets are predictive of ASD symptoms at the age of 24 months with areas under the curve (AUC) close or higher than 70%. rEEG, range EEG.

In TSC, dysregulation of mTOR signalling results in aberrations in cellular morphology, disturbed maturation of the migration patterns of the dysmorphic neurons, altered $GABA_A$, and AMPA receptor functions, which contributes to increased seizure susceptibility, epileptic synchronisation, altered synaptogenesis, and altered connectivity. The subsequent malformations of cortical development provide a neuroanatomical and functional substrate for the early appearance of seizures, and developmental, and psychiatric disorders seen in association with TSC (32–35). Neuropathological investigation of brain tissue of foetuses with TSC, stillborn between a GA of 23 to 38 weeks, suggested that mTOR overactivation during embryonic brain development, presumably between 10 and 20 weeks after conception, underlies the formation of brain lesions in patients with TSC (32). In addition, evidence was present for the involvement of the innate and adaptive immune system, which could be responsible for the dynamic changes occurring over time in tubers (32). Besides grey matter pathology in TSC, also the white matter is both on a structural and the neuropathological level affected. White matter radial migration lines have been seen in 20% of patients (9). Moreover, neuropathological studies found white matter pathology with depleted myelin and oligodendroglia in 62% of TSC patients (36). The underlying disturbed architecture and connectivity as a consequence of mTOR overactivation result in a hyperexcitable neural network. The early emergent EEG characteristics of this altered neural network, including dysmaturity, not only reflect the ongoing epileptogenesis in TSC, but can also assist to assess the risk of neurodevelopmental comorbidities.

Benova et al. showed a relation between EEG background abnormalities and ASD, and intellectual disability in a cohort of 22 children with TSC, who were followed from birth until the age of 12 years, although the latter association was not significant (15). Furthermore, in children with TSC aged above 3 years a significant association was found between ASD and the presence of IED, and the number of lobes with IED, but not with focal slowing (11). The abovementioned studies suggest that EEG characteristics, both IED and background abnormalities, are related to neurodevelopment. However, no prospective studies have been performed in neonates and young infants with TSC investigating the early EEG features and their prognostic value for developmental outcome. Neurodevelopmental outcome studies using maturational features of EEG are available in preterm infants. Several studies found that a dysmature EEG background was associated with a poor cognitive outcome (27, 28, 37–39). A recent meta-analysis of 255 young preterm infants born before a GA of 34 weeks and followed with EEGs until a GA of 43 weeks found that a dysmature, or a disorganised EEG pattern predicted cognitive outcome (assessed $\geq$ 3 months) (40). Although the included studies in the meta-analysis used different definitions, follow-up protocols, and neurodevelopment assessments, these results confirm that the absence of a dysmature or disorganised EEG background is a good predictor of normal cognitive development in preterm infants (40).

The most recent EEG study of Wu et al. enrolled infants with TSC that were older (average age of 3·6 months at enrolment) compared to our patients (average age of 1 month at enrolment) and did not assess the strength of association between EEG features and neurodevelopmental outcome (21). Wu et al. found that IED predicted subsequent epilepsy in 77% of the patients. They also reported that persistent seizures are associated with a decline on the Vineland and Mullen tests at 2 years of age (21). No specific data on ASD risk were reported in this paper (21).

Recent studies using quantitative EEG analysis also facilitate a better understanding of the neurobiological mechanisms of the altered brain maturation in patients with TSC and can help with the identification of infants requiring developmental interventions. Peters et al. found, using graph theory, in older children, adolescents and adults with ASD, with and without TSC, a decreased long- over short-range connectivity with local over-connection and decreased functional specialisation (34). Dickinson et al. showed in patients with TSC, using features of spontaneous alpha oscillations (alpha power, alpha phase coherence, and peak alpha frequency) in high density EEGs, a reduced interhemispheric alpha phase coherence between the left temporal and the right parietal area compared to controls at 12 months, suggesting a delayed or atypical maturation of white matter during infancy (41). In addition, within the group of patients with TSC the reduction in long range interhemispheric alpha phase coherence between the right parietal and left temporal region at the age of 24 months was more pronounced in children with ASD diagnosis (41).

Besides clinical and quantitative EEG features, other characteristics, such as the pathogenic TSC variant, the epilepsy course (including the development of refractory epilepsy or epileptic spasms), MRI features and early development, can help to identify young infants with TSC at risk of developmental comorbidities (6, 12, 16, 42–47).

One of the limitations of our study is that no measure of parental education or parental intelligence was included to predict developmental outcome. Second, the diagnostic power of the neurodevelopmental assessments at 24 months of age is perhaps not optimal, particularly in terms of the stability of diagnostic classification. It is theoretically possible that children who were classified with no ASD risk at 24 months of age are still diagnosed with ASD later in life. A follow-up study of EPISTOP patients with neurodevelopmental assessments at the age of 6 years is planned. Third, our cohort was relatively small, consequently including interaction terms in the multivariable models was not possible. Finally, the validity of the reported

models for quantitative EEG analysis should be further tested on an independent new dataset.

To conclude, in a prospectively studied cohort of neonates and young infants with TSC, an abnormal early EEG, and more specifically a dysmature EEG background was associated with a higher probability of ASD traits at the age of 24 months. A dysmature EEG background was also associated with lower DQs on cognitive, language, and motor BSID-III test results. Our findings suggest that detection of early EEG abnormalities in TSC infants can assist in the prediction of neurodevelopmental outcomes, facilitating early diagnosis and intervention.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Ethische Commissie UZ/KU Leuven. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

JD and LL contributed to study design. JV, KK, RM, PC, BW, KR, MF, PK, RN, AJ, KW, DD-P, MK-F, CH, CF, SS, BB, EA, DK, FJ, and SJ contributed to data collection. JD, ML, BV, JV, KL, SV, and LL contributed to data analysis. JD, ML, SV, and LL contributed to data interpretation and contributed to writing. JD contributed to literature search. All authors contributed to read 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.2020.582891/full#supplementary-material

Supplementary Material, including more detailed methods for quantitative EEG analysis and Supplementary Tables, are added in a separate file.

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TuberOus SClerosis registry to increAse disease awareness (TOSCA) Post-Authorisation Safety Study of Everolimus in Patients With Tuberous Sclerosis Complex

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This non-interventional post-authorisation safety study (PASS) assessed the long-term safety of everolimus in patients with tuberous sclerosis complex (TSC) who participated in the TuberOus SClerosis registry to increase disease Awareness (TOSCA) clinical study and received everolimus for the licensed indications in the European Union. The rate of adverse events (AEs), AEs that led to dose adjustments or treatment discontinuation, AEs of potential clinical interest, treatment-related AEs (TRAEs), serious AEs (SAEs), and deaths were documented. One hundred seventy-nine patients were included in the first 5

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years of observation; 118 of 179 patients had an AE of any grade, with the most common AEs being stomatitis (7.8%) and headache (7.3%). AEs caused dose adjustments in 56 patients (31.3%) and treatment discontinuation in nine patients (5%). AEs appeared to be more frequent and severe in children. On Tanner staging, all patients displayed signs of age-appropriate sexual maturation. Twenty-two of 106 female (20.8%) patients had menstrual cycle disorders. The most frequent TRAEs were stomatitis (6.7%) and aphthous mouth ulcer (5.6%). SAEs were reported in 54 patients (30.2%); the most frequent SAE was pneumonia (>3% patients; grade 2, 1.1%, and grade 3, 2.8%). Three deaths were reported, all in patients who had discontinued everolimus for more than 28 days, and none were thought to be related to everolimus according to the treating physicians. The PASS sub-study reflects the safety and tolerability of everolimus in the management of TSC in real-world routine clinical practice.

Keywords: everolimus, TOSCA, tuberous sclerosis complex, post-authorization safety study, mammalian target of rapamycin

INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare, genetic, multisystem disorder. TSC can affect almost any organ system, including the skin, central nervous system, kidneys, eye, heart, and lung. About 90% of the TSC patients experience neurological and renal abnormalities, which represent a major cause of morbidity and mortality (1, 2). The clinical presentation of TSC is heterogeneous, and the degree of severity is highly variable between individuals, even among the family members (1). The onset of clinical manifestations of TSC also typically varies with age, which further adds to the complexity to the disease (3, 4). These factors represent a significant challenge for the diagnosis and management of TSC. Current management guidelines are focused on early identification and close monitoring of lesion burden in combination with timely medical treatment of manifestations and early interventions for TSC-associated neuropsychiatric disorders (TANDs) (4). TSC is caused by pathogenic variants in either *TSC1* or *TSC2* genes, resulting in hyper-activation of the mammalian target of rapamycin (mTOR) signalling pathway and the subsequent development of hamartomatous lesions in patients with TSC (4).

Based on double-blinded, placebo-controlled, randomised, clinical trials that confirmed its safety and efficacy, the mTOR-inhibitor everolimus (Votubia®) was approved in Europe in 2011 for the treatment of subependymal giant cell astrocytoma (SEGA) and renal angiomyolipoma (5–13). Randomised clinical trial studies are required as "gold standard" for product licensing. However, they fail to reflect the "real-world" scenarios, particularly in terms of AE representation. The randomised clinical trials have shown that everolimus was generally well-tolerated in patients with TSC with manageable AEs, which were generally reversible and non-cumulative (14–16). However, since TSC is a rare disease, with a prevalence of 6.8–12.4 per 100,000 people (17), the three key registration trials included relatively small numbers of patients with TSC [ranging from 78 in EXIST-1 to 247 in EXIST-3 (5, 9, 12)].

The TuberOus SClerosis registry to increase disease Awareness (TOSCA) study was conducted to address existing lacunas in the diagnosis and management of TSC. Based on the request from European Medicines Agency (EMA) to use the TOSCA registry to collect data on long-term safety and reproductive abnormalities in patients taking everolimus for licensed indications, SEGA in children age 2–20 years, and angiomyolipoma in adults aged >18 years, the TOSCA post-authorisation safety study (PASS) was developed. Here, we report findings from this TOSCA sub-study.

METHODS

Study Design and Participants and Data Collection

The TOSCA clinical study methodology has been published previously (15). In brief, TOSCA was a large-scale non-interventional study in patients with TSC. The study was designed with a core section, six ancillary research projects (with more detailed focus on SEGA, renal angiomyolipoma and lymphangiomyomatosis, genetics, TAND, epilepsy, and patient's quality of life), and a PASS sub-study (EU PASS Register Number EUPAS3247).

The TOSCA-PASS sub-study was aimed at collecting prospective long-term safety data of treatment with everolimus prescribed for the indications licensed in Europe at time of enrolment data on AEs, therapeutic drug monitoring data, and the long-term reproductive abnormalities within routine clinical practice were collected. The PASS sub-study was conducted in 11 European Union countries participating in the TOSCA registry.

Patients

Patients who participated in the TOSCA registry and received everolimus treatment in the licensed indications (for SEGA or renal angiomyolipoma) in the European Union were eligible for inclusion in the TOSCA PASS, after providing additional written informed consent.

The data collection cut-off was 10 August 2017 for the TOSCA PASS sub-study. As per EMA indication (EMA/CHMP/59467/2014, 20 February 2014), data collection on sexual maturation and fertility is to be continued for all paediatric patients until they reach Tanner stage 5, or age 16 years for females and age 17 years for males, whichever occurs first.

For the TOSCA PASS sub-study, being a non-interventional and observational study, all treatment-related decisions (dose adjustments, treatment discontinuation) were at the discretion of the treating physicians. No treatment protocol, diagnostic/therapeutic procedure, or a formal visit schedule was mandated by the TOSCA PASS study protocol. However, the recommended data collection per study schedule was at 3-monthly intervals, which most likely mirrors the patterns of routine clinical care of patients treated with everolimus. Detailed management of each individual's AEs was not collected; however, general guidelines were followed by investigators (18). Data were collected for all patients who achieved Tanner stage 5 by the cut-off date. For patients who discontinued the study prematurely, the reason for discontinuation was determined. All patients were instructed regarding possible AEs and their possible treatment (19).

In this manuscript, we present an interim analysis of patient data up to 10 August 2017. The long-term safety data of these patients will be reported after termination of the TOSCA PASS study.

Outcome Measures and Data Analysis

Incidence of AEs, AEs that lead to dose adjustment or discontinuation, everolimus treatment-related AEs (TRAEs) as per the investigator assessment, AEs of special interest (AESIs), serious AEs (SAEs), and deaths were documented during the treatment (from day of enrolling into the PASS study to 28 days after the last dose of everolimus). AESIs were those AEs that were of specific clinical interest in connection with everolimus treatment. Potential AEs sought included non-infectious pneumonitis, severe infections, hypersensitivity (anaphylactic reactions), stomatitis, wound healing complications, increased serum creatinine/proteinuria/renal failure, hyperglycaemia, new onset of diabetes mellitus, dyslipidaemia, hypophosphatemia, cardiac failure, cytopenias, haemorrhages, thrombotic and embolic events, female fertility (including secondary amenorrhea), pre-existing infections (reactivation/aggravation/exacerbation), safety in patients with hepatic impairment, postnatal developmental toxicity, pregnant or breast-feeding women, male infertility, and muscle wasting/muscle loss. The relationship of the incidence of AESIs with everolimus blood levels was also noted. SAEs were defined as AEs that are fatal or life-threatening, result in persistent or significant disability/incapacity, constitute a congenital anomaly/birth defect, are medically significant, require medical or surgical intervention, or require inpatient hospitalisation or prolonged existing hospitalisation.

Data on everolimus (dose, interruption, dose change, and duration) and concomitant medication were captured. Concomitant medications entered into the database were coded using the World Health Organization (WHO) drug reference list, which employs the anatomical therapeutic chemical (ATC) classification system.

The analysis set consisted of all patients who had at least one post-baseline safety assessment and were exposed to at least one dose of everolimus after the enrolment. The AEs and SAEs were summarised by system organ class and preferred term using the Medical Dictionary for Regulatory Activities (MedDRA) version 20.

RESULTS

Patient Disposition and Baseline Characteristics

A total of 179 patients were enrolled in the study. Of 179 patients, 16 patients (8.9%) had discontinued participation in the study, and 31 patients continued to be followed up as part of the ongoing paediatric PASS. Of the 16 patients who discontinued the study, 12 were lost to follow-up, three died, and one patient was withdrawn as per investigator's decision (**Figure 1**). Everolimus was initiated for 73 (40.8%) patients with SEGA, 122 (68.2%) with renal angiomyolipoma, and 17 (9.5%) for both SEGA and renal angiomyolipoma.

The demographic and clinical characteristics of the enrolled patients are summarised in **Table 1**. Of 179 patients enrolled, 61 (34.1%) were children (<18 years), and 73 and 106 (59.2%) were female. The median age at consent was 27 years (range < 1–65 years). So far, all patients have reached their 1-year follow-up visit; while 175, 158, 75, and 37 patients have completed their second, third, fourth, and fifth annual follow-up visits, respectively. Mutation testing was performed on 92 patients (51.4%). The majority of patients, 71 (77.2%), had pathogenic variants in *TSC2*, 8 (8.7%) had pathogenic variants in *TSC1*, and 13 (14.1%) remained without genetic diagnosis (no mutation identified or NMI).

Safety

Overall, 118 of 179 patients (65.9%) had AEs of any grade, irrespective of its relationship with study drug (**Table 2**). The most common grade 3/4 AE that occurred in >3% of patients was pneumonia observed in 2.8% of patients (**Table 3**). The rate of AEs was higher in children compared with adults [75.4% ($n = 61$) vs. 61.0% ($n = 72$); $P = 0.0541$], and a decreasing trend on AE rate was noted with increase in age (**Table 2**).

The most frequent TRAEs were stomatitis (6.7%), aphthous mouth ulcer (5.6%), and hypercholesterolaemia (5%). Everolimus dose adjustments due to AEs were reported in 56 patients (31.3%). The most common AEs that led to dose adjustments in at least two patients were diarrhoea (five patients); stomatitis, pneumonia, common cold, and urinary tract infection (three patients each); and metrorrhagia, pyrexia, pyelonephritis, sinusitis, influenza, and otitis (two patients each). Haemorrhagic events leading to dose adjustments were haemorrhage on the left side of the brain, bleeding in angiomyolipoma, and renal haemorrhage (one patient each). AEs leading to everolimus discontinuation were reported in nine patients (5%) and included fatigue and amenorrhea (1.1% each); and anaemia,

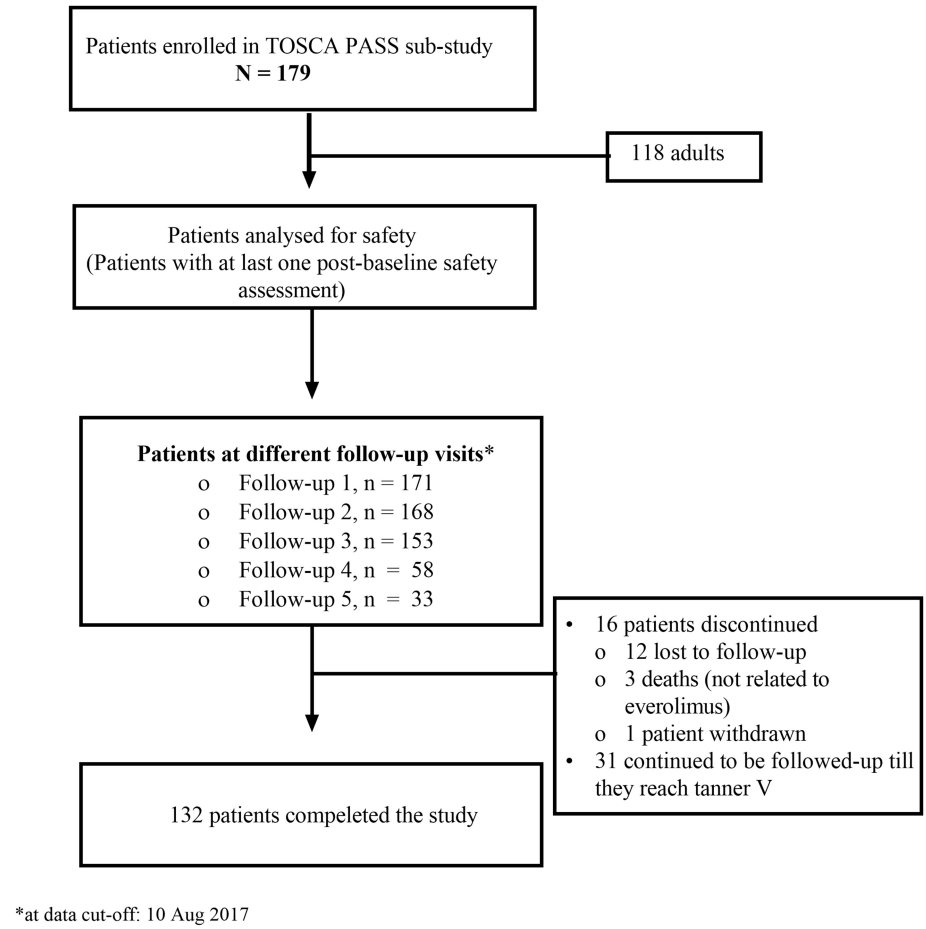

FIGURE 1 | Patient disposition in TOSCA PASS sub-study. TOSCA, TuberOus SClerosis registry to increase disease Awareness; PASS, post-authorisation safety study.

mouth ulceration, empyema, pneumonia, hyperglycaemia, type I diabetes mellitus, flank pain, intestinal adenocarcinoma, seizure, and alopecia (0.6% each).

SAEs were reported in 54 patients (30.2%). The most frequent SAE (>3% of patients) was pneumonia (grade 2, 1.1%; grade 3, 2.8%).

Three deaths were reported in the study. These deaths occurred after 28 days of everolimus discontinuation and were reported by study investigators as not related to everolimus treatment. Patient 1, male, aged 30 years, from the Netherland, died due to medically assisted death as per the local regulations on day 487 after commencement of everolimus administration. He had permanently discontinued everolimus treatment on day 205. Patient 2, male aged 52 years, died from influenza (grade 4) on day 1399; everolimus treatment was permanently discontinued on day 1359. Patient 3, male aged 46 years, died due to intestinal adenocarcinoma on day 74; everolimus was permanently discontinued on day 43. Autopsy was not performed for patients 1 and 2, whereas, this was unknown for patient 3.

