tsa
tuberous sclerosis association

INTERNATIONAL TUBEROUS SCLEROSIS COMPLEX RESEARCH CONFERENCE 2021

A VISION FOR THE FUTURE

PROUDLY ORGANISED BY THE
TUBEROUS SCLEROSIS ASSOCIATION

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Welcome

Dear Conference Participants,

On behalf of the Tuberous Sclerosis Association (TSA) UK, welcome to the International TSC Research Conference 2021: A Vision for the Future. We are so pleased you are joining us for the first virtual conference we have ever organised, and we know for many of you this will also be the first virtual conference you have attended. We are proud to be bringing you this event through an online platform hosted by the Royal College of Physicians, London.

The TSA is excited to host this year's conference, which will explore how academics, clinicians and researchers from across the globe are ambitiously driving new opportunities in TSC to advance scientific understanding, deliver innovation in clinical practice, and transform the lives of people living with TSC and their families.

The Conference Scientific Committee has worked incredibly hard over the past year to fashion a broad agenda covering seven key areas: Early diagnosis, early risk assessment, basic science and pre-clinical work, prevention, new and available therapies, social research and a vision for the future. The virtual conference features plenary presentations, poster sessions, an online exhibition, networking for delegates, and two industry-sponsored symposia for healthcare professionals and prescribers. We would like to thank the Committee for giving their time and expertise so generously to make this event a success.

Our opening keynote speaker is Professor Julian Sampson, an expert clinical geneticist who led the international consortium that identified TSC2 and worked as a member of the consortium that identified TSC1. Each day of the conference begins with a community speaker reminding us why it matters so much that the medical and research community continue to drive forward improvements in treatment and care, and we are grateful to Jennifer Flinn, Eva Schoeters and Vicky Whittemore for sharing their stories, as well as to Sanjay. The conference will wrap up on Saturday with a panel session focused on *'A vision for the future'* including Dr Xandra Breakefield who is investigating gene therapy as a potential therapeutic approach for TSC, together with Professor John Bissler and Professor Darcy Kreuger who will explore the latest research advances in TSC.

We would like to sincerely thank our research sponsors who have generously supported this year's conference, including GW Pharmaceuticals, Nobel Pharma, and Noema Pharma. We would also like to thank the following organisations who have taken exhibition space at this year's virtual event: Cambridge Rare Disease Network, Epilepsy Research UK, European Tuberous Sclerosis Complex Association, Gene People, GW Pharmaceuticals, Medics4RareDiseases, Nobel Pharma, Novartis, Tuberous Sclerosis Association, and Tuberous Sclerosis Complex International. We would like to say a huge thank you to our conference speakers and poster presenters, as we simply could not deliver this event without your vital contributions.

Finally, we thank all of our delegates for choosing to spend time with us – dialling in from your universities, labs, clinics and homes, with your colleagues, partners and children in the background, to share and understand the latest research into TSC. It is your ongoing passion for learning more about TSC that brings hope for the future to individuals and families affected by the condition.

Yours sincerely

Sanjay Sethi
Chair, TSA UK

Louise Fish
Chief Executive, TSA UK

Thank you for everything that you do

Dear TSC researchers and clinicians,

It is my great pleasure to open the International TSC Research Conference 2021 by saying a big 'Thank you' to you and all of the TSC researchers and clinicians attending this important event.

Because of your hard work, we can look to the future of TSC with hope.

During some periods of the past eighteen months, a time like no other in living memory, 'hope' has been in short supply. Determination, courage and kindness have helped us through the coronavirus pandemic. But, it is science and research that is allowing us to see a light at the end of the dark tunnel.

As Emily Dickinson memorably wrote:

Faith is a fine invention
When gentlemen can see –
But microscopes are prudent
In an emergency.

When my grandson was first diagnosed with TSC, it shook our family, as it shakes all families who receive similar news. We will always be incredibly grateful to the professionals, and families, who supported us during those early days, weeks and months. Their help gave us the information that we needed to understand my grandson's rare condition, and how we could support our amazing little boy.

It is also because of you – the researchers and clinicians who dedicate their professional lives to TSC – that families like mine can look to the future with hope.

It is only through your expertise, your personal passions, and your willingness to ask questions, that our shared understanding of TSC can move forward.

Every clue that you find, every piece of evidence that you unearth, and every breakthrough you discover, helps to solve the mystery of TSC, and takes us another step towards a cure.

On behalf of all of the grandfathers and grandmothers, mothers and fathers, brothers and sisters, aunts and uncles, cousins, and friends of those who live with TSC: Thank you.

Your incredible dedication gives my family hope for a better future for my grandson, and for everyone who lives with TSC.

With very best wishes,

Sir David Suchet CBE
Grandfather and actor

Welcome to the International TSC Research Conference 2021!

Dear Conference Participants,

On behalf of the conference committee, it is our pleasure to warmly welcome you to the International TSC Research Conference 2021.

When we gathered for the previous international conference, in Toronto, no-one could predict that the next time we would all come together would be virtually. We have worked hard to offer a virtual platform that can encourage scientific discussion and enquiry, and are confident that you will find the Royal College of Physicians' virtual presence a harmonious and impactful area to learn, discuss and and network with fellow TSC researchers and clinicians.

The past 18 months will live long in the collective memory of all of us – whether it has been personal loss, disruption to professional practices, or the impact of national lockdowns. However, the resilience, determination and belief of the TSC community – including families and also professionals – to get through this dark period together, has been incredible.

The global effort in science, research and healthcare provision, including the development of vaccination programmes, is helping the world push towards a brighter and more optimistic future. Similarly, TSC researchers and clinicians give direction and hope to the one million people worldwide affected by TSC. Let's embrace this conference's theme, *'A vision for the future'*, and shine a light to show everyone affected by TSC that there is reason for positivity and confidence in the diagnosis, treatment and management of TSC.

We were delighted to receive over 70 abstracts from researchers and clinicians keen to present their research at this year's conference. The abstract topics reflect the breadth of research that is underway at present, from understanding genetic risks to mosaicism detection rates, and from treating epilepsy to managing TAND symptoms. We would particularly like to thank Noema Pharma for sponsoring our Early Career Researcher awards, enabling six early career researchers to present their work and make useful contacts for potential future collaborations.

The sessions chosen for the International TSC Research Conference 2021 reflect this meeting's aims to push our understanding of TSC forward, through knowledge that is practical. We have chosen to focus on topics that will have the greatest impact on the individuals and families that live with the impact of TSC globally.

Thank you to all of our colleagues whose dedication and passion ensure that TSC research continues to progress at an exciting rate.

With our very best wishes,

Dr Chris Kingswood
ITSCRC2021 Co-Chair
St Geoge's University Hospital and TSA President

Dr Anna Jansen
ITSCRC2021 Co-Chair
UZ Brussel

Prof Andrew Tee
ITSCRC2021 Co-Chair
Cardiff University

Conference committees

Scientific committee (SC)

Co-Chairs: Professor Anna Jansen (UZ Brussel)
Dr Chris Kingswood (St George's University Hospital NHS Foundation Trust)
Professor Andrew Tee (Cardiff University)

Members: Dr Nicholas Annear (St George's University Hospital NHS Foundation Trust)
Professor Patrick Bolton (Kings College London)
Professor Elizabeth (Lisa) Henske (Harvard University)
Professor Simon Johnson (University of Nottingham)
Dr Angela Peron (University of Milan)
Dr Steve Roberds (TS Alliance)
Professor Julian Sampson (Cardiff University)
Professor Elizabeth Thiele (Harvard University)
Professor Petrus de Vries (University of Cape Town)

Lay representatives: Mr Perry James and Mrs Corinne Swainger

TSA representatives: Mrs Louise Fish (CEO), Dr Pooja Takhar (Head of Research) and Ms Bethan Vaughan (Research and Evidence Officer).

Early career researchers (ECR) committee

Dr Sam Amin (University of Bristol)
Dr Nicholas Annear (St George's University Hospital NHS Foundation Trust)
Dr Elaine Dunlop (Cardiff University)
Dr Charliaos (Harry) Filippakis (Harvard University)
Ms Fabienne Haslam (University of Manchester)
Dr Charlotte Tye (Kings College London)

TSA representatives: Dr Pooja Takhar (Head of Research) and Ms Bethan Vaughan (Research and Evidence Officer)

Internal (TSA) organising committee (OC)

Co-Chairs: Mrs Louise Fish (CEO) and Dr Pooja Takhar (Head of Research).

Members: Mrs Mikaela Conlin-Hulme (Head of Income Generation)
Mr Mike Dodson (Office Manager)
Mr Luke Langlands (Head of Communications and Support)
Miss Bethan Vaughan (Research and Evidence Officer)

Invited speaker biographies

Julian Sampson, MD, PhD

Clinical Geneticist; Professor of Medical Genetics, Institute of Medical Genetics, Cardiff University

Professor Sampson is an academic clinical geneticist at the School of Medicine, Cardiff University, UK. Professor Sampson's work on Tuberous Sclerosis Complex (TSC) started with international collaborative projects to map and then isolate the genes that cause TSC, followed by the characterisation of genotype-phenotype relationships, investigation of disease mechanisms and treatments in laboratory models, and finally clinical trials to help establish new and better treatments that are now used in the clinic. Along the way, Professor Sampson has had the good fortune to work with fantastic colleagues and with truly inspiring patients and carers who strive to understand and rise to the challenges of TSC.

Brendan Manning, MD, PhD

Professor of Molecular Metabolism, Harvard T.H. Chan School of Public Health, Boston, MA

Director of PhD Program in the Biological Sciences in Public Heath, Harvard University

Professor Brendan Manning is a Professor in the Department of Molecular Metabolism at the Harvard T.H. Chan School of Public Health and the Department of Cell Biology at Harvard Medical School, Director of the PhD Program in Biological Sciences in Public Health at the Harvard Graduate School of Arts and Sciences, and a Faculty Member of the Dana-Farber/ Harvard Cancer Center. Professor Manning received his BSc from the University of Massachusetts, Amherst and his PhD from Yale University. Professor Manning then joined the laboratory of Lewis Cantley at Harvard Medical School for his postdoctoral research. During this time, he discovered that Tuberous Sclerosis Complex (TSC) tumor suppressors serve as the molecular connection between the PI3K and mTOR pathways, thereby linking a signaling pathway activated in the majority of human cancers to a nutrient-sensing pathway that controls cell growth and metabolism. In 2004, Professor Manning joined the faculty of the then newly established Department of Genetics and Complex Diseases at Harvard (renamed Molecular Metabolism in 2019) to continue research at the interface of signaling and metabolism. Research in the Manning laboratory is particularly focused on understanding and treating TSC and LAM. Professor Manning has served on the Scientific Advisory Board (SAB) of the LAM Foundation since 2006, the International SAB of the TS Alliance since 2007, and the TS Alliance Board of Directors from 2014 to 2019, where he co-chaired the Science and Medical Committee. Professor Manning was an inaugural recipient of the National Cancer Institute's Outstanding Investigator Award.

Anna Jansen, MD, PhD

Head of Paediatric Neurology Unit, Head of Rare Diseases and Supervisor Resident Training Program Paediatric Neurology, UZ Brussel

Associate Professor and FWO Senior Clinical Investigator, Vrije Universiteit Brussel

Professor Anna Jansen is Head of Paediatric Neurology at Universitair Ziekenhuis Brussel where she coordinates the multidisciplinary TSC clinic for children and adults. With Professor Petrus de Vries, Professor Jansen is co-leading the TANDem project, which aims to reduce the TSC-Associated Neuropsychiatric Disorders (TAND) identification and treatment gap by empowering families through technology. Professor Jansen was co-investigator in the EPISTOP trial (FP7/2007-2013 GA 602391) and responsible for the dissemination of the project results (www.epistop.eu). Professor Jansen was the Vice-Chair of the TOSCA Scientific Advisory Board and coordinated the TuberOus SClerosis registry to increase disease Awareness (TOSCA) SEGA research project. Professor Jansen was part of the TSC-Associated Neuropsychiatric Disorders (TAND) committee of the 2012 and 2018 TSC Consensus Conferences and has contributed to the development of the TAND checklist which she continues to promote. Professor Jansen has participated in several clinical trials in TSC and epilepsy. Professor Jansen teaches in medicine and youth health, and is affiliated to the Neurogenetics Research Group at Vrije Universiteit Brussel, with a research focus on developmental brain malformations.

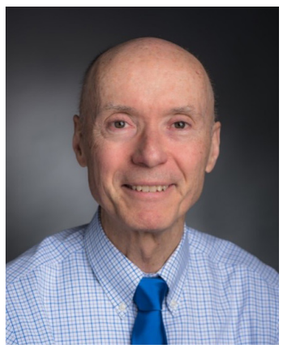
Darcy Krueger, MD, PhD

Professor of Clinical Paediatrics and Neurology, University of Cincinnati College of Medicine

Clack Endowed Chair in Tuberous Sclerosis; Director, Tuberous Sclerosis Clinic, Cincinnati Children's Hospital Medical Centre

Professor Darcy Krueger received his MD and PhD degrees from Saint Louis University, Missouri, and completed paediatrics and neurology training at Cincinnati Children's Hospital, Ohio. Dr Krueger is currently the Clack Endowed Chair in Tuberous Sclerosis and Director of the Tuberous Sclerosis Clinic at Cincinnati Children's Hospital Medical Center, and Professor of Clinical Paediatrics and Neurology at the University of Cincinnati College of Medicine. The TSC Clinic at Cincinnati Children's Hospital provides comprehensive, multidisciplinary clinical care to TSC patients of any age, and was a leader in efforts that resulted in the first ever FDA-approved medical therapy for TSC in 2010. Professor Krueger was the founding director of the TSC Clinical Research Consortium (TSCCRC) in 2012, now with 4 NIH-funded projects awarded to date: TSC Autism Center of Excellence Network (PI: Krueger, Sahin), TSC Epilepsy Biomarker Study (PI: Bebin), Preventing Epilepsy with Vigabatrin in Infants with TSC (PI: Bebin), and the Rare Diseases Clinical Research Network Developmental Synaptopathies Consortium (PI: Sahin). Professor Krueger also serves on the Board of Directors of the Tuberous Sclerosis Alliance patient advocacy organization, actively serving on committees focused on global outreach with TSC patients and specialists around the world, TSC research, and improved delivery of TSC patient clinical care. For contributions in TSC research and patient care, Professor Krueger was recipient of the Manuel R. Gomez award in 2019.

David Kwiatkowski, MD, PhD



Senior Physician, Brigham and Women's Hospital

Professor of Medicine, Harvard Medical School

Professor David Kwiatkowski and his lab are focused on Tuberous Sclerosis Complex (TSC), and cancers that have activation of the mTOR signalling pathway. Professor Kwiatkowski also has a broader interest in cancer genetics and its translational implications. Professor Kwiatkowski is best known for the identification of the TSC1 gene, and a long series of pioneering genetic studies in TSC. Professor Kwiatkowski's lab pursues multiple aspects of TSC research: use of massively parallel sequencing and computational methods to identify mosaic mutations in TSC1/TSC2 in 90% of individuals with a diagnosis of TSC in whom previous efforts at mutation identification had failed; examination of transcriptional pathways that are driver events in angiomyolipoma, including MITF; identification of a core expression gene set for TSC-mutant tumors, analysis of PEComa, and analysis of a novel therapeutic approach in TSC and use of bi-steric mTORC1 inhibitors.

Hope Northrup, MD

Director, Division of Medical Genetics and Professor, Department of Paediatrics, The University of Texas Medical School

Dr Hope Northrup is a Medical Geneticist (Clinical, Biochemical and Molecular) in the Department of Paediatrics at The McGovern Medical School, University of Texas Health Science Center at Houston. Dr Northrup is Vice Chairman of Academics in the Department of Paediatrics, and Professor and Director of the Division of Medical Genetics. Dr. Northrup is the Director of the Medical Genetics Residency Training Program (1993-present). Due to her clinical expertise, Dr Northrup has been selected numerous times for inclusion in various publications citing excellence in clinical care, including Best Doctors in America, America's Top Doctors, and Texas Super Doctors. In recognition of her teaching excellence, Northrup received the Regents Outstanding Teaching Award (ROTA) for the University of Texas System in 2016. Dr. Northrup was recently chosen by the Women Faculty Forum (WFF) of the McGovern Medical School to receive the Excellence in Clinical Science Award for 2020. Dr Northrup obtained a medical degree from the Medical University of South Carolina. Dr Northrup completed a Paediatric Residency at Children's Medical Center/Southwestern Medical School in Dallas, Texas, and a Medical Genetics Fellowship at the Institute for Molecular Genetics, Baylor College of Medicine in Houston, Texas. Dr Northrup's research focuses on unraveling the basis of neurogenetic diseases, specifically Tuberous Sclerosis Complex (TSC), and Spina Bifida, and developing therapies for genetic diseases. She was the 2003 winner of the Manuel R. Gomez Professional Recognition Award from the Tuberous Sclerosis Alliance for her research and clinical contributions in the field of TSC.

John Bissler, MD

Professor of Paediatrics; Director, TC Centre of Excellence, Memphis

Director, Division of Nephrology, St Jude Children's Hospital and LeBonheur Children's Hospital

Professor John Bissler saw his first patient with Tuberous Sclerosis Complex (TSC) over 35 years ago. Professor Bissler was struck by the lack of accurate information at that time, and has focused his career on the care of TSC patients with renal disease. Professor Bissler's work in this area includes developing a steroid protocol to greatly reduce the pain of the angiomyolipoma embolization procedure, and he was the principal investigator for the use of mTORC1 inhibitors TSC. Professor Bissler has seen over 1500 individual TSC patients in the United States, and many more than that internationally. Professor Bissler continues to focus his research on TSC renal disease, and is currently looking for therapies for the cystic disease, which has no approved therapies. Professor Bissler maintains a busy clinical practice as the Director of the TSC Center of Excellence in Memphis, Tennessee, one of the largest TSC Centers in the USA. Professor Bissler also specializes in the renal care of the child with cancer.

Elizabeth (Lisa) Petri Henske, MD

Medical Oncologist, Dana-Farber Cancer Institute

Professor of Medicine, Harvard Medical School

Director, Center for LAM Research and Clinical Care, Brigham and Women's Hospital

Professor Elizabeth (Lisa) Petri Henske is the Director of the Center for LAM Research and Clinical Care at Brigham and Women's Hospital. She is Professor of Medicine at Harvard Medical School, an Associate Member of the Broad Institute of MIT and Harvard, and a practicing medical oncologist at the Dana-Farber Cancer Institute. Professor Henske earned her undergraduate degree *summa cum laude* from Yale University, where she majored in Molecular Biophysics and Biochemistry, and her MD from Harvard Medical School. Professor Henske completed a residency in Internal Medicine and a fellowship in Hematology/Oncology at the Massachusetts General Hospital. Professor Henske's laboratory discovered that lymphangioleiomyomatosis (LAM) is caused by mutations in the Tuberous Sclerosis Complex genes. Professor Henske also was the first to discover that the TSC1 and TSC2 proteins physically interact. Professor Henske research laboratory is focused on the cellular, metabolic, and immunologic mechanisms underlying the pathogenesis of angiomyolipomas and LAM. She is a member of the American Society for Clinical Investigation and the Association of American Physicians, and serves on the Board of Directors of The LAM Foundation and the Professional Advisory Board of the Tuberous Sclerosis Alliance. Professor Henske has received awards for her research from the Tuberous Sclerosis Alliance, The LAM Foundation, the American Thoracic Society, and the Society for Women's Health Research (the Medtronic Prize).

Petrus J de Vries, MBChB, FRCPsych, PhD

Sue Struengmann Professor of Child and Adolescent Psychiatry, University of Cape Town

Professor Petrus de Vries is the Sue Struengmann Professor of Child & Adolescent Psychiatry, and Director of the Centre for Autism Research in Africa, and of the Adolescent Health Research Unit at the University of Cape Town. Professor de Vries trained in medicine at Stellenbosch University in South Africa, and he completed his clinical training in Psychiatry and Child and Adolescent Psychiatry, gaining a PhD in Developmental Neuroscience at the University of Cambridge. Professor de Vries currently focuses on child and adolescent mental health, and on implementation science in low- and middle-income settings. Professor de Vries was lead developer and author of the TAND Checklist, and closely involved in the phase II and III clinical trials of mTOR inhibitors and treatment-resistant epilepsy in TSC, leading to FDA and EMA approval for mTOR inhibitors as molecularly-targeted treatments in TSC. Professor de Vries was chairman of the Society for the Study of Behavioural Phenotypes (SSBP), and is the current President of the South African Association of Child and Adolescent Psychiatry and Allied Professions (SA-ACAPAP). Professor de Vries is chairman of the African Division of the Royal College of Psychiatrists, Secretary of the International Society for Autism Research (INSAR), and Treasurer of the International Association of Child and Adolescent Psychiatry and Allied Professions (IACAPAP), where he also coordinates the Helmut Remschmidt Research Seminars. He has been actively involved with many TSC user/carer organisations over the last 21 years, including the TSA UK, TS Alliance, and TS Deutschland. In 2014 he was awarded the Manuel R Gomez Award for his contributions to Tuberous Sclerosis Complex.

