**TAND Mini-symposium 2023 Abstracts**

## Title: One mutated allele in the *tsc2* gene is not enough to cause seizures but is responsible for TANDs in the zebrafish model of Tuberous Sclerosis Complex

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Introduction
Tuberous Sclerosis Complex (TSC) is a rare genetic disease that manifests with early symptoms, including childhood epilepsy and TSC-associated neuropsychiatric disorders (TANDs). The latter comprise anxiety, autism spectrum disorder, and intellectual disability, among others. We showed before that the homozygous *tsc2*$^{vu242/vu242}$ zebrafish recapitulate TSC pathology in human patients as we observed heterotopias and hyperactivation of the mTorC1 pathway in the pallial brain regions, commissural thinning responsible for brain dysconnectivity with delayed axon development and aberrant tract fasciculation, epileptogenesis that resulted in non-motor seizures, and anxiety-like behavior. However, as TSC patients are often heterozygotes, we analyzed also heterozygotic siblings of the accepted TSC zebrafish model.
Methods
We incrossed the *tsc2*$^{vu242/+}$ adult zebrafish, obtaining all three genotypes and we analyzed their i) brain morphology using wholemount immunofluorescence technique and single-plane illumination microscopy, and ii) brain function using behavioral assays.
Results
We discovered that the *tsc2*$^{vu242/+}$ mutants did not suffer from early epileptogenesis and seizures, yet they showed hyperactivation of the mTorC1 pathway in the pallial brain regions and commissural thinning responsible for brain dysconnectivity. Notwithstanding, the heterozygotic *tsc2*$^{vu242/+}$ mutants also exhibited increased anxiety-like behaviour, decreased learning and memory, and aberrant development of social behaviour, suggesting that loss of one allele of the *tsc2* gene is enough to cause TANDs-like phenotypes in the zebrafish model of TSC.
Conclusions
Our results are in line with the hypothesis that the majority of symptoms in TSC are inherited in an autosomal dominant manner – including TANDs – but for seizure development the loss of heterozygosity is needed.

**Title: Baby Talk in TSC**

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Introduction: Autism impacts up to 50% of those with TSC; however, speech and language difficulties seem to impact as many as 72%. Despite this, little is known about early language development in this population. In the field of vocal development, canonical babbling and volubility are two measures used to assess early infant language, i.e., baby talk. Canonical babbling (CB) is the repetitive use of consonant-vowel combinations, such as 'ba' and 'da'. Volubility is the number of syllables used per minute by an infant, and the canonical babbling ratio (CBR) is the number of canonical syllables/total syllables. In a preliminary study of 40 infants with TSC, we assessed CB, CBR and volubility and found that 12-month-old infants with TSC were delayed on all parameters. The goal of our current study *Baby Talk in TSC* is to expand upon this preliminary work by examining the same parameters in a larger cohort.

Methods: Using audio-video recordings of infants with TSC engaged in developmental testing from TACERN (TSC Autism Center of Excellence Research Network), we are near completion of analyzing all available remaining videos and analyzing the early infant vocalizations measured in the preliminary study.

Results: From the preliminary study, the mean CBR for infants with TSC was .117 (SE = .023) as compared to .346 for typically developing infants with a very large effect size. At 12 months, only 26% of the infants with TSC were in the canonical stage vs >90% of the typically developing infants. Volubility was the most distinguishing of the parameters. Typically developing infants used three times the syllables of those with TSC.
Conclusions: Little is known about the early language of infants with TSC. Our preliminary work suggests delays/deficits that we are currently following up in a larger cohort.

**Title: Tuberous Sclerosis Associated Neuropsychiatric Disorders in adolescents: case series**

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Introduction: Current research on Tuberous sclerosis Associated Neuropsychiatric Disorders (TAND) reveals there are different presentations of neuropsychiatric symptoms across life stages. This study aims to describe psychiatric assessment of three adolescents with Tuberous Sclerosis Complex (TSC), comparing results with the literature.

Methods: case series of three Brazilian adolescents diagnosed with TSC, without intellectual disability. Patients were assessed with K-SADS-PL DSM5 (Schedule for Affective Disorders and Schizophrenia); two participants also responded TAND checklist. Medical charts were reviewed. This study is part of a larger TSC research on TAND in the south of Brazil.