Everolimus Dosage and Exposure

Data on everolimus dosage and exposure were available for 150 patients. The mean duration of the everolimus exposure was 302.4 ± 105.04 days (median, 365 days; range, 7–669 days). The mean and median daily doses and the most commonly administered dosage (5 mg throughout the study) are shown in **Table 4**. The mean everolimus blood level at baseline was 6.27 ng/ml (median, 4.9 ng/ml; range, 1.4–35.9 ng/ml), with the maximum concentration reported at third follow-up visit (mean, 6.6 ng/ml) and the least at fourth follow-up visit (mean 4.937 ng/ml). Median duration of exposure in <18 years ($n = 59$) and ≥18 ($n = 91$) was 365 days ($P = 0.1735$) with a significant difference in mean daily everolimus dose of 6.4 mg (range, 1–13 mg) in <18 years vs. 7.7 mg (range, 1–20 mg) in ≥18 years ($P = 0.0144$), respectively. Changes in everolimus dosage were reported in 53 patients (35.3%), with dose increase reported in 44 patients (83%), dose interruptions in 20 patients (13.3%), and dose reductions in 14 patients (9.3%) (**Table 4**). The most common reasons for dose changes were side effects (21 patients, 14%); other reasons are specified in **Table 4**.

TABLE 1 | Demographic and clinical characteristics of the enrolled patients at baseline.

Characteristics	Patients *N* = 179
Sex, n (%)	
Male	73 (40.8)
Female	106 (59.2)
Age at consent, years	
Mean (SD)	27.1 (16.08)
Median (range)	27.0 (<1–65)
Age groups, n (%)	
≤2 years	7 (3.9)
>2 to ≤5 years	6 (3.4)
>5 to ≤9 years	21 (11.7)
>9 to ≤14 years	16 (8.9)
>14 to ≤18 years	11 (6.1)
≥18 to ≤40 years	77 (43.0)
>40 years	41 (22.9)
Geographic region	
Netherlands	75 (41.9)
Germany	43 (24.0)
France	19 (10.6)
Spain	17 (9.5)
Austria	12 (6.7)
Czech Republic	3 (1.7)
Slovenia	3 (1.7)
United Kingdom	3 (1.7)
Sweden	2 (1.1)
Denmark	1 (0.6)
Poland	1 (0.6)
Patients with molecular testing, n (%)	92 (51.4)
TSC1 mutation	8 (8.7)
TSC2 mutation	71 (77.2)
No mutation identified	13 (14.1)
TSC manifestations, n (%)	
Neurological	
SEGA	100 (55.9)
Cortical tuber	147 (82.1)
SEN	156 (87.2)
Cerebral white matter radial migration lines	24 (13.4)
Renal	
Renal angiomyolipoma	149 (83.2)
Multiple renal cysts	53 (29.6)
Polycystic kidneys	4 (2.2)
Impaired renal function	5 (2.8)
Renal malignancy	1 (0.6)
Pulmonary	
Lymphangioleiomyomatosis	32 (17.9)
Cardiovascular	
Cardiac rhabdomyoma	51 (28.5)
Dermatologic	
≥3 hypomelanotic macules	84 (46.9)
Facial angiofibroma	123 (68.7)
Shagreen patch	42 (23.5)
Ungual or periungual fibromas	44 (24.6)
Forehead plaque	19 (10.6)
Confetti lesions	16 (8.9)
Ophthalmologic	
Retinal hamartoma	32 (17.9)
Epilepsy	151 (84.4)

SD, standard deviation; SEGA, subependymal giant cell astrocytoma; SEN, subependymal nodule; TSC, tuberous sclerosis complex.

Correlations Between Everolimus Blood Levels and Adverse Events of Special Interest

AESIs were reported in 57 of 150 patients (38%) for whom data on everolimus dosage and exposure are available. AESIs were suspected to be everolimus-related in 40 patients (26.7%). The majority of the patients had grade 1 or grade 2 AEs. One patient reported grade 4 empyema. Most of the patients who experienced AESIs had everolimus concentration <8 ng/ml (24%). No significant correlation was observed between everolimus blood concentration and AESIs (**Table 5**).

Sexual Maturation and Menstrual Irregularities

Tanner staging was performed in 28 patients (15.6%; three male and 25 females). There were no significant delays in sexual maturation revealed (**Table 6**). Nineteen females (17.9%) used contraception, with the most commonly contraception being hormone-based contraception in 16 patients (84.2%). Overall, three patients (1.7%) had ovariectomy, and five (2.8%) used external sex hormones (**Table 6**).

Of the 179 patients enrolled, 22 of 106 (20.8%) female patients had menstrual cycle disorders. Amenorrhea was reported in nine patients (8.5%) and other abnormal reproductive conditions in three patients (1.7%). In the initial analysis, three patients (1.7%, one male and two females) were reported to have abnormal onset of puberty. However, on further analysis, it was noted that one female child had precocious puberty, which was treated successfully 5 years before starting everolimus treatment. The second patient, a male child, had developed behavioural problems during puberty, which were thought to be secondary to oxcarbazepine and predated everolimus treatment. The third patient, an adult female patient, had abnormal puberty before the start of everolimus too. Thus, abnormal puberty was found to be not related to everolimus treatment.

Abnormal hormone levels of thyroid-stimulating hormone were reported in four patients (2.2%); testosterone levels were abnormal in two patients (1.1%); luteinising hormone, follicular-stimulating hormone, and oestradiol were abnormal in one patient (0.6%) (**Table 6**).

TABLE 2 | Overall AE profile in overall population and across age groups.

	Overall (*N* = 179) n (%)	Age at consent, years				*P*-value
		≤2 (*N* = 7) n (%)	>2 to ≤ 9 (*N* = 27) n (%)	>9 to <18 (*N* = 27) n (%)	≥18 (*N* = 118) n (%)	
Overall, any AEs	118 (65.9)	6 (85.7)	21 (77.8)	19 (70.4)	72 (61)	
Patients with frequent (>3%) adverse events with CTC grade						
Grade 1	40 (22.3)	2 (28.6)	8 (29.6)	8 (29.6)	22 (18.6)	
Grade 2	27 (15.1)	1 (14.3)	2 (7.4)	4 (14.8)	20 (16.9)	
Grade 3	16 (8.9)	2 (28.6)	5 (18.5)	2 (7.4)	7 (5.9)	
Grade 4	2 (1.1)	0	0	0	2 (1.7)	
Patients with grade 3/4 AEs	43 (24.0)	4 (57.1)	8 (29.6)	6 (22.2)	25 (21.2)	
Patients with SAE	54 (30.2)	5 (71.4)	12 (44.4)	7 (25.9)	30 (25.4)	0.0567
AE requiring dose adjustment	56 (31.3)	5 (71.4)	10 (37.0)	9 (33.3)	32 (27.1)	0.1342
AE leading to discontinuation	9 (5.0)	1 (14.3)	1 (3.7)	1 (3.7)	6 (5.1)	0.8357
Treatment-related AE	76 (42.5)	6 (85.7)	15 (55.6)	16 (59.3)	39 (33.1)	0.0023
Deaths	3 (1.7)	0	0	0	3 (2.5)	

AE, adverse event; CTC, Common Terminology Criteria; SAE, serious adverse events.

TABLE 3 | Adverse events of any cause (by preferred term) reported in >3% of patients in overall population and across age groups.

Adverse events	Overall *N* = 179		Age at consent, years							
			≤2 (*N* = 7)		>2 to ≤9 (*N* = 27)		>9 to <18 (*N* = 27)		≥18 (*N* = 118)	
	All n (%)	Grade 3/4 n (%)	All n (%)	Grade 3/4 n (%)	All n (%)	Grade 3/4 n (%)	All n (%)	Grade 3/4 n (%)	All n (%)	Grade 3/4 n (%)
Stomatitis	14 (7.8)	1 (0.6)	1 (14.3)	0	3 (11.1)	1 (3.7)	5 (18.5)	0	5 (4.2)	0
Headache	13 (7.3)	0	0	0	0	0	3 (11.1)	0	10 (8.5)	0
Diarrhoea	12 (6.7)	1 (0.6)	1 (14.3)	0	3 (11.1)	0	1 (3.7)	0	7 (5.9)	1 (0.8)
Vitamin D deficiency	12 (6.7)	0	0	0	0	0	0	0	12 (10.2)	0
Aphthous ulcer	10 (5.6)	0	0	0	2 (7.4)	0	1 (3.7)	0	7 (5.9)	0
Hypercholesterolaemia	10 (5.6)	0	2 (28.6)	0	3 (11.1)	0	2 (7.4)	0	3 (2.5)	0
Urinary tract infection	10 (5.6)	1 (0.6)	1 (14.3)	1 (14.3)	0	0	1 (3.7)	0	8 (6.8)	0
Pyrexia	9 (5.0)	0	3 (42.9)	0	3 (11.1)	0	2 (7.4)	0	1 (0.8)	0
Hypertension	8 (4.5)	2 (1.1)	0	0	0	0	1 (3.7)	0	7 (5.9)	2 (1.7)
Pneumonia	8 (4.5)	5 (2.8)	2 (28.6)	1 (14.3)	2 (7.4)	2 (7.4)	1 (3.7)	0	3 (2.5)	2 (1.7)
Viral upper respiratory tract infection	8 (4.5)	0	0	0	1 (3.7)	0	1 (3.7)	0	6 (5.1)	0
Abdominal pain	7 (3.9)	1 (0.6)	0	0	2 (7.4)	1 (3.7)	0	0	5 (4.2)	0
Anaemia	7 (3.9)	1 (0.6)	0	0	1 (3.7)	0	1 (3.7)	0	5 (4.2)	1 (0.8)
Bronchitis	7 (3.9)	0	2 (28.6)	0	3 (11.1)	0	1 (3.7)	0	1 (0.8)	0
Oedema peripheral	7 (3.9)	0	0	0	1 (3.7)	0	0	0	6 (5.1)	0
Epilepsy	6 (3.4)	2 (1.1)	1 (14.3)	1 (14.3)	1 (3.7)	0	1 (3.7)	0	3 (2.5)	1 (0.8)
Hypertriglyceridaemia	6 (3.4)	2 (1.1)	0	0	0	0	4 (14.8)	2 (7.4)	2 (1.7)	0
Influenza	6 (3.4)	3 (1.7)	1 (14.3)	1 (14.3)	1 (3.7)	0	0	0	4 (3.4)	2 (1.7)
Vomiting	6 (3.4)	1 (0.6)	1 (14.3)	0	3 (11.1)	1 (3.7)	0	0	2 (1.7)	0

DISCUSSION

Based on the understanding of the TSC pathogenesis, the role of everolimus in the management of different TSC manifestations has been extensively evaluated. Studies evaluating everolimus in the treatment of SEGA, angiomyolipoma, and epilepsy have consistently demonstrated its efficacy and tolerability (6–8) which subsequently led to the approval of everolimus in the treatment of these TSC manifestations (14). Studies have also shown that even with prolonged treatment, no new toxicities or complications were observed (6, 10, 11, 13, 20). All these data were obtained from interventional controlled clinical trials.

TABLE 4 | Everolimus dosage and exposure.

	Baseline (*N* = 150)	FU1 (*N* = 171)	FU2 (*N* = 168)	FU3 (*N* = 153)	FU4 (*N* = 58)	FU5 (*N* = 33)
Pharmaceutical formulation						
Tablets	143 (95.3)	165 (96.5)	162 (96.4)	147 (96.1)	56 (96.6)	33 (100.0)
Dispersible tablets	9 (6.0)	9 (5.3)	8 (4.8)	10 (6.5)	3 (5.2)	0
Dosage						
2 mg	3 (2.0)	3 (1.8)	3 (1.8)	3 (2.0)	0	0
2.5 mg	16 (10.7)	13 (7.6)	19 (11.3)	17 (11.1)	6 (10.3)	2 (6.1)
3 mg	2 (1.3)	3 (1.8)	2 (1.2)	2 (1.3)	1 (1.7)	0
5 mg	118 (78.7)	131 (76.6)	128 (76.2)	114 (74.5)	43 (74.1)	31 (93.9)
10 mg	17 (11.3)	18 (10.5)	16 (9.5)	16 (10.5)	4 (6.9)	0
Other	34 (22.7)	37 (21.6)	25 (14.9)	18 (11.8)	6 (10.3)	0
Daily dose (mg)						
Mean (SD)	7.2 (3.11)	7.3 (3.14)	7.1 (3.28)	7.4 (4.27)	7.8 (3.40)	8.3 (3.99)
Median (min–max)	7.0 (1–20)	7.5 (1–20)	6.4 (0–20)	5.8 (0–35)	7.5 (3–15)	7.5 (3–15)
Patients with dose changes	53 (35.3)	55 (32.2)	52 (31.0)	34 (22.2)	19 (32.8)	0
Reductions	14 (9.3)	15 (8.8)	24 (14.3)	9 (5.9)	3 (5.2)	0
Interruptions	20 (13.3)	31 (18.1)	26 (15.5)	22 (14.4)	10 (17.2)	0
Increased	44 (29.3)	41 (24.0)	34 (20.2)	23 (15.0)	17 (29.3)	0
Reasons for changes						
Side effect	21 (14.0)	25 (14.6)	22 (13.1)	11 (7.2)	2 (3.4)	0
Dosing error	3 (2.0)	1 (0.6)	1 (0.6)	0	0	0
Lab test abnormality	2 (1.3)	4 (2.3)	1 (0.6)	0	0	0
Concomitant medication affecting drug exposure	1 (0.7)	2 (1.2)	0	0	0	0
Other	30 (20.0)	30 (17.5)	24 (14.3)	20 (13.1)	10 (17.2)	0

FUP, follow-up; SD, standard deviation.

TABLE 5 | Correlation between everolimus exposure and incidence of AESIs at baseline.

Time from baseline visit	Patients with AESI	Everolimus concentration (ng/ml), n (%)					*P*-value
		<3	3 to <7	7 to <9	9 to ≤15	>15	
Quarter 1 (*n* = 57)	Yes	4 (7)	10 (17.5)	0	1 (1.8)	0	0.1673
	No	12 (21.1)	15 (26.3)	7 (12.3)	4 (7)	4 (7)	
Quarter 2 (*n* = 59)	Yes	4 (6.8)	11 (18.6)	3 (5.1)	2 (3.4)	0	0.7195
	No	10 (16.9)	19 (32.2)	3 (5.1)	5 (8.5)	2 (3.4)	
Quarter 3 (*n* = 71)	Yes	5 (7)	9 (12.7)	0	5 (7)	1 (1.4)	0.2557
	No	8 (11.3)	24 (33.8)	9 (12.7)	7 (9.9)	3 (4.2)	
Quarter 4 (*n* = 67)	Yes	4 (6)	8 (11.9)	3 (4.5)	4 (6)	0	0.4535
	No	7 (10.4)	24 (35.8)	7 (10.4)	5 (7.5)	5 (7.5)	

Quarters 1 to 4 denote the quarter of year at the baseline visit. An event is mapped into Q j of Baseline if its start date is prior to Baseline date + 3 multiply by j months/Baseline date + (3 multiply by j + 12) months and {its stop date is on or after the Baseline date + 3 multiply by (j – 1) months/Baseline date + [3 multiply by (j – 1) + 12] months or the event is ongoing}, where j = 1, 2, 3, 4.
AESI, adverse event of special interest.

There was a need of real-world evidence on the safety of everolimus. Based on European Medical Agency indications to Novartis (EMA/CHMP/59467/2014, 20 February 2014), the PASS sub-study was performed as part of the TOSCA registry, to evaluate real-world evidence on the safety of everolimus in patients with TSC from 11 European countries. The number of patients recruited in this study varied between participating countries. In addition, as the recruitment was voluntary, the study population does not mirror the prevalent TSC population in each country.

In line with the previously reported everolimus in TSC studies (6–8), the most commonly reported AE was stomatitis, which

TABLE 6 | Sexual maturation and menstrual irregularities across age groups.

		Age at consent, years						
	Overall (*N* = 179)	**≤2 (*N* = 7)**	**2 to ≤5 (*N* = 6)**	**5 to ≤9 (*N* = 21)**	**>9 to ≤14 (*N* = 16)**	**>14 to ≤18 (*N* = 11)**	**>18 to ≤40 (*N* = 77)**	**>40 (*N* = 41)**
Total patients evaluated for Tanner stages	28 (15.6)	0	1 (6.7)	4 (19.0)	10 (62.5)	6 (54.5)	6 (7.8)	1 (2.4)
Male patients with Tanner stages evaluated	3 (10.7)	0	0	2 (50.0)	1 (10.0)	0	0	0
Male patients with genitalia stage	3 (4.1)	0	0	2 (14.3)	1 (20.0)	0	0	0
Stage 1	0	0	0	0	0	0	0	0
Stage 2	0	0	0	1 (50.0)	0	0	0	0
Stage 3	1 (33.3)	0	0	0	1 (100.0)	0	0	0
Stage 4	1 (33.3)	0	0	1 (50.0)	0	0	0	0
Stage 5	1 (33.3)	0	0	0	0	0	0	0
Patients with pubic hair stage	3 (4.1)	0	0	2 (14.3)	1 (20.0)	0	0	0
Stage 1	1 (33.3)	0	0	0	0	0	0	0
Stage 2	1 (33.3)	0	0	1 (50.0)	0	0	0	0
Stage 3	2 (66.7)	0	0	0	1 (100.0)	0	0	0
Stage 4	0	0	0	0	0	0	0	0
Stage 5	1 (33.3)	0	0	1 (50.0)				
Female patients with Tanner stages evaluated	25 (89.3)	0	1 (16.7)	2 (50.0)	9 (90.0)	6 (100.0)	6 (100.0)	1 (100.0)
Patients with breast stage	23 (21.7)	0	1 (33.3)	2 (28.6)	8 (72.7)	7 (70.0)	5 (10.2)	1 (4.0)
Stage 1	2 (8.7)	0	1 (100.0)	1 (50.0)	0	0	0	0
Stage 2	1 (4.3)	0	0	0	0	1 (16.7)	0	0
Stage 3	3 (13.0)	0	0	0	3 (37.5)	0	0	0
Stage 4	3 (13.0)	0	0	1 (50.0)	2 (25.0)	0	0	0
Stage 5	14 (60.9)	0	0	0	3 (37.5)	5 (83.3)	5 (100.0)	1 (100.0)
Patients with pubic hair stage	24 (22.6)	0	1 (33.3)	2 (28.6)	9 (81.8)	7 (70.0)	5 (10.2)	1 (4.0)
Stage 1	2 (8.3)	0	0	1 (50.0)	0	1 (16.7)	0	0
Stage 2	2 (8.3)	0	0	0	2 (22.2)	0	0	0
Stage 3	3 (12.5)	0	1 (100.0)	0	2 (22.2)	0	0	0
Stage 4	1 (4.2)	0	0	0	1 (11.1)	0	0	0
Stage 5	16 (66.7)	0	0	1 (50.0)	4 (44.4)	5 (83.3)	5 (100.0)	1 (100.0)
Contraception use								
Patients who used contraception	19 (17.9)	0	0	1 (14.3)	0	2 (25.0)	12 (24.5)	4 (16.0)
Patients with hormone-based contraception	16 (84.2)	0	0	1 (100.0)	0	2 (100.0)	10 (83.3)	3 (75.0)
Type of hormone-based contraception								
Ethinyl oestradiol/progestin combination								
Overall	8 (50.0)	0	0	0	0	1 (50.0)	5 (50.0)	2 (66.7)
>50 μg of ethinyl oestradiol	3 (37.5)	0	0	0	0	0	2 (40.0)	1 (50.0)
<50 μg of ethinyl oestradiol	5 (62.5)	0	0	0	0	1 (100.0)	3 (60.0)	1 (50.0)

(Continued)

