Tuberous Sclerosis Complex-Associated Neuropsychiatric Disorders (TAND): New Findings on Age, Sex, and Genotype in Relation to Intellectual Phenotype

Petrus J. de Vries 1*, Elena Belousova 2, Mirjana P. Benedik 3, Tom Carter 4, Vincent Cottin 5, Paolo Curatolo 6, Maria Dahlin 7, Lisa D’Amato 4,6, Guillaume Beure d’Augères 5, José C. Ferreira 12,13, Martha Feucht 11, Carla Fladrowski 10,12,13, Christoph Hertzberg 14, Sergiusz Joziwak 15,16, John A. Lawson 17, Alfons Macaya 18, Ruben Marques 8,19, Rima Nabbout 20, Finbar O’Callaghan 21, Jiong Qin 22, Valentin Sander 23, Matthias Sauter 24, Seema Shah 25, Yukitoshi Takahashi 26, Renaud Touraine 27, Sotiris Youroukos 28, Bernard Zonnenberg 29, John C. Kingswood 30 and Anna C. Jansen 31 on behalf of TOSCA Consortium and TOSCA Investigators

1 Division of Child and Adolescent Psychiatry, University of Cape Town, Cape Town, South Africa, 2 Research and Clinical Institute of Pediatrics, Progor Russian National Research Medical University, Moscow, Russia, 3 SPS Pediatrichna Klinika, Ljubljana, Slovenia, 4 TSA Tuberous Sclerosis Association, Nottingham, United Kingdom, 5 Hôpital Louis Pradel, Claude Bernard University Lyon 1, Lyon, France, 6 Tor Vergata University Hospital, Rome, Italy, 7 Astrid Lindgren Childrens Hospital, Stockholm, Sweden, 8 Novartis Farma S.p.A., Origgio, Italy, 9 Association Séroso Tuberéuse de Bourneville, Gradignan, France, 10 Centro Hospitalar Lisboa Ocidental, Lisbon, Portugal, 11 Universitätsklinik für Kinder-und Jugendheilkunde, Affiliated Partner of the ERN EpICEA, Vienna, Austria, 12 Associazione Tuberose Tuberosa ONLUS, Milan, Italy, 13 European Tuberous Sclerosis Complex Association, Datteln, Germany, 14 Vivantes-Klinikum Neukölln, Berlin, Germany, 15 Department of Child Neurology, Medical University of Warsaw, Warsaw, Poland, 16 Department of Neurology and Epileptology, The Children’s Memorial Health Institute, Warsaw, Poland, 17 The Tuberous Sclerosis Multidisciplinary Management Clinic, Sydney Children’s Hospital, Randwick, NSW, Australia, 18 Hospital Universitari Vall d’Hebron, Barcelona, Spain, 19 Institute of Biomedicine (IBOMED), University of Leon, León, Spain, 20 Department of Pediatric Neurology, Neckar Enfants Malades Hospital, Paris Descartes University, Paris, France, 21 Clinical Neurosciences Section, Institute of Child Health, University College London, London, United Kingdom, 22 Department of Pediatrics, Peking University People’s Hospital (PKUPH), Beijing, China, 23 Tallinn Children Hospital, Tallinn, Estonia, 24 Klinikverbund Kempten-Oberallgäu gGmbH, Kempten, Germany, 25 Novartis Healthcare Pvt. Ltd., Hyderabad, India, 26 National Epilepsy Center, Shizuoka Institute of Epilepsy and Neurological Disorders, NHO, Shizuoka, Japan, 27 Department of Genetics, CHU-Hôpital Nord, Saint-Etienne, France, 28 St. Sophia Children’s Hospital, Athens, Greece, 29 University Medical Center, Utrecht, Netherlands, 30 Cardiology Clinical Academic Group, Molecular and Clinical Sciences Research Centre, St Georges University of London, London, United Kingdom, 31 Pediatric Neurology Unit, Department of Pediatrics, UZ Brussel VUB, Brussels, Belgium

**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 TSCI 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 TSCI 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 TSCI-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 angiomiyolipoma and lymphangiomiyomatosis, 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 (TSCI 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 \( (P = 0.06) \). 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).
TAND Profiles and Intellectual Ability

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) | NoID (n = 395) | MID (n = 251) | M-PID (n = 248) | P-value* |
|--------------------|-----------------------------------------------|----------------|---------------|----------------|---------|
| **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 expressed 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.