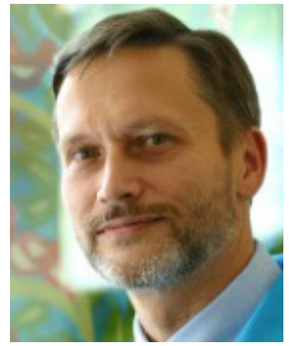
Xandra O. Breakefield, PhD

Geneticist (Neurology and Radiology), Massachusetts General Hospital

Professor of Neurology, Harvard Medical School

Professor Xandra Breakefield is a research scientist with a strong background in molecular genetics and neuroscience. Professor Breakefield has focused her efforts on identification of neurologic disease genes, gene therapy for neurologic diseases; and elucidation of the role of extracellular vesicles (EVs) in cell-to-cell communication and tumour progression. She is currently working on gene therapy for Tuberous Sclerosis Complex (TSC) in mouse models. Professor Breakefield has published over 500 scientific articles, and has received continuous support from the National Institutes of Health in the USA for her research over a 40-year period. Professor Breakefield did her undergraduate work at Wilson College, and her graduate work in Microbial Genetics at Georgetown University. She was a Postdoctoral Fellow with Nobel Prize winner Dr. Marshall Nirenberg at the NIH. She was appointed an Assistant Professor in the first Department of Human Genetics in the USA at Yale Medical School in 1974, and in 1984 moved to Harvard Medical School and Massachusetts General Hospital. Professor Breakefield has received a number of awards for her work, including a McKnight Foundation Neuroscience Development Award, two Javits Neuroscience Investigator Awards, the Society for Neuroscience Mika Salpeter Lifetime Achievement Award, the Harvard Medical School William Silen Lifetime Achievement Mentoring Award, and she is a recipient of NCI's Outstanding Investigator Award. Professor Breakefield is a member of the American Academy of Arts and Sciences, and a past president of the American Society of Gene and Cell Therapy.

Sergiusz Jóźwiak, MD, PHD



Head of Paediatric Neurology, Warsaw Medical University

Professor Sergiusz Jóźwiak is Head of the Department of Paediatric Neurology at Warsaw Medical University. Professor Jóźwiak received his medical degree from the Medical University Warsaw in 1983, and his doctoral degree from the Children's Memorial Health Institute in 1990. Professor Jóźwiak completed his studies at the same institute in 1995, and was appointed Head of the Paediatric Neurology and Epileptology Department in 1997. Professor Jóźwiak held this position until May 2015, when he moved to Warsaw Medical University. Between 2009-2014, Professor Jóźwiak served as a National Consultant in Paediatric Neurology. Professor Jóźwiak's research focuses mainly on neurocutaneous disorders and epilepsy, especially infantile spasms. For over 25 years, he has led a special programme for Tuberous Sclerosis Complex (TSC) patients, and worked out practical guidelines for TSC management. In 2009, Professor Jóźwiak received the prestigious Manuel Gomez Award established by Tuberous Sclerosis Alliance for "creative or pioneering efforts that have appreciably improved either the understanding of the disease or the clinical care available for individuals with Tuberous Sclerosis". Professor Jóźwiak is currently a coordinator of the large-scale European Commission Project EPISTOP, evaluating clinical and molecular biomarkers of epileptogenesis in a genetic model of epilepsy – Tuberous Sclerosis Complex. Professsor Jóźwiak is an active member of numerous international organisations, and has published more than 300 papers in national and international peer reviewed journals. He is on the editorial boards of several professional journals, including Paediatric Neurology (USA), European J. Paediatric Neurology (Amsterdam), Journal of Child Neurology (USA).

Conference agenda

Thursday 17 June – Saturday 19 June

Day One: Thursday 17 June

All times are shown in BST

Time	Agenda item	Duration
14:00 – 14:45	**Opening session** Welcome from the Tuberous Sclerosis Association (TSA) *Louise Fish (TSA Joint Chief Executive)* Thank you *Sir David Suchet CBE* Welcome (and housekeeping) from Conference Committee Co-Chairs *Chris Kingswood and Anna Jansen* Keynote speaker (sponsored by GW Pharma) Genetics of TSC: Past, present and future *Julian Sampson*	 5 mins 5 mins 5 mins 30 mins
14:40 – 15:50	**Session 1 – Early diagnosis** **Session Co-Chairs: Julian Sampson and Sam Amin** Sanjay Sethi (community speaker) *A parent's perspective on living with TSC* Professor Sergiusz Jozwiak *Early diagnosis and epilepsy prevention: Results of the EPISTOP study* Thomas Darling *Recognition of lesser-known skin findings for earlier diagnosis of Tuberous Sclerosis Complex* *Xavier Farre Ramon* *Shared genetic risk factors between lymphangioleiomyomatosis and pulmonary function* Katarzyna Klonowska (Early Career Researcher) *90% TSC1/TSC2 mosaicism detection rate in Tuberous Sclerosis Complex patients without mutation identified in commercial labs*	 10 mins 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
15:50 – 16:05	**Rapid-fire poster presentations 1** Robert Waltereit (#2) *mTOR inhibitor improves autistic-like behaviors related to TSC haploinsufficiency but not following developmental status epilepticus* Jade Marshall (#13) *Quality of life in patients with Tuberous Sclerosis Complex and epilepsy: Estimating patient and caregiver health state utilities* Dario R. Lemos (#20) *Modeling Tuberous Sclerosis Complex-sssociated renal angiomyolipoma tumors driven by TSC2 loss-of-heterozygosity using patient-derived hiPSCs*	15 mins (5x3 mins each)

	Muireann Ní Bhaoighill (#44) *Extracellular vesicles secreted by TSC2-deficient cells shuttle altered cargo to fibroblasts of the tumour microenvironment to promote pro-tumoral signalling* Eva Davis (#48) *Tuberous Sclerosis Complex, a service review and plan for the future*	
16:10 – 16:25	**Break and poster viewing***	15 mins
16:25 – 17:25	**Session 2– Early risk assessment** **Session Co-Chairs: Elizabeth Thiele and Anna Jansen** Jennifer Flinn (community speaker) *My TSC journey* David Kwiatkowski *Genetics and pathogenesis of kidney angiomyolipoma: role of MITF and other TFs* Laura S Farach *Creating a genetic risk prediction model for epilepsy in TSC* Angela Peron (Early Career Researcher) *Subependymal giant cell astrocytoma in adult patients with tuberous sclerosis: incidence rate, timing and causes of new diagnosis in adulthood*	 20 mins (+10 mins Q&A) 10 mins 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
17:25 – 17:40	**Rapid-fire poster presentations 2** Daniel Kummel (#4) *Insight into TSC protein complex function from structural biology* Tasmia Tahsin (#18) *Targeting mTORC1 overactivation and Nrf2 inhibition to overcome ferroptosis resistance in cancer cells* Linda Murphy (#55) *Quantification of healthcare, social care, and educational resource use in patients with Tuberous Sclerosis Complex (TSC)-associated epilepsy: Insights from a UK Delphi panel* Carmen Herranz (#67) *Histamine signaling and metabolism identify biomarkers and therapies for lymphangioleiomyomatosis* Stephanie Dooves (#27) *Tuberous Sclerosis Complex astrocytes affect neuronal synaptic balance through secreted factors*	15 mins (5x3 mins each)
17:40 – 18:00	**Break and poster viewing***	20 mins
18:00 – 19:00	**Industry symposium –** sponsored by **Nobel Pharma** *Current and future options in the treatment of facial angiofibromas* *This session is restricted to healthcare professionals and prescribers only to comply with the ABPI Code of Practice* **Poster networking session** (parallel session) *This parallel session is open to all delegates*	 60 mins 60 mins

Day Two: Friday 18 June 2021

Time	Agenda item	Duration
14:00 – 14:05	**Welcome from Conference Committee Co-Chairs** Chris Kingswood and Anna Jansen	5 mins
14:05 – 15:15	**Session 3 – Research into new and available therapies** **Session Co-Chairs: Chris Kingswood and Nicholas Annear** Eva Schoeters (community speaker) *TSC, life as we know it* Petrus de Vries *Research into new and available therapies for TAND* Elizabeth Thiele *Long-term safety and efficacy of add-on cannabidiol (CBD) for treatment of seizures associated with Tuberous Sclerosis Complex in an open-label extension trial (GWPCARE6)* Heng Du *Bi-steric mTORC1-selective inhibitors demonstrate improved potency and efficacy in tumors caused by TSC* Daniel Ebrahimi-Fakari (Early Career Researcher) *Prenatal sirolimus treatment for intrauterine rhabdomyomas in tuberous sclerosis*	 10 mins 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
15:15 – 15:30	**Rapid-fire poster presentations 3** Sam Amin (#9) *The metformin in Tuberous Sclerosis (MiTS) study: A randomised double-blind placebo-controlled trial* Farhad Sahebkar (#26) *Efficacy of Add-on Cannabidiol (CBD) treatment in patients with Tuberous Sclerosis Complex and a history of infantile spasms: Post hoc analysis of phase 3 trial GWPCARE6* Katharina Fitzian (#35) *Oligomerization of TSC1 and membrane binding via phosphatidylinositol-phosphates* Krinio Giannikou (#43) *Kidney angiomyolipomas are defined by a unique transcriptional profile and H3K27ac chromatin state* Charilaos (Harry) Filippakis (#56) *DGKA-dependent macropinocytosis supports tumorigenesis in Tuberous Sclerosis Complex*	15 mins (5x3 mins)
15:30 – 15:50	**Break and poster viewing***	20 mins

15:50 – 16:40	**Session 4 – Basic science and pre-clinical work (A)** **Session Co-Chairs: Elizabeth Henske and Elaine Dunlop** Professor Brendan Manning *New therapeutic strategies for eliminating TSC lesions* Jesse Champion *Drug inhibition of reduction-oxidation factor 1-apurinic/apyrimidinic endonuclease 1 restores the hypoxic-driven disease state of Tuberous Sclerosis Complex* Hilaire C Lam *Regulation of de novo serine synthesis by Interleukin 6 in Tuberous Sclerosis Complex (TSC)*	 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
16:40 – 17:30	**Session 5 – Basic science and pre-clinical work (B)** **Session Co-Chairs: Simon Johnson and Charilaos (Harry) Filippakis** Elizabeth Henske *TFEB and lysosomes: Roles in TSC pathogenesis and therapy* Constantinos Demetriades *A novel TSC-mTORC1-GRASP55 signalling axis controls unconventional protein secretion to reshape the extracellular proteome upon stress* Pranetha Baskaran (Early Career Researcher) *Unkempt is a novel downstream regulator of mTOR signalling in mammalian neurogenesis*	 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
17:30 – 17:45	**Rapid-fire poster presentations 4** Femke V.M. Mulder (#12) *The long-term effect of mTOR inhibition on lipid and glucose metabolism in Tuberous Sclerosis Complex: data from a nationwide registry.* Yan Tang (#21) *Single cell analysis of Tuberous Sclerosis Complex (TSC) reveals a stem-like tumor state linked to immune suppression* Mehdi Chihi (#32) *Tuberous Sclerosis Complex and cerebral aneurysms: The challenge of early detection* Katarzyna Klonowska (#53) *Millions of incipient angiofibromas in TSC facial skin* Montana Kay Lara (#62) *TSC-related phenotypes in C57BL/6J and DBA/2J mice with germline TSC1 haploinsufficiency*	15 mins (5x3 mins each)
17:45 – 18:00	**Break and poster viewing**	15 mins
18:00 – 19:00	**Industry symposium -** Sponsored by **GW Pharma** *Cannabinoids in the management of TSC-associated seizures* *This session is restricted to healthcare professionals and prescribers only to comply with the ABPI Code of Practice* **Poster networking session** (parallel session) *This parallel session is open to all delegates*	 60 mins 60 mins

Day Three: Saturday 19 June 2021

Time	Agenda item	Duration
14:00 – 14:05	**Welcome from Conference Committee Co-Chairs** Chris Kingswood and Anna Jansen	5 mins
14:05 – 15:25	**Session 6 – Prevention** **Session Co-Chairs: Petrus de Vries and Nicholas Annear** Vicky Whittemore (community speaker) *The Ups and Downs of Life with TSC* Hope Northrup *Tuberous Sclerosis Complex: Is prevention possible?* Stacey Bissell and Stephanie Vanclooster *Understanding the landscape of Tuberous Sclerosis Complex (TSC)-Associated Neuropsychiatric Disorders (TAND) research: A comprehensive scoping review* Gabrielle V Rushing *Evolution of the TS Alliance TSC biosample repository* Davide Caputo *Febrile seizures and febrile status epilepticus in Tuberous Sclerosis Complex: A retrospective analysis* Stacey Bissell (Early Career Researcher) *Exploring Sleep in Neurodevelopmental disorders through Online and Remote Evaluation (e-SNORE): Pilot and feasibility study in Tuberous Sclerosis Complex*	 10 mins 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A) 8 mins (+2 mins Q&A)
15:25 – 15:45	**Break and poster viewing***	20 mins
15:45 – 16:25	**Session 7 – Social research** **Session Co-Chairs: Patrick Bolton and Charlotte Tye** Anna Jansen *Towards socially responsive research in TSC* Jessica Martin (Early Career Researcher) *Investigating the impact of the pandemic on wellbeing in families of children with rare disorders: The CoIN Study*	 20 mins (+10 mins Q&A) 8 mins (+2 mins Q&A)
16:25 – 16:40	**Rapid-fire poster presentations 5** Kate Mowery (#17) *Pancreatic Neuroendocrine Tumors (PNETs) and utility of mTOR inhibitors as a treatment option* Krinio Giannikou (#42) *Spectrum of germline and somatic mitochondrial DNA variants in Tuberous Sclerosis Complex* Laura Geben (#61) *Mapping the time course of mTORC1-driven tumorigenesis in the developing brain* Samuel H. Barth (#40) *mTOR hyperactivity disrupts the molecular framework of inhibitory synapse formation* Katarzyna Klonowska (#52) *MHPA: a novel strategy enabling detection of extreme low-level mosaicism in TSC*	15 mins (5x3 mins each)
16:40 – 16:55	**Break and poster viewing***	15 mins
16:55 – 18:25	**Session 8 – A vision for the future** **Session Co-Chairs: Steve Roberds & Angela Peron** Xandra Breakefield *Gene therapy for Tuberous Sclerosis in mouse models* John Bissler *TSC renal cystic disease: Pathophysiology and treatment* Darcy Krueger *Changing the paradigm: Developing disease-modifying and preventative treatments for TSC* Panel session	 20 min (+10 misn Q&A) 15 mins (+5 mins Q&A) 15 mins (+5 mins Q&A) 20 mins
18:25 – 18:55	**Special session** Liesbeth De Waele *TANDem: An update on year 2*	 20 min (+10 mins Q&A)
18:55 – 19:00	**Thank you and close**	5 mins

*Posters will be available to view throughout the conference

Industry symposium sponsored by Nobel Pharma, 17 June 18:00

Current and Future Options in the treatment of Facial Angiofibroma

Chair

Dr Frances Elmslie MD FRCP

Consultant in genetics at St George's University Hospitals NHS Foundation Trust who specialises in paediatric genetics, the genetics of epilepsy and developmental brain malformations and tuberous sclerosis.

Speakers

Dr Lea Solman MD, MRCP, FRCPCH

Consultant in Paediatric Dermatology at Great Ormond Street Hospital. She is one of three doctors in the UK fully dually accredited both as a consultant paediatrician and a consultant dermatologist. She has written UK guidelines on the treatment of infantile haemangiomas (strawberry birthmarks).

'Safety and efficacy of topical sirolimus for Angiofibromas; 9 years' experience'

Dr Mari Wataya Kaneda MD

Department of Neurocutaneous Medicine, Division of Health Science, Graduate School of Medicine, Osaka University.

'Innovative Treatment Options in Japan for Facial Angiofibroma'

Dr Joyce Teng MD, PhD, FAAD

Professor in dermatology at Stanford University. She is affiliated with multiple hospitals in the area, including Lucile Salter Packard Children's Hospital (LPCH) at Stanford and Stanford Hospital and Clinics (SHC).

'Comprehensive Management of Facial Angiofibroma in the real World'

Symposium is sponsored by a grant from Nobelpharma Co Limited Japan.
This session is restricted to healthcare professionals only to comply with the ABPI Code of Practice.

PUP-UK-2021-001

Industry symposium sponsored by GW Pharma, 18 June 18:00

Cannabinoids in the management of TSC-associated seizures

Agenda and content overview		
Time	**Presenter**	**Title / Description**
18:05–18:10	**Prof. Finbar O'Callaghan, Chair**	Welcome and introductions
18:10–18:20	**Dr Chandni Hindocha**	What is cannabidiol (CBD)?
18:20–18:30	**Prof. Andrew Tee**	Scientific insights into CBD and TSC
18:30–18:45	**Prof. Finbar O'Callaghan**	TSC-associated epilepsy and GW CBD
18:45–19:00	**All**	Q&A / Meeting close

This session is organised and funded by GW Pharmaceuticals and is intended for an HCP or prescriber audience only. Prescribing information will be available at this session and from the GW virtual booth on the congress website.

Job code: VV-MED-18961

Date of preparation: May 2021

Abstract listings by number

1. The effect of pregnancy on renal angiomyolipoma in patients with tuberous sclerosis complex

Marlou Kluiving, E.F.H.I. Peeters, WL de Ranitz-Greven.

2. mTOR inhibitor improves autistic-like behaviors related to Tsc2 haploinsufficiency but not following developmental status epilepticus

Robert Waltereit, Tomas Petrasek, Iveta Vojtechova, Ondrej Klovrza, Klara Tuckova, Cestmir Vejmola, Jakub Rak, Anna Sulakova, Daniel Kaping, Nadine Bernhardt, Petrus J. de Vries, Jakub Otahal.

3. Successful combined treatment of the tuberous sclerosis complex with synergic effect (inhibitor mTOR + Vigabatrine) in child with 10 months

Julieta Sobreira Goes, Emilia Katiane Embiruau,de Araujo Leao, Joice Santana.

4. Insight into TSC protein complex function from structural biology

Daniel Kuemmel, Katharina Fitzian, Anne Brueckner, Laura Brohee, Constantinos Demetriades, Andrea Oeckinghaus.

5. Evolution of the TS Alliance TSC biosample repository

Gabrielle V. Rushing, Jo Anne Nakagawa, Zoë Fuchs, Dana R. Valley, Daniel C. Rohrer, Scott D. Jewell, Steven L. Roberds.

6. A mouse model of subependymal giant cell astrocytomas

Victoria A. Riley, Aidan M. Sokolov, David M. Feliciano.

7. Characterization of individuals with cutaneous manifestations associated with tuberous sclerosis complex in the United States: A sub-analysis of an international survey of caregivers and individuals with TSC

Steven L. Roberds, Sreedevi Boggarapu, Eric Beresford.

8. Characterization of individuals with facial angiofibroma associated with tuberous sclerosis complex in the United States and factors associated with use of topical mTOR inhibitors: a retrospective analysis of the Natural History Database

Jo Anne Nakagawa, Steven L. Roberds, Sreedevi Boggarapu, Eric Beresford.

9. The metformin in tuberous sclerosis (MiTS) study: A randomised double-blind placebo-controlled trial

Sam Amin, Andrew A Mallick, Hannah Edwards, Mario Cortina-Borja, Matthew Laugharne, Marcus Likeman, Finbar J.K. O'Callaghan.

10. Recognition of lesser known skin findings for earlier diagnosis of tuberous sclerosis complex

Thomas Darling, Joel Moss.

11. ABSTRACT WITHDRAWN

12. The long-term effect of mTOR inhibition on lipid and glucose metabolism in Tuberous Sclerosis Complex: data from a nationwide registry

Femke V. M. Mulder, Evelien F.H. Peeters, Jan Westerink, Wendela L. de Ranitz-Greven.

13. Quality of life in patients with tuberous sclerosis complex and epilepsy: estimating patient and caregiver health state utilities

Jade Marshall, Siu Hing Lo, Hanna Skrobanski, Andrew Lloyd.

14. Evaluation of renal disease in paediatric patients with tuberous sclerosis

Claire McFaul, Jenny Patterson; Shelagh Joss, Ihab Shaheen.

15. Shared genetic risk factors between lymphangioleiomyomatosis and pulmonary function

Xavier Farré Ramon, Miquel Angel Pujana, Roderic Espin, Alexandra Baiges, Eline Blommaert, Wonji Kim, Krinio Giannikou, Carmen Herranz, Antonio Antonio Román, Berta Sáez, Mireia Tena-Garitaonaindia, Fermín Sánchez de Medina, Francesca Mateo, Maria Molina-Molina, Sungho Won, David J. Kwiatkowski, Rafael de Cid.

16. Two generations of TSC: Impact of early diagnosis and interventions

Kate Mowrey, Mary Kay Koenig, Charles A. Szabo, Joshua Samuels, Shannon Mulligan, Deborah A. Pearson, Hope Northrup.