TABLE 6 | Continued

		Age at consent, years						
	Overall (*N* = 179)	**≤2 (*N* = 7)**	**2 to ≤5 (*N* = 6)**	**5 to ≤9 (*N* = 21)**	**>9 to ≤14 (*N* = 16)**	**>14 to ≤18 (*N* = 11)**	**>18 to ≤40 (*N* = 77)**	**>40 (*N* = 41)**
Progestin only								
Overall	8 (50.0)	0	0	1 (100.0)	0	1 (50.0)	5 (50.0)	1 (33.3)
Pill	4 (50.0)	0	0	1 (100.0)	0	0	3 (60.0)	0
Intrauterine devices	0	0	0	0	0	0	0	0
Depot injection	3 (37.5)	0	0	0	0	1 (100.0)	1 (20.0)	1 (100.0)
Implant	1 (12.5)	0	0	0	0	0	1 (20.0)	0
Use of external sex hormone that influence reproduction	5 (2.8)	0	0	0	0	1 (9.1)	2 (2.6)	2 (4.9)
Exogenous oestrogen	1 (20)	0	0	0	0	0	0	1 (50.0)
Progestin based to suppress menstruation	4 (80.0)	0	0	0	0	1 (100.0)	2 (100.0)	1 (50.0)
Patients with ovariectomy	3 (1.7)	0	0	0	1 (6.3)	0	2 (2.6)	0
Puberty abnormal	3 (1.7)	0	0	0	1 (6.3)	2 (18.2)	1 (1.3)	0
Male	1 (33.3)	0	0	0	0	1 (50.0)	0	0
Female	2 (66.7)	0	0	0	1 (100.0)	1 (50.0)	1 (100.0)	0
Menstrual cycle disorder (female > 11 years)	22 (20.8)	0	0	0	3 (27.3)	1 (12.5)	5 (11.6)	4 (16.0)
Amenorrhea (female > 11 years)	9 (8.5)	0	0	0	1 (9.1)	0	2 (4.7)	4 (16.0)
Amenorrhea lasted > 3 months	5 (55.6)	0	0	0	0	0	2 (100.0)	2 (50.0)
Other abnormal reproductive condition	3 (1.7)	0	0	0	2 (12.5)	0	1 (1.3)	0
Abnormal hormonal levels								
Thyroid-stimulating hormone	4 (2.2)	0	0	0	1 (6.3)	0	1 (1.3)	2 (4.9)
Follicular-stimulating hormone	1 (0.6)	0	0	0	0	0	0	1 (2.4)
Luteinising hormone	1 (0.6)	0	0	0	0	0	1 (1.3)	0
Oestradiol	1 (0.6)	0	0	0	0	0	1 (1.3)	0
Testosterone	2 (1.1)	0	0	0	0	0	2 (2.6)	0

is effectively managed to minimise the occurrence and severity in TSC patients. The incidence of stomatitis and infections was relatively low in this PASS sub-study (7.8 and 34%) than in the previous EXIST studies (5, 6, 9, 12). Rates of stomatitis in EXIST-1 and EXIST-2 were 31 and 48%, respectively (6, 8). Overall, 55–68% of patients had stomatitis in EXIST-3, which included stomatitis, mouth, aphthous, lip, and tongue ulcerations; mucosal inflammation; and gingival pain (5, 9, 12, 21). The decrease in AEs like stomatitis with longer follow-up could be attributed to better tolerability or better care or both (10). Additionally, the median dose of everolimus was similar to that in the EXIST interventional studies, whereas, the starting doses in the current study were lower, as suggested by 44 patients having their dose increased. In addition, outside of a strict trial protocol, physicians may have been able to interrupt and reduce the dose of everolimus to manage AEs. The rate of infections was reported in about 72% patients in EXIST-1 and 65% of patients in EXIST-2 (8, 11). Similarly, the rate of infections was higher in the EXIST-3 study with nasopharyngitis in 14–23.8%, upper respiratory tract infection in 13–22.4%, and pharyngitis 1–10.2% of the patients (12, 21). However, the incidence of diarrhoea (6.7%) was slightly higher than that reported in the EXIST-3 study (5%) but lower than that of the EXIST-1 and EXIST-2 studies (13% each) (6–8). Notably, PASS sub-study also showed a higher incidence of AEs in children than in the adults (**Table 2**), as previously reported in the EXIST-1 study (16, 22). It was unknown whether this was due to higher blood levels or increased susceptibility to everolimus.

No new safety signals were observed in the study. Most of the AEs observed in the study were manageable with dose adjustment and/or use of concomitant medication and were of modest severity, with grade 1 or 2 AEs observed in almost 42% of patients. The lower rate of stomatitis or aphthous mouth ulceration and some other AEs in this study compared with that expected from the literature could be due to several reasons including lower starting doses, early interruption or reduction of dose, better education and preparation of patients, or lower median blood concentration of everolimus in TOSCA PASS compared with those of the previous interventional trials (9, 12, 20). In general, there was likely to be a correlation between drug levels and AEs within individual patients as evidenced by the successful treatment of AEs by lowering the patients' dose (**Table 4**), but it appears that the different individuals have different sensitivities to any particular blood level of everolimus causing AEs. Three deaths were reported during the study. All occurred in adult patients and were deemed by the study investigators as not related to everolimus treatment.

The data on menstrual irregularities concur with the previous findings with respect to clinical features but at lower frequency (9) and confirm that everolimus can cause amenorrhoea and other menstrual irregularities. In most patients, sexual maturation was not affected by everolimus.

In conclusion, the findings from this study are confirmed the manageable safety profile of everolimus in patients with TSC with no new safety signal. The long-term safety data will continue to be collected as per study protocol for the paediatric patients.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by 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;

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Maintenance Therapy With Everolimus for Subependymal Giant Cell Astrocytoma in Patients With Tuberous Sclerosis – Final Results From the EMINENTS Study

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The aim of this EMINENTS prospective, single-center, open-label, single-arm study was to evaluate the cumulative efficacy and safety of reduced doses of everolimus (maintenance therapy) in patients with tuberous sclerosis and subependymal giant cell astrocytoma (SEGA).

Methods: The trial included 15 patients who had undergone at least 12 months of treatment with a standard everolimus dose. The dose of everolimus was reduced to three times a week, with a daily dose as in standard regimen. Data of 14 patients were analyzed. SEGA volume (SV) was evaluated at study entry and subsequent time points by an experienced radiologist. Adverse events (AEs) noted during maintenance therapy were compared to the AEs of standard dose period.

Results: Patients were followed over a mean duration 58.37 months (95%CI: 45.95–70.78). The differences in SEGA volume between subsequent time points (0, 3, 6,12, 18, 24, 36, 48, and 60 months) were not statistically significant ($p = 0.16$). At the end of the study, 7 out of 10 patients had stable SEGA volume. No clinical symptoms of progression were observed in any patients. No patient or tumor-related risk factors of progression were identified. Regarding AEs, infections (stomatitis, bronchitis, diarrhea) and laboratory abnormalities (neutropenia, anemia, hyperglycemia) occurred less frequently during maintenance therapy compared to the standard dose regimen.

Conclusions: Final results from EMINENTS study confirm that maintenance therapy with everolimus might represent a rational therapeutic option for patients TSC and SEGA after effective full dose treatment. It could be an option for patients who experienced everolimus-related AEs, instead of discontinuation of therapy. Careful evaluation of possible progression, especially concerning first six months of maintenance therapy should be advised.

Clinical Trial Registration: www.drks.de, identifier DRKS00005584.

Keywords: everolimus, MTOR inhibitor, maintenance therapy, subependymal giant cell astrocytoma, tuberous sclerosis

INTRODUCTION

Tuberous sclerosis (TSC) is an autosomal dominant genetic disorder in which mutation of *TSC1* or *TSC2* genes leads to increased activation of the mammalian target of rapamycin (MTOR) pathway. This results in the growth of benign tumors in multiple organs, including the brain, where the most severe clinical manifestation of TSC is the subependymal giant cell astrocytoma (SEGA). SEGAs develop in up to 20% of patients with TSC and when growing, may cause obstruction of cerebrospinal fluid flow leading to hydrocephalus. Currently, the recommended treatment options for SEGA associated with TSC are surgical resection or MTOR inhibitor (1).

Everolimus is an MTOR inhibitor, which has been recently approved in the United States by the Food and Drug Administration and in Europe by the European Medicines Agency for treatment of patients with TSC-related SEGA who require therapeutic intervention, but whose tumors cannot be curatively resected (2). Everolimus has recently demonstrated therapeutic efficacy and safety in patients with TSC in a number of trials (3, 4).

Evidence suggests that patients with TSC may require long-term treatment with MTOR inhibitors (5, 6). Some reports indicate that SEGAs may grow back after the cessation of MTOR inhibitor therapy (7) and the optimal duration of treatment with an MTOR inhibitor has yet to be determined: such treatment may be life-long. Therefore, safety issues connected to everolimus-related side effects must be taken into consideration. Although the short-term side effects related to everolimus therapy are generally considered acceptable, life-threatening events have also been reported (8). In addition, the long-term side effects are less known and require further research.

Treatment with everolimus results in a rapid initial reduction in SEGA volume, followed by a phase of slower reduction or stabilization of tumor size (9). However, once SEGA has been stabilized with MTOR inhibitor, it can be possible to reduce the dose of the drug in order to minimize any long-term adverse effects of the therapy. In 2014, Wheless et al. (2) proposed an algorithm for dose reduction intended to minimize the adverse effects of MTOR inhibitor therapy for SEGA cases that are stable or decreasing in size. This algorithm was tested for the first time in a clinical setting as part of the first results of the EMINENTS (Everolimus MaINtENance Therapy in SEGA) study published in 2017. The study followed 10 patients on a reduced dose of everolimus over at least 12 months (10). The recruitment was finished in October 2017, and the results of the subsequent ≥24-month analysis are presented herein.

Abbreviations: SV, SEGA volume; AEs, adverse events.

PATIENTS AND METHODS

Study Design

The design of the prospective, a single-center, open-label, single-arm study has been described in detail previously (10). The trial enrolled 15 patients who had undergone at least 12 months of treatment based on a standard everolimus dose. The recruitment was performed between December 19, 2013 and October 25, 2017, and the follow-up continued until January 3, 2020.

The study was approved by the Bioethics Committee of the Medical University of Lodz (# RNN/315/15/KE) and the study was registered in the German Clinical Trials Register (DRKS) (ID: DRKS00005584, http://apps.who.int/trialsearch/). It was conducted in compliance with good clinical practice guidelines and under the principles of the Declaration of Helsinki. All patients were treated at the Department of Pediatrics, Oncology and Hematology, Medical University of Lodz, Poland.

The primary aim of the study was to determine the proportion of patients with stable SEGA volume during reduced-dose everolimus treatment. Stabilization was defined as follows: no changes in the total volume of all SEGAs or an increase <50% relative to study entry or an increase to a volume not exceeding that observed before the start of standard everolimus treatment; no new lesions of 1 cm in diameter and no new hydrocephalus. The secondary objective included a safety profile of maintenance therapy with a comparison to standard everolimus therapy.

Patients

The patients were recruited from the whole of country. Following recruitment, they were treated and evaluated by an experienced team of pediatric oncologists at the study center. The inclusion criteria were as follows: patients with definite diagnosis of TSC with SEGA; previous treatment with everolimus at a standard dose for a minimum 12 months and a maximum 24 months, resulting in stabilization or reduction of SEGA volume; no signs of increased intracranial pressure and no hydrocephalus observed in brain MRI during evaluation prior to enrolment; signed informed consent by both the patient's parents, and the patients assent for participation in the trial.

Treatment

The standard treatment protocol for everolimus therapy consisted of oral everolimus administration once daily at the same time every day, consistently either with or without food. Dosing was titrated to attain trough concentrations of 5 to 15 ng/ml. It is described in details in a previous publication (10). After at least 12 months of standard treatment in the group of patients demonstrating reduction or stabilization of SEGA volume, the treatment regimen was changed to everolimus three times a week (Monday, Wednesday, Friday) with the same daily

dose (maintenance therapy). Everolimus (Votubia, Novartis, Germany) was provided by Polish National Health Fund for patients with TSC and SEGA diagnosis.

Evaluation of SEGA Volume

Magnetic resonance imaging (MRI) was conducted before the introduction of standard dose everolimus therapy, at the time of study entry, and then after 3, 6, 12, 18, 24 months of maintenance therapy, and once a year thereafter. All patients were examined using a 1.5-Tesla MRI scanner (Toshiba Medical System, Otawara, Japan) with a standardized protocol for brain examination. Measurements were obtained on enhanced T1-weighted images in three perpendicular planes.

Two methods of SEGA volume assessment were used. All scans were assessed by the same radiologist with 10 years' experience in brain MRI evaluation, who was blinded to the clinical history of the patients (standard dose treatment vs. maintenance therapy). SEGA volume was approximated as an ellipsoid, using the formula: 0.52 x a x b x c, where a, b, c are the maximum dimensions of the tumor measured in the axial, sagittal and coronal planes measured on MRI scans. To avoid potential overestimation of volume due to the ellipsoid approximation, a semi-automated method of volume measurement was also applied using ITK-SNAP software. These have been described in detail in previous publications (10, 11). Since a strong positive correlation was found between the manual and automatic methods of comparing tumor volumes at the same time points ($r = 0.8925$, $p < 0.0001$), only evaluations of the SEGA volume performed by the radiologist were incorporated in further analyses.

Safety Profile Assessment

The patients were clinically evaluated once per month for the initial 6 months and every 3 months thereafter. All clinical symptoms that occurred during therapy were recorded. Laboratory studies were performed at each study visit; these included complete blood count, fasting lipid profile, glucose level, liver and kidney function tests. Adverse events (AEs) were assessed with the use of the Common Terminology Criteria for Adverse Events (version 4.0) (12). The most severe grade of each AEs per patient per year were recorded. The number of patients with reported AEs during maintenance therapy per year were compared with those observed during standard everolimus therapy, and were statistically evaluated. Everolimus whole blood trough concentration was assessed at each study visit using ultra performance liquid chromatography/tandem mass spectrometry (UPLC/MS/MS) as described previously (13).

Statistical Analysis

The comparison between multiple groups was performed with the analysis of variance (ANOVA); if ANOVA yielded a significant difference, this was followed by between-group comparisons with the Unequal HSD *post hoc* test. Repeated measures ANOVA was used to evaluate time-dependent changes in tumor size. Mauchly's sphericity test was used to assess the assumption of data sphericity; comparisons that showed significant sphericity were subjected to the Greenhouse–Geisser correction, with an Unequal HSD *post hoc* test if needed. Cochrane's Q-test was applied to evaluate differences in global test for repeated measures with nominal data. Scores with p-levels < 0.05 were regarded as significant. Statistica version 13 (Dell Software) was used for statistical analysis.

RESULTS

Between December 2013 and November 2017, a total of 15 patients were enrolled into the study; however, one patient was excluded from the analysis due to diagnosis of malignant brain tumor (gliosarcoma grade IV acc. WHO classification) in SEGA location after 4 years of everolimus treatment (after 6 months on maintenance dose). Therefore, only 14 patients were included in the data analysis (**Figure 1**). The clinical characteristics of study group are presented in **Table 1**.

The mean duration of maintenance therapy was 58.37 months (95%CI: 45.95–70.78). The mean everolimus dose was 41 mg/m^2/week (95%CI: 34.22–47.77) at study entrance and 15.37 mg/m^2/week (95%CI: 12.82–17.91) at study end. The mean everolimus concentration during the study was 2.65 ng/ml (95%CI: 2.1–3.19) (**Table 1**).

Tumor Volume Evaluation

The mean SEGA volumes (SV) before and during the study are presented in **Supplementary Table 1**. The differences in SV between subsequent time points (0, 3, 6,12, 18, 24, 36, 48, and 60 months) were not statistically significant ($p = 0.16$) in the ANOVA repeated measures test.

Throughout the whole dataset, pretreatment SEGA size differed from that measured during the treatment period ($p = 0.003$). Pairwise comparisons between 0, 3, 6, 12, 18, 24, 36, 48, and 60 months and pretreatment values were significant (all p-levels < 0.0002).

During maintenance therapy, an increase in volume of 0.56 cm^3 per year was observed during the first six months and 0.14 cm^3 per year in the following 6 months. Further stabilization, i.e., an increase in volume < 0.07 cm^3 per year, was observed over subsequent months; however, a 0.14 cm^3 per year increase was observed in the third year.

The lowest proportion of patients with stable SV were observed at the time points 12 and 18 months (62%). This proportion then increased at time points: 36 (83%) and 48 months (82%) – **Supplementary Table 1**. At the end of the study, seven out of ten patients had stable SV.

The changes in SEGA volumes in individual patients are presented in **Figures 2A–C**. Seven patients demonstrated progression (**Supplementary Table 2**). Of these, three patients discontinued the study. One patient underwent neurosurgery after 6 months of maintenance therapy due to enlargement of ventricular volume and risk of hydrocephalus. This patient demonstrated the greatest SV of the group, which increased during the study from 2.27 cm^3 to 3.3 cm^3 (145%); however, the patient did not exactly meet the progression criteria. In this case, mean everolimus concentration during the study was extremely low 0.57 ng/ml (95%CI: 0.16–1.99) (**Supplementary Table 2**) indicating poor compliance with everolimus therapy. In the two

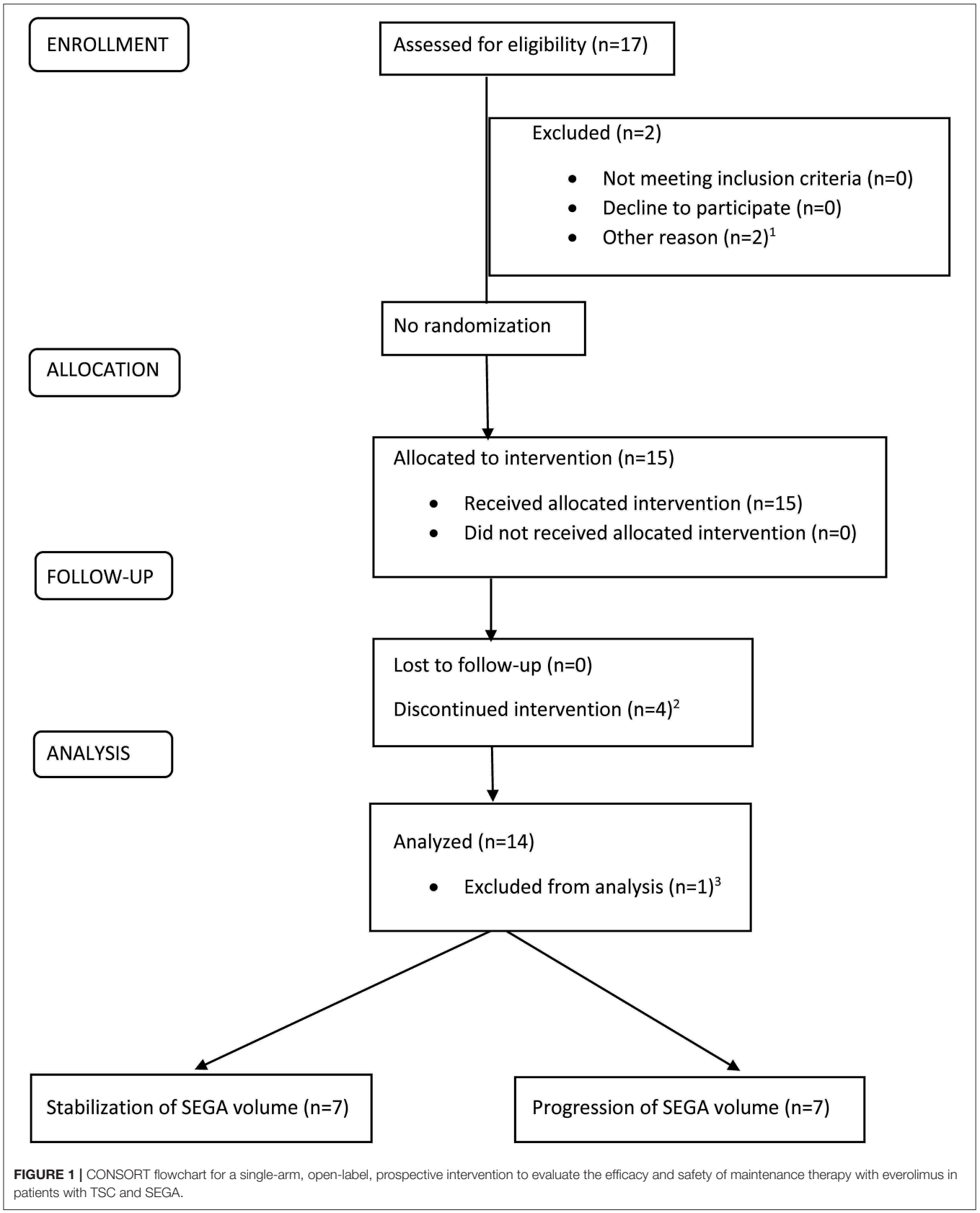

FIGURE 1 | CONSORT flowchart for a single-arm, open-label, prospective intervention to evaluate the efficacy and safety of maintenance therapy with everolimus in patients with TSC and SEGA.

