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2025 TSC International Research Conference: *Engage, Accelerate, Transform*

The 2025 TSC International Research Conference: *Engage, Accelerate, Transform,* presented by Drs. Bonnie and Jonathan Rothberg & Family, was held at the Bethesda North Marriott Hotel & Conference Center in North Bethesda, MD, June 26-28, 2025. The conference was hosted by the TSC Alliance and co-chaired by **Shafali Spurling Jeste, MD**, and **Carmen Priolo, MD, PhD.** Participants included 255 individuals from 25 different countries, and 12 percent of attendees self-identified as members of groups historically underrepresented in biomedical research. The conference featured 29 main-session oral presentations, 9 early-career symposium oral presentations, 56 poster presentations, and 3 mini-symposia sessions. The parallel mini-symposia, spanning 4 hours, allowed attendees to participate in interactive group discussions based on interests. The three topics were TSC-Associated Neuropsychiatric Disorders (TAND), mechanisms and genetics of TSC, lymphangioleiomyomatosis (LAM) and kidney manifestations of TSC. Each mini-symposium sought to bring experts together from different areas of expertise, along with members of the TSC community, to discuss basic biology through clinical care, identify ongoing challenges, and brainstorm future collaborative strategies.

The keynote address was presented by **Jeffrey Karp, BEng, PhD**, an Endowed Chair at Brigham and Women's Hospital and a Professor at Harvard Medical School and Massachusetts Institute of Technology. Dr. Karp specializes in bioinspired medical innovation, leading a research lab that focuses on harnessing lessons from nature. During his talk, Dr. Karp spoke of the importance of turning to the world around us for inspiration in our work and bringing focus to our goals, both personally and professionally. He encouraged attendees to integrate mindfulness practices into their work and to better focus their priorities and attention on what matters most to each person.

At the opening night dinner, the Manuel R. Gomez Award was presented to **Mustafa Sahin, MD, PhD**, in recognition of his extraordinary scientific and humanitarian efforts impacting our understanding of TSC in research delivery of clinical care for individuals with TSC. Dr. Sahin is a pediatric neurologist and developmental neurobiologist who has been running an NIH-funded basic

and translational research program for more than two decades, focused on the cellular mechanism(s) of axon guidance and its relationship to neurological disfunction, especially in childhood neurological diseases. Additionally, he was a leading force in creating the TSC Clinical Research Consortium and is a dedicated clinician who serves as the Clinic Director of the TSC Center of Excellence at Boston Children's Hospital. Seventeen early career researchers were also recognized with the Vicky H. Whittemore Travel Award based on their outstanding abstract submissions.

Mini-Symposia

TSC-Associated Neuropsychiatric Disorders (TAND)

Led by **Anna Jansen, MD, PhD**, and **Jamie Capal, MD**, this mini-symposium opened with a discussion of how TAND is identified, assessed, and managed in a clinical setting. Participants represented a variety of countries and institutions, so a variety of perspectives were shared. The group raised several challenges and barriers for delivering effective care for TAND and then brainstormed practical steps that could be taken to mitigate these challenges. Suggested actions clustered around concepts of education and training (for clinicians, individuals, and families), capacity building, use of technology to enhance collaboration, and advocating for changes in policies. The mini-symposium participants next identified which specific aspects of TAND were most under-studied or overlooked in research. Under-researched themes included TAND trajectories throughout the lifespan, depressive disorder, dementia, sexuality, sleep, and health-economic impact. The group brainstormed some actions that could be taken, such as developing a contact list for those interested in participating in TAND research, facilitating opt-in to research during routine care visits, and facilitating data sharing among projects.

Mechanisms and genetics of TSC

Led by **Lisa Julian, PhD**, and **Laura Farach, MD**, participants in this mini-symposium started by discussing knowledge gaps in the areas of TSC genetics and of understanding cellular mechanisms in TSC. The group then identified key questions to be addressed to begin filling each knowledge gap. To stimulate conversation, eight participants gave brief presentations to highlight ongoing work toward addressing some of the knowledge gaps. Some key areas for prioritizing research include understanding functional domains in the TSC2 protein, understanding how immune cells and pathways contribute to disease processes in TSC, and determining how cortical tubers and epileptic foci develop.

LAM and kidney manifestations of TSC

Led by **Lyndsay Harshman, MD**, and **Nishant Gupta, MD**, this mini-symposium addressed key future priorities for research into lymphangioleiomyomatosis (LAM) and renal angiomyolipoma. Although LAM and kidney manifestations were discussed separately, commonalities emerged. Priorities for both included facilitating clinical trials for treatments other than mTOR inhibitors, identifying and validating biomarkers for disease progression, improving clinical screening for LAM and angiomyolipoma, and understanding the cell of origin for these manifestations, which could open areas for new treatment research.