17. Pancreatic Neuroendocrine Tumors (PNETs) and utility of mTOR Inhibitors as a treatment Option

Kate Mowrey, Hope Northrup, Peyton Rougeau, S. Shahrukh Hashmi, Darcy A. Krueger, Daniel Ebrahimi-Fakhari, Alexander J. Towbin, Andrew T. Trout, Jamie K. Capal, David Neal Franz, David Rodriguez-Buritica.

18. Targeting mTORC1 overactivation and Nrf2 inhibition to overcome ferroptosis resistance in cancer cells

Tasmia Tahsin, Andrew Tee, Steve Conlan, Mark Davies, Gareth Healey, Stephen Hughes.

19. The association of co-morbidity (congenital heart defects, neurodevelopmental abnormalities and renal system) in patients with tuberous sclerosis complex I and II: a prospective cohort study

Jessica Robinson, Adrian Harwood, Jennifer Frances Gardner, Anurag Saxena, Orhan Uzun, Yasir Ahmed Syed.

20. Modeling tuberous sclerosis complex-sssociated renal angiomyolipoma tumors driven by TSC2 loss-of-heterozygosity using patient-derived hiPSCs

Dario R. Lemos, J.O.R.Hernandez, X. Wang, M. Vazquez-Segoviano, M.F. Sobral-Reyes, A. Moran-Horowich, M. Sundberg M., M. Lopez-Marfil M., D.O. Lopez-Cantu , G.U. Probst C.K., Ruiz-Esparza, K. Giannikou, E.P. Henske, D. J. Kwiatkowski, M. Sahin.

21. Single cell analysis of tuberous sclerosis complex (TSC) reveals a stem-like tumor state linked to immune suppression

Yan Tang, David J. Kwiatkowski, Elizabeth P. Henske.

22. HRQoL of TSC individuals in Hong Kong: a local study and comparison with literature using Health Utilities Index

Chung Cheuk Yee Coey, Dorothy C.C. Chan, Lorraine L.W. Chiang, William C.Y. Chu, Nicole W.L. Hon,

Wilfred H.S. Wong, Godfrey C.F. Chan.

23. Investigating the impact of the pandemic on wellbeing in families of children with rare disorders: the CoIN Study

Jessica Martin, Sarah Charles, Kate Baker, Ted Barker, Caroline Richards, Abigail Runicles, Gaia Scerif, CoIN Study Group and Charlotte Tye.

24. Time to onset of Cannabidiol (CBD) treatment effect and resolution of adverse events in the tuberous sclerosis complex phase 3 randomised controlled trial (GWPCARE6)

Farhad Sahebkar, Hannah Cock, Joyce Y. Wu, Orrin Devinsky, Charuta Joshi, Ian Miller, Colin M. Roberts, Rocio Sanchez-Carpintero, Daniel Checketts.

25. Prenatal Sirolimus treatment for intrauterine rhabdomyomas in tuberous sclerosis

Daniel Ebrahimi-Fakhari, Darcy Krueger, David Neal Franz.

26. Efficacy of add-on Cannabidiol (CBD) treatment in patients with tuberous sclerosis complex and a history of infantile spasms: post hoc analysis of phase 3 trial GWPCARE6

Farhad Sahebkar, Steven P. Sparagana, Patrick Kwan, Finbar J. O'Callaghan, Russell P. Saneto, James W. Wheless, Kerry Hyland.

27. Tuberous sclerosis complex astrocytes affect neuronal synaptic balance through secreted factors

Stephanie Dooves, Arianne JH van Velthoven; Linda G Suciati; Vivi M Heine.

28. The power of 1: N-of-1 studies to improve interventional research for tuberous sclerosis complex

Annelieke R. Muller, Marion M.M.G. Brands, Peter M. van de Ven, Kit C.B. Roes, Martina C. Cornel, Clara D.M. van Karnebeek, Frits A Wijburg, Joost G. Daams, Erik Boot, Agnies M. van Eeghen.

29. Aberrant DJ-1 expression underlies L-type calcium channel hypoactivity in tuberous sclerosis complex (TS) and Alzheimer's disease (AD)

Farr Niere, Luisa P. Cacheaux, Ayse Uneri, William C. Taylor, Suzanne Craft, C. Dirk Keene, Tao Ma, Kimberly F. Raab-Graham.

30. Neuropsychiatric disorders in tuberous sclerosis complex patients with epilepsy

Jeng-Dau Tsai, Hom-Yi Lee, Ji-Nan Sheu.

31. Seizure outcome after epilepsy surgery in tuberous sclerosis complex: results and analysis of predictors from a multicenter study

Chiara Vannicola, Laura Tassi, Carmen Barba, Clementina Boniver, Massimo Cossu, Marco de Curtis, Luca De Palma, Ignazio D'Errico, Giuseppe Didato, Renzo Guerrini, Francesca La Briola, Concetta Luisi, Roberto Mai, Francesco Mari, Carlo Efisio Marras, Massimo Mastrangelo, Angela Peron, Nicola Specchio, Irene Toldo, Katherine Turner, Aglaia Vignoli, Maria Paola Canevini.

32. Tuberous sclerosis complex and cerebral aneurysms: the challenge of early detection

Mehdi Chihi, Ulrich Sure, Ramazan Jabbarli.

33. The abnormality of white matter microstructure in the limbic system is correlated with TSC-associated neuro-psychiatric disorders (TAND)

Akemi Sato, Kuriko Kagitani-Shimono, Koji Tominaga, Yoshiko Iwatani, Yoko Kato, Mari Wataya-Kaneda, Masako Taniike.

34. Creating a genetic risk prediction model for epilepsy in TSC

Laura S. Farach, Melissa A. Richard, Philip J. Lupo, Kit Sing Au, Hope Northrup.

35. Oligomerization of TSC1 and membrane binding via phosphatidylinositol-phosphates

Katharina Fitzian, Katharina Fitzian, Anne Brueckner, Laura Brohee, Constantinos Demetriades, Andrea Oeckinghaus

36. Insights from a UK Delphi panel investigating the relationship between tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) and TSC-associated epilepsy

Jade Marshall, Linda Murphy, Catriona Crossan, Emily Jones, Dawn Lee, Chris Kingswood.

37. Exploring Sleep in Neurodevelopmental disorders through Online and Remote Evaluation (e-SNORE): Pilot and feasibility study in tuberous sclerosis complex

Stacey Bissell, Caroline Richards, Chris Oliver, Andrew Bagshaw; Caitlin Williams; Lucy Wilde; Petrus de Vries; Cathy Hill.

38. Subependymal giant cell astrocytoma in adult patients with tuberous sclerosis: incidence rate, timing and causes of new diagnoses in adulthood

Angela Peron, Roberto Previtali, Francesca La Briola, Katherine Turner, Chiara Vannicola, Aglaia Vignoli, Maria Paola Canevini.

39. Investigating the role of P/Q- and N-type voltage-gated calcium ion channels in a preclinical model of tuberous sclerosis

Hailey Egido-Betancourt, Kimberly F. Raab-Graham.

40. mTOR hyperactivity limits protein synthesis and reduces inhibitory synapse formation

Samuel H. Barth, Kimberly F. Raab-Graham.

41. Genotype-phenotype correlations between TSC and autism

Abigail Kacpura, Laura S. Farach, Melissa A. Richard, Philip J. Lupo, Kit Sing Au, Hope Northrup, Deborah A. Pearson.

42. Spectrum of germline and somatic mitochondrial DNA variants in tuberous sclerosis complex

Krinio Giannikou, Katie Martin, Ahmad Abdel-Azim, Thomas R. Hougard, Yan Tang, Jeffrey MacKeigan, David J. Kwiatkowski, Hilaire C. Lam.

43. Kidney angiomyolipomas are defined by a unique transcriptional profile and H3K27ac chromatin state

Krinio Giannikou, Clemens Probst, Xintao Qiu, Melissa Duarte, Nikolas Kesten, Mahsa Zarei, Zach Hebert, Raga Vadhi, Alba Font-Tello, Paloma Cejas, Charles H. Yoon, Chin-Lee Wu, Myles Brown, Elizabeth Henske, Henry Long, David J. Kwiatkowski.

44. Extracellular vesicles secreted by TSC2-deficient cells shuttle altered cargo to fibroblasts of the tumour microenvironment to promote pro-tumoral signalling

Muireann Ní Bhaoighill, Andrew R. Tee, Jason P. Webber, Elaine A. Dunlop.

45. Unkempt is a novel downstream regulator of mTOR signalling in mammalian neurogenesis

Pranetha Baskaran, Elin Vinsland, Simeon R. Mihaylov, Andrew R. Tee, Kriti Shah, Jernej Murn, Joseph M. Bateman.

46. Management of facial angiofibroma related to tuberous sclerosis complex and use of topical mTOR inhibitor in U.S. retrospective analysis of natural history database

Eric Beresford, Steven L. Roberds, Jo Anne Nakagawa, Sreedevi Boggarapu.

47. Facial angiofibroma related to tuberous sclerosis complex and the use of topical rapamycin in the United States: A survey of caregivers and individuals with TSC

Eric Beresford, Steven L. Roberds, Sreedevi Boggaparu.

48. Tuberous sclerosis complex, a service review and plan for the future

Eva Davis, Mary Vasseghi, Patrick Moloney, Sam Amin, Norman Delanty, Colin P. Doherty, Claire Behan.

49. A case study of neuropsychological intervention in a child diagnosed with tuberous sclerosis complex

Laís F. M. Cardozo, Ana Paula A. de Pereira, Isac Bruck, Sérgio A. Antoniuk.

50. Febrile seizures and febrile status epilepticus in tuberous sclerosis complex: a retrospective analysis

Davide Caputo, Francesca La Briola, Angela Peron, Eleonora Pollina, Fabio Bruschi, Aglaia Vignoli, Maria Paola Canevini.

51. 90% TSC1/TSC2 mosaicism detection rate in tuberous sclerosis complex patients without mutation identified in commercial labs

Katarzyna Klonowska, Joannes Grevelink, Krinio Giannikou, Magdalena Tyburczy, David Kwiatkowski.

52. MHPA: a novel strategy enabling detection of extreme low-level mosaicism in TSC

Katarzyna Klonowska, David Kwiatkowski.

53. Millions of incipient angiofibromas in TSC facial skin

Katarzyna Klonowska, Joannes Grevelink, Krinio Giannikou, Barbara Ogorek, David Kwiatkowski.

54. Bi-steric mTORC1-selective inhibitors demonstrate improved potency and efficacy in tumors caused by TSC

Heng Du, Yu Chi. Yang, Heng-jia Liu, Mallika Singh, David J. Kwiatkowski.

55. Quantification of healthcare, social care, and educational resource use in patients with tuberous sclerosis complex (TSC)-associated epilepsy: insights from a UK Delphi panel

Linda Murphy, Catriona Crossan, Emily Jones, Dawn Lee, Chris Kingswood, Jade Marshall.

56. DGKA-dependent macropinocytosis supports tumorigenesis in tuberous sclerosis complex

Kathryn Robertson, Abigail K. Runicles, Natasha Lindsay, Patrick Bolton, Tony Charman, Emily JH Jones, Mark Johnson, Charlotte Tye.

57. Infant sleep duration predicts autistic traits in toddlerhood in tuberous sclerosis complex

Kathryn Robertson, Abigail K. Runicles, Natasha Lindsay, Patrick Bolton, Tony Charman, Emily JH Jones, Mark Johnson, Charlotte Tye.

58. Metanalysis reveals tissue-dependent and independent transcriptomic aberrations upon loss of TSC1 and TSC2

Denis Qeska, Adam Pietrobon, Gareth Palidwor, William L. Stanford.

59. Understanding the landscape of Tuberous sclerosis complex (TSC)-Associated Neuropsychiatric Disorders (TAND) research: A comprehensive scoping review

Stacey Bissell, Stephanie Vanclooster, Agnies van Eeghen, Nola Chambers, Liesbeth De Waele, Anna Byars, Jamie Capal, Sebastián Cukier, Peter Davis, Jennifer Flinn, Sugnet Gardner-Lubbe, Tanjala Gipson, Tosca Heunis, Dena Hook, Chris Kingswood, Darcy Krueger, Aubrey Kumm, Mustafa Sahin, Eva Schoeters, Katie Smith, Shoba Srivastava, Megumi Takei, Robert Waltereit, Anna Jansen, Petrus J de Vries.

60. Amiodarone-sirolimus interaction in A neonate with tuberous sclerosis complex

Daniel Ebrahimi-Fakhari, Barbara Fiedler, Heymut Omran.

61. Mapping the time course of mTORC1-driven tumorigenesis in the developing brain

Laura Geben, Gabrielle V. Rushing, Asa A. Brockman, Mary B. Chalkley, Ethan Chervonski, Julia Gallagher, Serena R. Sweet, Zeljka M. Lanaghan, Kevin C. Ess, Jonathan M. Irish, Rebecca A. Ihrie.

62. TSC-related phenotypes in C57BL/6J and DBA/2J mice with germline Tsc1 haploinsufficiency

Montana Kay Lara, Khalil Abedrabbo, Amanda E. Hernan, Rod C Scott, J. Matthew Mahoney.

63. Long-term safety and efficacy of add-on Cannabidiol (CBD) for treatment of seizures associated with tuberous sclerosis complex in an Open-Label extension trial (GWPCARE6)

Elizabeth A. Thiele, E. Martina Bebin, Francis Filloux, Floor E. Jansen, Patrick Kwan, Rachael Loftus, Farhad Sahebkar, Steven Sparagana, James Wheless.

64. Economic burden associated with tuberous sclerosis complex in patients with epilepsy

Keith A. Betts, Karen M. Stockl, Lei Yin, Kelly Hollenack, Min-Jung Wang.

65. Epidemiology, healthcare resource use, and mortality in patients with tuberous sclerosis complex: A population-based study on German health insurance data

Adam Strzelczyk, Susanne Schubert-Bast, Johann Philipp Zöllner, Andreas Simon, Geoffrey Wyatt, Rowena Holland, Felix Rosenow.

66. A novel TSC-mTORC1-GRASP55 signaling axis controls unconventional protein secretion to reshape the extracellular proteome upon stress

Julian Nüchel, Marina Tauber, Janica L. Nolte, Matthias Mörgelin, Clara Türk, Beate Eckes, Constantinos Demetriades, Markus Plomann.

67. Histamine signaling and metabolism identify biomarkers and therapies for lymphangioleiomyomatosis

Carmen Herranz, Francesca Mateo, Alexandra Baiges, Simon R. Johnson, Suzanne Miller, Elżbieta Radzikowska, María Molina-Molina, Alvaro Casanova, Antonio Roman, Oscar Yanes, Miquel Angel Pujana.

68. Heterogeneity and cancer-related features in Lymphangioleiomyomatosis cells and tissue

Roderic Espín, Alexandra Baiges, Eline Blommaert, Carmen Herranz, Antonio Roman, Berta Saez, Álvaro Casanova, María Molina-Molina, Mireya Plass, Francesca Mateo, Joel Moss, Miquel Angel Pujana.

69. Drug inhibition of reduction-oxidation factor 1-apurinic/apyrimidinic endonuclease 1 restores the hypoxic-driven disease state of tuberous sclerosis complex

Jesse Champion, Kayleigh M. Dodd, Hilaire C. Lam, Sara Seifan, Ellie Rad, Melissa L. Fishel, Ann Ager, Elizabeth P. Henske, David M. Davies, Mark R. Kelley, Andrew R. Tee.

70. ABSTRACT WITHDRAWN

71. Regulation of de novo serine synthesis by Interleukin 6 in tuberous sclerosis complex (TSC)

Ji Wang, Harilaos Filippakis, Thomas Hougard, Heng Du, Chenyang Ye, Heng-Jia Liu, Long Zhang, Khadijah Hindi, Shefali Bagwe, Julie Nijmeh, John M. Asara, Wei Shi, Souheil El-Chemaly, Elizabeth P. Henske, Hilaire C. Lam.

Abstracts in full

1. The effect of pregnancy on renal angiomyolipoma in patients with tuberous sclerosis complex

Marlou Kluiving, E.F.H.I Peeters, W.L. de Ranitz-Greven

UMC Utrecht, Heidelberglaan 100, 3584 CX Utrecht.

Purpose: Clinicians see pregnancy as a risk factor for growth and hemorrhage in patients with Tuberous Sclerosis Complex (TSC). Our study tries to determine the effect of pregnancy on renal angiomyolipoma (AML) size and risk of haemorrhage in patients with TSC. Methods: We systematically reviewed published articles from the last 20 years with a focus on AML and pregnancy. Results: We found 42 case reports describing AML size during and after pregnancy. Two of these cases showed a decrease in renal AML size, two illustrated an increase in size and four cases depicted no change in size or variability in size over time. These case reports illustrated that complications were seen more frequently in the non-TSC group versus the TSC group. Haemorrhage occurred in 33% of the TSC patients and in 76% of the non-TSC group. Data from the retrospective study showed no significant difference between renal complications in pregnant versus non pregnant TSC patients (57% vs. 67%). Heamorrhage occurred in 30% of the pregnant group and in 11% of the never-pregnant group. Conclusions: The effect of pregnancy on size and renal complications of the renal angiomyolipoma's in TSC patients is unclear. Haemorrhage does not seem to occur more frequently in pregnant TSC patients. Additionally, AML size changes due to pregnancy appear to be variable. However, these results are mostly based on case reports, and therefore not considered reliable. In other words, more research is needed to get a more explicit answer.

2. mTOR inhibitor improves autistic-like behaviors related to Tsc2 haploinsufficiency but not following developmental status epilepticus

Robert Waltereit, Tomas Petrasek1, Iveta Vojtechova1,2, Ondrej Klovrza1,3, Klara Tuckova1,4, Cestmir Vejmola1, Jakub Rak1, Anna Sulakova1, Daniel Kaping1, Nadine Bernhardt5, Petrus J. de Vries6, Jakub Otahal7

Department of Child and Adolescent Psychiatry, University Medical Center Göttingen, Germany, 1National Institute of Mental Health, Klecany, Czech Republic; 2First Faculty of Medicine, Charles University, Prague, Czech Republic; 3Second Faculty of Medicine, Charles University, Prague, Czech Republic; 4Faculty of Science, Charles University, Prague, Czech Republic; 6Division of Child & Adolescent Psychiatry, University of Cape Town, Cape Town, South Africa; 7Department of Developmental Epileptology, Institute of Physiology CAS, Prague, Czech Republic.

Background: Tuberous Sclerosis Complex (TSC), a multi-system genetic disorder often associated with Autism Spectrum Disorder (ASD), is caused by mutations of TSC1 or TSC2, which lead to constitutive overactivation of mammalian Target of Rapamycin (mTOR). In several Tsc1+/- and Tsc2+/- animal models, cognitive and social behavior deficits were reversed by mTOR inhibitors. However, phase II studies have not shown amelioration of ASD and cognitive deficits in individuals with TSC during mTOR inhibitor therapy. We asked here if developmental epilepsy, common in the majority of individuals with TSC, but absent in most animal models, could explain the discrepancy. Methods: At postnatal day P12, developmental status epilepticus (DSE) was induced in male Tsc2+/- (Eker) and wild-type rats, establishing four experimental groups including controls. In adult animals (n = 36), behavior was assessed in the paradigms of social interaction test, elevated plus-maze, light-dark test, Y-maze and novel object recognition. The testing was carried out before medication (T1), during a 2-week treatment with the mTOR inhibitor everolimus (T2) and after an 8-week washing-out (T3). Electroencephalographic (EEG) activity was recorded in a separate set of animals (n = 18). Results: Both Tsc2+/- mutation and DSE caused social behavior deficits and epileptiform EEG abnormalities (T1). Everolimus led to persistent improvement of the social deficit induced by Tsc2+/-, while deficits related to DSE did not respond to everolimus (T2, T3). Conclusions: These findings may contribute to an explanation why ASD symptoms in individuals with TSC, where comorbid early-onset epilepsy is common, were in clinical studies not reliably ameliorated by mTOR inhibitors.

3. Successful combined treatment of the tuberous sclerosis complex with synergic effect (mTOR + Vigabatrine) in child with 10 months

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The Tuberous Sclerosis Complex (CET) is an autosomal dominant genetic disorder, with an incidence of 1: 5,000 to 10,000 live births, characterized by the development of benign tumors in various organs. Pathogenic variants in the TSC1 or TSC2 genes lead to dysregulation of the rapamycin pathway (mTOR), whose hyperactivation is associated with characteristic lesions (cortical tubers, white matter heterotopy, subependymal nodules, and subependymal giant cell astrocytomas). Up to 90% of patients with CET have epilepsy, and in more than 50%, control is not achieved with antiepileptic drugs. Refractory epilepsy appears to play a crucial role in patients' cognitive and behavioral development. Cognitive impairment and autism spectrum disorder (ASD) are frequent.

Method: Description of a clinical case of early treatment combined with a synergistic effect (mTOR inhibitor + vigabatrin) in a 10 m child and diagnosed with CET.