TABLE 1 | The clinical characteristics of the patients.

Characteristic	Mean (95%CI) or Number (%)
Age (years) at study entry	13.82 (11.35–16.29)
Full-dose treatment duration (months)	14.48 (11.63–17.34)
Maintenance therapy duration (months)	58.37 (45.95–70.78)
Sex: male	9/14 (64%)
Female	5/14 (36%)
TSC mutation status	4/14 (29%)
• *TSC1*	
• *TSC2*	8/14 (57%)
• No mutation identified	2/14 (14%)
Skin lesions	11/14 (79%)
• Facial angiofibroma	
• Fibrous cephalic plaque	3/14 (21%)
• Hypomelanotic macules	13/14 (93%)
• Shagreen patch	10/14 (71%)
Other features	8/14 (57%)
• Kidney angiomyolipomas	
• Multiple renal cysts	4/14 (29%)
• Cardiac rhabdomyoma	5/14 (36%)
• Retinal hamartomas	4/14 (29%)
TSC-associated neuropsychiatric disorders	10/14 (71%)
Epilepsy	11/14 (79%)
SEGA volume (SV) before treatment (cm^3)	2.1 (1.1 –3.1)
SEGA volume at study entry (cm^3)	0.84 (0.44– 1.23)
Percentage of SV at study entry compared to pretreatment (%)	51.47 (32.81– 70.14)
Everolimus dose at study entry (mg/m^2/week)	41 (34.22–47.77)
Everolimus dose at study end (mg/m^2/week)	15.37 (12.82–17.91)
Everolimus concentration at study entry (ng/ml)	8.32 (6.58– 10.05)
Everolimus concentration during the study (ng/ml)	2.65 (2.1– 3.19)

other patients, everolimus was escalated to daily treatment (full dose treatment).

The remaining four patients with progression continued the study with a reduced dose of everolimus; in two of these, SV decreased and they met the stabilization criteria. No clinical symptoms of progression were observed in any patients.

The patients who demonstrated stable SV during the whole study are compared with those presenting progression at any time in **Table 2**. No statistically significant differences were observed between groups with regard to in age, sex, TSC status, neurological status, pretreatment SV, SV at study entry, or in everolimus concentration and everolimus dose at study entry or during the study.

The other TSC lesions observed in the study group are presented in **Table 1**. The study safety concerns indicate that any of coexisting features did not deteriorate significantly during the study.

Adverse Events

Adverse events related to everolimus therapy are presented in **Table 3**.

AEs were divided into two groups: clinical AEs and laboratory abnormalities. Clinical AEs were observed in 11/14 patients during maintenance therapy. The most common were the infections which led to dose interruptions (9/14 patients). No grade three or four clinical AEs were noted. Clinical AEs related to everolimus administration did not lead to cessation of treatment in any patient.

Laboratory abnormalities were recorded in 13/14 patients during the maintenance dose. They were of mild or moderate severity (grade one or two) and they did not lead to dose interruption or cessation of treatment in any patient.

Of the clinical AEs, infections in total occurred less frequently during maintenance therapy compared to the standard dose regimen, with the most common ones being stomatitis, bronchitis and diarrhea. The same dependency was noted for some laboratory abnormalities, in particular neutropenia, anemia and hyperglycemia (**Table 3**).

DISCUSSION

This is the final report of the EMINENTS trial, which has previously been reported as an interim analysis for shorter follow-up for only 10 patients (10). Long-term maintenance therapy resulted in an insignificant increase of SEGA volume. Of 14 analyzed patients, eleven continued the study; of these, seven demonstrated stabilization of tumor volume during the whole observation period while the other four met the criteria of progression at some point in the study. Of those, four patients, who continued the reduced everolimus dosing regimen despite progression, SEGA volume decreased in two patients, meeting the stabilization criteria after a subsequent 24 and 30 months of maintenance therapy, while tumor size remained stable for the remainder of the therapy in the other two. No symptoms of progression were observed in any patient. Despite seven out of 14 patients experienced progression of SEGA, 11 patients (78%) continued a maintenance dose with subsequent stabilization or shrinkage of the tumor. These findings indicate that the continuation of a maintenance dose, even in case of growing SEGA, does not rule out a final good treatment effect in those patients under close clinical and imaging monitoring. As no differences in clinical characteristics were found between patients with progression and those who demonstrated stabilization of tumor volume, no risk factors of progression could be identified. In our study progression was defined as a tumor regrow comparing to the volume at study entry, which was a residual mass after 1 year of full dose everolimus therapy. This do not mean the same as progression used in oncology which is an increase of primary tumor volume.

Three patients discontinued the study. One patient underwent neurosurgery after 5 months of maintenance therapy with everolimus due to enlargement of ventricular volume and risk

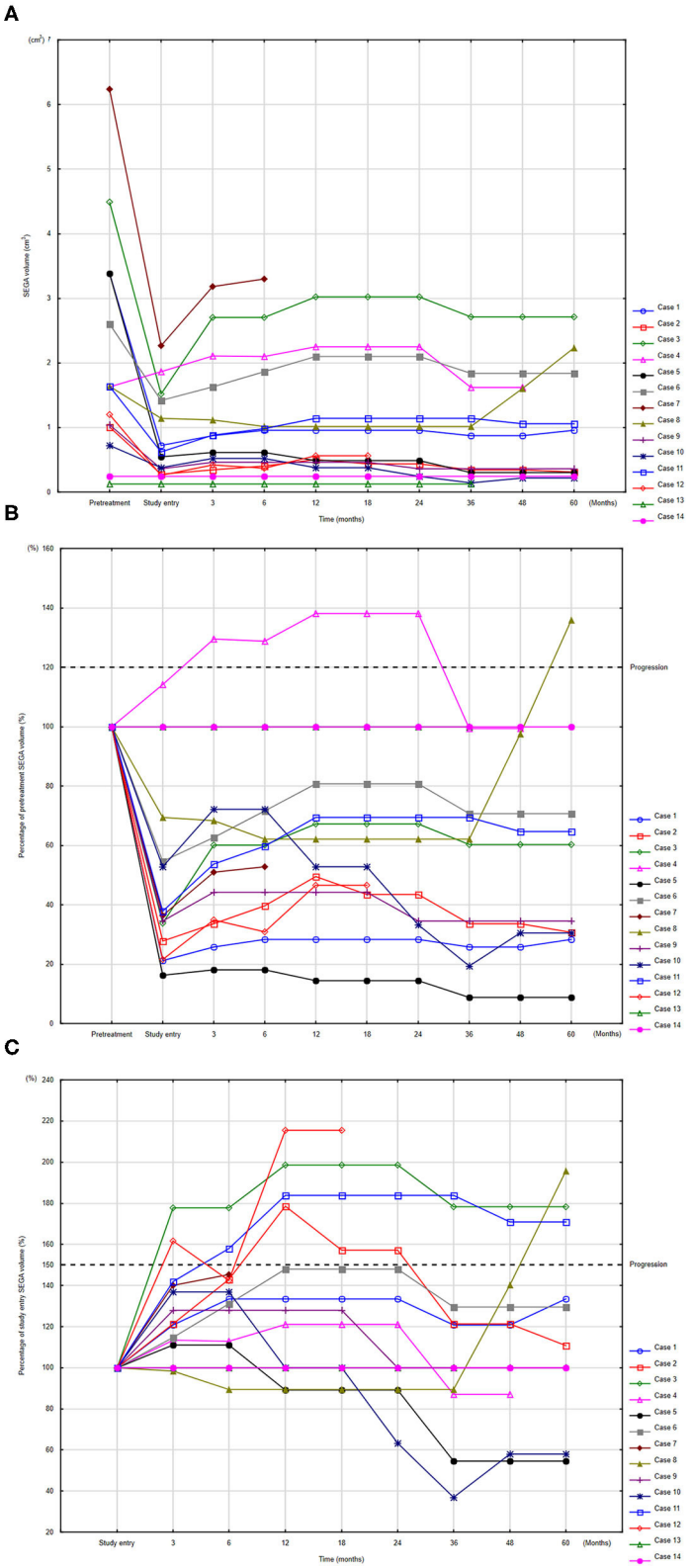

FIGURE 2 | Changes in SEGA volume (SV) compared to volume before everolimus treatment **(A)**, and percentages of SV change at study time point compared to pretreatment measurements **(B)** and to study entry **(C)**. To identify respective individuals, the indices for each patient are consistent among panels **(A–C)**. Progression defined according to RECIST **(B)** and according to EMINENTS study **(C)**.

TABLE 2 | Comparison of patients with stable SEGA volume (SV) vs. patients with progression of SEGA volume at any time during the study (two-tailed Fischer's exact test, UMW test according to distribution of the data).

Characteristic	Patients with stabilization of SV ($n = 7$)	Patients with progression of SV ($n = 7$)	*p*-value
Age (years)	14.08 (8.8–19.37)	13.56 (11.14–15.98)	0.46
Sex			1
• Male	4	5	
• Female	3	2	
TSC status			0.76
• TSC1	2	2	
• TSC2	4	4	
• No mutation	1	1	
TSC-associated neuropsychiatric disorders	5/7	5/7	1
Epilepsy	5/7	6/7	1
SV before everolimus treatment (cm^3)	1.64(0.31–2.98)	2.55 (0.7–4.41)	0.32
SV at study entry (cm^3)	0.54 (0.14–0.94)	1.14 (0.41–1.86)	0.16
Percentage of SV at study entry compared to pretreatment (%)	54.23 (22.41–86.04)	48.72 (18.58–78.86)	1
Everolimus concentration at study entry (ng/ml)	8.53 (5.67–11.4)	8.10 (5.2–11)	0.8
Everolimus concentration during the study (ng/ml)	2.93 (2.03–3.82)	2.37 (1.53–3.21)	0.38
Everolimus dose at study entry (mg/m^2/week)	43.06 (30.48–55.64)	38.93 (29.52–48.34)	0.62
Everolimus dose at study end (mg/m^2/week)	16.02 (12.22–19.83)	14.71 (10.15–19.27)	0.53
Treatment decision			–
• continuation of MT	7	4*	
• neurosurgery[a]	0	1	
• full-dose treatment[b,c]	0	2	

**Four patients with SV progression continued the study; of these, two patients with SV met stabilization criteria after 24 months (one patient) and 30 months (one patient) of maintenance dose. Three patients discontinued the study: [a]one patient - neurosurgical intervention; [b]one patient – returned to full-dose treatment due to progression of SV (parents' decision); [c]one patient – returned to full-dose treatment due to progression of SV (investigator's decision).*

of hydrocephalus. In two patients, the everolimus dose was escalated to full dose treatment: this change was the investigator's decision in one patient, and the parents' decision in the second, i.e., the parents withdrew their consent to participate in the study.

To summarize, of the three patients who discontinued the study, only one truly required dose escalation related to progression of SV after 60 months of MT: the other two left due to noncompliance in one case and withdrawal of consent in the other. However, even in this case with "true progression," no clinical symptoms of SEGA growth were noted and no signs of hydrocephalus were visible in the MRI.

SV progression has also been observed in other studies during full dose everolimus treatment: in 11.7% of patients in the EXIST-1 Study and 0.8% of patients in the EFECTS (6, 14). The SEGA regrowth was also observed in case series study by Weidman et al. in two out of four patients after cessation or reduction of sirolimus. In this limited series doses < 2.5 mg/m^2 were insufficient to maintain SEGA response (15). In our study, average dose of everolimus maintenance treatment was 15.37 mg/m^2/week (2.2 mg/m^2/day), showing therapeutic effect in 11 out of 14 patients. However, doses between 2 and 3 mg/m^2 may represent a gray zone where SEGA regrowth might be observed in some patients (15). A dose-response relation might be connected to the volume of the tumor, meaning that bigger volume needs higher dose. But this is only a hypothesis and the optimal dose of everolimus to maintain SEGA response requires further research.

Mean everolimus concentration was significantly lower during the study than at study entry (2.65ng/ml; 95%CI: 2.1 – 3.19 vs. 8.32ng/ml; 95%CI: 6.58 – 10.05). However, there were no differences in mean everolimus concentration between the patients demonstrating stabilization of SV during the whole study and those with progression at any time of the study. Final analysis of EXIST-Study reported that responses occurred despite a majority of patients had a median serum level of everolimus below or just within the usual therapeutic range. The authors stated that efficacy et a lower serum level may result in fewer adverse effects and better tolerability (5). In our previous report, no significant differences in SV reduction were observed between patients with everolimus trough level $<$ 5ng/ml and those with levels $\geq$ 5ng/ml, suggesting that drug dose titration according to

TABLE 3 | Adverse events related to everolimus in the study group during standard and maintenance therapy. NS, not statistically significant ($p > 0.05$) in a two-tailed Fisher's exact test.

	Standard therapy		Maintenance therapy										*p*-value
			1st year		2nd year		3rd year		4th year		5th year		
Adverse event	**All grade**	**Grade 3**	**All grade**	**Grade3**	**All grade**	**Grade 3**	**All grade**	**Grade 3**	**All grade**	**Grade 3**	**All grade**	**Grade 3**	
Infectious adverse events													
Stomatitis	9/14	0	2/14	0	1/12	0	4/12	0	2/11	0	2/10	0	0.001
Pharyngitis	8/14	0	6/14	0	5/12	0	6/12	0	5/11	0	2/10	0	0.25
Bronchitis	6/14	0	1/14	0	0	0	0	0	1/11	0	0	0	0.03
Diarrhea	6/14	0	0	0	0	0	1/12	0	0	0	0	0	0.0007
Skin/soft tissue infection	4/14	1/14	0	0	0	0	0	0	1/11	0	0	0	0.55
Otitis (media/externa)	1/14	0	0	0	0	0	0	0	0	0	1/10	0	0.55
Sinusitis	0	0	0	0	0	0	0	0	1/11	0	0	0	–
Urinary tract infection	1/14	0	0	0	1/12	0	1/12	0	0	0	2/10	0	0.21
Vulval infection	1/5	0	2/5	0	0	0	0	0	0	0	0	0	0.42
Infections - total	11/14	1/14	7/14	0	5/12	0	6/12	0	6/11	0	4/10	0	0.04
Infections other than pharyngitis	7/14	1/14	2/14	0	0/12	0	3/12	0	4/11	0	2/10	0	0.005
Other clinical/ Non-infectious adverse events													
Irregular menses	3/5 girls	0	2/4 girls	0	2/4 girls	0	2/4 girls	0	2/4 girls	0	2/4 girls	0	–
Hypertension	3/14	0	2/14	0	2/12	0	2/12	30	2/11	0	2/11	0	0.42
Sinus tachycardia	1/14	0	0	0	0	0	0	0	0	0	0	0	0.42
Constipation	1/14	0	1/14	0	1/10	0	0	0	0	0	0	0	0.42
Laboratory abnormality													
Hypercholesterolemia	9/14	0	10/14	0	9/12	0	6/12	0	7/11	0	7/10	0	0.19
Hypertriglyceridemia	9/14	0	7/14	0	9/12	0	5/12	0	6/11	0	6/10	0	0.31
Neutropenia	7/14	1/10	0	0	2/12	0	0	0	0	0	0	0	0.001
Anemia	6/14	0	2/14	0	3/12	0	2/12	0	1/11	0	1/10	0	0.05
Hyperglycemia	4/14	0	0	0	0	0	0	0	0	0	0	0	0.01
Gamma-glutamyltransferase increased	3/14	1/10	0	0	0	0	0	0	0	0	0	0	–
Leucopenia	2/14	0	0	0	2/12	0	0	0	0	0	0	0	0.16
Alanine/Aspartate aminotransferase increased	2/14	0	1/14	1/13	1/12	0	0	0	0	0	0	0	0.35
Thrombocytopenia	1/14	0	1/14	0	3/12	0	1/12	0	1/11	0	0	0	0.23
Bilirubin increased	1/14	0	1/14	0	2/12	0	2/12	0	1/11	0	1/10	0	0.42

blood concentration does not play a key role in achieving clinical success in SEGA treatment (9).

Although adverse events are very commonly observed during MTOR inhibitor treatment in patients with TSC, they are usually mild or moderate in severity (16, 17); however, life-threating events also have been reported (8). The AEs reported in this study were less severe and significantly less common than those observed during the standard dose period; this difference was particularly apparent after the exclusion of pharyngitis, which is a common health problem in children independent of immune status. The most common AEs were stomatitis, bronchitis and diarrhea, which are common reasons of treatment interruption. A recent meta-analysis found stomatitis and upper respiratory tract infections to be the most commonly-reported AEs (18).

The most important observation of our study was that no SAE was reported during MT and none of the patients discontinued the study due to AEs. This is in contrast to observations made during full dose everolimus treatment. In the EFFECTS-study, SAE was reported in 26.7% patients and AEs led to study drug discontinuation in 6.7% patients (14). In the EXIST-1 study, almost 10% of patients experienced an AE that led to everolimus discontinuation (6).

The number of patients with TSC receiving everolimus treatment is steadily growing as the numbers of clinical manifestations of TSC as indications for MTOR inhibitors, either approved or under controlled clinical trials, are also increasing (19). In addition, such treatment may be long-term or perhaps indefinite. Hence, there is a greater need to consider the safety issues associated with treatment. It is possible that maintenance therapy with everolimus might represent a rational therapeutic option for this growing population after effective full dose treatment. It could be an option for patients who experienced everolimus-related AEs, instead of discontinuation of therapy.

The present analysis is limited by the open-label and single-arm nature of its design.

However, comparison made with previous full-dose everolimus treatment period in terms of SV and AEs allowed to draw significant conclusions.

Another limitation is the small number of patients recruited to the study. However, as only 40 children with TSC and SEGA were treated with everolimus in Poland during the study period, our data represent a significant part of this population.

Although the research was conducted as a single-center study, the center was one of the most experienced in Poland for treating SEGA related to TSC. Thus, the decision about reduction of therapy was made by an experienced team, after close consideration of the situations of both patients and parents. Careful evaluation of possible progression, especially concerning first six months of maintenance therapy should be advised.

CONCLUSION

Maintenance therapy with everolimus might represent a rational therapeutic option for patients TSC and SEGA after effective full dose treatment, especially it could be an option for patients who experienced everolimus-related AEs, instead of discontinuation of therapy.

Our results suggest that progression of SEGA might be asymptomatic; in addition, no patient of tumor-related risk factors of progression could be identified. Therefore, close monitoring of SEGA volume on maintenance therapy should be recommended.

Continuation of a maintenance dose, even in case of slowly growing SEGA, does not rule out a final good treatment effect under strict clinical and imaging monitoring.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Komisja Bioetyczna przy Uniwersytecie Medycznym w Łodzi. Written informed consent to participate in this study was provided by the participants' legal guardian/next of kin.

AUTHOR CONTRIBUTIONS

KB, KKr, and WM (specialist of pediatric oncology) collected clinical data and wrote the draft of the manuscript and approved the final manuscript as submitted additionally. KB (specialist in statistic) performed statistical analysis. DB (specialist in radiology) evaluated MRI scans. KKa and SJ (specialist in neurology) provided some clinical and genetic data and contributed to writing of the manuscript and approved the final manuscript as submitted. JT designed the study and prepared the final version of the manuscript and approved the final manuscript as submitted. All authors contributed to the article and approved the submitted version.

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SUPPLEMENTARY MATERIAL

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Frequency, Progression, and Current Management: Report of 16 New Cases of Nonfunctional Pancreatic Neuroendocrine Tumors in Tuberous Sclerosis Complex and Comparison With Previous Reports

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Background: Tuberous sclerosis complex (TSC) is a genetic condition that causes benign tumors to grow in multiple organ systems. Nonfunctional pancreatic neuroendocrine tumors (PNETs) are a rare clinical feature of TSC with no specific guidelines outlined for clinical management at this time. Our purpose is to calculate the frequency of nonfunctional PNETs as well as characterize the presentation, current clinical management, and assess the impact of systemic mammalian target of rapamycin (mTOR) on nonfunctional PNETs in TSC.