Early Career Researcher Symposium

Prior to the start of the main research conference, the Early Career Researcher (ECR) Symposium was held the morning of June 26, 2025. This meeting served as a platform for early-stage career researchers, including graduate students, postdoctoral fellows and junior faculty, to network, present scientific findings and engage in career talks with established faculty and physicians.

Co-chairs **Nicole McDonald, PhD** and **Nicola Alesi, MD, PhD** kicked it off by inviting **Sara Chieffo**, mother of **Stella**, to share their family's TSC journey. She inspired the group with stories of Stella's unwavering perseverance and positive attitude. Sara expressed her deep appreciation for the next generation of TSC researchers. She especially encouraged them to get in "good trouble" and to aspire for a cure for TSC.

Thomas Li, PhD from UC Berkley, presented his latest work molecularly defining cortical tubers by using a TSC brain organoid model system. His systematic evaluation of TSC organoids throughout their development uncovered increased number of astrocytes in a disease-associated activated state. He then confirmed the expression of these activated astrocytic markers within primary TSC cortical tuber resection tissue donated by TSC patients following epilepsy surgery. His work gives the community a deeper understanding of cortical tubers which will be important for developing translational model systems, gaining a deeper understanding of the disease pathology and identifying new therapeutic targets.

Brian McGrath, PhD from Northwestern University discussed his work detailing the tissue microenvironment of lymphangioleiomyomatosis (LAM) lungs. He developed a 30-marker panel to recognize various LAM, immune and lung cell populations. This panel was applied to tissue sections from donated LAM and control lung transplants and imaged with a high-resolution spatial multiplex imaging system. His analysis uncovered LAM-specific intercellular relationships between the LAM cells and the surrounding tissue microenvironment. It also showed the first evidence of well-organized immunosuppressive and pro-immune structures near LAM tumor nodules which contribute to the ongoing inflammatory state. His work provides important insights into the unique immune-tumor landscape within LAM tissues which will lend itself to developing novel therapeutic strategies.

Joohwan Kim, PhD from UC Irvine shared his work that aims to identify novel biomarkers for early detection of TSC-associated epilepsy. To establish a potential biomarker associated with epilepsy, he performed metabolomic analysis on blood samples from close to 300 TSC patients with and without epilepsy. By molecularly characterizing close to 20,000 metabolites and comparing the signatures between patient groups, his team was able to identify about 50 metabolites which were significantly increased in patients with epilepsy. Notably, one of the consistently upregulated metabolites identified was previously implicated in seizure activity. This work provides a group of novel biomarkers that can be detected in a non-invasive way and potentially serve as diagnostic markers for TSC-associated epilepsy. After his presentation, the group was able to share memories about Joohwan's mentor, Gina Lee, who meant so much to the TSC community. We thank Joohwan for carrying Gina's great legacy forward and continuing the lab's important work.

Marco Ferniani, PhD from Baylor provided some exciting results from his investigation of therapeutically targeting TFEB in TSC kidney tumors. TFEB is a master regulator of protein processing and previously shown to contribute to tumorigenesis and cystogenesis in the TSC kidney. To measure TFEB activity, he established a fluorescent-reporter TSC kidney angiomyolipoma cell line. He then performed a high-throughput screen with over a thousand FDA approved compounds. From this screen, a group of compounds that could downregulate TFEB activity was identified. Further validation analysis demonstrated that this group of TFEB inhibitors impaired the growth of multiple TSC tumor cell lines. These exciting findings highlight a potential novel therapeutic strategy and bring forth new candidates that can be further investigated for therapeutic potential.

Tosca-Marie Heunis, PhD from the Mental Health and Wellbeing Research Group at Vrjie Universiteit Brussel reported the outcomes from evaluating the feasibility and acceptability of the TAND Toolkit app among TSC stakeholders. The at home app was developed in partnership with families to self-report symptoms from the TAND checklist (TAND-SQ). The app was developed to help close the gap between symptom identification and treatment and to assist patients and their families with communicating their disease state with health professionals. Feedback about the overall helpfulness and acceptability of the app was collected from various stakeholder groups from members of the TAND consortium, individuals with TSC and their caregivers, TSC Alliance and TSC International (TSCi). Amongst these feedback participants, 86% found the TAND toolkit 'helpful' or 'very helpful'. Furthermore 15/20 participants in a Belgian family cohort gave the app a 4 or 5 star. These positive results suggest the acceptability of the TAND Toolkit app as a resource for the TSC community.

Tarrant McPherson, PhD from Emory University updated the ECR symposium with the latest findings from the PREVeNT Trial. This trial evaluated the clinical efficacy of early treatment with vigabatrin prior to clinical seizures. He performed additional analysis with the data to investigate the effects of early vigabatrin on the number of EEGs with epileptiform activity. Electrophysiologists evaluated the 793 EEGs that were collected over the course of the study for the presence or absence of epileptiform activity. This in-depth analysis found that those individuals receiving early vigabatrin had fewer EEGs with interictal epileptiform discharge. However, deeper analysis showed the primary drivers of this decrease were children who never developed epileptic spasms. Early vigabatrin did not affect the number of epileptiform EEGs in individuals with epileptic spasms. These results provide important clinical data that can inform epilepsy treatment strategies for TSC patients.