*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
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
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
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.
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 Nationale de l'Informatique et des Libertés), CCTRIS (Comité Consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé); Comité Ético Investigación Clínica de Euskadi (CEIC-E); Consejería 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 komitter för medisinsk och helsefaglig forskningsetikk; Komisja Bioetyczna przy Instytucie “Pomnik Centrum Zdrowia Dziecka”; Ethikkommission bei der Ludwig-Maximilians-Universität München; Hokkaido University Hospital Independent clinical research Institutional Ethics Committee; Medical Juntendo University Institutional Ethics Committee; National Center for 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; 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 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 Zedeek Meidcla Center Helsinki Committee; Sheba Medical Center Helsinki Committee; Tel Aviv Sourasky Medical Center Helsinki Committee; 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; Dirección General de Regulación, Planificación i Recursos Sanitariat; 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 Ético Investigación Clínica del Hospital Universitario y Politécnico de La Fe; Dirección General de Farmacéuica i Productes Sanitaris, Generalitat de València; Comité de Ética de la Investigación de Centro de Granada; Instituto Aragonés de Ciencias de la Salud (IACS); Comité Ético Investigación Clínica Regional del Principado de Asturias; Comité Ético Investigación Clínica Hospital 12 de Octubre; Comité Ético Investigación Clínica Hospital Universitario Virgen de la Arrixaca; Sección de Ordenación e Inspección Farmacéutica Departamento de Salud; Comité Ético de Investigación Clínica del Hospital Universitario del Río Hortega de Valladolid; Comisión de Ética para a Saúde (CES), Centro Hospitalar de Lisboa Ocidental, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar do Porto, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar Lisboa Central, EPE; Comissão de Ética para a Saúde (CES), Hospital Garcia de Orta, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar de São João, EPE; Comissão de Ética para a Saúde (CES), Hospital Professor Doutor Fernando Fonseca, EPE; Comissão de Ética para a Saúde (CES), Centro Hospitalar do Algarve, EPE (Unidade de Faro); LUHS Kaunas Regional Biomedical Research Ethics Committee; Paula Stradiņa klinika Institute; Attīstības biedrības Kliniskās izpētes Etikas komiteja, Ethics Committee for Clinical Research; Komisija Republike Slovenije za medicinsko etiko; Comitato Etico Indipendente Presso La Fondazione Ptv Policlínico Tor
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.