Case description: Girl, 07 m, non-consanguineous parents, with cervical dyskinesia and eyelid myoclonus and developmental delay. On examination: hypopigmented macules, little visual interaction, motor stereotypes, and axial hypotonia. EEG: Organized base activity with potential focal epileptiform in the posterior and generalized quadrants and records the ictal activity of epileptic spasms. Cranial MRI: cortical tubers, radial bands, and subependymal nodules. Echocardiogram: rhabdomyoma without hemodynamic repercussions; Clinical Exome: 18 base pair deletion in exon 41 of the TSC2 gene (chr16: g.2088293_2088310del; Depth: 133x), in heterozygosis, which results in the in-frame deletion of six amino acids between codons 1746 and 1751 (p.His1746_Arg1751del ; ENST00000219476 .9) - pathogenic variant, related to the described phenotype. He started treatment with vigabatrin (VGA) and physical therapy, and speech therapy. There was a motor improvement, but it kept the crises and worsened social interaction and stereotypes. In the 6th week of VGB (200mg/kg/day), everolimus was associated (studies showed that up to 40% of patients with TEC and epilepsy had a relevant response to everolimus). On the 3rd week of everolimus + VGA presented crisis control and recovered language milestones. Final comments: Combined treatment of early CET (mTOR + VGA inhibitor) should be considered to control epilepsy and prevent the development of ASD, improving the prognosis.

4. Insight into TSC protein complex function from structural biology

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The TSC complex subunits TSC1, TSC2 and TBC1D7 form a large molecular machine. However, the functions of most parts of the complex are unknown. We use biochemical and structural characterization of different complex domains combined with cell biological studies to identify their role in physiology and pathology.

Recently, we have reported the structure of the catalytic GAP (GTPase activating protein) domain of TSC2 and elucidated the mechanism of catalysis and recognition of its substrate Rheb. Analysis of pathogenic TSC variants mapping to this domain revealed that mutations causing a partial functional defect in vitro showed manifestations in patients that do not fulfill the clinical criteria for TSC. Thus, mutations in TSC genes could be a more widespread cause of diseases than anticipated.

Furthermore, we have identified novel functions of TSC1, which so far has been considered primarily required for stabilization of the catalytic subunit TSC2. TSC1 contains a coiled-coil domain that binds TSC2 and a central helical domain that mediates oligomerization of TSC1. In addition, TSC1 facilitates recruitment of the TSC complex to the lysosome through binding with a HEAT repeat domain to PIP (phosphatidylinositol phosphate) lipids. We demonstrate that the TSC1 subunit mediates PIP-dependent lysosomal translocation of the TSC complex in vivo, which is required for inactivation of mTORC1 during starvation. Taken together, our data suggest a role of TSC1 in recruitment and formation of TSC supercomplexes on lysosomes.

Thus, gaining a molecular understanding of the TSC complex provides important insight into its function and the mechanisms that contribute to the development of disease.

5. Evolution of the TS Alliance TSC biosample repository

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The Tuberous Sclerosis Alliance Biosample Repository (BSR) began collecting biosamples in early 2016 and as of March 2021 houses a collection of more than 1,600 biospecimens including plasma, white blood cell pellets, DNA isolated from white blood cells and buccal cells, and remnant surgical tissue including brain, kidney, and liver, and a subset of postmortem tissues for use by researchers studying tuberous sclerosis complex (TSC). Many DNA and blood samples were collected in collaboration with the TSC Clinical Research Consortium's clinical trials. Our biosamples are linked to detailed clinical data in the Natural History Database (NHD), allowing applicants to request subsets of biosamples based on clinical phenotypes, age, biological sex, and other criteria. Additional data from the NHD relevant to the project may be requested for each sample. A condition of receiving biosamples is that data must be shared back with the TS Alliance, creating a continually growing dataset on these samples to be shared with researchers who request samples in the future. The BSR also provides researchers access to the TSC1- and TSC2-knockout HEK293T cell lines from the Nellist laboratory at Erasmus MC. All biosamples are stored at the Van Andel Institute in Grand Rapids, MI. More than 1000 samples have been shared with researchers, and a clinical trial was initiated in 2020 based on results from BSR plasma samples. The TS Alliance introduced mobile phlebotomy in December 2019 as part of the Waxlax Biosample Collection Initiative to permit anyone with a confirmed diagnosis of TSC to participate in our research projects regardless of where they live or where they go for their TSC care. As of March 2021, 80 constituents have provided samples this way, increasing the geographic diversity of the BSR. In this poster, we will present the latest BSR data as of June 1, 2021.

6. A mouse model of subependymal giant cell astrocytomas

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by inactivating mutations in either the TSC1 or TSC2 genes. Mutations in TSC1 or TSC2 cause hyperactivation of mTOR and subsequent formation of hamartomas within multiple organs including the brain. Growths in the brain, called subependymal nodules (SENs) and subependymal giant cell astrocytomas (SEGAs), are found around the lateral ventricles and may be a result of mutations in neural stem cells (NSCs) that normally reside in the subventricular zone (SVZ). Using neonatal SVZ electroporation and transgenic mice, we have developed models of SENs and SEGAs that resemble those seen in TSC. These models delete Tsc2 from NSCs, which led to the expansion of the SVZ and generated large distinct growths protruding into various regions of the lateral ventricle, indicating that regions of the SVZ do not differ in their susceptibility to hamartoma formation. Growths were enriched in NSC markers and contained few mature neurons. In addition to the growths found in the lateral ventricle, we also noted lesions within the striatum, rostral migratory stream (RMS), and olfactory bulb (OB). The SVZ, RMS, and OB were disorganized, displaying many of the classical cellular defects seen in TSC such as increased mTOR pathway activity, ectopic positioning, cytomegaly, increased dendrite complexity, and the presence of giant and balloon cells. To investigate the molecular consequences of Tsc2 inactivation, we performed RNA sequencing on OBs of these mice and found alterations in the MAPK and calcium signaling pathways, protein processing, and regulation of the actin cytoskeleton. Using this model, investigators will be able to gain mechanistic insight into the etiology and pathogenesis of TSC.

7. Characterization of individuals with cutaneous manifestations associated with tuberous sclerosis complex in the United States: A sub-analysis of an international survey of caregivers and individuals with TSC

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Cutaneous manifestations, such as facial angiofibroma, are predominant and often misdiagnosed early symptoms of tuberous sclerosis complex (TSC).

The TS Alliance conducted an online International Drug Development Survey between 15 May 2017 and 14 June 2017 by distributing a link to individuals with TSC and caregivers of dependent children or adults with TSC through various channels including social media.

Of the 420 caregivers and 133 individuals with TSC who responded from the US, 336 (80.0%) caregivers of dependents with TSC and 98 (73.7%) of individuals answering for themselves reported cutaneous manifestations (facial angiofibroma, ungual fibromas, etc.). The proportion of individuals with or without cutaneous manifestations were compared among various age groups. The proportion of those with or without cutaneous manifestations, respectively, were significantly different among three age categories as reported by caregivers: 0-23 months (6.5% vs. 22.0%, $p<0.0001$), 2-5 years (13.4% vs. 23.2, $p<0.05$), and 18-26 years (20.8% vs. 4.0%, $p<0.0001$). Age at diagnosis was not a strong determinant of the presence of cutaneous manifestations. The overall burden of TSC-related manifestations was significantly higher in individuals with cutaneous manifestations as reported by caregivers and individuals.

Individuals with cutaneous manifestations were older on average than individuals without cutaneous manifestations. The higher burden of TSC manifestations in patients with cutaneous manifestations illustrates the importance of accurate diagnosis of cutaneous manifestations by dermatologists and referral to a comprehensive multi-disciplinary TSC Clinic for surveillance and management of other TSC manifestations.

8. Characterization of individuals with facial angiofibroma associated with tuberous sclerosis complex in the United States and factors associated with use of topical mTOR inhibitors: a retrospective analysis of the Natural History Database

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The TS Alliance's Natural History Database was launched in 2006 and includes clinical data from 2279 individuals with tuberous sclerosis complex (TSC). From data of 2057 individuals from the US exported on 17th May 2020, facial angiofibroma (FA) was observed in 1329 (64.6%) individuals. Individuals with FA were older on average than those without FA (26.6 years vs. 16.7 years, p46 years), which is consistent with increased prevalence of FA with age. The TSC2 mutation was more common and TSC1 mutation was less common in individuals with versus without FA. The burden of other TSC-related manifestations was significantly higher in individuals with FA. A total of 329 (24.8%) individuals used topical mechanistic target of rapamycin inhibitors (mTORis) for the management of FA, despite no FDA-approved topical mTORis in the US. Topical mTORi use was more commonly associated with the 6-17-year-old age group, the age of diagnosis at 0-2 years of age, and the presence of TSC2 mutations. No difference in the use of topical mTORi by gender was observed. In conclusion, individuals with versus without FA were older and more frequently had a TSC2 mutation. The higher burden of TSC-related manifestations in individuals with FA illustrates the importance of accurate diagnosis by dermatologists and referral to a comprehensive multi-disciplinary TSC Clinic for surveillance and management of other TSC manifestations. The use of topical mTORi, which was observed in one-fourth of individuals with FA, was associated with the 6-17-year-old age group, the age of diagnosis at 0-2 years of age, and presence of TSC2 mutation. Access to FDA-approved mTORi could benefit many individuals with FA currently not receiving treatment.

9. The metformin in tuberous sclerosis (MiTS) study: A randomised double-blind placebo-controlled trial

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Background We investigated whether metformin would reduce growth of hamartomas associated with TSC. Metformin inhibits the mTOR pathway. Methods In this multicentre randomized, double-blind, placebo-controlled trial, patients with a clinical diagnosis of TSC, aged over 10 years were enrolled. Participants were randomly allocated (1:1) to receive metformin or placebo for 12 months. The primary outcome was percentage volume change of renal AML at 12 months compared to baseline. Secondary outcomes were percentage change at 12 months from baseline in volume of SEGA; appearance of facial and ungual hamartomas; frequency of epileptic seizures; and adaptive behaviour. Findings 72 patients were screened and 55 were randomly assigned to metformin (28) or placebo (27). All 51 patients who started therapy completed the trial and were assessed for outcome at 12 months. The median percentage change in AML volume was +7.6% (IQR -1.8% to +42.6%) for the placebo group and +8.9% (IQR 1.3% to 19.5%) for the metformin group (p = 0.28). Twenty-seven patients had SEGAs: 13 received placebo and 14 metformin. The median percentage change in SEGA volume was +3.0% (IQR -22.8% to +27.7%) for the placebo group and – 20.8% (IQR – 47.1% to - 5.0%) for the metformin group (p = 0.03). Twenty-one patients were assessed for seizure frequency: 9 received placebo and 12 received metformin. In the metformin group, a mean reduction of 43.7% from baseline in seizures was observed and in the placebo group a 3.1% mean reduction was observed, with a difference in response of 40.6% (95% CI -3.1% to +84.2%, p = 0.03). Interpretation Metformin did not reduce AML volume. Metformin did reduce SEGA volume and seizure frequency compared with placebo. There may be a role for metformin in slowing or reversing growth of some life-threatening hamartomas in TSC and for reducing seizure frequency.

10. Recognition of lesser known skin findings for earlier diagnosis of tuberous sclerosis complex

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A subgroup of individuals with tuberous sclerosis complex (TSC) present later in life, often with renal or pulmonary involvement and a remote or no history of seizures. TSC diagnosis can be delayed for decades in such individuals, despite the earlier presence of characteristic skin or oral lesions. These lesions include those comprising several of the major or minor features used in the clinical diagnosis of TSC, such as angiofibromas, fibrous cephalic plaque, hypomelanotic macules, confetti lesions, ungual fibromas, shagreen patch, oral fibromas, and dental pitting. Recognition of the typical and less common presentations of these lesions can lead to earlier diagnosis. Also, recognition of the pattern of skin involvement may point to mosaic rather than germline disease. For example, unilateral or asymmetric facial angiofibromas are a marker of mosaicism.

There are a variety of skin lesions that, although not listed as major or minor features, are associated with TSC with varying degrees of specificity. For example, angiofibromas may occur on the nipple or genitals. Poliosis circumscripta may involve scalp hair. Small collagenomas can be observed in the distribution of the fibrous cephalic plaque or shagreen patch. Large, protuberant and draining lesions called folliculocystic and collagen hamartomas may occur on head or trunk. Other skin lesions associated with TSC include molluscum pendulum, miliary fibromas, and nail changes such as red comets and splinter hemorrhages. Identification of these lesions should prompt consideration of TSC in undiagnosed individuals, along with consideration of other potential causes. Skin biopsy may be required for confirmation of the clinical diagnosis. The ability to recognize these lesions can be important to activate evaluation for TSC and may lead to earlier diagnosis. Also, a complete skin examination of relatives of an affected individual requires attention to the complete spectrum of possible skin lesions in TSC.

11. ABSTRACT WITHDRAWN

12. The long-term effect of mTOR inhibition on lipid and glucose metabolism in tuberous sclerosis complex: data from a nationwide registry

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Objective: MTOR inhibition has proved to be an effective treatment for many manifestations of tuberous sclerosis and its use is increasing. Because MTOR inhibition is a disease modifying therapy, lifelong use will most likely be necessary. This study addresses the long term effects of mTOR inhibition on lipid and glucose metabolism and aims to provide better insight in the incidence and time course of these metabolic adverse effects in treated TSC patients.

Methods: All patients that gave informed consent for the nationwide TSC Registry and were ever treated with mTOR inhibitors (sirolimus and/or everolimus) were included. Lipid profiles, HbA1c and medication were analysed in all patients during mTOR inhibitor treatment.

Results: We included 141 patients, median age 36 years, median use of mTOR inhibitors 5.1 years (aimed serum levels 3.0-5.0µg/l). Total cholesterol, LDL- and HDL-cholesterol levels at baseline were similar to healthy reference data. Hypercholesterolemia increased from 10% at baseline to 29% within 6 months of mTOR inhibition therapy. Although the incidence of hypercholesterolemia did not increase further during longer follow-up, the proportion of patients using lipid lowering medication did triple over 5 years. Incidence of diabetes was rare, as only 2.5% of patients developed an increased HbA1c ≥48 mmol/mol after 5 years of mTOR inhibitor use. Conclusion: Hypercholesterolemia is a frequent side effect of MTOR inhibition in TSC patients, and predominantly occurs within the first half year of treatment. Although hyperglycemia is a frequent side effect in other indications for mTOR inhibition, incidence of diabetes mellitus in TSC patients was low. This may reflect the difference of mTOR inhibition in patients with normal mTOR complex functioning versus patients with overactive mTOR complex signaling due to a genetic defect (TSC patients).

13. Quality of life in patients with tuberous sclerosis complex and epilepsy: estimating patient and caregiver health state utilities

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Objectives: Tuberous sclerosis complex (TSC) is often associated with treatment-resistant epilepsy. Studies exploring the relationship between seizure activity and health-related quality of life (HRQL) in TSC are limited. This study estimated the impact of seizure frequency and type on HRQL for patients with TSC and their caregivers.

Methods: A vignette-based utility survey was designed to estimate the impact of different seizure types (focal seizures with impaired awareness, generalised seizures, or a combination of both) and different seizure frequencies on patient/caregiver HRQL. Sixteen health state vignettes were developed to describe the holistic experience of a patient with TSC (n=8) or caring for a child with TSC (n=8), including aspects such as physical health, personal care, emotional wellbeing, and social functioning. A targeted literature review and qualitative interviews with TSC caregivers and healthcare professionals informed vignette development. To generate health state utility scores, time trade-off (TTO) methodology and a visual analogue scale were used to evaluate vignettes in 200 videoconference interviews with the UK general population (n=100 each for patient and caregiver vignettes).

Results: Patient TTO utility scores (mean), based on daily seizure frequency, were: TSC with no seizures: 0.725; focal seizures: 1–2/day, 0.504; 3–4/day, 0.282; 5–14/day, 0.074; generalised seizures: 1/day, 0.183; 2/day, 0.089; 3–14/day, −0.113. The impact on HRQL was greatest in those with a combination of seizure types (5–14 focal and 3–14 generalised seizures: −0.234). Similar to patient TTO utility scores, caregiver utility scores decreased with increasing seizure frequency, ranging from 0.905 for TSC with no seizures to 0.221 (5–14 focal and 3–14 generalised seizures).

Conclusions: Patients with TSC-associated epilepsy and their caregivers have significantly impaired HRQL, which worsens with increasing seizure frequency. Generalised seizures are expected to be more detrimental for HRQL than focal seizures.

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14. Evaluation of renal disease in paediatric patients with tuberous sclerosis

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Introduction: Renal disease is an established cause of morbidity and mortality in tuberous sclerosis complex (TSC). The main manifestations; angiomyolipomas (AML) and cystic disease contribute to premature loss of renal function. Targeted therapy in the form of mTOR inhibitors has enabled a nephron-sparing approach to TSC-related AMLs. Current UK clinical guidelines recommend active imaging surveillance to identify and provide early opportunity for intervention.

Objective: To review the renal evaluation of paediatric patients with TSC attending the Royal Hospital for children TSC multi-disciplinary clinic.

Method: Electronic patient records of children attending the national TSC MDT clinic were audited regarding renal evaluation and sequelae.

Results: Data were obtained for 45 children. Current age range: 6 months to 18 years (median 10 years). On renal imaging 16/45 (35%) had renal cystic disease and 17/45(37%) AMLs. Median age of diagnosis of renal cysts or AMLs was 8 years. The majority of individuals with renal cysts had TSC2 pathogenic variants (14/16, 87%) and one has a contiguous TSC2/PKD1 deletion. Six individuals had a documented BP >95th centile, all had renal cystic disease. No individuals had documented CKD. Surveillance imaging was predominantly performed by ultrasound. Only 14/45 of the cohort had undergone MRI abdomen though the majority had previously had cranial MRI. Seven of our cohort are currently treated with mTOR inhibitors; renal indication 2/7, neurological indication 5/7.

Conclusion: Renal manifestations of TSC as demonstrated by our cohort, occur at a young age. The pathogenesis of renal cystic disease in TSC is less well-characterized than AMLs. Evaluation of renal sequelae in paediatric TSC cohorts permits early intervention and identification of modifiable progression factors such as BP. Further detailed characterization may also aid future development of novel therapeutics for cystic disease.

15. Shared genetic risk factors between lymphangioleiomyomatosis and pulmonary function

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Introduction: Lymphangioleiomyomatosis (LAM) is a rare low-grade metastasizing disease characterized by cystic lung destruction. LAM can occur as an isolated disease (sporadic LAM, S-LAM) or in association with tuberous sclerosis complex (TSC-LAM). Biallelic inactivation of TSC1 or TSC2 (more frequently) gene occurs in LAM cells. However, the genetic basis of LAM risk remains incompletely determined, and the disease cell-of-origin is uncertain. We analyzed the shared genetic basis of LAM with cancers and pulmonary function to further decipher LAM etiology.

Methods: The results of genome-wide association studies (GWASs) of S-LAM, 17 cancer types, and spirometry measures (FEV1), FVC, FEV1/FVC ratio, and PEF) were analyzed for genetic correlations, shared genetic variants, and causality. Genomic regulatory and transcriptomic data were examined, and immunohistochemistry assays were performed to evaluate potential pleiotropic genes.

Results: There were no significant overall genetic correlations between LAM and cancers, but LAM correlated negatively with FVC and PEF, and a trend in the same direction was observed for FEV1 (i.e., higher LAM risk - lower pulmonary function). Twenty-two shared genetic variants were uncovered between LAM and pulmonary function, while only seven shared variants were identified between LAM and cancer (gastric, kidney, and prostate cancer). Mendelian randomization analysis suggested a causal relationship between LAM and FEV1. The shared genetics of LAM and pulmonary function identified four pleiotropic gene candidates that were previously recognized in a LAM single-cell transcriptome signature: ADAM12, BNC2, NR2F2, and SP5. The NR2F2 locus corresponded to the primary LAM GWAS results, and we identified its functional partner NR3C1 as another potential pleotropic factor. NR3C1 expression was confirmed in LAM lung lesions.

Conclusions: This study proposes common etiology between LAM and pulmonary function. The LAM-cancer risk association appears to be less relevant. Further studies may be warranted to deepen knowledge into the depicted LAM genetic and molecular factors.

16. Two generations of TSC: Impact of early diagnosis and interventions

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The establishment of TSC surveillance guidelines and increased utilization of early intervention therapies has greatly impacted the overall quality of life of individuals with TSC. Here, we compare two family members' experiences with TSC to highlight the impact of early diagnosis and intervention. Patient 2 is the great paternal uncle to Patient 1. Of note, Patient 1 and Patient 2 are confirmed to have different pathogenic variants in TSC2.

Patient 2 is currently 58 years old and was given a clinical diagnosis prior to the age of 10 years. His clinical features include a unilateral retinal hamartoma, skin manifestations, seizures starting in childhood, SENs, SEGA diagnosed at 19 years, hypertension, angiomyolipomas, and mild intellectual disability. He obtained speech therapy at school from first to sixth grade and required significant academic intervention. Patient 2 took multiple AEDs throughout childhood and suffered his last seizure in August 2016 while on lamotrigine monotherapy. Lastly, he underwent right frontal craniotomy for tumor resection and ventriculoperitoneal shunt secondary to increased intracranial pressure in his early 20s.