Ali Valdrighi, MD from Lucille Packard Children's Hospital at Stanford shared her work to identify biomarkers of seizure recurrence following resection surgery. Her analysis of post-operative surgical outcomes focused on determining if recurrence occurs near the resected tissue or in distant regions that showed interictal epileptiform discharges (IEDs). Patient data from Stanford TSC patients suggested that most recurrences came from tissue near the resected tissue, though recurrence from new foci with prior IEDs was observed. The data suggests that there are epileptogenic networks which may contribute to the source of recurrence and disrupting the network improves the response. Her work will be essential for identifying biomarkers to assist with surgical planning and identifying epileptogenic tubers to improve clinical outcomes.

Camilia Ferrario, MS from UCLA presented findings from the RAINBOW study that investigated disruptive behaviors in pre-school aged children with TSC. The aim of this study was to develop a fully remote assessment that captures the types and frequency of problematic behavior present

during this developmental stage. She shared analysis of the baseline results that provided insights into the most common maladaptive behaviors and the child characteristics that could help predict parent-related stress and caregiver burden. Disruptive behavior was the most shared behavioral issue among participants, followed by externalizing and then internalizing behavior. Adaptive skills were the strongest predictor of caregiver burden. Notably, higher externalizing and disruptive behaviors were associated with poorer perceived quality of parent-child interaction. These results will be important for developing improved intervention methods and future integration of additional clinical data such as seizure status will allow for better understanding of the correlation with behavioral outcomes for individuals with TSC.

Samantha Verling, MS from the National Heart, Lung, and Blood Institute presented her results of a natural history study of periungual fibromas. These skin lesions which appear during adolescence often result in nail distortion, can be painful and consistently regrow after surgical resection. To collect natural history, she performed a retrospective cohort study of 21 TSC patients representing over 50 periungual fibromas. Periungual fibroma size was evaluated across a variety of conditions including regrowth after biopsy and with or without sirolimus treatment. Regrowth following biopsy was a common occurrence independent of treatment status, however the regrowth size relative to baseline was variable. The trends suggested that periungual fibroma size stabilized during adulthood and persist with sirolimus treatment. These studies provide important clinical information for one of the more understudied TSC-associated skin manifestations.

Our Early Career Researcher Symposium concluded with a funding and career development Q&A with a panel which included, **Mackenzie Catron, PhD**, PsychoGenics, Inc., **David Feliciano, PhD**, Clemson University, **Lyndsay Harshman, MD**, University of Iowa, and **Rajsekar Rajaraman**, MD, UCLA. Our panelist began by sharing their career journeys and how they became involved with the TSC research community. During the Q&A session, ECR attendees asked questions related to starting their own independent research careers, building resilience and versatility, and identifying keys to mentorship and advice for navigating the challenges that naturally arise within research. The fruitful discussion left all who attended with many great ideas to pursue moving forward. Overall, the ECR symposium was filled with high energy and great discussion to set a positive tone and kick off the 2025 meeting.

Translational Research in LAM, Renal Disease, and Topics Relevant to Adult Care in TSC

William L. Stanford, PhD presented the generation of TSC human pluripotent stem cells to study the mechanisms underlying renal angiomyolipomas and LAM development. This cellular model of TSC, which can be differentiated into any cell type, was used to generate *Tsc*-mutant renal organoids that exhibited characteristic cystic and angiomyolipoma features both *in vitro* and in xenograft models. Using single-cell RNA sequencing, various cell populations were identified as contributors to this process, including glial-derived Schwann cell precursors—an abnormally present cell type in LAM and angiomyolipoma tissues. Interestingly, *Tsc2* gene deletion specifically in Schwann cells led to renal cysts and lung lesions in female mice. Finally, through drug and CRISPR screenings using the new TSC model, the HDAC inhibitor vorinostat and two genetic targets were identified as potential therapeutic candidates for TSC. Notably, vorinostat administration was effective in a mouse model of TSC affecting the kidney, while antisense downregulation of the two novel targets induced apoptosis and inhibited proliferation of TSC mutant cells both *in vitro* and *in vivo*. In conclusion, this cellular model of TSC, combined with high-throughput screening technologies, represents a promising platform for the discovery of new therapeutic targets for TSC.

Yan Tang, PhD described a new gene therapy approach for pulmonary lymphangioleiomyomatosis (LAM). LAM is a genetic disease characterized by mTORC1 hyperactivation in the lungs and is often associated with mutations in the *Tsc1* or *Tsc2* genes. As such, the reintroduction of functional *Tsc* genes via gene replacement therapy represents a promising therapeutic strategy. Lipid nanoparticles (LNPs) are currently the most widely used method for systemic mRNA delivery. To achieve lung-specific targeting, a lipid library screen was performed, leading to the identification of specific lipid composition modifications that enhance delivery to pulmonary tissue. These modified LNPs, carrying functional *Tsc2* mRNA, were tested in a preclinical LAM model, demonstrating reduced disease progression and tumor burden. Alongside CRISPR- and AAV-based strategies, this LNP-based delivery system represents a promising therapeutic platform for the treatment of LAM.