Methods: This retrospective chart review was performed by a query of the TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database for patients with nonfunctional PNET. Clinical data from these two groups was summarized for patients identified to have a nonfunctional PNET and compared to previously reported cases with TSC and nonfunctional PNETs.

Results: Our calculated frequency of nonfunctional PNETs is 0.65%. We identified 16 individuals, nine males and seven females, with a median age of 18.0 years (interquartile range: −15.5 to 25.5). Just over half (56.3%, $n = 9$) of the patients provided results from genetic testing. Six had pathogenic variants in *TSC2* whereas three had pathogenic variants in *TSC1*. The average age at PNET diagnosis was 15.0 years (range: 3–46 years). Almost all individuals were diagnosed with a PNET during routine TSC surveillance, 56.3% ($n = 9$) by MRI, 12.5% ($n = 2$) by CT, 25% ($n = 4$) by ultrasound, and 6.2% ($n = 1$) through a surgical procedure. Follow up after diagnosis involved 68.8% ($n = 11$)

having serial imaging and nine of the sixteen individuals proceeding with surgical removal of the PNET. Eight individuals had a history of using systemic mTOR inhibitors. Tumor growth rate was slightly less in individuals taking an mTOR inhibitor (−0.8 mm/yr, IQR: −2.3 to 2.2) than those without (1.6 mm/yr; IQR: −0.99 to 5.01, $p > 0.05$).

Conclusions: Nonfunctional PNETs occurred at younger ages in our TSC cohort and more commonly compared to ages and prevalence reported for the general population. PNETs in patients on systemic mTOR inhibitors had lower rates of growth. The outcome of this study provides preliminary evidence supporting the use of mTOR inhibitor therapy in conjunction with serial imaging as medical management for nonfunctional PNETs as an alternative option to invasive surgical removal.

Keywords: pancreatic neuroendocrine tumor, nonfunctional, tuberous sclerosis, surveillance, abdominal imaging

INTRODUCTION

Tuberous sclerosis complex (TSC) is a rare, multi-system genetic condition that causes benign tumors to grow in the brain and in other vital organs, such as skin, kidneys, heart, lungs, and eyes. The estimated incidence of TSC is one in ∼11,000 live births (1). The clinical presentation of TSC has significant inter- and intra-familial variability that leads to a spectrum of severity seen amongst affected individuals (2, 3). TSC is caused by mono-allelic pathogenic variants in *TSC1* or *TSC2,* which encode for hamartin and tuberin, respectively (4, 5) *TSC1* pathogenic variants causes 26% of the cases and *TSC2* pathogenic variants causes 69% of the cases. In general, *TSC2* pathogenic variants tend to be associated with a more severe clinical presentation and account for the majority of *de novo* cases of TSC (6). Surveillance recommendations for TSC were established in 2012 and called for all individuals with TSC to have routine brain MRIs to monitor for the emergence of subependymal giant cell astrocytomas (SEGA) and abdominal MRIs to monitor for the emergence and/or progression of kidney findings, which include renal cysts and angiomyolipomata (2). Serial abdominal imaging of this patient population has led to an increase in the identification of pancreatic masses that are presumed to reflect neuroendocrine tumors (PNETs) (7–12).

According to the recent 2019 WHO classification system, pancreatic neuroendocrine neoplasias (PNEN) are classified as either PNET or poorly differentiated Pancreatic Neuroendocrine carcinoma (PNEC) (13). These are two different entities are determined based on the degree of cellular differentiation (i.e., Ki-67 value) that present with different genetic etiologies, as well as clinical presentation, radiological features, treatment and prognosis (12, 14). Sporadic PNETs are extremely rare with an annual incidence of 0.43 in 100,000 (15–17). Ten percent of PNETs present in association with genetic syndromes including Multiple Endocrine Neoplasia Type I (MEN-1), Neurofibromatosis Type 1 (NF-1), TSC, and von Hippel-Lindau (VHL) (18–22). Tumor grading and staging are the two most important aspects that define management for PNETs. Depending upon their hormone production, PNEN can also be classified as functional or nonfunctional, with the former presenting earlier during the clinical course due to the symptoms associated with abnormal hormonal production and often require special molecular imaging techniques for localization (23). Insulinomas are the most common functional PNETs, followed by gastrinomas and VIPomas (12, 24). Nonfunctional tumors present later during their clinical course, and rarely present with symptoms. When they are present, they are usually associated with local invasion. Often, nonfunctional tumors are diagnosed incidentally during surveillance for other conditions, such as the one established for TSC (25–27). Somatostatin receptor scintigraphy (SRS) and single-photon emission computed tomography (SPECT) are good techniques for initial staging of PNETs with ^{68}Ga-labeled somatostatin analogs having the highest sensitivity and specificity for noninsulinoma PNETs and glucagon-like peptide 1 (GLP-1) receptor analogs scintigraphy for patients with insulinomas (24). For well-differentiated grade 1 and 2 that are bigger than 2 cm, growing faster than 0.5 cm in 6–12 month time frame, or Grade 3 is an indication for surgery. mTOR inhibitors seem promising in the medical management of Grade 3 tumors. Surgical resection is indicated in cases of large tumors, rapidly growing or poorly differentiated Grade 3 PNEC (14). For well-differentiated, Grade 1 and 2 nonfunctional PNETs <2 cm and growing <0.5 cm during 6–12 month time frame conservative management is recommended with follow up imaging (CT or MRI); however, prospective studies are needed to further define surgical intervention, as early removal of this lesions may be associated with better long term outcomes (24, 28). The 5-year progression free survival for incidentally diagnosed nonfunctional PNETs is 86%, compared to 59% in symptomatic functional tumors (26).

More than 40 cases of PNETs have been reported in association with TSC. Of those, 21 are functional and 19 are nonfunctional with more than 15 nonfunctional PNET cases being reported after 2012 (7–10, 29–37). Previous case series reported on the presence of both functional and nonfunctional PNETs in association with TSC (9, 34). Extrapolating data from these case series, the estimated prevalence of both functional and nonfunctional PNETs in patients with TSC is 4–9% (9, 34). Previously, a prevalence of 1% of PNETs in association with TSC (38). Since the publication of the 2012 surveillance recommendations, however, there has been a rise in the number

of case reports of nonfunctional PNETs in individuals with TSC. As there is a lack of surveillance guidelines for management of nonfunctional PNETs specifically in relation to TSC, individuals may be receiving surgical intervention unnecessarily or earlier than needed based on size and growth rate. Unlike the previously mentioned genetic conditions, the use of mammalian target of rapamycin (mTOR) is indicated for TSC to reduce the size of TSC-related tumors. The use of mTOR inhibitors may be impact the growth or size of nonfunctional PNET growth. Unfortunately, there is limited data available about impact of mTOR inhibitors on TSC- associated PNETs.

This report focuses on nonfunctional PNETs in association with TSC, primarily diagnosed as incidental findings on routine surveillance for renal angiomyolipomas after the establishment of the surveillance guidelines in 2012. As functional PNETs are often diagnosed before being apparent on abdominal MRI and management depends upon presence of symptoms, we did not focus our analysis on these tumors. Our overall objective is to raise awareness and educate clinicians on the emerging pancreatic phenotype observed in the TSC population in the hopes of improved clinical decision making regarding nonfunctional PNETs in TSC, thereby optimizing clinical outcomes that can lead to an improved quality of life. The purposes of our study were to: (1) Clinically characterize nonfunctional PNETs in a large population of patients with TSC, (2) Evaluate the impact of mTOR inhibitors on tumor growth, and (3) Review medical management reported in our case series, in conjunction with the reports in the medical literature, to summarize the current management and treatment regimens for nonfunctional PNETs.

MATERIALS AND METHODS

In this case series, we extracted individuals with a diagnosis of TSC and nonfunctional PNET from two separate databases. The study design and data gathering process were developed by the authors and approved by the Institutional Review Board of University of Texas Health Science Center at Houston (HSC-MS-19-0273) and Cincinnati Children's Hospital Medical Center (IRB #2012-2317). Data collection was performed from June 2019–August 2019. For individuals in the case series, each respective clinic site completed our author-designed questionnaire. All data was de-identified prior to being exported to the authors.

Study Sample

Based on the prevalence of TSC (1:11.000) and the anticipated low frequency of PNETs in TSC subjects, participants were obtained through two databases: TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database (**Figure 1**). Specifically, the TS Alliance's Natural History Database is comprised of individuals with TSC among 18 U.S-based clinical sites. For each database, a single person extracted individuals that met our inclusion criteria. Our inclusion criteria include a clinical and/or molecular diagnosis of TSC as well as the presence of a nonfunctional PNET. Individuals were excluded from our study if they did not have a clinical or molecular diagnosis of TSC, functional PNET, or had a secondary diagnosis of a condition that is associated with an increased risk of PNETs, such as NF-1, VHL, or MEN-1. Once identified as eligible for inclusion, we cross-compared multiple, individual-specific data points between the two clinical databases as well as the medical literature to ensure each subject in the new cohort was unique.

Data Collection

The questionnaire completed by each respective clinic site included questions regarding supplemental demographics, PNET characteristics, serial imaging of nonfunctional PNETs, as well as previous or current use of a systemic mTOR inhibitor. Specifically, demographic data included current age, sex, age of TSC diagnosis, and clinical vs. molecular diagnosis. PNET data included age of diagnosis, imaging modality that lead to the PNET diagnosis, location of PNET, functionality, number of tumors, and reported clinical vs. surgical management. For available individuals, serial images were obtained for the nonfunctional PNET. All available imaging reports were reviewed to extract the date of evaluation, modality (CT vs. MRI vs. ultrasound) and the diameter of the tumor(s) in mm. Lastly, we obtained the start and stop date for any individual with a history or current use of a systemic mTOR inhibitor.

Statistical Analysis

Descriptive statistics were performed with categorical data described as frequencies (and percentages) and continuous data described as medians (interquartile ranges (IQR) and ranges). Pearson correlation coefficients was calculated to assess the linear correlation between the nonfunctional PNET size at time of diagnosis of the PNET and patient age. The data from patients that had nonfunctional PNET size information from two or more scans was set up as panel data and analyzed as a longitudinal dataset. Generalized linear mixed models were utilized to identify temporal changes in the size of the PNET (dependent variable) while adjusting for age of the patient, pancreatic location of the PNET and the use of mTOR inhibitors at the time of the scan. Estimates temporal trends were described for the variables along with 95% confidence intervals. Statistical significance was assumed at a Type I error rate of 5%. All analyses were performed in Stata (v.14, College Station, Texas).

RESULTS

Eighteen TSC patients with nonfunctional PNET were identified in the TS Alliance's Natural History Database ($n = 14$) and the Cincinnati Children's Hospital TSC Database ($n = 4$). We excluded one report from the TS Alliance's Natural History Database ($n = 13$) from the final analysis due to lack of information, but this report was included in the frequency calculation. Furthermore, a duplicated report was identified between the TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database. The duplicated report was removed from the TS Alliance's Natural History Database ($n = 12$) and from the frequency calculation. In our series, 0.65% (17/2,580) of patients have a nonfunctional PNET

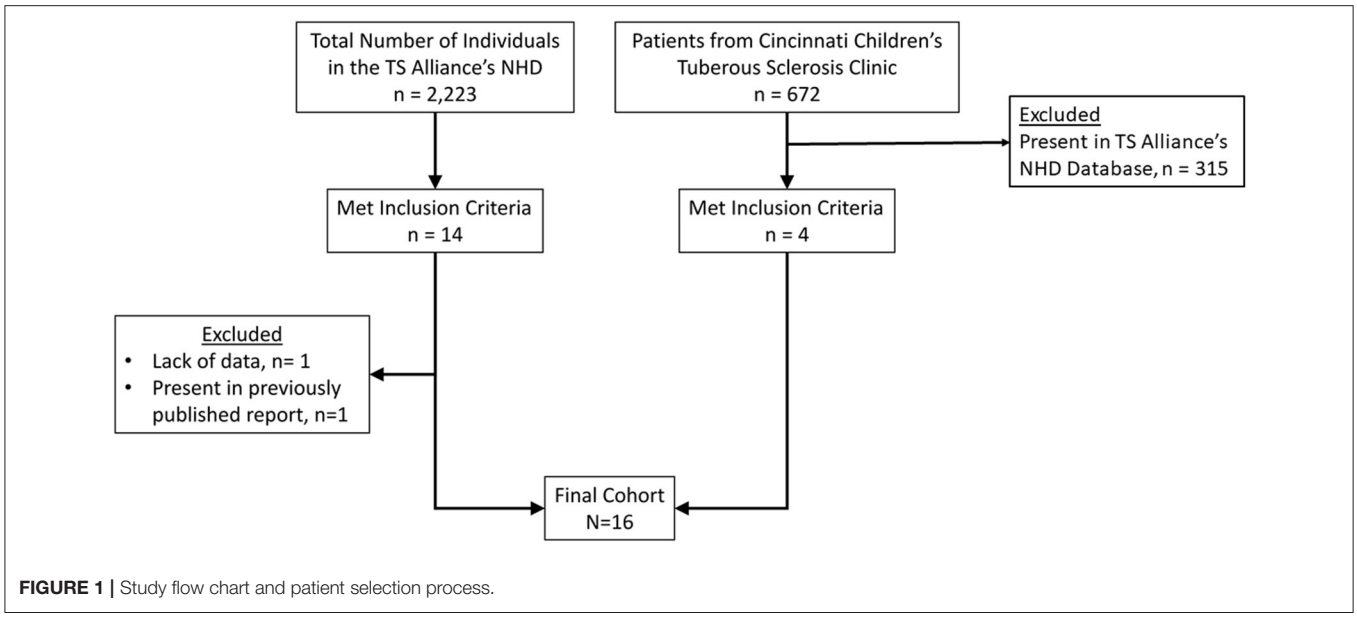

FIGURE 1 | Study flow chart and patient selection process.

TABLE 1 | Clinical information of subjects with TSC diagnosed with nonfunctional PNET in our case series.

Patient #	TSC1/TSC2	Variant	Variant classification	Age of at PNET Dx (years)	Sex	Location	Diameter at Dx	Hx of mTOR	Surgery	Serial MRI	Serial CT	Ultrasound
1	TSC1	9>0insT12956	Pathogenic	8	M	Head	1.0 cm	Yes	No	✓	X	X
2	–	–	–	13	M	Tail	1.4 cm	Yes	No	✓	X	X
3	TSC2	3 bp deletion of AAG	VUS	16	F	Body	0.7 cm	Yes	No	✓	X	X
4	TSC1	c.228 C>T	Pathogenic	3	F	Body	0.7 cm	No	No	✓	X	X
5	TSC2	c.3281C>A	Pathogenic	15	F	Body	1.9 cm	No	No	✓	X	X
6	–	–	–	46	M	Body	Unknown	No	Yes	X	✓	X
7	TSC2	c.4279delA	Pathogenic	12	M	Tail	1.7 cm	No	Yes	✓	X	X
8	TSC2	3 bp deletion of CAT; c.1108C>T	Pathogenic; Benign	21	F	Body	4.1 cm	Yes	Yes	–	–	–
9	TSC1	c.330insT	Pathogenic	7	M	Tail	1.2 cm	No	Yes	✓	✓	X
10	–	–	–	9	F	Tail	1.5 cm	No	Yes	✓	X	✓
11	–	–	–	6	M	Tail	2.0 cm	Yes	Yes	✓	X	X
12	–	–	–	10	F	Body	1.0 cm	Yes	Yes	–	–	–
13	TSC2	c.4646 A>G	Pathogenic	18	M	Body	1.1 cm	No	Yes	✓	X	X
14	–	–	–	15	M	Tail	2.3 cm	No	Yes	–	–	–
15	TSC2	c.5238_5255del18	Pathogenic	9	F	Head	1.6 cm	Yes	No	✓	X	X
16	–	–	–	32	M	Head	3.8 cm	Yes	Unknown	–	–	–

✓, receiving imaging follow up; X, not receiving imaging follow up; –, no information provided.

(**Table 1**). **Table 2** summarizes the 13 publications that describe 19 patients with TSC and nonfunctional PNET.

Demographics

At the time of data extraction, the median age was 18.0 years (range 3–55 years, IQR: 15.5–25). **Table 3** shows the demographics, age of PNET diagnosis, and method of diagnosis in the case series and the individuals reported in the medical literature with a nonfunctional PNET and TSC. In the case series, sex was relatively equal with 56.3% ($n = 9$) male and 43.7% ($n = 7$) female. The age of TSC diagnosis was available for 14 of the patients. Most ($n = 9$, 64%) were diagnosed with TSC at <1 year of age with a median age of diagnosis of 8.5 months (range: 1 month to 7 years; IQR 3.5 months to 1 year). Results from genetic testing were available for just over half (56.3%, $n = 9$) of the patients. Information on *TSC1* and *TSC2* variants is in **Table 1**.

In the case series, half of the individuals ($n = 8$) had a history of treatment with a systemic mTOR inhibitor (oral everolimus or sirolimus). Of those, 63% ($n = 5$) used a systemic mTOR inhibitor

TABLE 2 | Clinical information from individuals with TSC diagnosed with nonfunctional PNETs reported in the medical literature.

Publication	TSC1/TSC2	Exon/c.	Age at PNET Dx	Sex	Location	Single/Multiple	Diameter	Management
Ilgren et al. (35)	Unknown	–	23 years	F	–	Single	–	Discovered during autopsy
Verhoef et al. (36)	TSC2	Exon 12	12 years	M	Tail	Single	9.5 cm	Malignant, surgical removal
Francalanci et al. (30)	TSC2	Exon 33	6 years	M	Body/Tail	Single	–	Malignant, surgical removal
Merritt et al. (29)	TSC2	1 bp insertion at position 45-46	39 years	M	Body/Tail	Multiple	–	Surgical removal
Larson et al. (34)	Unknown	–	39 years	M	Tail	Single	4.8 cm	Cystic
	Unknown	–	48 years	M	Tail	Single	3.7 cm	Cystic and enlarged by 25% in 5 years
	Unknown	–	51 years	F	–	–	–	Discovered during autopsy
van den Akker et al. (33)	Unknown	–	–	M	Unknown	Single	–	Surgical removal
Diaz et al. (32)	Unknown	–	31 years	M	Tail	Single	2.3 cm	Surgical removal
Arva et al. (31)	TSC2	Transition A>G in IVS17-2	15 years	M	Body, Tail	Multiple	8.2 cm 1.2 cm	Malignant, surgical removal
Bombardieri et al. (7)	TSC2	c.5160+2_5160+3insT	10 years	M	Head	Single	3.3 cm	Surgical removal
Mortaji et al. (8)	TSC1	Exon 15 c.1530_1531delCA	35 years	F	Tail	Single	1.1 cm	Surgical removal
Koc et al. (9)	Unknown	–	12 years	M	Tail	Single	1 cm	Surgical removal
	Unknown	–	5 years	M	Tail	Single	2.6 cm	Reduced in size on everolimus and then surgical removal
	Unknown	–	19 years	F	Body	Single	2.7 cm	Clinical observation; stable size on everolimus
	Unknown	–	13 years	M	Tail	Single	4.0 cm	Clinical observation; reduced in size on everolimus
	Unknown	–	14 years	M	Tail	Single	0.2 cm	Clinical observation
Mehta et al. (10)	TSC1	Exon 10 c.989dupT	3 years	M	Body	Single	0.4 cm	Surgical removal at 1 cm
Amarjothi et al. (11)	Unknown	–	17 years	F	Head	Single	2.5 cm	Surgical removal

during the time of image acquisition in which the nonfunctional PNET was identified and/or followed serially (**Figure 2**).

Genetic Testing Results

Just over half (56.3%, $n = 9$) of the patients provided results from genetic testing and 43.7% ($n = 7$) either did not undergo genetic testing and/or did not provide results from genetic testing (**Table 1**). Of the nine individuals who provided results from genetic testing, four variants were found in *TSC1,* and six variants were identified in *TSC2*. There was one individual who was found to have a one pathogenic variant and one benign variant in *TSC1*. Of the 4 variants identified in *TSC1*, three were classified as pathogenic and one was classified as benign. Of the six variants identified in *TSC2*, five were classified as pathogenic and one was classified as a variant of uncertain significance (VUS).