TOSCA Investigators

Japan: Nobuo Shinohara, Shigeo Horie, Masaya Kubota, Jun Tohyama, Katsumi Imai, Mari Kaneda, Hideo Kaneko, Yasushi Uchida, Tomoko Kirino, Shoichi Endo, Yoshikazu Inoue, Katsuhisa Uruno; Turkey: Ayse Serdaroglu, Zuhal Yapi, Banu Anlar, Sakir Altunbasak; Russia: Olga Lyova, Oleg Valeryevich Belyaev, Oleg Agronovich, Elena Vladislavovna Levitina, Yulia Vladimirovna Maksimova, Antonina Karas; China: Yuwu Jiang, Liping Zou, Kaifeng Xu, Yushi Zhang, Guoming Luan, Yuxin Zhang, Yi Wang, Meiling Jin, Dingwei Ye, Weiping Liao, Liemian Zhou, Jie Liu, Jianxiang Liao, Bo Yan, Yanchun Deng, Li Jiang, Zhisheng Liu, Shaoping Huang, Hua Li; Korea: Kijoong Kim; Taiwan: Pei-Lung Chen, Hsiu-Fen Lee, Jeng-Dau Tsai, Ching-Shiang Chi, Chao-Ching Huang; Australia: Kate Riney, Deborah Yates, Patrick Kwan; Thailand: Surachai Likasitwattnakul, Charcinr Nabangchang, Lunilya Thampratankul Krinsachai Chomtho, Kamornwan Katanyuwong, Somjit Srijomdakjorn; South Africa: Jo Wilmshurst; Israel: Reeval Segel, Tal Gilboa, Michal Tzadok, Aviva Fattal-Valevski; Greece: Panagiotis Papathasanopoulos, Antigone Syrigou Papavasiliou, Stylianos Giannakodimos, Stylianos Gatzonis, Evangelos Pavlou, Meropi Tzoufi; Netherlands: A. M. H. Vergeer; Belgium: Marc Dhooghe, Hélène Verhelst, Filip Roelens, Marie Cécile Nossogne, Pierre Defresne, Liesbeth De Waele, Patricia Leroy, Nathalie Demonceau, Benjamin Legros, Patrick Van Bogaert, Berten Ceulemans, Lina Dom; France: Pierre Castelnau, Anne De Saint Martin, Audrey Riquet, Mathieu Milh, Claude Cances, Jean-Michel Pedespan, Dorothee Ville, Agathe Roubertie, Stéphane Avuin, Patrick Berquin, Christian Richelman, Catherine Allaire, Sophie Gueden, Sylvie Nguyen The Tich, Bertrand Godet; Spain: Maria Luz Ruiz Falco Rojas, Jaime Campistol Planas, Antonio Martinez Bermejo, Patricia Smeyers Dura, Susana Roldan Aparicio, Maria Jesús Martínez Gonzalez, Javier Lopez Pison, Manuel Oscar Blanco Barca, Eduardo Lopez Laso, Olga Alonso Luengo, Francisco Javier Aguirre Rodriguez, Ignacio Malaga Dieguez, Ana Camacho Salas, Ixtaso Marti Carrera, Eduardo Martinez Salcedo, Maria Eugenia Yoldi Petri, Ramon Cancho Candela; Portugal: Ines da Conceicao Carrilho, Jose Pedro Vieira, Jose Paulo da Silva Oliveira Monteiro, Miguel Jorge Santos de Oliveira Ferreira Leao, Catarina Sofia Marceano Ribeiro Luis, Carla Pires Mendonca; Lithuania: Milda Endziniene; Latvia: Jurgis Strautmanis; Estonia: Inga Talvik; Italy: Maria Paola Canevini, Antonio Gambardella, Dario Pruna, Salvatore Buono, Elena Fontana, Bernardo Dalla Bernardina; Romania: Carmen Burloiu, Iuliu Stefan Bacos Cosma, Mihaela Adela Vintan, Laura Popescu; Czech Republic: Karel Zitterbart; Slovakia: Jaroslava Payerova, Ladislav Bratsky, Zuzana Zilinska; Austria: Ursula Gruber-Sedlmayr, Matthias Baumann, Edda Haberlandt, Laura Popescu; Hungary: Judit Kelemen, Zsuzsanna Helyes, Zsuzsanna Kovacs, Anna Béres; Sweden: Katarina Sohl, Therese Svanberg, Anna Strömberg, Susanne Åkesson; Denmark: Peter Uddall; Sweden: Paul Uvebrant, Olof Rask; Norway: Marit Bjornvold, Eylert Brodtkorb, Andreas Sloerdahl, Ragnar Solhoff, Martine Sofie Gilje Jaatun; Poland: Marek Mandera, Elżbieta Janina Radzikowska, Mariusz Wysocki; Germany: Michael Fischereder, Gerhard Kurleman, Bernd Wilken, Adelheid Wiemer-Kruel, Klemens Buddle, Klaus Marquard, Markus Knuf, Andreas Hahn, Hans Hartmann, Andreas Merkenschlager, Regina Trollmann.

FUNDING

This study was funded by Novartis Pharma AG. Novartis has contributed to study design, data analysis, and the decision to publish. Novartis authors reviewed the draft for submission.

ACKNOWLEDGMENTS

We thank patients and their families, investigators, and staff from all participating sites. The authors thank Pranitha Akula...
(Novartis Healthcare Pvt. Ltd.) and Manojkumar Patel (Novartis Healthcare Pvt. Ltd.) for providing medical writing support, which was funded by Novartis Pharmaceutical Corporation in accordance with Good Publication Practice (GPP3) guidelines (http://www.ismpp.org/gpp3).

**SUPPLEMENTARY MATERIAL**

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fneur.2020.00603/full#supplementary-material

**REFERENCES**

1. Curatolo P, Moavero R, de Vries PJ. Neurological and neuropsychiatric aspects of tuberous sclerosis complex. *Lancet Neurol.* (2015) 14:733–45. doi: 10.1016/s1474-4422(15)00069-1

2. de Vries PJ, Belousova E, Benedik MP, Carter T, Cottin V, Curatolo P, et al. TSC-associated neuropsychiatric disorders (TAND): findings from the TOSCA natural history study. *Orphanet J Rare Dis.* (2018) 13:157. doi: 10.1186/s13023-018-0901-8

3. de Vries PJ, Wilde L, de Vries MC, Moavero R, Pearson DA, Curatolo P. A clinical update on tuberous sclerosis complex-associated neuropsychiatric disorders (TAND). *Ann J Med Genet C Semin Med Genet.* (2018) 178:309–20. doi: 10.1002/ajmg.c.31637

4. Leceizo L, de Vries PJ. Advances in the treatment of tuberous sclerosis complex. *Carr Opin Psychiatry.* (2015) 28:113–20. doi: 10.1097/yco.0000000000000136