Flaviane de Fatima Silva, PhD presented a novel mechanism underlying renal cystic disease driven by Rag GTPases. Rags are key regulators of mTORC1 activity, and their downregulation would typically be expected to attenuate mTOR signaling. Surprisingly, however, Rag downregulation resulted in a cystic phenotype resembling that of TSC, despite the absence of strong mTORC1 activation. This phenotype was found to be driven by the nuclear translocation of TFEB, a transcription factor that regulates lysosome biogenesis and promotes cyst formation when localized in the nucleus. Notably, deletion of TFEB reversed the cystic phenotype. Similarly, deletion of Raptor—a core component of mTORC1—also prevented cyst development, but without inducing TFEB nuclear translocation. This represents a paradoxical effect, as TFEB nuclear localization is generally promoted by mTORC1 inhibition. This work uncovers a novel mechanism of renal cystogenesis independent of mTORC1 hyperactivation, highlighting potential therapeutic opportunities through modulation of Rag GTPases, TFEB, or Raptor function.

Simon Johnson, DM, FRCP presented evidence that mTOR dysregulation drives a senescent phenotype in LAM. In both animal and cellular models of LAM, *Tsc2* deletion led to increased levels of senescence markers p21 and p16, along with elevated expression of cathepsin K and metalloproteases in alveolar type 2 cells. These findings indicate heightened lung injury and impaired repair capacity in the context of LAM. Furthermore, IL-6 was found to be upregulated in LAM patients and identified as a key driver of the senescent phenotype. LAM cells produce IL-6, which in turn induces p21 and p16 expression in alveolar type 2 cells, thereby inhibiting wound repair processes. Treatment with tocilizumab, an IL-6 antagonist, was able to block this mechanism—similarly to rapamycin—suggesting that IL-6 expression is mTOR-dependent. These findings highlight senescence in alveolar type 2 cells as a central mechanism of lung damage and impaired regeneration in LAM, and position IL-6 as a promising therapeutic target for both LAM and TSC.

Manoocher Soleimani, MD described the role of the proto-oncogene c-KIT in kidney cystogenesis in TSC. Previous studies have shown that the transcription factor Foxi1 is upregulated in TSC, and that its deletion prevents cyst formation originating from intercalated cells in the kidney. Through RNA sequencing, c-KIT was identified as upregulated in TSC mice, and its expression was abolished by Foxi1 downregulation. Importantly, genetic deletion of c-KIT in TSC mice completely prevented cysts formation and inhibited mTORC1 activation. Treatment with imatinib, a clinically approved c-KIT inhibitor, produced similar protective effects, blocking renal cystogenesis. Mechanistically, c-KIT upregulation was found to enhance Erk1/2 and RSK signaling pathways both in vitro, using M-1 cortical collecting duct cells, and in vivo in the kidneys of TSC mice. Notably, c-KIT downregulation abolished this signaling hyperactivation in TSC models. In conclusion, these findings identify c-KIT as a key driver of renal cystogenesis in TSC and support imatinib as a promising therapeutic candidate for TSC-related kidney disease.

Lara Friel, MD, PhD reported updates from the TSC Alliance Reproductive and Perinatal Health (RPH) Initiative Task Force, whose goal is to establish consensus recommendations for preconception and pregnancy care of women with TSC. The RPH Task Force has updated the TSC Alliance Natural History Database to include pulmonary, kidney, and maternal complications during pregnancy. It held an RPH Workshop in 2024 and is currently conducting a community survey series to gather women's experiences with TSC before and during pregnancy. High-quality, objective outcome-based longitudinal research is needed to address the gaps in evidence-based care, including early identification and ongoing monitoring for women with TSC/LAM before, during and after pregnancy.

David Ritter, MD, PhD discussed how reproductive health, similar to TAND, has been under-researched in TSC due to the invisibility of issues but is of critical importance to women with TSC. He reported findings from a clinic-based survey that found a high incidence of menstrual irregularities and an increased risk of pre-eclampsia. History of mTOR inhibitor treatment increased the risk of pre-eclampsia, but further research is needed to understand the nature of this relationship, as this may be reflecting the severity of TSC within individuals rather than medication effects.

Diego Maciel-Lima, MSc, presented findings from a single-case experimental design study developing a career guidance program for adults with TSC. Three individuals participated in a 20-week Building Career Path intervention, utilizing neuropsychology assessment, psychoeducation, professional identity, career exploration, decision-making and planning skill development. Results indicated feasibility and acceptability of the program with positive impact on professional identity and career planning in adults with TSC, particularly in individuals with an average intellectual and language ability. He is currently conducting a larger cohort study to further assess the program.