Patient 1 is currently a three-year-old and was diagnosed with TSC at 2 months old. Routine surveillance revealed cardiac rhabdomyomas, hypomelanotic macules, bilateral retinal hamartomas, bilateral renal cysts, and cortical tubers. Patient 1 elected to participate in the PREVeNT trial and underwent serial EEGs and neurodevelopmental screening. Patient 1 has experienced 3 seizures between ages of 13 to 16 months. Vigabatrin was initiated after her first seizure and levetiracertam was added after her second seizure. She started to fall behind developmentally at this time and speech therapy was initiated. At 24-month, the Bayley-III noted that she was in the low-average range for language and motor and average range in cognition. With the appropriate interventions and optimized seizure control, Patient 1 is currently developmentally appropriate at 36 months.

17. Pancreatic neuroendocrine tumors (PNETs) and utility of mTOR inhibitors as a treatment option

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Nonfunctional pancreatic neuroendocrine tumors (PNETs) are a rare clinical feature of TSC with no specific guidelines on clinical management. Since the establishment of the TSC surveillance guidelines in 2012, there has been an increase in case reports of nonfunctional PNETs in the medical literature. Given this emerging phenotype, it is becoming increasingly important to consider treatment options. This study set out to determine the frequency of nonfunctional PNETs, current clinical management,

and assess the impact of systemic mammalian target of rapamycin (mTOR) on nonfunctional PNETs. Data was gathered through a retrospective chart review at the TS Alliance's Natural History Database and the Cincinnati Children's Hospital TSC Database. Sixteen individuals were identified and included in the study. The calculated frequency of nonfunctional PNETs is 0.65%. The average age at PNET diagnosis was 15.0 years (range: 3 - 46 years). Almost all individuals were diagnosed with a PNET during routine TSC surveillance; 56.3% by MRI, 12.5% by CT, 25% by ultrasound, and 6.2% through a surgical procedure. Clinical follow up after PNET diagnosis indicated 68.8% underwent serial imaging and 56.3% eventually proceeding with surgical removal. Eight individuals in this study had a history of using systemic mTOR inhibitors. After reviewing serial imaging of the nonfunctional PNETs, the tumor growth rate was noted to be slightly less in individuals taking an mTOR inhibitor (-0.8 mm/yr, IQR: -2.3 to 2.2) than those without (1.6 mm/yr; IQR: -0.99 to 5.01, p >0.05). Nonfunctional PNETs occurred at younger ages in our TSC cohort and more commonly compared to ages and prevalence reported for the general population. The outcome of this study provides preliminary evidence supporting the use of mTOR inhibitor therapy in conjunction with serial imaging as medical management for nonfunctional PNETs as an alternative option to invasive surgical removal.

18. Targeting mTORC1 overactivation and Nrf2 inhibition to overcome ferroptosis resistance in cancer cells

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Ferroptosis is a newly identified iron-mediated necrosis characterised by oxidative damage of phospholipids and is regulated by cellular metabolism, redox homeostasis, and various signalling pathways related to cancer. Abnormal activation of mTORC1 due to the loss of TSC1-TSC2 complex triggers impaired autophagy and activation of the Kelch-like ECH-associated protein 1 (Keap1)-nuclear factor E2-related factor 2 (Nrf2) antioxidant defence mechanism, which protects cells against ferroptotic cell death. Consequently, this study utilised drug combinations that can induce ferroptosis and inhibit the Nrf2 compensatory survival pathway in our in vitro models of mTORC1 activation.

Viability in the presence of Ferroptosis inducers Erastin [0-5uM] and (1S,3R)-RSL3 [0-0.1uM], either alone or with an Nrf2 inhibitor, Trigonelline [500uM], was measured by MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay. Lipid peroxidation and Reactive oxygen species levels were assessed by C11-Bodipy 665/676 [1M] and CM-H2DCFDA [10M], respectively as markers of Ferroptosis. Samples were run on a BD Accuri C6 flow cytometer.

MTT revealed dose-dependent viability decreases in both cell lines. TSC(-/-) cells showed a greater resistance to ferroptosis induction (EC50: Erastin 1.4uM, (1S,3R)-RSL3 0.4uM) compared to TSC(+/+) cells (EC50: Erastin 0.7uM; (1S,3R)-RSL3 0.2uM) but the resistance was significantly reduced (p 0.5) in TSC(-/-) cells with concomitant Nrf2 inhibition (EC50: Erastin 0.8uM, (1S,3R)-RSL3 0.2uM).

Lipid peroxidation and ROS measurements correlated with the decreased MTT viability, suggesting ferroptosis as the mode of viability loss. There was a dose-dependent increase in lipid peroxidation and ROS in both cell lines but there were more lipid peroxidation positive cells in TSC(+/+) compared to TSC(-/-) cells. Decreased viability in Nrf2-inhibited TSC(-/-) cells resulted in increased numbers of lipid peroxidation positive cells, consistent with increased susceptibility to ferroptotic cell death.

Our data confirms that Nrf2 is a putative therapeutic target in overcoming ferroptotic cell death resistance in TSC-driven cancers.

19. The association of co-morbidity (congenital heart defects, neurodevelopmental abnormalities and renal system) in patients with tuberous sclerosis complex I and II: a prospective cohort study

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Tuberous sclerosis complex (TSC) is a genetic syndrome associated with variable phenotype that affects heart, brain, and kidney development. The correlation between TSC1 and TSC2 mutations and incidence of both congenital heart diseases (CHDs) in the form of rhabdomyomas, poorer neurodevelopmental outcome in children, and renal disorders (RDs) have long since been investigated independently of one another, but few studies have attempted to establish an association between pleiotropic phenotype of the three organs, despite organogenesis sharing a similar developmental timelines in the developing human foetus and sharing common genetic pathways. The genes that cause TSC1/2 are already known, many downstream molecular pathways have been identified, and the resulting perturbations of cellular events are becoming increasing clear. However, the association between congenital heart disorders, brain neurodevelopmental disorders (NDDs), and renal disorders in context of TSC is unknown. The aim of this study is to evaluate the abnormalities in heart, brain, and kidney development in patients with TSC1 and TSC2 mutations using clinical data from TSC patient databases at Cardiff and Vale University Health Board (CAV UHB) collected over the last 30 years. Our data demonstrates that TSC1/TSC2 patients have a much greater prevalence for brain effects than heart or kidney. Further our result demonstrates a positive correlation between a brain effect and likelihood of an accompanying heart defect in both TSC1 and TSC2 patients, but a weaker association between co-occurance of heart and kidney effects. The outcomes of this study may inform future clinical recommendations for TSC patient management for comorbidity between CHDs, NDDs, and RDs and direct future clinical strategy.

20. Modeling tuberous sclerosis complex-associated renal angiomyolipoma tumors driven by TSC2 loss-of-heterozygosity using patient-derived hiPSCs

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Angiomyolipomas (AMLs) are the most common renal manifestation in patients with Tuberous Sclerosis Complex. The recapitulation of AMLs experimentally has remained a challenge, precluding the study of tumor mechanisms. Here we derived renal organoids with AML characteristics from genome-edited human induced pluripotent stem cells (hiPSCs) carrying bi-allelic inactivating mutations in the TSC2 locus. Organoids derived from TSC2-/- hiPSCs but not from isogenic TSC2+/- or TSC2+/+ hiPSCs shared a common transcriptional signature and a myomelanocytic cell phenotype with kidney AMLs, and developed epithelial cysts, suggesting a central role for TSC2 loss-of-heterozygosity (LOH) in the etiology of both AML and cystic disease. TSC2-/- AML organoids formed growing tumors upon transplantation into the kidneys of immunodeficient rats, where they were efficiently ablated by rapamycin-loaded nanoparticles. Collectively, our findings support the notion that kidney AMLs originate from cells of the renal lineage and establish a novel, reproducible and scalable bioengineering strategy for modeling AMLs using hiPSCs, in vitro and in vivo.

21. Single cell analysis of tuberous sclerosis complex (TSC) reveals a stem-like tumor state linked to immune suppression

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We performed integrative analysis of single cell RNA-sequencing (scRNA-seq), paired single cell T cell receptor sequencing (scTCR-seq), and spatial transcriptomic profiling on samples from 15 Tuberous Sclerosis Complex (TSC) associated tumors and matched normal tissues, and identified a stem-like tumor cell state linked to T cell dysfunction via tumor-modulated immunosuppressive macrophages.

Two distinct cell states in the tumor cell population were identified: stem-like state and inflammatory state. Stem-like state tumor cells are characterized with high stemness and dormancy, whereas inflammatory state tumor cells showed high expression of inflammatory genes and pathways. Intriguingly, CD8+ T cells derived from "stem-like state"-dominant tumors exhibited much higher exhaustion and lower cytotoxicity compared to those from "inflammatory state"-dominant tumors. Integrative analysis of paired scRNA-seq and scTCR-seq revealed that clonal expansion and T cell velocity were almost completely suppressed in "stem-like state"-dominant tumors, which is not directly suppressed by tumor cells.

Immunosuppressive myeloid cells, such as tumor-associated macrophages (TAMs), are considered major barriers to immunotherapy, due to their potent suppressive function and high abundance in the tumor microenvironment. In TSC tumors, enrichment of macrophages represented the most striking immune infiltration. Specifically, "stem-like state"-dominant tumors were enriched with M2-like macrophages with high expression of TREM2 and TYROBP, a receptor complex on macrophages recently shown to suppress T cell function in tumor microenvironment. Integrative scRNA-seq and spatial transcriptomics revealed a immunosuppressive regulatory axis from stem-like state tumor cells to TAMs via APOE-TREM2/TYROBP interaction, with APOE as a putative ligand for the TREM2/TYROBP complex in TSC tumor microenvironment.

Taken together, these data reveal differential immune remodeling by previously unrecognized distinct cells states in TSC tumors, and provide a rationale for precision immunotherapy in TSC.

22. HRQoL of TSC individuals in Hong Kong: a local study and comparison with literature using Health Utilities Index

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Background:

The heterogeneous nature of Tuberous Sclerosis Complex (TSC) mandates long-term follow-up. This may incur adverse health-related quality of life (HRQoL) on TSC patients. We performed a comprehensive review on the HRQoL of the ethnic Chinese TSC population in Hong Kong as data in Asia remains to be a paucity.

Methods:

Health Utilities Index Mark 2 (HUI2) and Health Utilities Index Mark 3 (HUI3) were adopted to assess the HRQoL of adult and pediatric TSC patients. The indices were derived from HUI-Ch, a validated Chinese multiple-choice questionnaire. Proxy-data encompassing items on socio-demographics, common chronic health conditions and multiple attributes that assess functional level were extracted. Data was analysed using multiple imputation with two-sample t-test and multiple regression to predict variations in HRQoL and compared with other disease entities.

Results:

In the study sample of 27 patients, our TSC patients had a higher proportion of "severe disability" on HUI2 and HUI3 (81.5% and 77.8%) compared to local Down syndrome (DS) patients (60.0% and 72.0%). Behavioral problems on HUI2 and attention deficit hyperactivity disorder (ADHD) on HUI3 were statistically significant predictors of poorer HRQoL. It was found that behavioural problems affect cognition (0.88 vs 0.98, $p<0.01$) and self-care (0.96 vs 1.00, $p<0.01$) while ADHD affects cognition (0.71 vs 0.95, $p<0.01$) and speech (0.87 vs 0.95, $p<0.01$). Subgroup HUI3 scores of our TSC patients with epilepsy, autism, anxiety problems and chronic kidney diseases were lower than that of local DS patients and the general population in the literature.

Conclusions:

Hong Kong TSC patients display poorer HRQoL than DS and other chronic health conditions. Findings can serve as a pivotal guide for the evaluation of disease burden and management outcomes in TSC patients of similar cultural backgrounds.

23. Investigating the impact of the pandemic on wellbeing in families of children with rare disorders: the CoIN Study

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Families of children with rare neurogenetic syndromes are a unique vulnerable group, who prior to the outbreak of the Covid-19 pandemic experience poorer mental health and wellbeing. Sudden pandemic-induced changes in stability, including established routines, relationships, and personal and professional support networks, combined with an elevated risk of Covid-19 infection, are likely to significantly impact the wellbeing of children and their parents, and magnify pre-existing difficulties. The Covid-19 impact on wellbeing in families of children with rare neurogenetic disorders (CoIN) Study is an ongoing longitudinal survey study of UK-based families of children aged 0-16 years old with a rare neurodevelopmental and/or genetic disorder during Covid-19. The study aims to: (1) identify the specific challenges of Covid-19 for these families; (2) identify predictors of parental mental health and child behaviour; and (3) investigate interactions between child characteristics and family wellbeing over time. Between May-July 2020, 122 parents of children with over 75 different rare neurogenetic conditions completed the baseline survey, with tuberous sclerosis complex (TSC) being the largest group (n=18). Among several domains selected to match large general population surveys, each timepoint includes standardised measures of parental mental health (Depression, Anxiety and Stress Scale – 21 Items [DASS-21]) and child behaviour (Strengths and Difficulties Questionnaire [SDQ]). Emerging findings suggest parents of children with rare neurogenetic disorders experience poorer mental health compared to parents of typically developing children during the pandemic. The majority of parents also report increased behavioural, emotional and attentional difficulties amidst Covid-19 versus the general population. Survey responses have enabled the immediate provision of specialised resources and coping tips for families. Combined with in-depth qualitative interviews and community involvement in study design and dissemination, the results from this study will inform preparedness and planning for future disruptive emergencies.

24. Time to onset of cannabidiol (CBD) treatment effect and resolution of adverse events in the tuberous sclerosis complex phase 3 randomised controlled trial (GWPCARE6)

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Introduction: Add-on CBD significantly reduced seizures associated with tuberous sclerosis complex (TSC) across the 16-week double-blind treatment period in GWPCARE6 (NCT02544763). A post hoc analysis was conducted to estimate time to onset of CBD treatment effect and resolution of adverse events (AEs).

Methods: Patients received GW Pharmaceuticals' formulation of plant-derived highly purified CBD (100 mg/mL oral solution) at 25 mg/kg/day (CBD25) or 50 mg/kg/day (CBD50), or placebo for 16 weeks. Treatment started at 5 mg/kg/day for all groups, reaching 25 mg/kg/day on Day 9 in CBD25 and 50 mg/kg/day on Day 29 in CBD50. Percentage change from baseline in primary endpoint TSC-associated seizures (countable focal or generalised) was calculated by cumulative day (i.e., including previous days). Time to onset and resolution of AEs were evaluated.

Results: Overall, 224 patients were randomised 1:1:1 to CBD25 (n=75), CBD50 (n=73), and placebo (n=76). The median (range) age was 11 (1–57) years. Patients had discontinued a median of 4 antiepileptic drugs (AEDs) and were currently taking a median of 3 AEDs. Differences in seizure reduction between CBD and placebo emerged on Day 6 (when titration reached 15 mg/kg/day) and became nominally significant ($p<0.05$) by Day 11 (CBD50) or Day 12 (CBD25). Over 90% of patients had an AE, with onset during the first 2 weeks of the titration period in 63%. AEs resolved within 4 weeks of onset in 42% of placebo and 27% of CBD patients and by end of study in 78% of placebo and 51% of CBD patients; most frequent AEs - diarrhoea, somnolence, decreased appetite - resolved in 69-88% of CBD patients.

Conclusions: Findings suggest that onset of treatment effect (efficacy and AEs) occurred within the first 2 weeks. AEs lasted longer for CBD vs. placebo but resolved within the 16-week study in most patients.

FUNDING: GW Research Ltd.

25. Prenatal sirolimus treatment for intrauterine rhabdomyomas in tuberous sclerosis

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BACKGROUND: In tuberous sclerosis most cardiac rhabdomyomas regress spontaneously. In some cases, the tumors can cause life-threatening hemodynamic comprise requiring subsequent surgical resection. The mTOR inhibitors everolimus and sirolimus have shown to be effective treatments for multiple conditions. There are four cases reporting off-label treatment with transplacental sirolimus in tuberous sclerosis for fetal rhabdomyomas. The optimal dosing regimen is unknown.

METHODS: Retrospective chart review of all patients treated prenatally with sirolimus for rhabdomyomas. All fetuses had a clinically defined diagnosis of tuberous sclerosis (2012 Consensus Diagnostic Criteria, including a positive genetic test). Clinical history, mTOR inhibitor dosing and levels, outcome and adverse events were reviewed after initiation of sirolimus treatment.

RESULTS: Three fetuses were treated with maternal sirolimus. Dosing regimens and subsequent trough levels differed from 1 mg/day to 6 mg/day and & 1.0 ng/mL to 12.2 ng/mL. Cardiac rhabdomyomas gradually shrunk in all patients. Growth restriction was noted in one patient. No severe adverse events occurred during the treatment period.

CONCLUSIONS: Maternal sirolimus appears to be a safe treatment option in prenatally detected rhabdomyomas with possible need for intervention. Follow-up visits with fetal ultrasound, echocardiography and laboratory work should be performed weekly during the treatment period. The optimal dosing and trough level timepoints remain unclear. Based on our results we recommend a sirolimus starting dose of at least 2 mg/m2/day, preferably 3-3.5mg/m2/day to achieve a target trough level of 10-12 ng/mL.

26. Efficacy of add-on cannabidiol (CBD) treatment in patients with tuberous sclerosis complex and a history of infantile spasms: post hoc analysis of phase 3 trial GWPCARE6

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Introduction: This post hoc analysis of a phase 3, randomised, placebo-controlled trial (GWPCARE6/ NCT02544763) compared response to add-on CBD in patients with tuberous sclerosis complex (TSC) and treatment-resistant epilepsy with and without a history of infantile spasms (IS).

Methods: Patients received GW Pharmaceuticals' formulation of plant-derived highly purified CBD (100 mg/mL oral solution) titrated to 25 mg/kg/day (CBD25) or 50 mg/kg/day (CBD50) or placebo for 16 weeks. Negative binomial regression effect modification analysis assessed whether IS history affected CBD efficacy.

Results: 138/224 (62%) patients had IS history. Median (range) age: 12.2 years (1.1 - 56.8) for patients with IS history, 10.5 years (1.6 - 55.8) for those without; 74% <18 years. Median (Q1, Q3) baseline monthly TSC-associated seizure frequency: 59 (28, 117) and 51 (29, 96) for patients with and without IS history. CBD reduced TSC-associated seizures vs. placebo regardless of IS history (interaction p-value: 0.803 for CBD25, 0.561 for CBD50). For patients with IS history, percent reduction in seizures from baseline: 45% for CBD25, 43% for CBD50, and 23% for placebo; placebo-adjusted reduction (95% CI): 29% (6%–45%) for CBD25 and 25% (3%–43%) for CBD50. For patients without IS history, reduction was 54% for CBD25, 55% for CBD50, and 32% for placebo; placebo-adjusted reduction (95% CI) was 32% (5%–52%) for CBD25 and 34% (7%–54%) for CBD50. AE incidence: 93% for CBD25, 100% for CBD50, and 95% for placebo; 8 patients (11%) on CBD25, 10 (14%) on CBD50, and 2 (3%) on placebo discontinued treatment because of an AE. Common AEs: diarrhoea and somnolence, occurring more frequently with CBD than placebo. ALT/AST elevations (>3×ULN): 9 (12%) patients on CBD25, 19 (26%) on CBD50, none on placebo; 79% were on concomitant valproate.

Conclusions: CBD produced consistent reductions in TSC-associated seizures in patients with and without IS history.

FUNDING: GW Research Ltd.

27. Tuberous sclerosis complex astrocytes affect neuronal synaptic balance through secreted factors

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Neurological symptoms and neurodevelopmental issues cause a significant burden on TSC patients. It is hypothesized that changes in the excitation/inhibition (E/I) balance may underlie epilepsy and autism, which are both common in TSC patients. Indeed, previous studies have shown changes in E/I balance in TSC models. Although studies have traditionally focused on neuronal abnormalities, both neurons and glial cells are affected by TSC mutations. Astrocytes are important for neuronal development, and dysfunction of astrocytes can cause E/I balance changes. We studied the role of astrocytic dysfunction in neuronal development in TSC using patient-derived induced pluripotent stem cells (iPSCs). TSC and control iPSCs were differentiated into astrocytes according to established protocols. TSC astrocytes showed increased proliferation compared to control astrocytes, and changes in gene expression related to EGF signaling and secreted/transmembrane proteins. To study the effect of astrocyte secreted factors, control neurons were cultured in astrocyte conditioned medium (ACM) of TSC or control astrocytes. After culture in TSC ACM, the balance between GABAergic and glutamatergic synapses was altered, showing that TSC astrocytes affect neuronal development. In a 3D organoid model, containing neurons and glial cells, a similar alteration of synaptic balance was observed. In conclusion, TSC patient-derived astrocytes show changes in signaling pathways and secreted factors from these astrocytes alter the synaptic balance in vitro. This shows that astrocyte secreted factors may provide a new therapeutic target for TSC.