5. Waltereit R, Feucht M, de Vries MC, Huemer J, Roessen V, de Vries PJ. Neuropsychiatric manifestations in Tuberous Sclerosis Complex (TSC): diagnostic guidelines, TAND concept and therapy with mTOR inhibitors. *Z Kinder Jugendspsychiatr Psychother.* (2019) 47:139–53. doi: 10.1024/1422-4917/a000604

6. de Vries PJ, Hunt A, Bolton PF. The psychopathologies of children and adolescents with tuberous sclerosis complex (TSC): a postal survey of UK families. *Eur Child Adolesc Psychiatry.* (2007) 16:16–24. doi: 10.1007/s00787-006-0570-3

7. Leceizo L, Jansen A, Whitemore VH, de Vries PJ. Pilot validation of the tuberous sclerosis-associated neuropsychiatric disorders (TAND) checklist. *Pediatr Neurol.* (2015) 52:16–24. doi: 10.1016/j.pediatrneurol.2014.10.006

8. de Vries PJ, Whitemore VH, Leceizo L, Byars AW, Dunn D, Ess KC, et al. Tuberous sclerosis associated neuropsychiatric disorders (TAND) and the TAND Checklist. *Pediatr Neurol.* (2015) 52:25–35. doi: 10.1016/j.pediatrneurol.2014.10.004

9. Lewis JC, Thomas HV, Murphy KC, Sampson JR. Genotype and psychological phenotype in tuberous sclerosis. *J Med Genet.* (2004) 41:203–7. doi: 10.1136/jmg.2003.012757

10. Pulss F, Winterkorn ER, Thiele EA. Psychological profile of adults with tuberous sclerosis complex. *Epilepsy Behav.* (2007) 10:402–6. doi: 10.1016/j.ybbeh.2007.02.004

11. Trickett J, Heald M, Oliver C, Richards C. A cross-syndrome cohort comparison of sleep disturbance in children with smith-magenis syndrome, angelman syndrome, autism spectrum disorder and tuberous sclerosis complex. *J Neurodev Disord.* (2018) 10:9. doi: 10.1186/s11689-018-0215-0

12. Joinson C, O’Callaghan FJ, Osborne JP, Martyn C, Harris T, Bolton PF. Learning disability and epilepsy in an epidemiological sample of individuals with tuberous sclerosis complex. *Psychol Med.* (2003) 33:335–44. doi: 10.1017/s0033291702007929

13. Kingswood JC, d’Augereas GR, Belousova E, Ferreira JC, Carter T, Castellana R, et al. Tuberous SCerosis registry to increase disease Awareness (TOSCA) - baseline data on 2093 patients. *Orphanet J Rare Dis.* (2017) 12:2. doi: 10.1186/s13023-016-0553-5

14. de Vries PJ. Neuropsychiatric and cognitive aspects of tuberous sclerosis complex. In: Kwiatkowski DJ, Whitemore VH, Thiele EA, editors. *Tuberous Sclerosis Complex.* Weinheim: Wiley-Blackwell (2010). p. 229–68.

15. Tye C, McEwen FS, Liang H, Underwood L, Woodhouse E, Barker ED, et al. Long-term cognitive outcomes in tuberous sclerosis complex. *Dev Med Child Neurol.* (2020) 62:322–9. doi: 10.1111/dmcn.14356

16. Nabbout R, Belousova E, Benedik MP, Carter T, Cottin V, Curatolo P, et al. Epilepsy in tuberous sclerosis complex: findings from the TOSCA study. *Epilepsia Open.* (2019) 4:73–84. doi: 10.1002/epio.22286

17. de Vries PJ. Targeted treatments for cognitive and neurodevelopmental disorders in tuberous sclerosis complex. *Neurotherapeutics.* (2010) 7:275–82. doi: 10.1016/j.nurt.2010.05.001

18. de Vries PJ, Howe CJ. The tuberous sclerosis complex proteins—a GRIPP on cognition and neurodevelopment. *Trends Mol Med.* (2007) 13:319–26. doi: 10.1016/j.molmed.2007.06.003

19. Emerson E, Hatton C, Baines S, Robertson J. The physical health of British adults with intellectual disability: cross sectional study. *Int J Equity Health.* (2016) 15:11. doi: 10.1186/s12939-016-0296-x

20. Solomon MB, Herman JP. Sex differences in psychopathology: of gonads, adrenals and mental illness. *Physiol Behav.* (2009) 97:250–8. doi: 10.1016/j.physbeh.2009.02.033