Laura Gorecki, MS described findings from an anonymous online survey aimed at describing patterns of TSC diagnosis disclosure to romantic partners. Results suggested that individuals with TSC who had a greater number of relationships disclosed TSC less often. Those with depression were more likely to fall into the more frequent disclosure group, whereas individuals with ADHD and seizure history were more likely to be in the less frequent disclosure group. Ethical obligations, family planning, fear of rejection, and previous disclosure experience influenced disclosure decisions. Overall, many young adults with TSC found romantic disclosure and dating challenging and want disclosure-specific support and resources.

Amber Goedken, PharmD, PhD presented research on the patterns of renal imaging within a US population-based TSC cohort using the Merative MarketScan database. The 2021 International TSC Consensus guidelines recommended that individuals with TSC have renal imaging at least once every three years, and this is especially important for pregnant women with TSC, as increased estrogen during pregnancy increases risk of complications of AML and LAM. However, the results suggest that although women were more likely to receive renal surveillance imaging than men, potentially up to 68% of women do not have renal imaging in the three years before pregnancy. This indicates that the consensus guidelines are not being followed which may be contributing to a higher risk of AML-associated complications and hospitalizations in pregnant women with TSC.

Research to Address Challenges of Controlling Epilepsy in TSC

Lisa Julian, PhD presented a model system in which she differentiated CRISPR-edited TSC2-/- cells and isogenic controls into 2D neural cultures, 3D cerebral organoids, and 3D scaffolded

neuroepithelial tissues (scNETs). In 2D cultures, increased gliogenesis over 5 weeks was observed, as well as increased neurite length, branching, soma size, and spontaneous activity in neurons, all of which are reversible with rapamycin if treated at early stages. In cerebral organoids, there was increased neurogenesis at day 30, but increased gliogenesis at day 60. Using RNA sequencing, an increase in GO terms related to ER stress and immune signals was seen, with a downregulation of terms related to brain development. In addition, TSC2-/- cells activated the unfolded protein response at neural lineage entry, as shown by higher levels of GRP78. Finally, Julian showed her method for generating single lumen neural tube tissue constructs, using a micropatterned size-restricted area to restrict differentiation to a neural tube shape. These scNETs generate a single, ring-shaped ventricular zone. Compared to isogenic controls, TSC2-/- cells have increased proliferation, generating scNETs with less lumen circularity, increased overall volume, and increased folding. After removing the cultures from the micropatterned surface, scNETs can be maintained as organoids in suspension. At days 14 and 28 in culture, TSC2-/- scNET organoids show increased surface folding and visibly increased neurogenesis.

Darcy Krueger, MD, PhD provided an overview of the use of mTOR inhibitors (mTORi) to treat epilepsy in TSC and Smith-Kingsmore syndrome. He outlined current gaps in knowledge about **mTORi** for TSC treatment, such as how to identify which patients would have the best response, which TSC manifestations are best addressed by this class of drugs, and what ideal dosage and durations of treatment would be. In addition to sharing the usage of mTORi at Cincinnati (2009-2024, with 603 patients on either everolimus, sirolimus, or both), he discussed the EXIST-3 and STOP-2 clinical trials, which tested everolimus and sirolimus respectively. The EXIST-3 trial showed that while there is a dose dependent relationship between mTORi and improvement in outcomes, not all patients respond the same way, and the response happens over a timeframe as long as 18 weeks. Side effects from mTORi included mouth sores (stomatitis), and hypercholesterolemia/hypertriglyceridemia. The STOP-2 trial showed that treatment with sirolimus to prevent epilepsy in infants with TSC showed generally favorable outcomes with 4 of 5 patients having a low risk of autism by age 2. These results led to the initiation of the TSC-STEPS trial, which is a randomized controlled trial to study the efficacy and safety of sirolimus from ages 0-12 months. Finally, Krueger suggested uses for mTORi beyond TSC, including in diseases such as segmental overgrowth, intellectual disability, and Smith-Kingsmore, with a caveat that sirolimus consistently results in circadian rhythm sleep-wake cycle disruption at higher doses.

Zin-Juan Klaft, MD, discussed an AAV-mediated gene therapy approach for restoring functional tuberin in a mouse model of TSC. Because the full-length human tuberin sequence is too long to fit in an AAV, he used a condensed version of tuberin, named cTuberin (cTUB). Transfection of cTUB into human patient derived TSC2-/- neural progenitor cells caused a reduction in cell size. To assess the functionality of AAV-cTuberin, he generated a mouse model with stochastic, postnatal and cerebral knockout of TSC2, generated using a mouse floxed for TSC2 (TSC2 fl/fl) and with an Ai9 reporter. Upon injection of AAV1-cre at p0, these mice develop phenotypes including neuronal hypertrophy, reduced survival over time, and spontaneous seizures (measured using long-term ECoG recordings). In particular, these mice have epileptiform interictal patterns, termed High Amplitude Epileptiform Activity (HAEA), and will die during seizures and interictal HAEA. Injection of high-titer AAV9-cTUB into the brains of these mice 3 weeks before recording significantly reduced the number of seizures and instances of HAEA.