Results: Patient 1 - 14-year-old male, diagnosed by the age of 3, history of epilepsy (uncontrolled seizures), presented past enuresis and present minor mood symptoms. Patient 2 – 14-year-old female, diagnosed by the age of 9, history of Ischemic cerebrovascular disease and TSC kidney lesions, presented past Somatic Symptom Disorder and present Social Anxiety Disorder, Enuresis and Attention Hyperactivity Disorder. Patient 3 - 17-year-old male, diagnosed by the age of 5, history of epilepsy (controlled seizures), presented major depressive episode in the past and present minor mood symptoms. TAND checklist results were compatible with the psychiatric structured interview (for patients 1 and 3).

Conclusion: These case series results are in accordance with literature description of anxiety and mood symptoms in adolescents with TSC. Two participants presented current or past enuresis, a condition that can be further investigated in the psychiatric assessment of TSC patients.

**Title: How will my child grow up? Adult functioning and childhood predictors in Tuberous Sclerosis Complex: a cross-sectional survey**

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Introduction:
As the largest population with TSC is now adult, there is a great need for more knowledge about adult TSC manifestations and functioning. We aimed to investigate factors present in childhood affecting physical and mental health and functioning of adults with TSC. More insights can help predict prognosis, and provide targets for prevention, monitoring and treatment.

Methods:
In this cross-sectional survey, a recently validated TSC-specific patient-reported outcome measure (TSC-PROM) instrument was distributed in the Netherlands, Belgium and USA. To analyse the data, we explored adult functioning on domains of physical health, mental health, functioning and participation. Asssociations between childhood predictors and these outcomes were performed using univariate testing and a regression analysis, including age, sex, age of diagnosis TSC, mutation type (TSC1 or TSC2), organ involvement, presence of refractory epilepsy, autism, ADHD, intellectual functioning, and level of education.

Results:
In total 163 adult participants with TSC completed the TSC-PROM, of which 85 as a self-report and 78 caregivers completed the proxy-version. Both refractory epilepsy and level of intellectual functioning were found to be significantly associated with reduced physical and mental health, as well as participation and functioning. Early TSC manifestations such as cardiac, neurologic and/or dermatologic were significantly associated with reduced physical health, while the presence of autism and AD(H)D were associated with lower scores on mental health. ADHD significantly influenced the domain of functioning. The educational level was significantly influenced by presence of refractory epilepsy, autism, ADHD and level of intellectual functioning.

Conclusion
Early childhood predicting factors of lower adult functioning scores were identified, including age of diagnosis TSC, TSC2 mutation type, organ involvement, presence of refractory epilepsy, autism, ADHD, intellectual functioning, and level of education. Screening, prevention and early treatment and support during whole life of individuals with TSC could lead to better quality of life on all life domains.

**Title: Understanding the impact of TSC: Development and validation of the TSC-PROM**

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Introduction: More insight into the impact of TSC on functioning will help improve care and surveillance, which can be measured by patient-reported outcome measures (PROMs). The aim of this study was to develop a TSC-specific PROM for adults that captures the impact of TSC on physical functions, mental functions, activity and participation, the social support patients receive, and quality of life.

Methods: COSMIN methodology was used to develop a self-reported and proxy-reported version. In the developmental phase, relevant themes were identified using patient-driven data, literature and expert groups. The International Classification of Functioning and Disability was used as a

framework. Content validity was examined by a multidisciplinary expert group and cognitive interview study. Structural and construct validity, and internal consistency were examined in a large cohort.

Results: The study resulted in an 82-item self-version and 75-item proxy-version with four subscales. Sufficient results were found for structural validity. With regard to construct validity, 82% of the hypotheses were met for the self-version and 59% for the proxy version. The PROM showed good internal consistency (Cronbach's alpha 0.78-0.97).
Conclusion: We developed a PROM for adults with TSC, named TSC-PROM, showing sufficient evidence for internal consistency reliability and validity that can be used in clinical and research settings to systematically gain insight into patients' experiences. It is the first PROM in TSC that addresses the impact of specific TSC manifestations on functioning, providing a valuable, patient-centered addition to the current clinical outcomes.