Imaging Modality of PNET Identification

In the case series, the median age of nonfunctional PNET diagnosis was 12.5 years with a range from 3 to 46 years of age (IQR: 8.5–17) (**Table 3**). Most individuals (93.8%, $n = 15$) had their tumors incidentally identified on routine imaging. MRI was the modality on which PNET was more commonly identified ($n = 9$, 56.3%), followed by ultrasound ($n = 4$, 25%) and CT ($n = 2$, 12.5%). One individual's tumor was diagnosed during a distal pancreatectomy and splenectomy. The location of the PNET was 43.8% ($n = 7$) in the body of the pancreas, 37.5% ($n = 6$) in the tail of the pancreas, and 18.7% ($n = 3$) in the head of the pancreas. Of note, the diagnosis of the nonfunctional PNET was delayed in 3 patients. Retrospective review of prior imaging revealed the presence of the nonfunctional PNET on a previous imaging series. In these 3 cases, the sizes of the missed PNETs were 8 mm on MRI (diagnosed a year later at 11 mm on MRI), 13 mm on MRI (diagnosed 3 years later at 15 mm on MRI) and 43 mm on MRI (diagnosed a year later at 41 mm on CT). **Figures 3A–D** represents the initial and subsequent abdominal MRIs of a nonfunctional PNET that was not diagnosed on the original imaging.

Follow-Up Management and Tumor Growth

Over half of the individuals ($n = 9$) underwent surgical intervention for their nonfunctional PNET. Surgical status was unknown for one patient. Due to the retrospective nature of this study, the clinical indication for the surgeries was not reported to the authors. Clinical imaging follow-up was available for

TABLE 3 | Demographic information of patients in this case series and published case reports.

	Frequency, *n* (%)†	
	Case series (*n* = 16)	**Reported cases (*n* = 19)**
Age at data collection		
≤19 years	10 (62.5)	62.5 (11)
20–39 years	4 (25)	25 (5)
40–59 years	2 (12.5)	12.5 (2)
Sex		
Male	9 (56.3)	56.3 (14)
Female	7 (43.7)	43.7 (5)
Age at TSC diagnosis		
0–11 months	8 (57.1)	57.1 (2)
1–3 years	5 (31.3)	31.3 (2)
≥4 years	1 (6.2)	6.2 (4)
Unknown	2 (12.5)	12.5 (11)
Molecular diagnosis of TSC		
Yes	8 (50.0)	50.0 (7)
No or unknown	8 (50.0)	50.0 (12)
Age at PNET diagnosis, years, median (IQR)*	12.5 (8.5 - 17)	16 (12 - 34)
Age of PNET diagnosis		
≤10 years	7 (43.8)	43.8 (4)
11–20 years	6 (37.5)	37.5 (7)
21–30 years	1 (6.2)	6.2 (1)
31–40 years	1 (6.2)	6.2 (4)
≥41 years	1 (6.2)	6.2 (2)
Initial diagnosis method		
MRI	9 (56.3)	56.3 (8)
CT	2 (12.5)	12.5 (5)
Ultrasound	4 (25.0)	25.0 (3)
Other	1 (6.2)	6.2 (2)

†*Unless otherwise stated; *IQR, interquartile range.*

12 patients. Of these patients, six individuals did not undergo surgical intervention and only had serial imaging follow-up (**Table 1**). MRI was used for follow-up imaging in all but one patient. CT was solely utilized in one subject and ultrasound was utilized for follow-up for only one patient but was used in combination with MRI.

In the case series, data on the size of the nonfunctional PNET at the time of diagnosis was available for 15 patients. The median size of the PNET at that time was 15 mm (interquartile range: 11–19 mm; range: 7–40 mm). There was a strong positive correlation ($\rho = 0.74$, 95% confidence interval = 0.37–0.91; $p = 0.002$) between the age at diagnosis of the PNET and its size in our cohort (**Figure 4**). A linear mixed model of our cohort data suggested that for every year increase in the age of diagnosis, the PNET size at the time of diagnosis was increased by 1.04 mm (95% confidence interval = 0.47–1.60 mm).

Twelve of the sixteen total patients had two or more imaging studies performed where the PNET could be visualized and measured longitudinally. The average rate of change of the PNET was an increase of 2.0 mm/year but varied considerably from person to person and even within a person over time (**Figure 2**). Overall, the median rate of change per patient was 1.02 mm/year (IQR: 0.0–5.02) and ranged from a decrease in size of 5.7 mm/year to an increase of 13 mm/year, with standard deviations for these individual patient size changes ranging from 1.4 to 11.0 mm/year. Panel data analysis using mixed models that adjusted for age of the patient and use of mTOR inhibitors, identified an independent effect of time on the size of the PNET, with an average increase of 0.95 mm per year (95% CI: 0.54–1.36 mm). When adjusted for the age of the patient and location of the PNET, the mixed models also demonstrated an association between mTOR use and the size of the PNET with patients on mTOR inhibitors having PNETs that were smaller compared to the size measured in patients not taking mTOR inhibitors (difference of 5.5 mm, 95% CI: 2.1–9.0 mm) (**Figure 2**).

We had information on 12 subjects regarding tumor growth over time. Of those, five were on systemic mTOR therapy. Three demonstrated a decrease in the tumor size (subjects 1, 11, and 15), one was stable (subject 16), and one demonstrated an increase in tumor size (subject 3). Of the 7 without any mTOR treatment, 4 showed a spontaneous decrease in tumor size (2, 8, 9, and 13) at some points in time but growth at other time points. Tumor growth rate was slightly less in individuals taking an mTOR inhibitor (−0.8 mm/yr, IQR: −2.3 to 2.2) than those without (1.6 mm/yr; IQR: −0.99 to 5.01) but the difference was not statistically significant ($p > 0.05$).

DISCUSSION

This case series reports 16 new cases of nonfunctional PNETs in association with TSC. New cases were identified from the TS Alliance's Natural History Database that follows 2,223 TSC subjects across the United States and from Cincinnati Children's Hospital TSC Database that follows an additional 357 subjects. We documented rate of growth, age of diagnosis of nonfunctional PNETs, location, management and growth rate in the presence and absence of mTOR inhibitors. Our study adds to the growing literature of reported nonfunctional PNETs in hopes to encourage the creation of consensus guidelines and utilization of mTOR inhibitor therapy for this rare clinical feature of TSC.

Frequency

The number of reported functional and nonfunctional PNETs associated with TSC has increased over the last decade, likely coinciding with 2012 consensus recommendations for abdominal MR imaging every 1–3 years to detect and monitor renal angiomyolipoma (2). Indeed, nearly all our cases were diagnosed as unexpected findings during recommended surveillance. The estimated frequency of nonfunctional PNET in our case series is 0.65%. The reported prevalence in the general population of 0.003% (15, 16, 39). Whereas, the prevalence reported for PNET in association with other genetic syndromes such as MEN-1, VHL, and NF-1 is 80%, 9–17%, and <10%, respectively (18–22, 40).

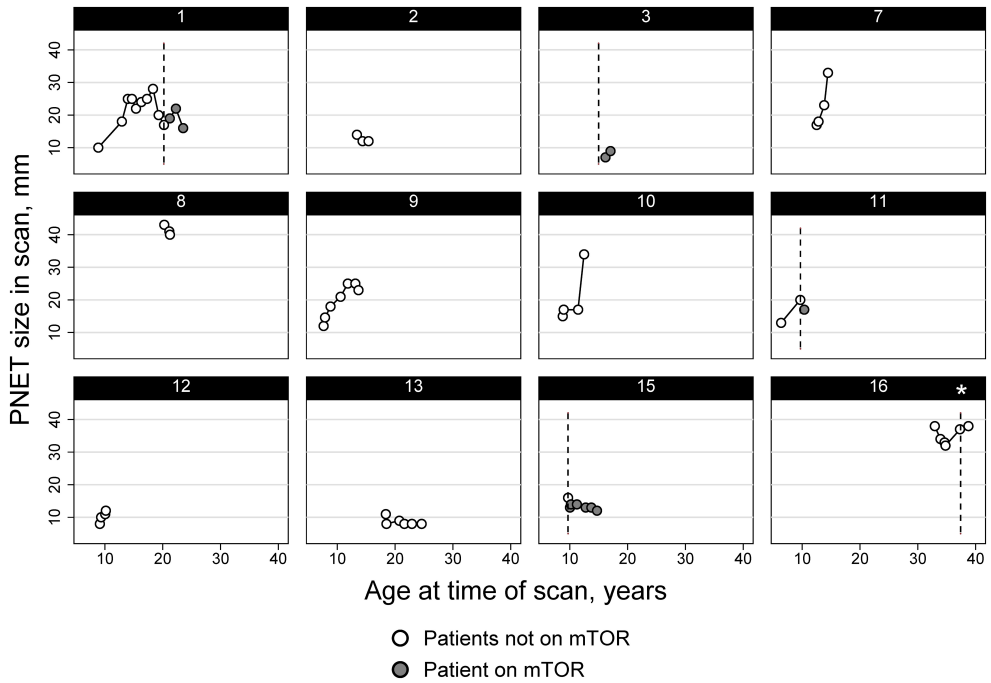

FIGURE 2 | Diameter trend of PNET in subjects from the TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database. Asterisk indicates that Subject 16 started and stopped an mTOR inhibitor therapy between imaging and never had any imaging performed while actively taking an mTOR inhibitor.

Delayed Diagnosis

Prior single center TSC studies have estimated PNET prevalence of nonfunctional and functional PNETs to be between 4 and 9% (9, 34). The TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database utilized in our study provided a starting cohort much larger in size and representative of patients with TSC across many different TSC centers in the United States, but did have the limitation that identification of PNET cases in our study depended on numerous outside radiologists' recognition and reporting of PNET in final imaging reports rather than study-specific review of the individual's imaging. As a result, our study identified that 3/16 (19%) tumors in our cohort were retrospectively found to be present on prior imaging but were not identified or reported initially. The tumor diameter differences between measurements was larger than MRI spatial resolution. Therefore, the frequency in our study likely underestimates true frequency of nonfunctional PNET in TSC. Under-recognition of PNETs is important to acknowledge, as pancreatic findings are not commonly expected in TSC, and the primary purpose for abdominal MRI is for diagnosis and surveillance of renal findings rather than pancreatic. Under these circumstances, radiologists may miss small pancreatic tumors or misdiagnose them as being pancreatic angiomyolipomas, which have been reported in association with TSC (41). This information particularly highlights the importance of paying special attention to the pancreas on surveillance abdominal MRIs of individuals with TSC and compare to previous images.

Age of Nonfunctional PNET Diagnosis

Younger individuals with TSC included in our cohort were more likely to have abdominal MRIs in childhood given their current age in relation with the establishment of the surveillance guidelines in 2012. Given this, it is possible that the older individuals with TSC could have received their diagnosis of a nonfunctional PNET earlier through what is now standardized surveillance recommendations. Even when considering this limitation, PNETs in the general population occur with peak prevalence between 70–80 years of age and are highly associated with malignancy. Surprisingly, the average age of PNET in TSC appears to be young (42, 43). The median age in our cohort was 18 years at diagnosis, with 88% under the age of 40 years and no one diagnosed older than 60 years. Prior published cases demonstrate similar age prevalence (**Table 3**). Also, it is notable that none of the previously mentioned genetic syndromes have surveillance protocols with abdominal imaging for individuals younger than 16 years of age; therefore, their PNET prevalence may be under-estimated due to lack of surveillance guidelines for younger subjects in comparison to individuals with TSC. In comparable studies of nonfunctional PNETs associated with MEN-1 diagnosed by endoscopic ultrasonography, the mean age of diagnosis is 30 years (range: 13–65 years) and reports that 18% of MEN-1 males had a diagnosis by 20 years of age (42, 43).

Genotype-Phenotype Correlations

In this study, it was difficult to arrive at conclusions on genotype-phenotype correlations in relation to nonfunctional PNETs due

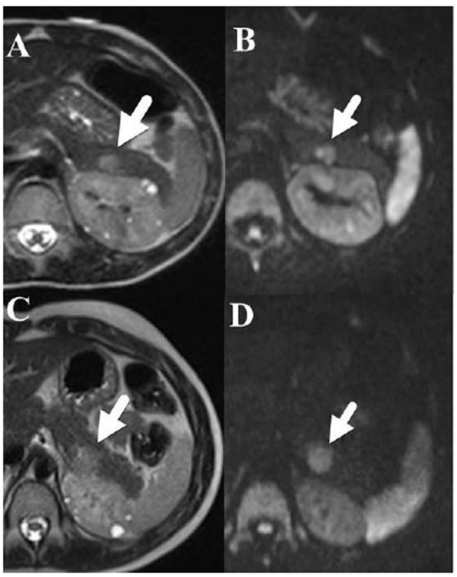

FIGURE 3 | (A) [Upper left] Axial T2-weighted fast spin echo and **(B)** [Upper right] diffusion weighted MRI images performed in a 6-year-old boy show a 1.2 × 0.8 cm hyperintense mass (arrow) in the tail of the pancreas that restricts diffusion. Note that small cysts are also present within the left kidney. **(C)** [Lower left] an Axial T2-weighted fast spin echo and **(D)** [Lower right] diffusion weighted MRI images performed 3 years later show that the pancreatic tail mass (arrow) has grown slowly and now measures 1.4 × 1.5 cm.

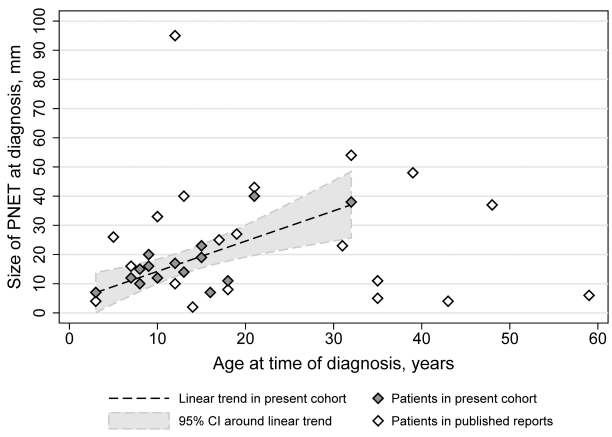

FIGURE 4 | PNET diameter as a function of age in TSC subjects of the TS Alliance's Natural History Database, the Cincinnati Children's Hospital TSC Database, and previously published cases.

to the limited size of this case series. Genotype information was available in nine subjects. Six had *TSC2* pathogenic variants and 3 had *TSC1* pathogenic variants (**Table 1**). In previous publications, molecular diagnosis was available in 7 out of the 19 cases (**Table 2**). Five cases were reported having pathogenic variants in *TSC2* and two cases had *TSC1* pathogenic variants. Based on the data available from previous publications and our case series, the proportion of nonfunctional PNET cases due to *TSC2* compared to *TSC1* reflects the mutation spectrum described in TSC rather than suggests an increased occurrence of nonfunctional PNETs with *TSC2* pathogenic variants. Furthermore, there was not any correlation between location or type of pathogenic variants with the likelihood of development or clinical characteristics of nonfunctional PNETs in our series or previously reported cases.

Management of Nonfunctional PNETs

It is difficult to ascertain if the development of TSC-associated nonfunctional PNETs is an age-related phenomenon that eventually will resolve as many individuals in our cohort had their nonfunctional PNET surgically removed. The current surgical recommendations for sporadic PNETs are to only remove tumors over 20 mm in size or that are doubling faster (24, 44). In our cohort of patients, we documented reduction of the size of the tumors in several individuals over time, but there are no reports of fully self-resolved pancreatic TSC-associated nonfunctional PNETs after introduction of surveillance protocols. This unresolved issue becomes particularly relevant for TSC, a condition for which resolution of neonatal cardiac rhabdomyosarcomas is routinely observed and lack of malignant transformation for most TSC-related tumors (30, 36). In our cohort, there were no instances of malignant nonfunctional PNETs, but they have been three separate case reports by Francalanci et al. (30), Arva et al. (31), and Verhoef et al. (36) that have documented this occurrence. All three cases were males from 6 to 15 years old with germline *TSC2* pathogenic variants. Two of these cases documented loss of heterozygosity (LOH) in tumor tissue (30, 36). Based on the rare occurrence of PNET and the predominance of *TSC2* cases, it is difficult to conclude on specific associations between *TSC2* and male-sex as risk factors for malignant PNETs. Like sporadic PNETs, the recognition of malignant PNETs in association with TSC supports the recommendations of the PNET guidelines for long-term clinical follow up and intend surgical intervention for tumors larger than 2.5 cm in diameter or rapidly growing tumors (24, 44).

Interestingly, we had one documented recurrence of nonfunctional PNET that was confirmed by the clinic site to the authors. This instance occurred 4 years after resection of the initial tumor. To the best of our knowledge, there are no other reported cases of recurrent nonfunctional PNETs in association with TSC. This highlights the importance of continuing the surveillance for these tumors despite initial resection.

There are no reports in the medical literature of functional transformation of nonfunctional PNETs in association with TSC. Additionally, we have not seen this phenomenon documented in the TS Alliance's Natural History Database or Cincinnati Children's Hospital TSC Database. However, there are six reports of transformation of nonfunctional PNETs into functional in cases of sporadic PNETs (18, 45–48). Nahmias et al. (47) described on three adults, two with nonfunctional PNETs and one with a gastrinoma. All three individuals progressed to insulinomas. Two of these cases, the individual with the gastrinoma and one with the nonfunctional PNET, demonstrated tumor reduction with the use of everolimus for a short period

of time, but eventually required surgical removal. Sayki Arslan et al. (48) reported on a PNET measuring 3.1 × 2.7 cm that transformed into a malignant insulinoma in a 62-year-old male. His diagnosis of nonfunctional PNET occurred 3 years before the transformation into an insulinoma. Functional transformation remains a possibility for PNET in association with a TSC diagnosis. Interestingly, these publications provide additional data that argues in favor for the use of mTOR inhibitor treatment for PNETs associated with TSC, in particular for functional PNETs.

Tumor Growth and mTOR Inhibitor Therapy

We had information on 12 subjects regarding tumor growth over time. Of those, five were on systemic mTOR therapy during varying portions of the study period. It is well-known that mTOR inhibitors have showed reduction in TSC-related tumors across many organ systems. The tumor growth rate was slightly less in individuals taking an mTOR inhibitor than those without. Although, our case-series was not powered for this comparison and the difference was not statistically significant, the trend observed was consistent with the reported effect of mTOR inhibitors on PNET growth in TSC and is the basis for the recommendation of its use on advanced PNETs (49). To better understand the natural history of these tumors and their response to mTOR treatment, prospective studies are needed in TSC with standardized imaging paradigms with special attention to potential confounding factors regarding tumor growth.

Study Limitations

This descriptive report is a case series, and as such wasn't powered for comparative analyses, especially for comparisons to other studies. The few comparisons we did perform within our cohort (e.g., tumor growth rate in patients on mTOR inhibitors and those without) were exploratory in nature, and are presented as such. Although the analyses, including the adjusted mixed regression models, are appropriate means of more thoroughly describing our cohort, it should be noted that due to the exploratory nature we have not performed any other statistical corrections for multiple testing. Additionally, our study design does not allow for full recognition of the presence of age-related penetrance of PNETs in TSC and the natural history of these tumors. Additionally, the lack of uniformity across radiologists reading the images and modalities used could introduce measurement error regarding the nonfunctional PNETs size. Lastly, the source of our study sample was composed of individuals followed at specialized centers across multiple clinical sites in the United States, who voluntarily chose to participate in large natural history databases. This might result in a selection bias due to exclusion of individuals with milder disease.

Conclusions

Based on our data, TSC-associated nonfunctional PNETs are slow growing, and the majority appear to be benign or nonmalignant in nature. This study provides preliminary evidence supporting the use of mTOR inhibitor therapy in conjunction with serial imaging as medical management for nonfunctional PNETs as an alternative option to invasive surgical removal. This can easily be integrated into the TSC surveillance recommendations for abdominal MRIs every 1–3 years for monitoring of angiomyolipomas and renal cysts (2). Furthermore, patients with TSC often have other indications for the use of mTOR inhibitors, such as large angiomyolipomas, SEGAs, lymphangioleiomyomatosis, or refractory epilepsy. As described here, mTOR inhibitors may slow the rate of growth of nonfunctional PNETs, but it remains unanswered if the use of mTOR inhibitors should be the initial method of medical management for nonfunctional PNETs or an alternative to surgical removal. Other unresolved issues include the possibility of self-resolution or associated risk factors for these tumors including genotype-phenotype associations, age-related penetrance, and rate of malignancy. Only then will solid evidence-based surveillance and treatment recommendations be possible for nonfunctional PNET occurring in the setting of TSC.