28. The power of 1: N-of-1 studies to improve interventional research for tuberous sclerosis complex

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Aim: Approximately one million of people worldwide are affected by Tuberous Sclerosis Complex (TSC), one of the nearly 6000 rare genetic disorders. Interventional research with conventional randomized controlled trials (RCTs) is challenging due to the small, heterogeneous and vulnerable patient population. An alternative study design is the N-of-1 study, which is a randomized, controlled, multiple crossover trial within a single patient. To improve the use of N-of-1 studies, we systematically reviewed the literature to provide recommendations. In addition, we present a trial protocol of an N-of-1 study for TSC to illustrate the power of an N-of-1 design in TSC research.

Methods: EMBASE and MEDLINE were searched for N-of-1 studies in rare genetic neurodevelopmental disorders. Information was recorded on types of interventions, outcome measures, validity, strengths and limitations and recommendations were formulated with an expert group. An N-of-1 study was designed to study the effectiveness of cannabidiol (CBD) on behavioral manifestations in seven children and adults with TSC based on a power analysis. The primary outcome is the Aberrant Behavior Checklist. Secondary outcome measures include personalized measures, the Anxiety, Depression and Mood Scale, and seizure frequency.

Results: In the systematic review, twelve N-of-1 studies were identified, including both single-patient trials and series. Main strengths were the use of personalized and clinically relevant outcomes. Heterogeneity, rarity, vulnerability and intensity were considered as challenging to both generalizability and treatment compliance. Generalizability was compromised due to limited use of validated and generalizable outcome measures. To illustrate how these limitations can be overcome, we present an N-of-1 study to investigate the effectiveness of CBD on behavioral problems in TSC.

Conclusion: Properly executed N-of-1 studies may provide a powerful alternative to larger RCTs in rare disorders. We provide recommendations for future N-of-1 studies with a focus on patients with TSC, ultimately optimizing evidence-based and personalized care.

29. Aberrant DJ-1 expression underlies L-type calcium channel hypoactivity in tuberous sclerosis complex (TS) and Alzheimer's disease (AD)

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L-type voltage-dependent Ca2+ channels (L-VDCC) integrate synaptic signals to facilitate a plethora of cellular mechanisms. L-VDCC dysfunction is implicated in several neurological and psychiatric diseases. Despite their importance, signals upstream of L-VDCC activity that regulate their channel density, however, are poorly defined. In disease models with overactive mammalian target of rapamycin complex 1 (mTORC1) signaling (or mTORopathies), including tuberous sclerosis (TS) and Alzheimer's disease (AD), we report a novel mechanism downstream of mTORC1 signaling that results in a deficit in dendritic L-VDCC activity. Deficits in L-VDCC activity are associated with increased expression of the mTORC1-regulated RNA-binding protein DJ-1. DJ-1 binds the mRNA coding the auxiliary Ca2+ channel subunit α2δ2 responsible for shuttling L-VDCC to the membrane and represses its expression. Moreover, this novel DJ-1/α2δ2/L-VDCC pathway is disrupted in human AD and preclinical models of AD and TS. Our discovery that DJ-1 directs L-VDCC activity and L-VDCC-associated protein α2δ2 at the synapse suggests that DJ-1/α2δ2/L-VDCC is a common, fundamental pathway disrupted in TS and AD that can be targeted in clinical mTORopathies.

30. Neuropsychiatric disorders in tuberous sclerosis complex patients with epilepsy

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BACKGROUND: Epilepsy and neuropsychiatric disorders are the two most common issues in tuberous sclerosis complex (TSC) with psychological burden on the affected individuals and caregivers. This study aimed to describe the correlation between epilepsy and neuropsychiatric disorders.

METHODS: Information on participant demographics, genotype, seizure history, and neuropsychiatric

disorders of TSC were collected. A subsequent questionnaire for neuropsychiatric disorders was performed for individuals and caregivers.

RESULTS: Twenty-one (36.4%) adults (>18 years) and 19 (63.6%) children were enrolled. Among psychiatric disorders, intellectual disability and autistic spectrum disorder are correlated with the history of epilepsy. Epilepsy history in TSC is statistically significant in attention, dual tasking/multi-tasking, visuo-spatial tasking, and executive skills in neuropsychological skills. Epilepsy in TSC is associated with extreme shyness, language delay, poor eye contact, repetitive behavior, inattention, and restlessness.

CONCLUSION: It is crucial to continue the surveillance of neuropsychiatric features and their burden on daily life through basic questionnaire and screening procedures during clinical follow-up.

31. Seizure outcome after epilepsy surgery in tuberous sclerosis complex: results and analysis of predictors from a multicenter study

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Epilepsy surgery is recommended in selected patients with Tuberous Sclerosis Complex (TSC). However, reports on predictive factors of seizure outcome are controversial.

Here we report on seizure and cognitive outcome of 35 TSC patients who received surgery for refractory epilepsy in 7 Italian centers over a period of 22 years (1997-2019). The Engel Epilepsy Surgery Outcome Scale was used to measure the outcome after surgical treatment (Engel I-IV).

The rate of seizure-free individuals at last follow up (mean 7.5 years, range 1-21 years) was 51%. Patients with longer follow-up (10 years) had a lower rate of Engel I outcome (11.1%) than those who received surgery in the last 10 years (65.4%, p=0.003). Factors associated with Engel II, III or IV outcome in our cohort included: high number of cortical tubers (≥5); presence of subependymal nodules (SENs); seizure onset before age 1 year; and multifocal epileptic discharges on electroencephalogram (EEG). A subset of patients evaluated with Vineland Adaptive Behaviour Scales (VABS) showed developmental gains, in line with their developmental trajectories, but no improvement in standard scores after surgery was noted.

Our study demonstrates that the rates of successful seizure outcome of epilepsy surgery in TSC have improved in the last 10 years. More than half of the patients achieved seizure freedom, and a high proportion of affected individuals experienced a reduction in seizure burden and in antiseizure medications. A comprehensive assessment after surgery should be performed in TSC patients to evaluate the overall neurodevelopmental outcome, as measures that are based only on seizure control do not adequately identify the benefits of surgery on global functioning in these patients.

32. Tuberous sclerosis complex and cerebral aneurysms: the challenge of early detection

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Introduction: Tuberous sclerosis complex (TSC) is a rare multiorgan neuroendocrine disease. Aneurysms were well described in the extracranial vasculature of patients with TSC, where anomalies of the vascular connective tissue have been histopathologically and genetically investigated. In contrast, cerebral aneurysms have not yet been addressed and their incidence is still totally unknown.

Methods: A systematic review including 3 databases (PubMed, Scopus and Web of Science) was performed in accordance with the PRISMA guidelines. In addition, we screened our observational database containing records of all consecutive patients with cerebral aneurysms treated at our institution since 2003, which yielded 2 additional cases of cerebral aneurysms associated with TSC, and these cases were included in our analysis.

Results: Thirty-three patients with 42 cerebral aneurysms were found, and seemed to have distinct characteristics compared to other syndromal and non-syndromal cerebral aneurysms. Indeed, TSC patients with cerebral aneurysms were found to be young male individuals that present with large/giant, fusiform, mostly asymptomatic and unruptured aneurysms, and located on the internal carotid artery unrelated to branching zones. Also, 3 cases of subarachnoid hemorrhage were reported. In the pediatric subgroup of patients (n=22), cerebral aneurysms were all unruptured, large/giant and fusiform in 63.3%, multiple in 27,2% and originated from the internal carotid artery in 60% of the cases. Also, they were diagnosed whether incidentally (n=10, 45.4%) or due to a new onset of a neurological deficit (n=6, 27.2%). A rapid aneurysmal growth was described in 2 patients.

Conclusion: Prospective screening, genetic and histopathological studies are urgently needed to improve the understanding of the pathogenesis and epidemiology of cerebral aneurysm formation in TSC. This cannot be achieved without enhancing the recommendations of the 2012 International TSC Consensus Conference with a cranial time-of-flight magnetic resonance angiography at diagnosis and all regular screening consultations.

33. The abnormality of white matter microstructure in the limbic system is correlated with TSC-associated neuro-psychiatric disorders (TAND)

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Aim: The subjects with tuberous sclerosis complex (TSC) show a wide variety of the TSC-associated neuro-psychiatric disorders (TAND). Although TAND affects more or less the TSC patients on their quality of lives, the particular regions in brain associating with TAND remain unknown. We investigated the white matter microstructure using diffusion tensor imaging (DTI) in TSC and analyzed the association between white matter and neuro-psychological assessment.

Methods: Twenty-two patients(11±6years) with TSC and 23 age-matched controls(11±2years) participated in this study. DTI were acquired on a 3.0 T MRI and compared with tract-based spatial statistics (TBSS). Cognitive and social-behavioral ability of TSC were assessed by Wechsler Intelligence scale, TAND checklist, Vineland Adaptive Behavior Scale-2, and Social Responsiveness Scale-2.

The history or severity of epilepsy was evaluated by retrospective review of the medical records.

Results: TBSS showed lower fractional anisotropy (FA) in the right cingulum(hippocampus),right inferior longitudinal fasciculus and higher mean diffusivity (MD) in the left superior longitudinal fasciculus in TSC group. Correlation analysis revealed FA in the body of fornix negatively correlated with the Vineland maladaptive behavior score(ρ=-0.53,p=0.02). In addition, FA in the right uncinate fasciculus was positively correlated with the Vineland socialization score (ρ=0.52,p=0.01).

Conclusion: The fornix is connecting the hippocampus with the mammillary body, the anterior thalamic nuclei, and the hypothalamus,

which form the Hippocampal-diencephalic-cingulate networks and associated with memory, emotion and psychiatry.

The uncinate fasciculus is one of the important white matter connections between the orbitofrontal cortex, temporal pole, insula, and

amygdala which are associated with social-emotional function. Our study showed the abnormality of white matter microstructure in the limbic system was correlated with the behavior problems and socio-emotional impairment, which is related to TAND.

34. Creating a genetic risk prediction model for epilepsy in TSC

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Background: Identifying patients with TSC who will develop epilepsy is imperative to improve neurocognitive function, yet there are few strategies for predicting adverse outcomes. We previously developed a clinical risk prediction model for epilepsy in TSC and hypothesize incorporating modifier genes could further improve prediction. As a first step, we evaluated the association of common single nucleotide polymorphisms (SNPs) mapped to epilepsy candidate genes.

Study Design: We performed a nested case-control study of epilepsy among 377 TSC patients with phenotype information and array-based genotyping. SNPs mapped to 22 candidate epilepsy-associated genes and 68 mTOR pathway genes were compared in TSC subjects with epilepsy to those without epilepsy. We used logistic regression to test log-additive genetic associations, adjusting for sex, principal components of genetic ancestry, TSC1/TSC2 mutation, and tuber presence.

Results: We identified SNPs in 12 of 22 epilepsy candidate genes and 22 of 68 mTOR pathway genes that were significantly (P<0.05) associated with epilepsy. We did not identify additional common variants mapped to TSC1 or TSC2 associated with epilepsy. The top variants in epilepsy candidate genes mapped to GABRA2 (rs62304124, P=0.0042), GABBR2 (rs13440299, P=0.0004), GRIN2B (rs17834134, P=0.0075), CACNA1G (rs2079231, P=0.0090), and CACNA1A (rs116654215, P=0.0043). The top SNPs in mTOR pathway genes mapped to PIK3R1 (rs185180, P=0.0040), PIK3R4 (rs78034521, P=0.0067), PIK3C2G (rs7980686, P=0.0033), GRB10 (rs2715139, P=0.0040), and RPTOR (rs62069421, P=0.0004).

Discussion/Conclusion: SNP analysis of genes other than the causative TSC1/TSC2 pathogenic variants demonstrate that modifier genes could explain some epilepsy variability, with common alleles at 34 genes demonstrating significant effect of either increasing or decreasing the risk of epilepsy.

Future: Our next step is to augment our clinical risk prediction model with a genetic risk score based on common variants in epilepsy candidate genes and the mTOR pathway.

35. Oligomerization of TSC1 and membrane binding via phosphatidylinositol-phosphates

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Tuberous sclerosis complex is caused by mutations in the genes encoding TSC1 and TSC2, which together with TBC1D7 form the TSC protein complex. This complex was established to act via TSC2 as GTPase activating protein in the inactivation of the small GTPase Rheb and the downstream effector mTORC1. The loss of function as a negative regulator of the mTORC1 signaling pathway is the main cause for the development of TSC. The molecular mechanisms and the role of TSC1 in this context, however, remains unclear. We combined structural and cell biology studies to gain a better understanding of TSC1 function.

Initially, we characterized TSC1 from the thermophile Chaetomium thermophilum and identified three domains in vitro. We demonstrated that TSC1 assembles as a dodecamer or more precisely a hexamer of dimers, and is the driving force of oligomerization of the TSC complex. We determined a linker helix as a main oligomerization interface. This linker helix connects the N-terminal HEAT repeat domain with the parallel C-terminal coiled-coil region. Furthermore, we demonstrate the binding of TSC1 to a vacuolar/lysosomal lipid mix by recognizing phosphatidylinositol-phosphates (PIPs). Upon TSC1 oligomerization the overall binding affinity for membranes is increased. We could also show that these activities are conserved in human TSC1 and designed point mutations that failed to interact with PIPs. Importantly, these mutants also failed to restore TSC complex function in TSC1 ko cell lines, demonstrating a requirement of TSC1 lipid binding in mTORC1 regulation. In cells, we identified PI3,5P2 generated by PIKfyve as a key factor in this process.

Our data thus suggests an additional mechanism of complex recruitment to the lysosomal membrane and a new role for TSC1 in TSC complex function and regulation.

36. Insights from a UK Delphi panel investigating the relationship between tuberous sclerosis complex (TSC)-associated neuropsychiatric disorders (TAND) and TSC-associated epilepsy

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Objectives

To obtain expert opinion on key aspects of TAND in patients with TSC-associated epilepsy, and to determine the potential effect of reducing seizure frequency on TAND progression.

Methods

Two-round Delphi panel composed of 10 UK healthcare professionals experienced with TSC (predefined consensus threshold: 70%, where consensus was sought).

Results

10/10 and 9/10 experts in Rounds 1 and 2 provided responses. There was consensus that increased seizure frequency and type (focal seizures with impairment of awareness or generalised seizures) impact the progression of TAND (both 90%, Round 1). Experts agreed a treatment that reduces seizure frequency would impact the following aspects of TAND: delayed development, behavioural issues, intellectual disability (70% consensus, Round 1), autism spectrum disorder, attention deficit hyperactivity disorder (ADHD), and anxiety disorder (>70% consensus, Round 2). Experts agreed early seizure control was beneficial in reducing/preventing each aspect of TAND (90%, Round 1). In Round 2, whilst some experts indicated that controlling seizures at older ages (>12 years) has beneficial effects on TAND (particularly behavioural issues and anxiety disorders), experts agreed seizures would need to be controlled at a young age (<2 years) to have most impact (delayed development, intellectual disability, and ADHD; 90%). There was also consensus that controlling seizures at <5 years would be beneficial for behavioural issues and anxiety disorders (80%, Round 2). Consensus was not achieved on the level of seizure reduction required by a new medicine to impact TAND; 60% of experts agreed ~50% seizure reduction would be required.

Conclusions

Seizure control at a young age is beneficial in reducing/preventing aspects of TAND, such as delayed development, behavioural issues, and anxiety disorders. This study provides robust clinical consensus on the relationship between seizures and TAND, an area with limited evidence. Quantification in natural history studies would further strengthen these data.

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37. Exploring Sleep in Neurodevelopmental disorders through Online and Remote Evaluation (e-SNORE): Pilot and feasibility study in tuberous sclerosis complex

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Background: Given the prevalence of seizures, painful health conditions, co-occurring autism, challenging behaviours and intellectual disability, there are several mechanisms hypothesised to exacerbate poor sleep in tuberous sclerosis complex (TSC). Based on caregiver report, children with TSC evidence significant settling problems, night-waking and daytime sleepiness compared to typically developing (TD) children. However, informant-report questionnaires (which can have poor correspondence with objective measures of sleep) have limited scope to identify potential causal mechanisms underpinning sleep difficulties in TSC.

Method: This study aimed to model the cause and maintenance of poor sleep in children aged 4-15 years with TSC, utilising both subjective and objective measures of sleep, and via comparisons to an age and gender matched TD group. Caregivers completed informant-report measures, including the Modified Simonds and Parraga Sleep Questionnaire (MSPSQ), and sleep, pain, seizure and behaviour diaries across consecutive mornings and evenings via a mobile app. Actigraphy was employed as an objective measure of sleep for a minimum of seven nights.

Results: Children with TSC obtained higher scores on the MSPSQ daytime sleepiness subscale than the TD group ($p = .005$, $r = -.638$; medium effect size). However, groups did not significantly differ on any other MSPSQ subscales (e.g. sleep onset delay, night-waking) or any objective sleep parameters measured using actigraphy (e.g. sleep efficiency, wake after sleep onset). When exploring seizure activity as a potential mechanism underlying poor sleep, objective sleep parameters did not differ between nights of highest and lowest seizure activity in the TSC group.

Conclusions: This is the first study to utilise overnight actigraphy to explore the profile of sleep in children with TSC. Although groups did not differ on the majority of subjective and objective measures of sleep, potential mechanisms underlying higher levels of daytime sleepiness in TSC (e.g. adverse effects of antiepileptic medications) warrants further investigation.

38. Subependymal giant cell astrocytoma in adult patients with tuberous sclerosis: incidence rate, timing and causes of new diagnoses in adulthood

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Aims: Little is known about subependymal giant cell astrocytomas (SEGAs) in adults with TSC, except for data from the TOSCA study. We aimed at evaluating the incidence rate of new diagnoses of SEGA in a cohort of adult TSC patients and why the diagnoses were made in adulthood.

Methods: Of 385 TSC individuals, we selected those who received a diagnosis of SEGA in adulthood. SEGA was defined as a lesion at the caudothalamic groove with size >1 cm or a subependymal lesion showing serial growth on consecutive imaging regardless of size.

Results: 21 individuals (47.6% males; 52.4% females) received a diagnosis of SEGA at age ≥18 years (range 18-46): 5.5% of all the TSC patients; 7.7% of all adults; 30.0% of all the patients with SEGAs. In 2/21 (9.5%) individuals acute symptoms of endocranial hypertension prompted the diagnosis of SEGA (29 and 44 yrs) and subsequent diagnosis of TSC, which was previously unknown. 3/21 (14.3%) patients had a previously known TSC diagnosis, but did not undergo follow-up and presented with acute signs of hydrocephalus requiring surgery (18-24 yrs). 8/21 (38.1%) individuals, who had a diagnosis of TSC with irregular surveillance, were diagnosed with asymptomatic SEGA on brain imaging (22-46 yrs). 8/21 (38.1%) patients, who had a TSC diagnosis and were receiving regular follow-up, had onset of SEGA in adulthood (18-28 yrs). Details about growth, treatment and outcome will be presented.

Conclusions: Irregular surveillance explains half of the diagnoses of SEGA in adulthood in TSC individuals. Nevertheless, 1/3 of the patients were diagnosed in adulthood despite appropriate periodic surveillance (one diagnosed at age 28 years), confirming that SEGAs can occur in adulthood. These results stress the need of strictly abiding to the international recommendations and raise the question whether to consider extending brain imaging to the age of 30 years.

39. Investigating the role of P/Q- and N-type voltage-gated calcium Ion channels in a preclinical model of tuberous sclerosis

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Tuberous Sclerosis Complex (TSC) is a neurodevelopmental disorder caused by mutations in the TSC1 or TSC2 gene, which causes the mammalian/mechanistic target of rapamycin complex to become overactive. This overactivity causes downstream complications that manifest into a variety of neurological disorders within TSC. Notably, 90% of TSC people suffer from epilepsy. Currently, there is no cure for TSC, and available antiepileptic medications only address some of the physical symptoms, but not their origin. Thus, determining these underlying mechanisms for epilepsy in TSC is crucial. Indeed, it is known that voltage-gated calcium ion channels (VGCC), such as P/Q- and N-type channels, are associated with epilepsy, although their exact role in TSC remains elusive. Therefore, my work will examine the role of P/Q- and N-type VGCCs in a preclinical model of TSC. My project assayed P/Q- and N-type VGCCs in a preclinical mouse model of TSC. Dissociated hippocampal neurons were utilized for calcium imaging. Herein, we show that KCl-induced depolarization of TSC1-KO (knockout) neurons increased calcium activity in the proximal dendrites, a region that serves as a gatekeeper to control synaptic signal integration to regulate neuronal firing. To determine the source of calcium, P/Q- and N-type channels were blocked prior to KCl administration with ω-agatoxin-IVA or ω-conotoxin-MVIIA (conotoxin), respectively. TSC1-KO neurons show conotoxin insensitivity while the wildtype (WT) neurons show decreased calcium activity, with no changes in P/Q-type calcium activity. Loss of conotoxin sensitivity would suggest deficiency in N-type channels in TSC-KO neurons. Unexpectedly, western blotting demonstrates increased N-type channel protein in hippocampal synaptoneurosomes with no changes in P/Q-type channels. Immunohistology on hippocampal slices from TSC1-KO mice further substantiates the N-channel protein increase, and compared to WT mice, indicates N-type channels are mislocalized in TSC1-KO slices. Together, these studies demonstrate calcium channel expression and function are disrupted in preclinical models of TSC.