21. McLean CP, Asnaani A, Litz BT, Hofmann SG. Gender differences in anxiety disorders: prevalence, course of illness, comorbidity and burden of illness. *J Psychiatr Res.* (2011) 45:1027–35. doi: 10.1016/j.jpsychires.2011.03.006

22. Wilcutt EG. The prevalence of DSM-IV attention-deficit/hyperactivity disorder: a meta-analytic review. *Neurotherapeutics.* (2012) 9:490–9. doi: 10.1016/j.nurt.2012.01-0135-8

23. Fombonne E. Epidemiology of autistic disorder and other pervasive developmental disorders. *J Clin Psychiatry.* (2005) 66(Suppl 10): 3–8

24. Arnett BD, Pennington BF, Willcutt EG, DeFries JC Olson RK. Sex differences in ADHD symptom severity. *J Child Psychol Psychiatry.* (2015) 56:632–9. doi: 10.1111/jcpp.12337

25. Clarke A, Cook P, Osborne JP. Cranial computed tomographic findings in tuberous sclerosis are not affected by sex. *Dev Med Child Neurol.* (1996) 38:139–45. doi: 10.1111/j.1469-8749.1996.tb12085.x

26. Hunt A, Shepherd C. A prevalence study of autism in tuberous sclerosis. *J Autism Dev Disord.* (1993) 23:323–39. doi: 10.1007/bf01062283

27. Kingswood JC, Bruzzi P, Curatolo P, de Vries PJ, Fladrowski C, Hertzberg C, et al. TOSCA - first international registry to address knowledge gaps in the natural history and management of tuberous sclerosis complex. *Orphanet J Rare Dis.* (2014) 9:182. doi: 10.1186/s13023-014-0182-9

28. Matson JL, Shomaker ME. Psychopathology and intellectual disability. *Carr Opin Psychiatry.* (2011) 24:367–71. doi: 10.1097/YCO.0b013e328242424a

29. Einfeld SL, Piccinin AM, Mackinnon A, Hofer SM, Taffe J, Gray KM, et al. Psychopathology in young people with intellectual disability. *JAMA.* (2006) 296:1981–9. doi: 10.1001/jama.296.16.1981

30. Krueger DA, Northrup H, on behalf of the International Tuberous Sclerosis Consensus Group. Tuberous sclerosis complex surveillance and management: recommendations of the 2012 international tuberous sclerosis complex consensus conference. *Pediatr Neurol.* (2013) 49:255–65. doi: 10.1016/j.pediatrneurol.2013.08.002
Conflict of Interest: PV, EB, TC, VC, PC, GB, JK, JE, MF, CF, CH, SJ, RN, FO'C, JQ, MS, RT, MD, JL, AM, SY, MB, BZ, and AJ, received honoraria and support for the travels from Novartis. VC received personal fees for consulting, lecture fees and travel from Actelion, Bayer, Biogen Idec, Boehringer Ingelheim, Gilead, GSK, MSD, Novartis, Pfizer, Roche, Sanofi; grants from Actelion, Boehringer Ingelheim, GSK, Pfizer, Roche; personal fees for developing educational material from Boehringer Ingelheim and Roche. PV has been on the study steering group of the EXIST-1, 2, and 3 studies sponsored by Novartis, and co-PI on two investigator-initiated studies part-funded by Novartis. RN received grant support, paid to her institution, from Eisai and lectures fees from Nutricia, Eisai, Advicenne, and GW Pharma. YT received personal fee from Novartis for lecture and for copyright of referential figures from the journals, and received grant from Japanese government for intractable epilepsy research. SJ was partly financed by the EC Seventh Framework Programme (FP7/2007–2013; EPISTOP grant agreement no. 602391), the Polish Ministerial funds for science (years 2013–2018) for the implementation of international cofinanced project and the grant EPIMARKER of the Polish National Center for Research and Development No. STRATEGMED3/306306/4/2016. JK, PC, CH, JL, and JQ received research grant from Novartis. RM and SS are employees of Novartis. LD'A was employee of Novartis at the time of manuscript concept approval.

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

Copyright © 2020 de Vries, Belousova, Benedik, Carter, Cottin, Curatolo, Dahlin, D'Amato, Beaure d'Augères, Ferreira, Feucht, Fladrowski, Hertzberg, Jozwiak, Lawson, Macaya, Marques, Nabbout, O'Callaghan, Qin, Sander, Sauter, Shah, Takahashi, Touraine, Yourouchko, Zonnenberg, Kingwood and Jansen. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) and the copyright owner(s) are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.