Eleonora Aronica, MD, PhD discussed the emergence of epileptogenic networks in TSC, with a focus on interneurons and GABAergic signaling. Using single-nuclei RNA sequencing of patient

tubers, she found the greatest changes in GABA-ergic neurons from CGE and MGE regions, which had downregulation of PVALB, CALB1, RELN, VIP, and CCK compared to controls. Both MGE and CGE lineage cells show downregulation of GABAA subunit a1, but only MGE lineage cells show upregulation of a2. In MGE-derived interneurons the a1/a2 ratio is decreased, suggesting a more immature phenotype. SST-expressing interneurons showed the most immature phenotype of all GABAergic interneuron subtypes, suggesting functional dysregulation. Microtransplantation of GABAA receptors from pediatric patients to Xenopus oocytes showed a more depolarized EGABA and reduced affinity for GABA. *In vitro*, stem cell derived cultures containing mixtures of excitatory and inhibitory neurons and astrocytes, neurons with TSC2 mutations were shown to be hyperactive. Finally, proteomic and transcriptomic studies in primary astrocytes revealed changes in calcium signaling, including mitochondria dysfunction, reduced calcium influx, lower oxygen consumption, and reduced respiration capacity.

Nina Gruetzmacher, PhD presented a mechanism for aberrant neuronal excitability in TSC. She presented two mouse models, one in which TSC1 is floxed, and another in which TSC1 is floxed and S6K1;2 is knocked out. In both systems, AAV9-cre is injected at p0. Gruetzmacher demonstrates that knock-out of TSC1 results in increased phospho-S6 levels and the presence of giant cells, as well as reduced survival and epileptic seizures. These phenotypes were reversed by the knock-out of S6K. A phospho-proteomic analysis identified a number of novel S6K substrates, primarily relating to transmission and synapse GO terms. One particular substrate of interest was TREK-1, the permeability of which is modulated by mTOR. In HEK293 and 3D neurospheres expressing TREK1, they observe increases in pTREK1(ser333), which is abolished by knock-out of S6K.

S. Katie Ihnen, MD, PhD showed that electrical stimulation of induced seizures (ESIS) can help localize epilepsy for surgical purposes. ESIS is able to generate habitual seizures, as well as aura and non-habitual seizures. In both children with and without TSC, ESIS is able to generate a seizure in 90% of patients, and 71% generate a habitual seizure. There were a number of differences in ESIS in patients with and without TSC. In TSC, the region with the most induced seizures was the insula, most patients have exclusively induced habitual seizures, stimulation frequency does not affect yield, and there is no significant relationship between charge density and age. In non-TSC patients, the region with the most induced seizures was the mesial temporal lobe, stimulation frequency does affect yield, and there is a positive correlation between charge density and age. In terms of surgical outcomes, habitual seizures are not associated with surgical outcome for TSC patients, but they are in non-TSC.

Darcy Krueger, MD, PhD gave a second talk focused on the efficacy and safety of vigabatrin. There is one RCT that shows the effectiveness of vigabatrin for treating infantile spasms in TSC (Chiron 1997), and since then, vigabatrin is increasingly recommended for TSC treatment. While there were initial concerns regarding vision abnormalities in animal models and individual reports, 37% of all patients reviewed showed pathology that reflected their underlying condition and therapy, unrelated to vigabatrin, with only 2% of patients with pathology that might be related to vigabatrin. A phase 4 vigabatrin vision study in adults showed that many patients (60%) had baseline vision abnormalities. There were no patients with a confirmed decrease in acuity using vigabatrin, and there were no statistically significant changes in vision. Additionally, while vigabatrin-associated brain abnormalities seen on MRI were reported, abnormalities were also observed in patients not treated with vigabatrin and MRI findings felt to be associated with vigabatrin resolved after the treatment was discontinued.

Impacting TAND Through Basic, Translational, and Clinical Research

Mustafa Sahin, MD, PhD presented research on investigating TAND and epilepsy using human neurons derived from pluripotent stem cells with the aim to better understand the biology of TSC in order to develop safe and effective treatments for TSC and TAND. Key questions addressed were 1) timing and whether neuronal dysfunction becomes mTORC1-independent, and 2) gene dosage effects and non-cell autonomous effects on cellular differentiation. Conclusions were that the presence of TSC2-/- cells alters differentiation of surrounding cells; TSC2-/- cells secrete factors that impair neurogenesis; TSC2-/- organoids secrete extracellular vesicles containing cytoskeletal proteins that inhibit YAP1; knockdown of AMOT in TSC2-/- cells restores neuronal differentiation (TBR1 expression); and cortical tubers contain neurons that show similarities to neurons from mixed genotype organoids.