40. mTOR hyperactivity limits protein synthesis and reduces inhibitory synapse formation

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Up to 90% of Tuberous Sclerosis Complex (TSC) patients present with seizures, and between 25-50% of those with TSC are later diagnosed with an autism spectrum disorder (ASD). Changes in the synaptic excitation/inhibition (E/I) balance have been hypothesized to contribute to epilepsy and ASD. A key regulator of synaptic number and efficacy is the kinase mammalian target of rapamycin complex 1 (mTORC1). While it is known that mTORC1 activation is required for the formation of excitatory synapses, mTORC1's role in inhibitory synapse formation has not been established. Moreover, the molecular mechanism responsible for inhibitory synapse formation remains elusive. Herein, we report that gephyrin, a protein integral to the postsynaptic stabilization and function of inhibitory synapses, and vesicular GABA transporter (vGAT), a presynaptic protein involved in packaging GABA into synaptic vesicles, are regulated by mTOR. We identified gephyrin and vGAT as putative mTOR sensitive proteins through an unbiased screen using mass spectrometry and bioinformatics. vGAT and gephyrin puncta number increase and return back to wild-type (WT) levels when TSC1 knockout (KO) cortical neurons are treated with rapamycin (a potent mTORC1 antagonist), suggesting that mTORC1 represses vGAT synthesis and gephyrin clustering. Importantly, this increase is blocked when neurons are pretreated with cycloheximide (CHX), a protein synthesis inhibitor. Moreover, vGAT and gephyrin puncta number decrease with increasing TSC1 gene loss. Using a novel in vitro fluorescent method to detect pre- and postsynaptic engagement, we have determined that vGAT-gephyrin synapse number changes with mTOR activity. Treating rat cortical neurons with either rapamycin or MHY1485 (an mTOR agonist) increases and decreases, respectively, the number of vGAT-gephyrin puncta number. Altogether, these results indicate that an overactive mTOR environment represses inhibitory synapse formation in TSC, bolstering the E/I imbalance hypothesis thought to underline ASD and epilepsy.

41. Genotype-phenotype correlations between TSC and autism

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Background: Genotype-phenotype correlations are clinically useful in predicting risk of manifestations such as epilepsy and intellectual disability in tuberous sclerosis complex (TSC). Multiple studies have consistently shown that patients with TSC2 pathogenic variants (PVs) are more likely to have epilepsy and intellectual disability. There are a few studies that demonstrate those with TSC2PVs also have an increased risk for autism; however, further studies are needed to replicate the results in larger TSC cohorts, and determine whether the association of TSC2 PVs and autism is independent of epilepsy. In order to address these questions, we will investigate TSC cohorts of larger sample size, along with well-defined autism, epilepsy, and other phenotypic data based on objective testing.

Methods: Our assembled cohorts (n=207) is the largest genotype-phenotype study of autism in TSC to date. We characterized the prevalence of PVs in TSC1, PVs in TSC2, and NMI between TSC patients with and without autism and performed some descriptive analyses. We will use logistic regression to calculate unadjusted odds ratios, 95% confidence intervals, and p-values to compare TSC causal gene prevalence between patients with and without autism. Significant associations will be determined by logistic regression p-values < 0.05. Due to the likely association of TSC2 PVs with both autism and epilepsy, additional adjusted analyses will be conducted to determine whether the genotype-phenotype correlation for autism is independent of epilepsy genotype-phenotype correlations.

Results thus far: Autism was present is 27%, 35%, and 29% of the TSC1, TSC2, and NMI groups, respectively.

Discussion/Conclusion: Our results thus far appear to support results seen in similar studies; demonstrating that those with TSC2 PVs are at increased risk for autism. We are continuing analyses to determine if the risk is independent of epilepsy and other covariates and if there are genotype-phenotype correlations for autism severity.

42. Spectrum of germline and somatic mitochondrial DNA variants in tuberous sclerosis complex

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Background: Tuberous Sclerosis Complex (TSC) is caused by genetic alterations in TSC1 or TSC2. While the nuclear genome has extensively been studied in TSC, there is limited knowledge regarding the role of mitochondrial DNA (mtDNA) in TSC pathogenesis.

Aim: To examine the prevalence and spectrum of mtDNA variants in TSC patients and correlate them with clinical features and disease severity since mtDNA variants may act as a disease modifier contributing to phenotypic heterogeneity and tumor development in TSC.

Methods: We analyzed mtDNA from buccal swabs from 102 TSC patients (44 male, 54 female, 4 unknown, median age: 31 years; 11 familial cases) using deep coverage amplicon massively parallel sequencing (median read coverage: 7,349). mtDNA analysis was also performed in 100 TSC related tumors (58 kidney angiomyolipoma, 24 SEGA, 11 cortical tubers, 2 LAM, 5 TSC-RCC) with matching normal sample (n=9) from 70 patients; 80 tumors had exome data available. Alterations in mitochondrial copy number were determined by qPCR in tumor-normal samples.

Results: A median of 21 non-synonymous mtDNA variants were identified in 102 buccal swabs, with high homoplasmy (median: 99.62% allele frequency) mainly missense of unknown significance. A pathogenic variant (UUR;MT-TL1; m.3243A>G, heteroplasmy 12%) was identified in one male TSC patient. Five VUS small indels with >97% heteroplasmy were identified in five individuals. Large mtDNA deletions were not detected. Analysis of TSC tumors demonstrated similar spectrum of mtDNA variants as seen in buccal swabs. mtDNA variants did not correlate with any pathological TSC features. qPCR analysis did not reveal changes in mitochondrial content between tumors and normal tissue.

Conclusions: Our study provides insight into the mtDNA landscape of TSC for first time, demonstrating that mtDNA genome is stable within the tumors analyzed and across different tissues.

43. Kidney angiomyolipomas are defined by a unique transcriptional profile and H3K27ac chromatin state

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Background: Kidney angiomyolipomas (AML) are commonly seen in TSC, and also occur sporadically, and due to TSC1/TSC2 biallelic loss. We hypothesized that the chromatin state and master transcription factors (TFs) are also drivers of AML growth, alongside mTORC1 activation.

Material and Methods: We performed RNA-Seq on 28 AML tumors, and H3K27ac ChIP-seq (marks open chromatin) on 25 kidney AMLs, the 621-101 line, and a melanoma cell line (SK-MEL30). MITF ChIP-Seq was also performed on five AMLs and the SK-MEL30 line.

Results: Expression analyses of AMLs compared to both TCGA tumor data and normal tissues identified 347 differentially expressed genes (DEG), including 18 TFs (FDR<0.05). Among these TFs, MITF and PPARG were the only known oncogenes identified, both highly expressed in kidney AML (4th and 1st out of 27 TCGA tumor types, respectively). In addition, 6 of 10 top DEGs in kidney AML are known MITF targets including CTSK, PMEL, and GPNMB. ROSE and regulatory potential analysis of H3K27ac ChIP-seq data compared to human normal tissues, identified MITF-A (near TSS of A isoform), PPARG, CTSK and GPNMB as genes with extended open regulatory chromatin regions, suggesting they are critical for AML development. Pathway analysis of all 347 DEGs showed enrichment in pathways for epithelial-mesenchymal transition, myogenesis, adipogenesis, and estrogen response (all q-values< 6.54x10-9). Immunohistochemistry demonstrated positive staining for nuclear MITF, ARID5B, MEIS2, NR2F2, NFIC TFs, and cytoplasmic GPNMB in AML sections and other TSC tumors, compared to adjacent normal tissue. High levels of ARID5B, MEIS2, NR2F2 expression were confirmed by western plot in protein lysates from AML tumors. siRNA for selected top TFs MEIS2 and ARID5B demonstrated reduction in cell growth in vitro.

Conclusions: Our studies have identified unique chromatin signatures, and several highly expressed TFs which likely are essential for AML development, enabling potential novel treatment strategies.

44. Extracellular vesicles secreted by TSC2-deficient cells shuttle altered cargo to fibroblasts of the tumour microenvironment to promote pro-tumoral signalling

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Loss-of-function Tuberous Sclerosis Complex 1 or 2 (TSC1/2) mutations enable mammalian target of rapamycin complex 1 (mTORC1) hyperactivation, causing multi-organ tumour growth in patients with Tuberous Sclerosis Complex (TSC). Although our knowledge of intracellular signalling downstream of TSC1/2 mutations is increasingly well elucidated, knowledge about the mechanisms by which TSC tumours signal intercellularly to the tumour microenvironment to support tumour growth is limited. It is known that extracellular vesicles (EVs) – nanometre-sized vesicles that shuttle biological cargo between cells – can promote tumour growth and facilitate angiogenesis in the microenvironment of some solid tumours. Investigating the role of EVs released from TSC tumour cells may therefore identify a new tumour-supporting mechanism, thus improving our understanding of TSC tumour progression and revealing potential therapeutic targets. Therefore, our aim was to assess the characteristics, cargo composition, and functional capacity of EVs secreted from TSC2-deficient angiomyolipoma (disease) and TSC2-expressing (control) cell lines. We assessed EV protein cargo, and selected targets were validated by ELISA. EV-mediated signalling activation in recipient stromal fibroblasts was assessed, and tumour-supporting functions of EV-activated fibroblasts were explored. We show increased secretion of EVs from TSC2-deficient cells compared to TSC2-expressing cells. We observe that EVs from TSC2-deficient cells have distinct proteomic profiles, enriched for cancer- and mTORC1 signalling-associated markers. Several of these markers are novel, and not previously associated with TSC. Furthermore, we show that TSC2-deficient EVs can modulate tumour-supporting signalling and promote pro-tumoral fibroblast phenotypes in non-transformed fibroblasts of the microenvironment, to aid tumour development. Importantly, rapamycin treatment could, at least partially, reverse disease-associated characteristics and tumour-supporting functions of TSC2-deficient EVs. Together, this work addresses important knowledge gaps about EV number and cargo from TSC cells, and how these EVs can promote TSC tumour growth by modifying the tumour microenvironment.

45. Unkempt is a novel downstream regulator of mTOR signalling in mammalian neurogenesis

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Tuberous sclerosis complex (TSC) is a genetic disorder affecting cellular proliferation, migration and differentiation early in development, resulting in the formation of hamartomas in different organs of the body. TSC is caused by pathogenic variants in TSC1 or TSC2, leading to mechanistic target of rapamycin (mTOR) pathway hyperactivation. mTOR is a protein kinase that regulates anabolic cellular processes and plays a key role in neurogenesis. mTOR dysregulation is associated with several neurological disorders including epilepsy, autism and focal cortical dysplasia. Very little is understood about the factors that act downstream of mTOR during nervous system development. Unkempt was identified as the first neurogenic component of the mTOR pathway in Drosophila. The aim of this work is to

investigate the mechanistic relationship between Unkempt and mTOR and its functional role in mammalian neurogenesis. Unkempt is a zinc finger/RING domain protein that negatively regulates neuronal differentiation in Drosophila. In mammals, Unkempt regulates the translation of several hundred target mRNAs and negatively regulates their translation. We show that Unkempt is directly phosphorylated by mTOR complex 1 (mTORC1) and this phosphorylation is regulated by nutrient levels and growth factors. We have identified mTORC1-dependent phosphorylation sites on Unkempt and show that the phosphorylation state of Unkempt is required for its ability to control cell morphology. We also show that Unkempt directly binds to Raptor through its C-terminal serine rich region. Unkempt is required for early neuronal development. We find that overexpression of Unkempt in mice rescues the aberrant neuronal migration phenotype caused by expression of constitutively active Rheb - the canonical activator of mTORC1. Investigation of novel mTOR pathway components regulating neurogenesis, like Unkempt, will provide new insight into the mechanism by which mTOR regulates neural development. This understanding could aid the development of targeted therapies for the neurological manifestations of diseases like TSC/FCD.

46. Management of Facial Angiofibroma Related to Tuberous Sclerosis Complex and use of Topical mTOR Inhibitor in U.S. Retrospective analysis of Natural History Database

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Facial angiofibroma is the most predominant (74.5% to 83%) cutaneous manifestation of tuberous sclerosis complex (TSC), a genetic disorder impacting the mechanistic target of rapamycin (mTOR) signalling pathway. Invasive therapeutic modalities (e.g., surgery and laser therapy) used for the treatment of facial angiofibroma are associated with pain, bleeding, and recurrence. Effectiveness of various topical rapamycin compounded formulations for the management of facial angiofibroma is well established. however, lack of FDA-approved formulation in the United States is a major limitation. This retrospective analysis of the data from the TSC Alliance's Natural History Database aimed to evaluate current treatment approaches for the management of facial angiofibroma.

The TSC Alliance's Natural History Database, the largest repository of longitudinally studied TSC patients, is an IRB-approved research database implemented in 2006. In this retrospective analysis, data from patients with facial angiofibroma (n=1329) enrolled in the 18 US-based clinical sites until 17th May 2020 were included.

The median (range) age of participants included in this analysis was 22 (3–86) years, of whom 58.8% were 18–45 years old. Of the 798 participants who had genetic testing, 517 (64.8%) had TSC2 mutations and 164 (20.6%) had TSC1 mutations. Testing was inconclusive in the other 14.7%. Facial angiofibroma was diagnosed more frequently (28.0%) at 6–17 years of age. Topical rapamycin was used by 329 (24.8%) participants for the management of facial angiofibroma. Systemic mTOR inhibitor for the management of facial angiofibroma was used by 163 (12.3%) participants. However, after excluding other eligible conditions, only 16 (1.2%) participants received systemic mTOR inhibitor exclusively for the management of facial angiofibroma. Additionally, 222 (16.7%) participants used systemic mTOR inhibitor for other conditions. Abrasive (2.6%) or laser (17.1%) therapy had been used by a few participants. A total of 601 (45.2%) participants received no treatment for facial angiofibroma, perhaps due to unavailability of approved formulations or relatively mild cases.

Despite the lack of an FDA-approved formulation, nearly 25% of individuals with TSC have used a topical mTOR inhibitor for the management of facial angiofibroma, and a few individuals (1.2%) have used a systemic mTOR inhibitor solely for the management of facial angiofibroma. This analysis emphasizes the unmet need for an FDA-approved topical mTOR inhibitor formulation, access to which could benefit many individuals with angiofibroma currently not receiving treatment.

47. Facial Angiofibroma Related to Tuberous Sclerosis Complex and the use of Topical Rapamycin in the United States: A Survey of Caregivers and Individuals with TSC

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Facial angiofibroma, a predominant cutaneous manifestation of tuberous sclerosis complex (TSC), considerably impairs quality of life causing psychological and social distress. A major limitation in the US is lack of FDA-approved treatment for facial angiofibroma, although several studies reported effectiveness of topical rapamycin. This analysis was aimed to study the impact of facial angiofibroma on patients with TSC and its management, focusing on topical rapamycin use (as a compounded formulation) in the US.

The TSC Alliance conducted an online International Drug Development Survey between 15 May 2017 and 14 June 2017 by distributing a link to pts with TSC and caregivers (to respond on behalf of their dependent child(s) or adult patients) through various channels including social media. Of the 420 caregivers and 133 patients responded from the US, 336 (80.0%) caregivers and 98 (73.7%) patients reported cutaneous manifestations (facial angiofibroma, ungual fibromas, etc.).

Of the 336 caregivers, 11 (3.3%) and 10 (3.0%) caregivers were taking care of 2 and ≥3 pts, respectively. Cutaneous manifestations lead to a minor, moderate, or severe impact on the lifestyle as reported by caregivers (32.7%, 11.9%, and 11.9%, respectively) and patients (31.6%, 19.4%, and 11.2%, respectively). Surgical removal of cutaneous manifestations was reported by 28.6% of caregivers and 61.2% of patients. Use of ≥1 mechanistic target of rapamycin inhibitor (mTORi) that include systemic/topical rapamycin or everolimus was reported by a total of 191 (56.8%) caregivers and 64 (65.3%) patients. A total of 129 (38.4%) caregivers and 18 (18.4%) patients did not report the use of any mTORi treatment.

Of the total 105 caregivers and 23 patients who reported the use of topical rapamycin, 68 (64.8%) and 16 (69.6%) responders, respectively, reported an improvement in skin condition. Improvement with the use of topical rapamycin alone was reported by 35/53 (66.0%) caregivers and 5/9 (55.6%) patients. Compared with surgical removal alone (6.3%) or systemic mTORi use alone (33.0% to 47.0%), more responders reported improvement with the use of topical rapamycin alone (56.0 to 66.0%). Topical rapamycin was reported to be moderately or very effective by 82.9% of caregivers and 73.9% of patients.

Use of topical rapamycin for the management of facial angiofibroma was reported by 105 (31.3%) caregivers and 23 (23.5%) patients, despite the lack of an approved topical rapamycin formulation. Improvement in facial angiofibroma was more common with topical rapamycin than other treatment approaches, and most (>70%) caregivers and patients rated topical rapamycin as moderately effective or very effective. Access to an FDA-approved topical rapamycin formulation could benefit even more people living with facial angiofibroma and TSC.

48. Tuberous sclerosis complex, a service review and plan for the future

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Background

In the Republic of Ireland, there are no TSC clinics. TSC care in Ireland is fragmented, difficult to navigate and wasteful of resources due to the complex nature of this disease and no formal clinical

setting to manage it.

Methods

A clinical audit to

• Assess the care received by patients registered on the National Epilepsy Electronic Patient Record, measuring their care against the UK guidelines.

• To identify the gaps in care and harness this data to improve care.

• Formulate an inter-discipline network for each patient to create improved communication, care and ease of health care navigation.

The audit included 46 questions under the following headings: Patient characteristics, genetic, central nervous system, kidney, lung, heart, eyes, skin, liver and pancreas and access. All TSC (n38) patients attending two epilepsy services in Dublin were included.

Results

Data shows that many patients exhibit numerous TSC manifestations and face a lack of coordinated disease management. Although many baseline investigations are carried out, not all have had diagnostic genetic testing. Many patients have neurological symptoms; however, neuropsychiatry is largely neglected, and completion of neuropsychiatric assessments checklists is inadequate. Access to treatments is limited. Access to specialist physicians relies on interdisciplinary referral. The service gaps echo the demand for an improved care system.

Discussion

The absence of a specialist TSC clinic compounds the complexity of navigating care for individuals with TSC, families and healthcare professionals. This project highlights the gaps in two large epilepsy services in Dublin, Ireland. Following these findings, an individual patient care network is in development for each patient. A research group has been established, and a PhD project has been submitted to investigate TSC care in Ireland further and explore a virtual MDT TSC clinic model. This work is transferable to other countries without specific TSC clinics.

49. A case study of neuropsychological intervention in a child diagnosed with tuberous sclerosis complex

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In Tuberous Sclerosis Complex (TSC), cognitive deficits that impact learning and behavior are commonly observed, and it is essential to develop practices that reduce long-term cognitive impairments, contributing to a better quality of life. This case study aimed to develop a neuropsychological intervention to improve cognitive skills in children with TSC. The initial project was carried out with a third-grade, 8-year-old, girl diagnosed with TSC and epilepsy. The intervention was organized in two parts: (1) based on single case experimental design (ABA) with multiple baselines, involved 15 sessions, six of which were baseline. Cognitive training tasks were created to stimulate attention, memory, planning, and organization. A variety of activities was developed including electronic and board games; and (2) fifteen sessions with the participation of her parents. On each of these days, an ecological task was chosen to be performed at home. The child was assessed by neuropsychological tests before the beginning of the first part and after the end of the second part. The results were evaluated based on the comparison among baselines, the description of her parents, and the comparison between pre and post neuropsychological assessment scores. Results showed there was a reduction in the response time, as well as an improvement in the scores of sustained and alternating attention tests. Parents reported improvement in attention and planning at home and described an increase in academic development. On the other hand, difficulties in visual perception were observed and neuropsychological assessment pointed to deficits in visual skills, which may indicate the importance of interventions in this area. This pilot study showed that the single case experimental design could be an appropriate strategy for the neuropsychological intervention with TSC patients. Sessions with the family are important to the generalization of cognitive skills.

50. Febrile seizures and febrile status epilepticus in tuberous sclerosis complex: a retrospective analysis

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Purpose: 1. To retrospectively assess the prevalence of febrile seizures (FS) and Febrile Status Epilepticus (FSE) in patients with Tuberous Sclerosis (TSC) followed at San Paolo Hospital in Milan; 2. To describe their characteristics and evolution; 3. To compare the results with those of the general population.

Method: We reviewed the medical records of 378 TSC patients followed, and selected the individuals with FS and FSE. FSE was defined as seizures triggered by fever lasting more than 30 minutes.

Results: 46 patients (12%) exhibited FS and/or FSE. Four had a pathogenic variant in TSC1 (8.7%), 34 in TSC2 (74%), 6 (13%) had no mutation identified (NMI), and in 2 (4%) analyses were pending. At time of FS/FSE onset 39/46 (85%) patients suffered from epilepsy, which was drug-resistant in 24%; 21/46 (46%) patients were already on antiepileptic treatment. Mean age at FS/FSE onset was 20 months (8-72). The last episode occurred at 24 months (8-72). 7/46 (15%) individuals exhibited simple FS, 24/46 (52%) showed complex FS, which were focal in 16/24 (66%) and prolonged or in series in 8/24 (33%). FSE was observed in 21/46 (46%). 4/46 (9%) patients showed both FS and FSE. In 74% of the patients FS were limited to a single episode, whilst in 13% they were recurrent. 37/46 (80%) individuals had intellectual disability (mild to severe).