**Title: Understanding and supporting families with TSC-Associated Neuropsychiatric Disorders (TAND) in India**

Author: Shoba Srivastava

Introduction Tuberous sclerosis complex (TSC) is a rare genetic disease with a birth incidence of ~1:6,000 and is seen all over the globe. TSC is an autosomal dominant genetic disorder characterized by non-cancerous (benign) tumors in the brain and several areas of the body, including the spinal cord, nerves, eyes, lung, heart, kidneys, and skin. In addition, TSC has many neurodevelopmental manifestations and often causes disabling disorders like epilepsy, intellectual disabilities, and autism, which have a significant impact on the quality of life of those with TSC, as well as their caregivers and families. These neuropsychiatric manifestations are known as 'TAND' (TSC-Associated Neuropsychiatric Disorders), a term coined in 2012. A recent scoping review of all TAND-related publications in the peer-reviewed literature found that despite the fact that TAND are very common in individuals with TSC, and causes a heavy psychosocial burden for individuals and their caregivers, very little TAND-related research had been done in India which is one of the largest countries in the world with a population of ~1.4 billion people. The TAND scoping review identified only 9 peer-reviewed publications from India, all case reports. It is therefore clear that there is an enormous knowledge gap about TSC and TAND in the country. This project will aim to understand and address some of these knowledge gaps in India.

Recently, an international group of research, clinical, and family experts developed a self-report, quantified TAND checklist (TAND-SQ) designed to allow families and individuals with TSC identify and track their TAND profiles. As part of the TANDem Project, this checklist was built into a smartphone application that links TAND profiles with a TAND toolkit, which consists of evidence-informed tips and supports for families and individuals with TSC,. This tool has great potential to address the knowledge gaps in TAND in India but needs to be assessed for its feasibility and acceptability in the Indian context. In addition, caregivers of individuals with TSC need evidence-based support to manage their well-being as they support their loved ones with TSC in light of the significant psychological burden this caregiving can have.

Aims

1) To explore the perspectives and experiences of Indian families and clinicians regarding TAND, including whether TAND were recognized, assessed, or treated in their loved one with TSC, or in their clinical practice;
2) To evaluate the feasibility and acceptability of the new TAND-SQ and the TAND Toolkit App to families in India;
3) To evaluate the feasibility, acceptability, and potential effectiveness of a web-based caregiver wellbeing program for caregivers of individuals with TSC in India

Methods The study will use mixed methods to achieve the aims.

Aim 1: Results will be derived by qualitative analysis of data obtained from purposefully sampled focus group discussions (FGDs) with families and clinicians in India.

Aim 2: 50 Indian families will be asked to complete the TAND-SQ and to use the TAND Toolkit app. Feasibility data will be collected through a combination of quantitative feedback (all families) via a feedback form, and qualitative feedback via focus group discussions (a subset of families).

Aim 3: A 3-session web-based caregiver wellbeing programme adapted from the WHO Caregiver Skills Training Programme for caregivers of children with developmental disabilities will be administered to 16 families. Mixed-methods will be used to evaluate the programme. Feedback forms and FGDs will be used to evaluate acceptability, cultural appropriateness, and accessibility (to inform adaptation) of the programme and programme materials. A pre-posttest waitlist crossover design with repeated measures will be used for limited efficacy-testing.

Conclusions The expected outcomes of the study include an improved understanding of the needs of families living with TSC and TAND in India. The feasibility data regarding the TAND-SQ, TAND Toolkit app and the Caregiver Wellbeing programme will provide a foundation for potential scale-up of these tools and interventions in the country.

Key words: Tuberous Sclerosis Complex, TAND Checklist, Family needs; Caregiver well-being

**Title: Understanding caregiver experiences of sleep management difficulties in individuals with tuberous sclerosis complex: A qualitative approach**

Stacey Bissell

Background: Sleep difficulties have been identified as the second most common behavioural presentation of TAND, with a 44% overall prevalence based on findings from the TuberOus SClerosis registry to increase disease Awareness. However, across all TAND clusters, the eat/sleep cluster is the most under-researched in cohort studies based on findings from a recent TAND scoping review (35/153; 23%), with a specific absence and need for qualitative research identified. Methods: Researchers from the University of Birmingham (Stacey Bissell) and Aston University (Georgie Agar) recently received seed funding from the 2022 TANDem Seed Grants Funding Round to conduct interviews with caregivers of children, adolescents, or adults with TSC currently experiencing sleep difficulties (e.g. night waking, nocturnal seizures, early morning waking, daytime sleepiness). These interviews aim to document current sleep management strategies and capture caregiver lived experiences of access to services, avenues and barriers to support, and priorities for sleep management and treatment. Findings: Initial findings from the Patient and Public Involvement processes in this study leading to interview schedule design and development will be discussed.

**Title: The Role of Developmental and Behavioural Paediatrics in a TSC Clinic**

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Introduction: Tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) encompass a range of cognitive, behavioural, and psychiatric symptoms that can impact quality of life. The following highlights our early conceptualization and initiation of a multidisciplinary TSC clinic, plus ideas for future research and outcome measures.