Kirstin Risgaard, MS presented a study on the relationship between sleep and TSC in adults. Sleep difficulties are a common feature of TAND but are poorly characterized. This study aimed to determine the prevalence of sleep disorders in adults with TSC in the U.S. and examine the role of clinical features and treatment regimens. The Pittsburgh Sleep Quality Index (PSQI) was used to quantitatively analyze sleep. Results showed that 64% of the 84 participants had poor sleep, and sleep problems were more likely in participants with mental illness or anxiety and in individuals who self-reported using certain medications (mTOR inhibitors, antiepileptic drugs, or antidepressants). Future research will include a larger study with a more diverse TSC sample and further investigation into how medications in TSC affect sleep. This study emphasizes the need for healthcare providers to assess sleep in adults with TSC and to consider possible interactions of clinical features and medications.

Jamie Capal, MD presented Regulating Together in Tuberous Sclerosis Complex (RT-TSC), a non-pharmacological group intervention study to address emotion dysregulation in children and teenagers (8-17 years) with TSC and TAND. The goals are to better understand emotion regulation (ER) difficulties in TSC, determine the efficacy of RT-TSC on ER in TSC, understand who benefits most from this intervention (e.g. age, intellectual functioning, TAND symptomology, etc.), evaluate the feasibility of remote/virtual delivery, and refine the curriculum for broader implementation. The curriculum includes child and caregiver sessions and uses the Emotion Dysregulation Inventory (EDI), a caregiver-report questionnaire, to collect data. Feedback included that children stay well engaged throughout the sessions, and families liked the virtual format. Future plans involve adapting the curriculum for young adults (18+) and lower functioning individuals with TSC, developing videos and resources on different skill topics, and coordinating quarterly meetings with the larger Regulating Together team to discuss what has been learned across ages and conditions. (www.regulatingtogether.com)

Nola Chambers, PhD presented results from the TANDem-1 project validating the Cluster Severity Scores (CSS) and Total TAND Severity Scores (TTSS) derived from the TAND-SQ Checklist. The internal consistency of the severity ratings for items within each TAND cluster, relationships between the TAND-SQ CSS and TTSS with relevant clinical diagnoses, and behavioral measures were examined in two samples from the TSC Alliance Natural History Database (n=69) and the RDCRN (n=23). Findings provide support for the internal consistency and validity of the CSS and TTSS in the TAND-SQ and support their use in clinical decision-making and future research. The TAND-SQ (available in multiple languages soon) and more information on the TANDem-1 and TANDem-2 projects can be found at www.tandconsortium.org.

Kate Fifield, BSc reported findings on the development of a smartphone ecological momentary assessment (EMA) to help young adults with TSC self-report experiences or symptoms in the moment, rather than retrospectively over the last months. The project was co-designed with an advisory group consisting of two young adults with TSC. The study recruited young adults, 18 with TSC and 18 without TSC. Feasibility findings showed an average completion rate of 88,8% for TSC participants, and 89.8% for participants without TSC, showing no differences in completion rate. Conclusions were that EMA could be helpful to support young adults with TSC, and that all stages of research could benefit from patient and public involvement (PPI).

Elizabeth Thiele, MD, PhD, shared insights from 20 years of experience at the Herscot Center for TSC, focusing on the neurobehavioral aspects of TSC. In this cohort, 85% of individuals with TSC develop epilepsy and the presence of refractory epilepsy and infantile spasms in TSC is significantly associated with cognitive impairment, autism, psychiatric disorders including self-injurious behaviors, and sleep disorders. Adults with TSC are at much greater risk of psychiatric disorders than the general population. Future research at the Herscot Center will explore the impact of CBD and dietary therapies (e.g. the low glycemic index treatment) on TAND symptoms, focusing on anxiety and OCD. Eligibility criteria for the trial will be determined by the TAND-SQ Checklist. Results from the EpiCom study, investigating behavioral outcomes following adjunctive cannabidiol for the management of TAND, are expected mid-2026. Thiele has also co-authored the book 'Epilepsy for Dummies'.

Nicole McDonald, PhD, presented an overview of the RAINBOW study, a remote, parent-mediated intervention using Parent-Child Interaction Therapy (PCIT) to address behavior problems in young children with TSC (3-6 years of age). The study aims to assess the prevalence of behavior issues in children with TSC, the feasibility and effectiveness of PCIT, and which children benefit most. PCIT focuses on reducing disruptive behaviors and improving parent-child relationships in early childhood by teaching effective behavior management and positive parenting skills. Initial findings (n=28) support the feasibility and acceptability of PCIT in TSC families, showing high parent satisfaction (4.39/5) and good attendance (14.62/20 sessions). Using telehealth improved access to the trial and treatment, but challenges remained in reaching underrepresented communities.