DATA AVAILABILITY STATEMENT

The raw data supporting the conclusions of this article will be made available by the authors, without undue reservation.

ETHICS STATEMENT

The studies involving human participants were reviewed and approved by Institutional Review Board of University of Texas Health Science Center at Houston and Cincinnati Children's Hospital Medical Center. Written informed consent from the participants' legal guardian/next of kin was not required to participate in this study in accordance with the national legislation and the institutional requirements.

AUTHOR CONTRIBUTIONS

KM and DR-B: conceptualization and study design, manuscript writing, data collection, statistical analysis, and manuscript editing. HN: conceptualization and study design, data review, and manuscript editing. PR: manuscript writing, data collection, statistical analysis, and manuscript editing. SH: statistical analysis and manuscript editing. DK, JC, ATT, and AJT: data collection, data review for Cincinnati subjects, and manuscript editing. DE-F: data collection for Cincinnati subjects and critical revision of the manuscript. DF: data collection and data review for Cincinnati subjects. All authors contributed to the article and approved the submitted version.

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Historical Patterns of Diagnosis, Treatments, and Outcome of Epilepsy Associated With Tuberous Sclerosis Complex: Results From TOSCA Registry

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Background: Epilepsy is the most common neurological manifestation in individuals with tuberous sclerosis complex (TSC). However, real-world evidence on diagnosis and treatment patterns is limited. Here, we present data from TuberOus Sclerosis registry to increase disease Awareness (TOSCA) on changes in patterns of epilepsy diagnosis, treatments, and outcomes over time, and detailed epilepsy characteristics from the epilepsy substudy.

Methods: TuberOus Sclerosis registry to increase disease Awareness (TOSCA) was a multicentre, international disease registry, consisting of a main study that collected data on overall diagnostic characteristics and associated clinical features, and six substudies focusing on specific TSC manifestations. The epilepsy substudy investigated detailed epilepsy characteristics and their correlation to genotype and intelligence quotient (IQ).

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Results: Epilepsy was reported in 85% of participants, more commonly in younger individuals (67.8% in 1970s to 91.8% in last decade), while rate of treatments was similar across ages (>93% for both infantile spasms and focal seizures, except prior to 1960). Vigabatrin (VGB) was the most commonly used antiepileptic drugs (AEDs). Individuals with infantile spasms showed a higher treatment response over time with lower usage of steroids. Individuals with focal seizures reported similar rates of drug resistance (32.5–43.3%). Use of vagus nerve stimulation (VNS), ketogenic diet, and surgery remained low.

Discussion: The epilepsy substudy included 162 individuals from nine countries. At epilepsy onset, most individuals with infantile spasms (73.2%) and focal seizures (74.5%) received monotherapies. Vigabatrin was first-line treatment in 45% of individuals with infantile spasms. Changes in initial AEDs were commonly reported due to inadequate efficacy. TSC1 mutations were associated with less severe epilepsy phenotypes and more individuals with normal IQ. In individuals with TSC diagnosis before seizure onset, electroencephalogram (EEG) was performed prior to seizures in only 12.5 and 25% of subsequent infantile spasms and focal seizures, respectively.

Conclusions: Our study confirms the high prevalence of epilepsy in TSC individuals and less severe phenotypes with *TSC1* mutations. Vigabatrin improved the outcome of infantile spasms and should be used as first-line treatment. There is, however, still a need for improving therapies in focal seizures. Electroencephalogram follow-up prior to seizure-onset should be promoted for all infants with TSC in order to facilitate preventive or early treatment.

Keywords: epilepsy, registry, TOSCA, TSC, tuberous sclerosis complex

INTRODUCTION

Epilepsy is a common manifestation of tuberous sclerosis complex (TSC), affecting 80–90% of individuals (1, 2). It usually presents during the first year of life with infantile (epileptic) spasms or focal seizures. Focal seizures remain the most frequent type after the first year of life, but individuals with TSC may develop almost all seizure types. In about two-thirds of individuals with TSC, seizures are refractory to anticonvulsant treatment (3), a much higher proportion than the 23% reported in the general epilepsy population (4). Epilepsy is associated with a wide range of TSC-associated neuropsychiatric disorders (TAND) including intellectual disability (ID), autism spectrum disorder (ASD), and attention deficit hyperactivity disorder (ADHD), as well as impaired health-related quality of life (HRQoL) (5–9).

Treatment options for TSC-associated epilepsy in the first year of life are specific to infantile spasms because of high rates and individuals' responsiveness to vigabatrin (VGB), a first-line treatment option. Antiepileptic drug (AED) recommendations in TSC after the age of 1 year are the same as in the general epilepsy population based on seizure types. Candidates for epilepsy surgery should be identified early in the course of the disease. Other non-pharmacological treatment options including ketogenic diet, and vagus nerve stimulation (VNS) should also be considered early if the epilepsy is refractory (10). Evidence supports the use of mammalian target of rapamycin (mTOR)-inhibitors as adjunctive treatment to AEDs for treating focal epilepsy in TSC individuals, with a higher response rate in the younger subgroup aged below 6 years (11–13). Given the early onset, severity and significant impact of TSC-associated epilepsy on quality of life (QoL) (3, 5, 6), there is value in longitudinal population-based studies of detailed epilepsy characteristics.

The TuberOus Sclerosis registry to increase disease Awareness (TOSCA), which included individuals from 170 sites in 31 countries, was conceived to expand our understanding of different TSC manifestations, treatment patterns, and outcomes (14). TuberOus Sclerosis registry to increase disease Awareness consisted of a main study representing the diagnostic characteristics and associated clinical features, and six substudies, each focusing on specific TSC manifestations. In our initial publication, we reported characteristics of TSC-associated epilepsies (2). The key observations were (a) a typical onset pattern of focal seizures and infantile spasms in the first two years of life, (b), high rates of drug resistance in focal seizures compared to infantile spasms, and (c) a low proportion of individuals treated with non-pharmacological therapies, including epilepsy surgery. Here, we present data from the TOSCA final analysis, describing rates of epilepsy, treatment interventions, and outcomes over time. We also report findings

from the epilepsy substudy, a TOSCA research project, aimed at reporting more detailed epilepsy characteristics including time to epilepsy diagnosis, electroencephalogram (EEG) patterns, and therapies.

METHODS

TuberOus Sclerosis registry to increase disease Awareness was a multicentre, international disease registry. The study methods have been reported in detail previously (14). In the main study, general background information (i.e., demographic data, family history, pre-natal history, and disease features such as neurological and neuropsychiatric, renal, cardiovascular, pulmonary, dermatological, and others) were collected retrospectively at baseline (first inclusion visit) followed by prospective data collection during an observation period of up to 5 years. Follow-up visits were scheduled according to the standard practice of the site and per the treating physician's best judgement, but at minimum intervals of 12 months. Data were retrieved from clinical records, electronic medical records, individuals' questionnaires, and *ad-hoc* clinical databases. Research projects were designed to record additional, more detailed data related to specific disease manifestations [i.e., subependymal giant cell astrocytoma (SEGA), renal angiomyolipoma, lymphangioleiomyomatosis, genetics, TAND, QoL, and epilepsy].

Participants and Procedure

Individuals of any age who fulfilled clinical criteria for TSC diagnosis and a documented clinical visit for TSC within the past 12 months or newly diagnosed with TSC were enrolled in the main study. Investigators, specialized in epilepsy care, from 27 sites across nine countries (Belgium, France, Germany, Italy, Poland, Slovenia, Spain, Japan, and Turkey) participated in the epilepsy substudy.

Given the observational nature of the study, both diagnostic and treatment/management were performed according to local best practice. The study protocol, therefore, did not request any particular additional clinical or laboratory investigations.

Both main and substudy were 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 individuals, parents, or guardians prior to enrolment, with prior endorsement by the local human research ethics committee.

Data Analyses

From the main study, we present epilepsy characteristics, emphasizing changes in the rates of epilepsy diagnosis, treatment, and outcome over time. From the epilepsy substudy, we report characteristics [age of onset, frequency, tuber numbers, treatments and treatments outcomes, and intelligence quotient (IQ) level] of individuals with infantile spasms and focal seizures and correlated them to genotype. We report the impact of epilepsy characteristics and EEG foci on intellectual ability, date of EEG compared to the date of the seizure onset in individuals with focal seizures and infantile spasms with TSC diagnosis prior to seizure onset, number of AEDs used at epilepsy diagnosis, and the reasons for changes in the AED regimen. Intellectual ability was categorized as normal (IQ $>$ 70), mild ID (IQ 51–70), moderate ID (IQ 36–50), severe ID (IQ 20–35), and profound ID (IQ $<$ 20). The response of individuals with infantile spasms was defined as follows: spasm-free + hypsarrhythmia resolved + normalized EEG or spasms free with disappearance of hypsarrhythmia, but persistent EEG anomalies. Efficacy in focal seizures was defined as $>$50% decrease in seizure frequency with rates of seizure freedom and response of $>$75%.

All eligible individuals enrolled in the TOSCA registry and epilepsy substudy, without any major protocol deviations, were included. As the study was observational in nature, primarily descriptive statistic methods were used. Continuous variables were evaluated quantitatively (frequency, mean, standard deviation, median, range), and categorical variables (presence/absence of a manifestation) were analyzed in terms of frequency distribution at baseline and at follow-up visits.

RESULTS

Findings From the Final Analysis of the Main Study

Clinical Characteristics of Epilepsy

Of the 2,211 individuals enrolled in the TOSCA main study, 1,879 (85%) were reported to have epilepsy. Of these, 942 (50.1%) were female and 937 (49.9%) were male (**Table 1**).

TABLE 1 | Demographics and characteristics of individuals with TSC and epilepsy.

Characteristics	Individuals
No. of individuals with epilepsy, *n* (%)	1,879 (85.0)
Sex, *n* (%)	
Male	937 (49.9)
Female	942 (50.1)
Age of individuals in years, *n* (%)	
≤2	257 (13.7)
>2 to ≤5	282 (15.0)
>5 to ≤9	311 (16.6)
>9 to ≤14	276 (14.7)
>14 to ≤18	126 (6.7)
>18 to ≤40	499 (26.6)
≥40	128 (6.8)
Type of epilepsy, *n* (%)	
Infantile spasms	735 (39.1)
Focal seizures	1,343 (71.5)
Other seizures	537 (28.6)
Median (range) age at diagnosis, years	
Infantile spasms	<1 (0–30)
Focal seizures	1 (0–66)
Genetic analysis	
Individuals with mutational analysis data available, *n* (%)	849 (45.2)
TSC1	155 (18.3)
TSC2	587 (69.1)
No mutation identified	107 (12.6)

SD, standard deviation.

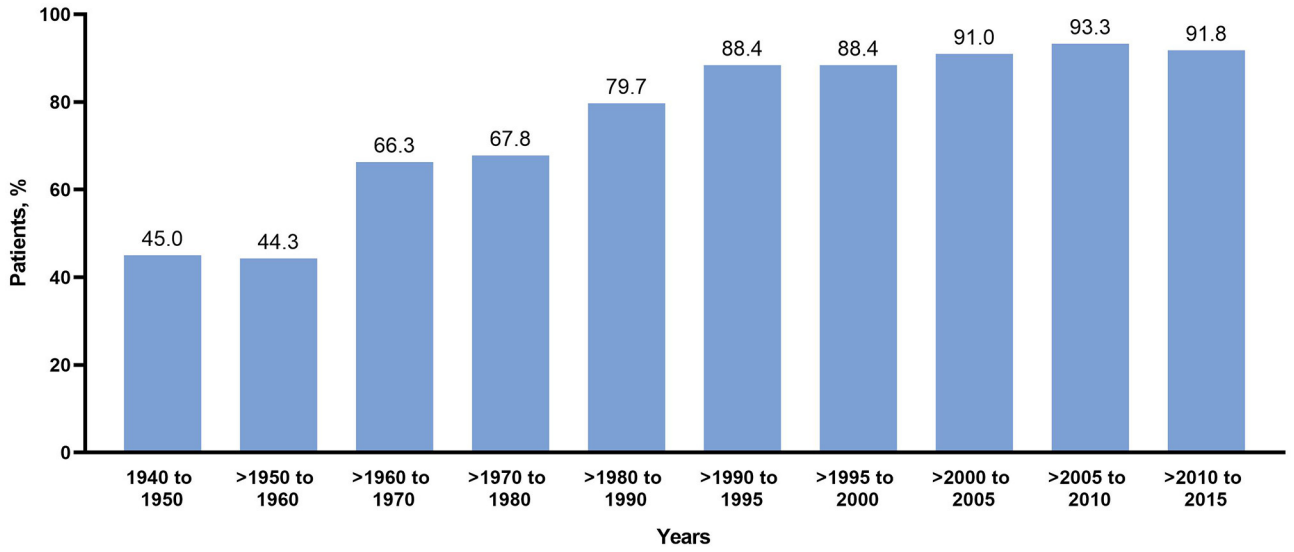

FIGURE 1 | Rates of diagnosis of infantile spasms and focal seizures over time.

Focal seizures were reported in 1,343 individuals (71.5%), while infantile spasms were reported in 735 (39.1%). Five hundred and thirty-seven individuals (28.6%) were reported to have other seizure types. The median age at diagnosis was 1 year (range 0–66) for focal seizure and <1 year (range 0–30) for infantile spasms, respectively. Genetic data were available for 849 individuals with epilepsy; 587 (69.1%) had pathogenic mutations in the *TSC2* gene, while 155 (18.3%) had mutations in the *TSC1* gene. In 107 individuals (12.6%), no mutations were identified.

Epilepsy Diagnosis, Treatment, and Outcome Patterns Over Time

Epilepsy diagnosis was more common in younger individuals, ranging from 67.8% in the 1970s to 91.8% in the last decade (**Figure 1**, **Table 2**). The rates of infantile spasms diagnosis increased from 24.6% in the 1960s to about 41.4% from the 1990s. The rates of focal seizures diagnosis increased from 29.6% in the 1950s to about 84% in 2000s (**Figure 1**).

More than 93% reported treatment for infantile spasms or focal seizures after 1960 (**Table 2**). Vigabatrin was the most commonly used AEDs in TSC individuals with infantile spasms and focal seizures in any year, with usage increasing over time and a clear shift after the late 1990s (>1950–1960: 33 and 50%; >1960–1970: 37.5 and 42.3%; >1970–1980: 40.7 and 50.9%; >1980–1990: 68.2 and 61.8%; >1990–1995: 62.9 and 66.7%, >1995–2000: 83.3 and 77.1%; >2000–2005: 86.2 and 69.1%; >2005–2010: 88.1 and 73.3%; >2010–2015: 91.2 and 76.9%). In contrast, usage of steroids for infantile spasms was at a peak (reported in 33.9%) between 1990 and 1995, decreasing thereafter (>1995–2000:16.7%; >2000–2005: 13.8%; >2005–2010: 14.2%; >2010–2015: 10.0%).

Epilepsy surgery for infantile spasms was reported in only one of 66 individuals (1.5%) between 1980 and 1990. Epilepsy surgery appeared as an alternative treatment for infantile spasms since 2000 and reached a peak in recent years (8.0% during 2005 and 2010). In individuals with focal seizures, use of the ketogenic diet was first reported in two individuals (1.5%) in 1980 with more regular use since 1995. Use of VNS was first reported in the late 1980s in patients with infantile spasms. Use of VNS showed a peak at the beginning of the 2000s (reported in 6% of individuals with infantile spasms and 7.8% in those with focal seizures), but there was a clear decrease thereafter. In contrast, the ketogenic diet showed a slow increase since its first use in this cohort but did not exceed 9% for infantile spasms and 12% for focal seizures. The use of mTOR inhibitors was reported in 17.1% of individuals with infantile spasms and 18.1% of individuals with focal seizures between 2010 and 2015.

Over time, individuals with infantile spasms responded better to treatment than those with focal seizures (**Table 2**); those with infantile spasms achieved a high response rate with a plateau since the late 1990s. This correlated to an increased use of VGB and a decreased use of steroids (**Figure 2**, **Table 2**). Outcome of focal seizures did not vary much since the 1960s, plateauing between 56 and 64% (**Table 2**).

Findings From the Epilepsy Substudy

A total of 162 individuals (65 adults and 97 children) from 27 sites across nine countries were enrolled into the epilepsy substudy; 74 (45.7%) were males and 88 (54.3%) were females. The median age at enrolment was 14 years (range 2–63 years). Median duration of epilepsy prior to enrolment was 12 years (range 1–63 years).

Information about the type of treatment at epilepsy diagnosis was available in 68 of 71 individuals with infantile spasms and in 88 of 94 of those with focal seizures; 52 individuals (73.2%) with infantile spasms and 70 (74.5%) with focal seizures received monotherapies, while 16 (22.5%) with infantile spasms and 18 (19.1%) with focal seizures received polytherapies.

TABLE 2 | Rates of epilepsy and treatments over time among individuals with TSC and epilepsy.

Characteristics	1940 to 1950 *N* = 20	>1950 to 1960 *N* = 61	>1960 to 1970 *N* = 104	>1970 to 1980 *N* = 183	>1980 to 1990 *N* = 265	>1990 to 1995 *N* = 172	>1995 to 2000 *N* = 241	>2000 to 2005 *N* = 323	>2005 to 2010 *N* = 461	>2010 to 2015 *N* = 380
Individuals ever had epilepsy, *n* (%)	9 (45.0)	27 (44.3)	69 (66.3)	124 (67.8)	212 (79.7)	152 (88.4)	213 (88.4)	294 (91.0)	430 (93.3)	349 (91.8)
Type of epilepsy[a]										
Infantile spasms	0	4 (14.8)	17 (24.6)	29 (23.4)	70 (33.0)	63 (41.4)	80 (37.6)	122 (41.5)	178 (41.4)	172 (49.3)
Focal seizures	5 (55.6)	8 (29.6)	27 (39.1)	57 (46.0)	135 (63.7)	93 (61.2)	168 (78.9)	246 (83.7)	361 (84.0)	243 (69.6)
Other seizures	4 (44.4)	19 (70.4)	42 (60.9)	66 (53.2)	79 (37.3)	64 (42.1)	52 (24.4)	54 (18.4)	84 (19.5)	73 (20.9)
Infantile spasms										
Individuals received treatment, *n* (%)	0	3 (75.0)	16 (94.1)	27 (93.1)	66 (94.3)	62 (98.4)	78 (97.5)	116 (95.1)	176 (98.9)	170 (98.8)
Type of treatment, *n* (%)[b]	–									
VGB		1 (33.3)	6 (37.5)	11 (40.7)	45 (68.2)	39 (62.9)	65 (83.3)	100 (86.2)	155 (88.1)	155 (91.2)
ACTH	–	0	3 (18.8)	6 (22.2)	20 (30.3)	21 (33.9)	13 (16.7)	16 (13.8)	25 (14.2)	17 (10.0)
Ketogenic diet	–	0	1 (6.3)	0	1 (1.5)	0	2 (2.6)	10 (8.6)	7 (4.0)	13 (7.6)
Fructose derivatives	–	0	0	0	0	2 (3.2)	2 (2.6)	3 (2.6)	5 (2.8)	4 (2.4)
Vagus nerve stimulation	–	0	0	0	1 (1.5)	2 (3.2)	1 (1.3)	7 (6.0)	7 (4.0)	0
mTOR inhibitors	–	0	1 (6.3)	1 (3.7)	4 (6.1)	1 (1.6)	4 (5.1)	4 (3.4)	16 (9.1)	29 (17.1)
Surgery	–	0	0	0	1 (1.5)	0	0	9 (7.8)	14 (8.0)	12 (7.1)
Other	–	2 (66.7)	13 (81.3)	17 (63.0)	39 (59.1)	35 (56.5)	36 (46.2)	39 (33.6)	83 (47.2)	78 (45.9)
Treatment outcome, *n* (%)										
Resolved spontaneously	–	0	1 (6.3)	0	2 (3.0)	7 (11.3)	0	2 (1.7)	9 (5.1)	1 (0.6)
Controlled	–	3 (100.0)	9 (56.3)	19 (70.4)	44 (66.7)	43 (69.4)	69 (88.5)	99 (85.3)	138 (78.4)	137 (80.6)
Not-controlled	–	0	5 (31.3)	3 (11.1)	14 (21.2)	8 (12.9)	7 (9.0)	12 (10.3)	26 (14.8)	31 (18.2)
Unknown	–	0	1 (6.3)	5 (18.5)	6 (9.1)	4 (6.5)	2 (2.6)	3 (2.6)	3 (1.7)	1 (0.6)
Focal seizures										
Individuals received treatment, *n* (%)	5 (100.0)	8 (100.0)	26 (96.3)	53 (93.0)	131 (97.0)	90 (96.8)	166 (98.8)	243 (98.8)	360 (99.7)	238 (97.9)
Type of treatment [b]										
VGB	1 (20.0)	4 (50.0)	11 (42.3)	27 (50.9)	81 (61.8)	60 (66.7)	128 (77.1)	168 (69.1)	264 (73.3)	183 (76.9)
ACTH	0	0	0	1 (1.9)	4 (3.1)	2 (2.2)	3 (1.8)	9 (3.7)	13 (3.6)	9 (3.8)
Ketogenic diet	0	0	0	0	2 (1.5)	0	6 (3.6)	26 (10.7)	16 (4.4)	28 (11.8)
Fructose derivatives	0	0	4 (15.4)	4 (7.5)	8 (6.1)	7 (7.8)	7 (4.2)	15 (6.2)	19 (5.3)	19 (8.0)
Vagus nerve stimulation	0	0	0	2 (3.8)	8 (6.1)	10 (11.1)	8 (4.8)	19 (7.8)	13 (3.6)	3 (1.3)
mTOR inhibitors	0	0	1 (3.8)	9 (17.0)	17 (13.0)	10 (11.1)	24 (14.5)	40 (16.5)	37 (10.3)	43 (18.1)
Surgery	0	0	0	2 (3.8)	8 (6.1)	6 (6.7)	10 (6.0)	25 (10.3)	35 (9.7)	21 (8.8)
Other	5 (100.0)	5 (62.5)	23 (88.5)	39 (73.6)	96 (73.3)	61 (67.8)	116 (69.9)	179 (73.7)	244 (67.8)	166 (69.7)
Treatment outcome, *n* (%)										
Resolved spontaneously	0	0	0	0	0	1 (1.1)	1 (0.6)	2 (0.8)	6 (1.7)	0
Controlled	3 (60.0)	5 (62.5)	18 (69.2)	30 (56.6)	75 (57.3)	50 (55.6)	106 (63.9)	139 (57.2)	212 (58.9)	134 (56.3)
Not-controlled	2 (40.0)	3 (37.5)	7 (26.9)	21 (39.6)	52 (39.7)	39 (43.3)	54 (32.5)	98 (40.3)	137 (38.1)	100 (42.0)
Unknown	0	0	1 (3.8)	2 (3.8)	4 (3.1)	0	5 (3.0)	4 (1.6)	5 (1.4)	4 (1.7)