Methods: Our multidisciplinary team (consisting of a TSC nurse/ clinic coordinator, child neurologist, developmental-behavioural paediatrician (DBP), and a paediatric nephrologist) created a weekly multidisciplinary TSC clinic in March 2023. We aim to provide comprehensive evaluations and management plans for TSC patients. DBPs --with experience identifying/ treating the behavioural manifestations of autism, ADHD, and anxiety--are uniquely poised to use the TAND checklist as a framework for evaluating TSC patients, and then to offer recommendations for behavioural strategies and/or medications. Our model includes evaluations with DBP every 6-12 months, with brief developmental testing when appropriate, behavioural/ emotional screening, and recommendations for services, medications, and/or further evaluations. We hope to see a measurable effect on caregiver-reported child behaviours and mood (with improvements on ADHD and anxiety rating scales) and/or quality of life/ stress levels as reported on the TAND checklist (questions 8-9).

Conclusion: The goal of our TSC/ TAND clinic is to support patients and families by addressing the patients' physical, emotional, social, and cognitive development. Given the experience of DBPs in both children's medical care and mental health, we make the case for including DBPs in a multidisciplinary TSC clinic.

**Title: The Assessment and Treatment of Behavior Problems in TSC: A Telehealth Approach**

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Children with TSC are at significantly increased risk of externalizing behavior problems, such as hyperactivity and aggression. Unsurprisingly, these behavior problems are associated with elevated levels of stress in parents of children with TSC (Ebrahimi-Fakhari et al., 2019; Kopp et al., 2008), which may amplify the overall challenges of managing the care of a medically complex child (Zollner et al., 2020). There has been increased attention to TAND in recent years (de Vries et al., 2018), but our scientific understanding of the prevalence and impact of externalizing behavior problems as they first arise in early childhood in TSC remains limited.

We will present an overview of a new study in which we apply a telehealth approach to remotely assess and treat challenging behaviors in young children with TSC. We focus on the preschool-age period when externalizing behavior problems are expected to increasingly interfere in daily life, yet remain malleable. We selected an empirically-supported intervention, Parent-Child Interaction Therapy (PCIT), that is ideally suited to remote use and has shown initial efficacy in neurodevelopmental populations. We are first assessing the nature, frequency, and impact of externalizing behavior problems in preschool-aged children with TSC, contributing to general knowledge of TAND and providing an avenue for selecting children with elevated behavior problems requiring treatment. We are then examining feasibility, acceptability, and preliminary efficacy in a pilot clinical trial of internet-delivered PCIT in children with TSC and externalizing behavior problems.

**Title: The DNA-V Model of Acceptance and Commitment Therapy for Young People Living with Tuberous Sclerosis Complex: Study Protocol and Early Findings for an Acceptability and Feasibility Randomised Control Trial**

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Background: The psychological impact of Tuberous Sclerosis Complex (TSC) poses significant challenges to the quality of life and overall well-being of individuals. However, there is limited accessibility to psychological support for young people with TSC. This study explores the feasibility and acceptability of remotely delivered model of Acceptance and Commitment Therapy (ACT) developed specifically for adolescents and teenagers (DNA-V), as a potential intervention for young people with TSC.

Methods: A two-arm, parallel group, randomized controlled trial, comparing DNA-V with a waitlist control group is being conducted. The trial will recruit 15 participants (11-24 years old) with TSC to receive 12 weeks of DNA-V either immediately or after a 12-week waiting period. Feasibility and acceptability will be assessed based on participant recruitment, completion rates, and clinical outcome measures evaluating physical and mental health, everyday functioning, quality of life, and service usage at the 12-week follow-up. The durability of treatment effects will be evaluated at multiple time points up to 48 weeks. Qualitative interviews are being conducted to explore experiences of receiving remotely delivered DNA-V.

Early results: Of 14 patients screened, eight participants were enrolled, with two declining to participate and four not meeting eligibility criteria. Four participants have completed treatment to date. Three were excluded during the course of the study due to accessing outside psychological support. Early screening and baseline measures highlight the pressing need for mental health services for young people with TSC. Current figures demonstrate a high uptake rate of the intervention among eligible patients (72%) and promising acceptability. However, further analysis is needed due to the limited sample size. Early data shows promise of the intervention based on participant feedback, outcome rating scales and session suitability ratings. Qualitative feedback will provide additional insights.