Insights into TSC Biology from Basic Science, Cell Biology, Immunology, and Neuroscience

Rebecca Ihrie, PhD explored how epigenetic regulation influences cell fate in TSC. While DNA methylation typically increases during cell differentiation, early neural progenitor cell (NPC) cultures derived from TSC mutant iPSCs exhibited widespread hypomethylation at hundreds of loci. Notably, one of these loci included the potassium channel KCNC3, which is a key factor in neuronal differentiation. Analysis of patient tuber tissue also showed a variable mixed-lineage phenotype expressing combinations of proteins associated with progenitor and mature cells. These data suggest that a single TSC2 mutation may not select for a single cell identity but rather unlock a range of possible cell identities.

Elizabeth Henske, MD presented her research on the immune microenvironment in TSC and LAM. TSC-associated tumors, including LAM and angiomyolipomas, show elevated levels of T cell exhaustion markers. The Henske lab focused on B7-H3, a PD-L1 homolog, which is significantly upregulated in TSC. Knocking down B7-H3 reduces tumor growth in immunocompetent mice as well as inhibits kidney tumors in $TSC2^{+/-}$ mice, indicating that B7-H3 inhibits T cells in TSC. Single-cell RNA sequencing confirmed T cell dysfunction in TSC and further revealed an important

function of M2-like immunosuppressive macrophages in renal angiomyolipomas. These tumor-associated macrophages promote disease progression in LAM and TSC and represent another mechanism of T cell dysfunction in TSC. Currently, the Henske lab investigates whether targeting these M2-like macrophages could decrease disease progression in TSC and LAM.

Brendan Manning, PhD presented the latest unpublished findings of the metabolic effects of aberrant mTORC1 signaling in the tumor microenvironment. In TSC mice treated with 1mg/kg rapamycin for three days, tumor weight remained unchanged but there were significant alterations in the metabolic composition of the tumor interstitial fluid compared to control mice. Inhibiting mTORC1 by rapamycin stimulates lysosomal biogenesis and promotes catabolism of membrane phospholipids either to store or generate energy from the resulting free fatty acids. These fatty acids are stored as triglycerides within the tumor but not in the plasma, of rapamycin-treated mice. Tumor lipidomic analysis further revealed an accumulation of acyl-carnitine species, which are substrates for fatty acid oxidation. Overall, these findings suggest that mTOR inhibition triggers a metabolic shift in tumors from glucose utilization to fatty acid oxidation, highlighting the need to better understand how mTOR inhibitors used in TSC treatment influence tumor metabolism and the tumor microenvironment.

Weibo Niu, PhD reported an integrated multi-omics analysis to decipher the impact of TSC mutations on the lipid metabolism in microglia. Microglia are activated in the brain of TSC patients, and emerging evidence suggests a connection between microglia activation and epilepsy. Therefore, they differentiated microglia from TSC patient-derived iPSCs and performed extensive multi-omics analysis. It was shown that in TSC microglia, TSC2 mutations alone are sufficient to disrupt lipid metabolism, leading to increased phagocytosis and inflammation. This dysregulation was driven by an upregulation of the lipoprotein lipase (LPL) pathway in TSC. As a result, microglia in TSC were found to exert a direct impact on neuronal development, excitability, and neuronal network activity, underscoring the mTOR-LPL pathway as a promising therapeutic target in TSC.

Peter Crino, MD, PhD provided an overview of the development of the epileptic network in TSC, summarizing key advances made over the last two decades. The TSC brain is characterized by chronic inflammation, and potential contributors to epilepsy include alterations in cellular architecture, regional brain organization, and epigenetic regulation. Despite these insights, the precise mechanisms driving epilepsy in TSC are not fully understood yet. A comprehensive meta-analysis of existing multi-omics data might identify converging, targetable pathways. One intriguing candidate is a signaling hub linking amino acid metabolism and mTOR regulation, which may play a critical role in seizure initiation. The so-called GATORopathies, conditions caused by mutations in the GATOR1 complex that result in mTOR hyperactivation, have been linked to the development of epilepsy in some TSC patients and could present a targetable target to treat TSC-related epilepsy. Further research will address new strategies for therapeutic development in TSC patients.

TSC International

TSC International (TSCi) held its annual meeting on Thursday, June 26. This meeting brought together key stakeholders in the TSC space, including TSCi member organizations, clinicians, researchers, and industry partners to discuss global clinical trials in TSC and hear

an update on the TANDem project. 35 representatives from 18 countries attended the meeting. It was sponsored by Aeovian Pharmaceuticals, Biogen, Grin Therapeutics, Jazz Pharmaceuticals, and Lundbeck.

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Authorship

Vicki H. Whittemore Travel Award Winners Francesco Avanzi, Kate Fifield, Nina Gruetzmacher, Tosca Heunis, Tommy Li, and Brian McGrath wrote scientific summaries for all the individual presentations in this report. Their contributions are greatly appreciated! Sam Metzger, Katie Smith, and Steve Roberds of the TSC Alliance wrote all other portions of this report.