ACTH, adrenocorticotropic hormone; mTOR, mammalian target of rapamycin; VGB, vigabatrin.

[a] *Individuals may have more than one type of epilepsy.*

[b] *Individuals may have received treatment as monotherapy or as combination therapy.*

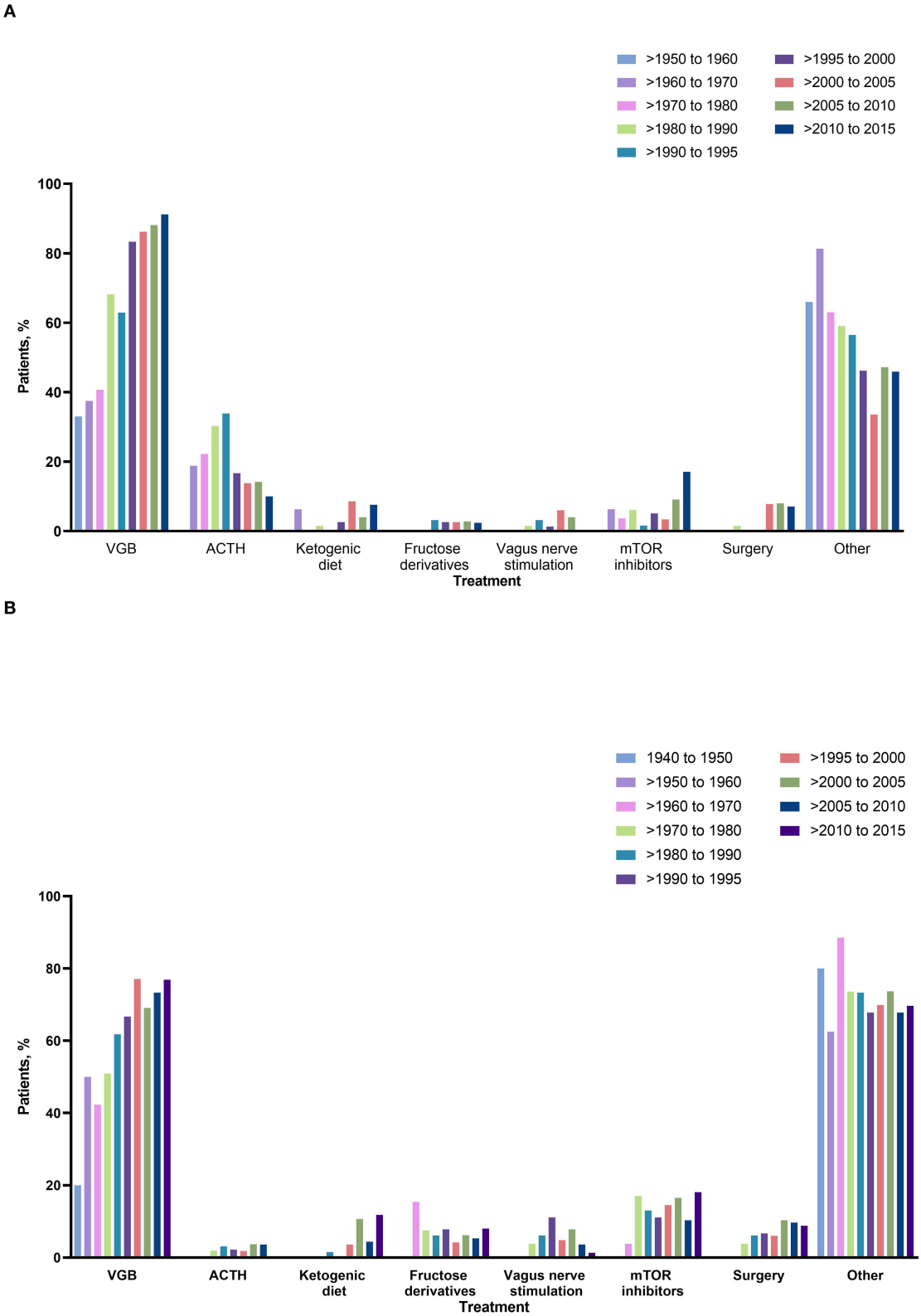

FIGURE 2 | Types of intervention over time in individuals with **(A)** infantile spasms and **(B)** focal seizures. ACTH, adrenocorticotropic hormone; mTOR, mammalian target of rapamycin; VGB, vigabatrin.

Changes of the initial antiepileptic treatment were reported in 64 (90.1%) individuals with infantile spasms and 64 (68.1%) with focal seizures. Most frequently reported reasons for change of treatment were partial or lack of efficacy of the first therapy. Vigabatrin was used as first-line therapy in individuals with infantile spasms in less than half of cases (45.1%) and was the most frequent second line treatment option (62.5%, **Table 3**).

Characteristics of Epilepsy in TSC by Genotype

Of 63 individuals with available genetic data, 10 had pathogenic mutations in *TSC1* and 53 had pathogenic mutations in *TSC2*. Median age of epilepsy onset was 8 years in individuals with pathogenic variants in *TSC1* and <1 year in those with pathogenic variants in *TSC2*.

Infantile spasms were not reported in individuals with *TSC1*, but in 16 individuals (30.2%) with *TSC2*. Focal seizures were reported for most individuals (90% of *TSC1* individuals and 69.8% of *TSC2* individuals). The median frequency of focal seizures per week was 3.5 in individuals with pathogenic variants in *TSC1* and 1 in individuals with pathogenic variants in *TSC2*. MRI showed a mean number of three tubers in individuals with *TSC1* and 9.2 tubers in in *TSC2*.

Focal seizures were controlled with treatment in 60% of individuals with *TSC1* compared with only 22.6% of those with *TSC2*. Infantile spasms were controlled with treatment in 28.3% of individuals with *TSC2*.

Association of Epilepsy Foci With IQ Level

The association between epilepsy and IQ was examined in 102 individuals at baseline (69 had normal intellectual ability and 33 had various degrees of ID). Regarding IQ and focal spikes on last EEG recording, EEG showed temporal focal spikes in 52.3% of individuals with normal IQ and frontal focal spikes in 68% of individuals with moderate to severe ID.

Correlation of IQ Level and Genotype

Sixty-two of 102 individuals showed normal IQ level. The IQ level was normal in 70% of individuals with *TSC1* and in 20.8% of those with *TSC2*; moderate ID was found in 20% of individuals with *TSC1* and in 22.6% of those with *TSC2*; severe ID was observed in 15.1% of individuals with *TSC2*, but none of those with *TSC1*.

EEG in Individuals With TSC Diagnosis Before Seizures Onset

Diagnosis of TSC was established in 28 individuals before seizure onset. In this group, 16 individuals developed infantile spasms and 12 developed focal seizures. Median age at first EEG was 6 months in individuals with infantile spasms and 11 months in those with focal seizures. The first EEG was performed in 12.5% of individuals before the onset of infantile spasms and in 25% of individuals before the onset of focal seizures. Electroencephalogram was performed the same day seizures occurred in 18.8 and 16.7% of individuals with infantile spasms and focal seizures, respectively. In the remaining cases, 68.8 and 58.3%, EEG was performed after the onset of infantile spasms and focal seizures, respectively.

DISCUSSION

This study provides final data or information on epilepsy characteristics in a large cohort of TSC individuals who participated in the TOSCA registry and in the epilepsy substudy.

Findings from the main study emphasize the changes in both diagnosis and treatment patterns of TSC-associated epilepsies over time. Overall, a diagnosis of epilepsy was reported in approximately 85% of all individuals with TSC included, equally affecting both sexes. Infantile spasms were reported in about 39% of individuals with a median age of <1 year at diagnosis, and focal seizures in two-thirds of the individuals with a median age of 1 year at diagnosis. These findings were consistent with our previous report and also with other studies (2, 3, 15–17).

In our study, epilepsy diagnosis rates, especially diagnosis of infantile spasms, were higher in younger individuals (67.8% in 1970s to 91.8% in last decade). Since infantile spasms were reported as the seizures types of West syndrome by William West in 1841 (18), followed by Gibbs and Gibbs' description of the characteristic EEG pattern of hypsarrhythmia in 1952 (19), clinicians have made remarkable progress in recognizing this syndrome. The first proposal of classification of patients with epilepsies in syndromes published in the "Guide Bleu" (Blue Guide) in 1984 added to this knowledge (20). In addition, the better recognition of infantile spasms in TSC and their focal nature might have changed the delineation of focal seizures and infantile spasms in the recent years. Although we believe that there was an improvement in the diagnosis of infantile spasms and that this major improvement in clinical epileptology guarantees earlier and better seizure and developmental outcomes. We should be cautious about the concept of an increased rate of epilepsy diagnosis because older individuals in TSC clinics often have a lower rate of epilepsy as they present with angiomyolipoma or being the parent of a child with TSC.

Our data showed a better treatment response rate in individuals with infantile spasms over time, but not in those with focal seizures. This seems to be due to VGB specifity in infants with West syndrome and its growing usage since the 1990s. A decrease in the use of steroids after VGB also clearly shows the specific efficacy of VGB and the lack of a need to add steroids as practiced in infantile spasms due to other etiologies (21). Vigabatrin is an established first-line therapy for individuals with infantile spasms (10, 22). This precision medicine approach in individuals with infantile spasms in TSC is a major example of how an early diagnosis of TSC can help to better target the therapy and to avoid therapeutic failures and ineffective polytherapies. In addition, VGB is recommended as first-line treatment for focal seizures in individuals with TSC in the first year of life (10), aiming to prevent transition into infantile spasms. However, its use for focal seizures in older individuals does not seem to be superior to other AEDs licensed for focal seizures. Indeed, there has been no change in responder rates for focal seizures for the past 45 years despite availability of over 30 new AEDs (23, 24). This finding is also in line with the high percentage of drug resistance reported in individuals with TSC-associated focal seizures in recent reports (3, 25). Despite the

TABLE 3 | Initial and change in the treatment and reason for change in the epilepsy substudy.

	Infantile spasms	Focal seizures
Number of individuals	71 (43.8)	94 (58.0)
Type of initial treatment reported at the epilepsy diagnosis		
Monotherapy	52 (73.2)	70 (74.5)
VGB	32 (45.1)	33 (35.1)
ACTH	8 (11.3)	2 (2.1)
Other	12 (16.9)	33 (35.1)
Polytherapy	16 (22.5)	18 (19.1)
GABAergics and other	5 (7.0)	3 (3.2)
Change of first treatment	64 (90.1)	64 (68.1)
Median time from first to second treatment, days	214.0 (0–5,480)	288.0 (0–8,402)
Type of second treatment		
VGB	40 (62.5)	31 (48.4)
ACTH (steroids)	10 (15.6)	3 (4.7)
Ketogenic diet	1 (1.6)	0
Fructose derivates	1 (1.6)	1 (1.6)
Vagus nerve stimulation	0	0
mTOR inhibitors	0	0
Other	42 (65.6)	49 (76.6)
Reason for change of drugs	62 (96.9)	62 (96.9)
Partial efficacy	20 (31.3)	24 (37.5)
No efficacy	20 (31.3)	18 (28.1)
Side effects	1 (1.6)	4 (6.3)
Other	21 (32.8)	16 (25.0)

ACTH, adrenocorticotropic hormone; mTOR, mammalian target of rapamycin; VGB, vigabatrin.
Values are expressed as n (%) unless otherwise mentioned.

high response to VGB in individuals with infantile spasms, it was not always the first therapy in individuals with infantile spasms (only 45% received VGB as first-line monotherapy). This finding is unexpected, especially in epilepsy centers, but emphasizes the need for more education about the use of individualized treatment options for specific etiologies.

Surprisingly, other non-pharmacological therapies such as VNS and the ketogenic diet were not used in this highly drug resistant population (range 1.5–8.6%). This might be due to the lack of randomized controlled trials in both therapies and evidence often based on retrospective small series (26) from one hand and the lack of expertise in both therapies on the other hand. The use of VNS in this cohort decreased during recent years after a peak in the 2000 and might be still underused although recommended as last resort in patients with refractory seizures.

Early evaluation for epilepsy surgery candidates in individuals with drug resistant TSC-associated epilepsies should be performed in expert centers in order to prevent/minimize developmental consequences of ongoing seizures (27). In our study, only a few individuals had epilepsy surgery. However, we did not ask in the study protocol how many had undergone pre-surgical evaluation.

Epilepsy surgery shows a relevant rate of 8–10% in our study but might not reflect yet the number of patients that were good candidates for epilepsy surgery and that can benefit from such therapy. Additionally, not all of the epilepsy centers participating in the study were also surgery centers trained in TSC-associated epilepsy. Therefore, additional training and education are needed and additional collaboration with expert surgery centers should be established for individuals with drug-resistant epilepsy with TSC in order to promote early identification of surgery good candidates.

Individuals with TSC and epilepsy are prescribed with multiple AEDs or undergo multiple surgical procedures to manage epileptic seizures (28, 29). However, we have observed in our epilepsy substudy that a large number of individuals were initiated on AED monotherapies as recommended by the ILAE (International League Against Epilesy). This might be related to the use of VGB in the first year of life in both infantile spasms and focal seizures or epilepsy combining both seizure types.

Our results also showed the increased use of disease-modifying treatment with mTOR inhibitors. The efficacy of this therapy was reported in late 2010 and its use in case of failure of initial treatment could be the rational approach. Its use increased and reached 18% in the last reports from the TOSCA study in 2015, showing the need for more efficient therapies in focal seizures associated with TSC. This increased use of approved mTOR inhibitor, everolimus, and the wider evaluation of surgery candidates in the management of TSC-associated focal seizures and in some individuals with drug-resistant infantile spasms might improve responder rates in

the future and could help to achieve a better cognitive TAND outcomes.

In our substudy, in infants with TSC diagnosis prior to seizure onset, EEG was performed mainly after the onset of clinical seizures, both for infantile spasms (in 68.8%) and focal seizures (in 58.3%). Curatolo et al. recommended in 2012 (22) and later in 2018 (10) to use EEG in infants with TSC before seizure onset to early identify individuals at high risk of developing epilepsy. This was also reported in the international recommendations (guidelines) in 2013 (30), based on studies showing that TSC individuals who were diagnosed and treated before the onset of seizures had less severe epilepsy and better neurodevelopmental outcomes (31). Abnormal EEG patterns and/or in some instances subclinical seizures recorded on the EEG should urge the use of AED therapy without waiting for the onset of overt clinical seizures. The results of the research project are in contrast with these recommendations and emphasize the need for more information for clinicians about the key role of sequential EEG recordings to early recognize individuals at high risk of developing early onset seizures and preventive AED treatment. Parents should be educated to recognize seizures earlier and most importantly EEG recordings should be performed—with an ultrasound of cardiac rhabdomyoma, pre-natal, or post-natal MRIs—in cases with family history of TSC with signs of TSC or with cutaneous hallmarks of TSC.

The place of this pre-symptomatic diagnosis strategy for epilepsy in TSC and the preventive therapy might be better implemented after the recent validation of this approach with the first results of the EPISTOP study (32, 33). Individuals receiving early preventive treatment showed a later epilepsy onset and a less severe epilepsy compared to those receiving standard therapy started after the onset of clinical seizures. The cognitive outcome might need further validation and longer follow-up (32, 33).

Our data show no significant correlation between the spikes focus and the IQ levels as for frontal or temporal focus. More severe cognitive but mainly psychiatric disorders as ASD are reported with temporal lesions (34). However, we did not report ASD testing and TAND results were mostly missing.

Finally, our study showed that individuals with *TSC1* had less severe phenotypes than those with *TSC2*. This finding is in accordance with the literature (3, 25, 35), but, importantly, we were able to validate it on a very large cohort probably less biased than mono-center studies and smaller series. A higher proportion of individuals with *TSC1* had normal IQ levels than those with *TSC2*. Compared to individuals with *TSC2*, they had fewer numbers of tubers, later onset of epilepsy, and higher rates of controlled seizures. The tuber load, usually higher in individuals with *TSC2*, might have a role in creating more complex and diffuse abnormal networks, with fewer regions showing normal brain cortex, leading more frequently to drug resistant epilepsy and higher rates of co-morbidities.

In conclusion, our study highlights that despite the improvement in diagnosis and in some aspects of treatment of TSC-associated epilepsy over time, especially for infantile spasms, there are still some major improvements to be made. Better epilepsy control is urgently needed, mainly for focal seizures. A more targeted use of available therapies and the promotion of innovative therapies and of evaluating surgery candidates should continue. Despite the established guidelines, the need for further education of clinicians in order to provide earlier diagnosis of epilepsy based on serial EEGs before the onset of seizures in patients with TSC should be promoted and to use VGB as first monotherapy for infantile spasms established as the first line therapy. Pre-seizure diagnosis will also help to use timely or even preventive therapies and could be a major step toward changing the natural history of epilepsy in individuals with TSC. Finally, the use of new targeted therapies such as mTOR inhibitors, or cannabidiol (36), and earlier and better definition of candidates for epilepsy surgery may lead to better outcomes, especially for focal seizures where the seizure control rates have plateaued in the last decade.

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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. 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

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AUTHOR CONTRIBUTIONS

RN, EB, MB, PC, JF, MF, CH, SJ, JK, JL, AM, MS, RT, BZ, and AJ: designing the study, patient accrual, clinical care, data interpretation, drafting, revising, final review, and approval of the manuscript. PdV, 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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