Conclusion: Data about the prevalence of FS and FSE in TSC patients are scarce. Our analysis showed that, although the mean age at onset and at last febrile event is comparable with that of the general population, TSC patients seem more likely to develop FS and FSE. A better characterization of these patients is desirable, in order to achieve effective clinical management and to obtain clearer data about prognosis, in terms of cognitive outcome and development of drug-resistant epilepsy.

51. 90% TSC1/TSC2 mosaicism detection rate in tuberous sclerosis complex patients without mutation identified in commercial labs

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Introduction: Our past Massively Parallel Sequencing (MPS) studies have shown that mosaicism is a common phenomenon in TSC patients who had 'no mutation identified' (NMI) by conventional testing.

Materials and Methods: We used MPS for analysis of 144 samples [different TSC tumors/tissues/fluids: facial angiofibroma (FAF), ungual fibroma, shagreen patch, hypomelanotic macule, angiomyolipoma (AML), normal skin, buccal swab, saliva, blood, semen and amniotic fluid] from 30 NMI TSC patients (median age:33), including 5 with mild TSC manifestation (2 clinical features only). Mutations were detected using hybrid-capture MPS (median coverage:740x) and validated by our new MPS Multiplex High-sensitivity PCR Assay (MHPA) method utilizing Unique Molecular Identifier (UMI) based error suppression [sensitivity:0.05% variant allele frequency (VAF)]. Combining these results with previous analysis of 81 former NMI/mosaic TSC patients, we performed genotype-phenotype correlations.

Results: TSC1/TSC2 mutations were identified in 27 of 30 patients (90%) [21(78%) in TSC2; 6(22%) in TSC1]; 25 patients had mosaicism [blood VAF:0-19%, median:2.8%]. VAFs of the identified mosaic mutations were enriched in TSC tumors in comparison with normal tissues/fluids. Number of TSC-related clinical features in subjects with TSC1 and TSC2 mosaicism was highly variable, with a median of 5 features for both TSC1 and TSC2. We identified 7 novel TSC1/TSC2 mutations, including 5 large mutations, a large (221kb) inversion, and a de novo deep intronic deletion. We also identified an individual with minimal TSC features and two unique TSC2 mutations in each of AML and FAF, suggesting that both tumor events were sporadic.

Conclusions: We have developed an analysis approach for very sensitive TSC1/TSC2 mutation detection that is capable of a 90% detection rate on NMI cases and define the spectrum of mosaic mutations and associated clinical features in greater detail than previously.

The study was funded by Engels Family Fund and FY2020 Tuberous Sclerosis Alliance Postdoctoral Fellowship Award (KK)

52. MHPA: a novel strategy enabling detection of extreme low-level mosaicism in TSC

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Introduction: Detection of low frequency TSC1/TSC2 mutations in mosaic Tuberous Sclerosis Complex (TSC) patients is challenging due to high error rate of massively parallel sequencing (MPS).

Methods: Recently, we have developed Multiplex High-sensitivity PCR Assay (MHPA) that can detect TSC2 mutations at variant allele frequency (VAF) of 0.01-0.05% providing 10-fold improvement in sensitivity, in comparison with our previous MPS method for mosaicism detection.

Results: MHPA employs barcoding of single DNA molecules, which enables compression of the MHPA data to consensus reads that permits error-suppression of MPS artifacts. 40 short amplicons were designed for multiplex amplification of TSC2 exons, focusing on mutation hotspots (total coverage:75% of all TSC2 mutations). MHPA enables extremely high read depth [median depth before and after compression: 203000x and 23000x, respectively], with very low DNA input (10-50ng) required. To assess the reliability of the method, we performed MHPA analysis of 44 samples (TSC tissues/fluids, including blood, angiofibroma, angiomyolipoma) from patients with low-mosaic and heterozygous germline TSC2 mutations identified by our previous MPS approach. MHPA confirmed all previously identified TSC2 mutations (n=47), with very high correlation of VAFs ($r=0.97$, $p<0.0001$). MHPA also enabled identification of new low-frequency mosaic somatic mutations, which were not identified by previous method (VAF=0.01-0.4%). Replicate MHPA analysis (MHPA sequencing performed twice) was conducted for 7 samples and showed very good concordance for mutation detection [100% for VAF ≥ 0.08%].

Conclusions: MHPA enables identification of low level TSC2 mutations with higher sensitivity than any previous method. MHPA has many potential uses for research and clinical situations. It will enable reliable detection of low-level mosaicism in clinical labs (current limit for reporting TSC mosaicism in most clinical labs: VAF 2-10%), enhancing the care of mosaic TSC patients.

The study was funded by FY2020 Tuberous Sclerosis Alliance Postdoctoral Fellowship Award (KK) and Engels Family Fund.

53. Millions of incipient angiofibromas in TSC facial skin

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Introduction: Previously we had shown by Massively Parallel Sequencing (MPS) that UV-induced mutation is a consistent second hit mechanism in TSC facial angiofibromas (FAFs).

Methods: Recently, we have developed a novel MPS method, Multiplex High-sensitivity PCR Assay (MHPA), that has a sensitivity of 0.01-0.05% variant allele frequency (VAF) in the most commonly mutated regions of TSC2.

Results: MHPA analysis of 24 FAFs, including 19 with low level systemic mosaic and 5 with heterozygous germline TSC2 mutations, led to the identification of 101 low VAF (0.01–8%, median:0.1%,) somatic indels/point mutations. Remarkably, two or more somatic TSC2 mutations were identified in 19 of 24(79%) FAFs, suggesting that these small (2mm diameter) FAF biopsies contained at least two angiofibroma clones. 34 of 101(34%) mutations were CC:GG>TT:AA indicative of UV radiation causation. 30 of 522(6%) CC:GG sites in the region sequenced were affected by CC:GG>TT:AA mutation in one or more FAFs. Point mutations were predominantly C:G>T:A, with more C than G mutated, reflecting transcription-coupled nucleotide excision repair occurring for UV mutations (untranscribed strand bias, $p=0.002$). We also developed an MHPA assay for TP53, and identified an average of 8.7 TP53 mutations (likely in keratinocytes) per FAF biopsy. The nonsynonymous to synonymous mutation ratio [TSC2: 12(86/7); TP53: 21(189/9)] is indicative of strong selective pressure.

Conclusions: Considering the number of mutations, the mosaic/germline VAF in FAFs, and the size of the total facial skin, we estimate that 5-10,000,000 polyclonal fibroblast proliferations due to second hit mutations in TSC2 occur in the skin of TSC patients, a small proportion of which develop into observable FAF lesions. These observations highlight the importance of sunblock and other measures to limit facial UV exposure for TSC individuals of all ages.

The study was funded by FY2020 Tuberous Sclerosis Alliance Postdoctoral Fellowship Award (KK) and Engels Family Fund

54. Bi-steric mTORC1-selective inhibitors demonstrate improved potency and efficacy in tumors caused by TSC

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Rapalogs exhibit modest benefit for TSC patients, possibly due to their lack of effect on 4EBP1, which is a key target downstream of mTORC1. A new class of selective mTORC1 inhibitors has been developed and termed 'bi-steric', which comprises a rapamycin-like core moiety covalently linked to an mTOR active-site inhibitor. RMC-4627 and RMC-6272 are representative bi-steric tool compounds that exhibit potent and selective (>10-fold) inhibition of mTORC1 over mTORC2. RMC-5552 is the first clinical candidate of this class, and clinical testing is planned in 2021.

RMC-4627 and RMC-6272 induced more effective growth inhibition in multiple TSC1 or TSC2 mutant tumor cell lines than rapamycin. Both RMC-4627 and RMC-6272 at ~1 nM showed near

complete inhibition of p4EBP1T37/46, which was not seen with rapamycin treatment, while inhibition of pS6S240/244 levels was similar for rapamycin and bi-steric mTORC1 inhibitors. RMC-6272 had prolonged anti-proliferative activity in TSC-null cells, as shown by washout studies. Rapamycin, MLN0128 and both bi-steric mTORC1 inhibitors markedly reduced kidney tumor burden in Tsc2+/- A/J mice after four weeks of treatment. RMC-6272 significantly inhibited tumor regrowth after two-month of treatment cessation as compared to rapamycin and MLN0128. Furthermore, RMC-6272 treatment led to a greater induction of apoptosis in kidney tumor cells relative to rapamycin. In a standard preclinical mouse model of lymphangioleiomyomatosis (by tail vein injection of TTJ cells derived from Tsc2-null mouse kidney tumor), RMC-6272 also demonstrated a strong and durable in vivo anti-tumor activity.

In summary, RMC-4627 and RMC-6272 demonstrated more potent and durable inhibition of mTORC1 through near complete inhibition of p4EBP1 as compared to rapamycin and induced more cell death in Tsc2-null tumors in vivo. These preclinical data indicate the potential of bi-steric mTORC1-selective inhibitors as a novel therapeutic strategy to treat patients with tumors caused by TSC.

55. Quantification of healthcare, social care, and educational resource use in patients with tuberous sclerosis complex (TSC)-associated epilepsy: insights from a UK Delphi panel

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Objectives

To quantify healthcare, educational, and social care resource use of UK patients with TSC-associated epilepsy in the context of seizure frequency, seizure type, and age (paediatric vs. adult).

Methods

Two-round Delphi panel composed of 10 UK healthcare professionals experienced with TSC (predefined consensus threshold: 70%).

Results

10/10 and 9/10 experts in Rounds 1 and 2 provided responses. Higher seizure frequencies were associated with greater healthcare resource use (HCRU). Paediatric patients with 8−50 seizures/week had additional healthcare attendances/year vs. seizure-free patients: general practitioner (3.4 times as many), physician (x3.6), psychiatry (x5.3), physiotherapy (x7.6), occupational therapy (x10.4), Accident and Emergency (A&E, x10.5), and hospital admissions (x5.9). Across HCRU considered, patients with seizures had increased healthcare attendances vs. seizure-free patients: 1–2 seizures/week, x2.2 (paediatric), x2.7 (adult); 3–7 seizures/week, x4.0 (paediatric), x4.3 (adult); 8–50 seizures/week, x6.5 (paediatric), x6.8 (adult). Higher seizure frequencies (vs. seizure-free patients) were associated with higher percentages of paediatric patients requiring educational support (91% vs. 43%), adult patients requiring day-care support (57% vs. 14%), and increased paid caregiver support hours (paediatric: 16.0 vs. 1.6; adult: 26.7 vs. 2.8). Patients who experienced focal seizures with impaired awareness alone vs. those who also experienced generalised seizures typically had 39% fewer A&E visits, 27% fewer hospital admissions, 20% shorter hospital stays, and 39% fewer patients received rescue medication. Paediatric patients typically accessed more physiotherapy (x3.2), speech and language therapy (x4.0), occupational therapy (x1.9), and epilepsy nurse phone calls (x2.0) per year vs. adult patients.

Conclusions

Higher seizure frequency is associated with higher healthcare, educational, and social care resource use. HCRU is typically greater in paediatric (vs. adult) patients and in those with generalised seizures (vs. those with only focal seizures with impaired awareness). Quantitative studies would further strengthen these data, which are based on expert opinion.

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56. DGKA-dependent macropinocytosis supports tumorigenesis in tuberous sclerosis complex

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Tuberous Sclerosis Complex (TSC) is an autosomal dominant genetic disease that affects multiple organ systems including the brain, kidney, heart, skin, and lung. Lymphangioleiomyomatosis (LAM), the pulmonary manifestation of TSC, is a rare destructive lung disease affecting primarily women. In TSC and LAM, biallelic loss of TSC1/2 leads to mTORC1 hyperactivation and autophagy inhibition. To determine how the metabolic vulnerabilities of TSC2-deficient cells can be targeted, we performed a high throughput screen utilizing the "Repurposing" library at the Broad Institute, with or without the autophagy inhibitor chloroquine. We identified ritanserin, an inhibitor of Diacylglycerol Kinase alpha (DGKA), as a selective inhibitor of the proliferation of Tsc2-/- MEFs with no impact on Tsc2+/+ MEFs. DGKA is a lipid kinase that metabolizes diacylglycerol (DAG) to phosphatidic acid (PA), a key component of plasma membranes. Macropinocytosis, an endocytic process for nutrient uptake, is known to be upregulated in TSC2-deficient cells. Surprisingly, ritanserin decreased macropinocytic uptake of albumin by 80%, limited the number of lysosomes in TSC2-/- MEFs by 60%, and reduced lysosomal activity by 50%. Lipidomic analysis revealed that phosphatidic acid levels are increased 5-fold in TSC2-/- MEFs compared to TSC2+/+ MEFs. Treatment of TSC2-/- MEFs with ritanserin led to depletion of phosphatidic acid by 90% and rewiring of phospholipid metabolism. Ritanserin treatment decreased the cyst numbers and volume in a mouse model of TSC, and genetic downregulation of DGKA prevented alveolar destruction and airspace enlargement (30%) in a mouse model of LAM. Collectively, these data indicate that DGKA upregulates macropinocytosis in TSC2-deficient cells to maintain phospholipid homeostasis and promote proliferation. Targeting macropinocytosis with ritanserin may represent a novel therapeutic approach for the treatment of TSC and LAM.

57. Infant sleep duration predicts autistic traits in toddlerhood in tuberous sclerosis complex

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Background: Individuals with Tuberous Sclerosis Complex (TSC) have an increased likelihood of experiencing an array of physical, cognitive and behavioural challenges. Up to 60% of individuals receive a diagnosis of autism spectrum disorder (ASD) and up to 90% develop epilepsy. Individuals with TSC often experience sleep difficulties, which are associated with ASD and epilepsy in other populations. It is not known when differences in sleep emerge and whether they predict neurodevelopmental outcome.

Methods: The Early Development in Tuberous Sclerosis (EDiTS) Study is a prospective, longitudinal study of infants with TSC (total n=32) and age-matched typically developing infants (total n=32) from birth to 24 months of age. We assessed behaviour and development across up to 7 time points. This included a standardised observational measure of developmental ability (Mullen Scales of Early Learning) and parent-reported epilepsy severity (E-Chess), sleeping habits (sleep diaries) and emerging autistic traits in toddlerhood (Q-CHAT). Here, we focused on daytime and nighttime sleep duration in the first year of life.

Results: While no group differences were observed at 5 months, at 10 months nighttime sleep duration was significantly shorter (t=3.25, p=.003) in the TSC cohort (M=9.9 hours, SD=82.9) compared to typically

developing controls (M=11.2 hours, SD=62.05). No group differences were observed in daytime sleep. Shorter duration of night-time sleep at 10 months was associated with higher Q-CHAT scores (rho= -.39, p= .26). There were no significant associations with developmental ability or epilepsy severity.

Conclusion: Infants with TSC have a shorter duration of night-time sleep, which is associated with autistic traits in toddlerhood. Early sleep differences may be a key marker of likelihood for ASD. Further analyses will examine change in sleep duration over time, and associations with other behaviours. These findings support interventions targeting sleep aimed at improving neurodevelopmental outcomes for young children with TSC.

58. Metanalysis reveals tissue-dependent and independent transcriptomic aberrations upon loss of TSC1 and TSC2

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Tuberous sclerosis complex (TSC) is caused by loss-of-function mutations in either TSC1 or TSC2. Despite a monogenetic etiology, manifestations of TSC vary widely across affected tissue types, underscoring context-specific consequences of loss of TSC1/2. Furthermore, it remains unknown which cellular programs induced by loss of TSC1/2 are conserved across tissues types. Here, we present a metanalysis of transcriptomic studies comparing wildtype versus TSC1/2-/- cells to identify tissue-dependent and independent aberrations. We compiled public RNA-seq datasets studying various organs in human and mouse then collectively analyzed for differentially expressed genes. Publicly available human and mouse RNA-sequencing data was obtained from the Gene Expression Omnibus. Our systematic search yielded six studies in humans and twenty-one in mice, containing 40 and 346 samples, respectively. Differential gene expression analysis demonstrated loss of TSC1/2 results in a conserved transcriptomic signature. Consistent with published work, pathways differentially expressed independent of organ source included tyrosine kinase receptors, organ development, T-cell immunity, and ECM remodeling with metalloprotease. Treatment with sirolimus attenuated the transcriptomic signature upon loss of TSC1/2. From principal components analyses, organ source dominated clustering, suggesting a strong contribution to transcriptomic profile. As such, although a strong influence of organ source on transcriptomic signature and tissue-specific mechanisms were observed, persistence of tissue agnostic signals suggest a conserved transcriptomic aberration upon loss of TSC1 or TSC2.

59. Understanding the landscape of tuberous sclerosis complex (TSC)-Associated Neuropsychiatric Disorders (TAND) research: A comprehensive scoping review

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Background: TAND describes the behavioural, psychiatric, intellectual, scholastic, neuropsychological and psychosocial manifestations of TSC. Although these TAND manifestations and their natural TAND clusters are of significant concern to individuals and caregivers, they are relatively under-researched. This scoping review aimed to: a) describe the current TAND research landscape, and b) identify existing knowledge gaps, in order to prioritise recommendations for future TAND research.

Method: Twelve electronic databases were systematically searched in February and March 2020. Search terms encompassed the six levels of TAND (behavioural, psychiatric, intellectual, scholastic, neuropsychological and psychosocial). The study was conducted in accordance with the five stages of the scoping review framework: outlining research questions, study identification, study selection, data charting, analysis and interpretation.

Results: Of the 2,841 returned searches, 230 articles were included (animal studies: n = 30, case studies: n = 47, cohort studies: n = 153). Year of publication ranged from 1987-2020, with 52% of included studies published since 2013 alone. TAND studies were identified from 45 countries; however, 41% of all TAND research was derived from just two countries (USA = 26%; UK = 15%). The autism spectrum disorder-like cluster was the most widely researched, while the scholastic cluster was the most under-researched. Across 153 cohort studies, only 15 were interventional; eight were pharmacological and four were everolimus clinical trials. No published TAND study to date has utilised a behavioural intervention.

Conclusions: Although TAND research has increased exponentially since the term was coined in 2013, this review has identified a number of key knowledge gaps to guide future research. Recommendations include TAND research that reflects the cultural context of low/middle income countries, exploring TAND in older adulthood, interventional research (particularly non-pharmacological), and direct behavioural assessments/observations of scholastic outcomes. Research utilising remote technologies would directly address many of the TAND knowledge gaps identified.

60. Amiodarone-sirolimus interaction in a neonate with tuberous sclerosis complex

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BACKGROUND: Tuberous-sclerosis-complex (TSC) is a genetic condition with multiple organ manifestations, including the heart. Cardiac rhabdomyomas (CR) may be detected in utero or after birth. Typically, CR regress spontaneously. However, they can cause life-threatening left or right outflow tract obstruction and arrhythmia, depending on size and localization, prompting poor neonatal outcome. mTOR-inhibitors represent a causal treatment option in TSC. Nevertheless, they are currently approved for other TSC manifestations. Amiodarone represents a symptomatic treatment for ventricular and supraventricular arrhythmias. Concomitant administration may result in increased sirolimus levels, possibly due to inhibition of sirolimus metabolism through CYP3A4 by amiodarone (Nalli et al, 2006).

CASE REPORT: A 35-year-old female gave vaginal birth to a female infant at 38+6 weeks gestational age after the detection of fetal arrythmias. After good adaptation, the infant presented with distress and supraventricular tachycardia (260bpm). Echocardiography revealed a 1,69x1,26cm sized tumor with relevant right ventricular outflow obstruction. The infant was intubated and stabilized, including amiodarone infusion. On day 13 of life, she had two episodes of ventricular tachycardia, resistant to electric and pharmacological cardioversion. After consent, we started sirolimus treatment with 0,4mg/m2/dose, twice daily, with target trough level of 10-12ng/mL. Two days after administration sirolimus level was 11.2ng/mL. Two days later the level was 31.9ng/mL. We held sirolimus for several days, until the level drifted down to 7.9ng/mL and restarted sirolimus with reduced dose. CR shrunk, there were no further relevant arrythmias detected. The patient continued sirolimus treatment until 3 months of age.

She is now one year old, her cardiac status is stable, she reaches all her developmental milestones while she is treated with vigabatrin for focal seizures and hypsarrhythmia.

CONCLUSIONS: Amiodarone and mTOR inhibitors, such as sirolimus may interact significantly with increased risk of sirolimus toxicity. This case highlights this important interaction and may help treating physicians.

61. Mapping the time course of mTORC1-driven tumorigenesis in the developing brain

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Subependymal giant cell astrocytomas (SEGAs) are large tumors that grow in the brains of patients with TSC. SEGAs preferentially present near the ventral region of the lateral ventricles – a position that makes them difficult to surgically resect and can block the flow of cerebral spinal fluid – and are thought to be derived from neural stem/progenitor cells. However, the precise time window during development when a neural stem cell is susceptible to tumorigenesis is unmapped. Neural stem cells exist in heterogeneous populations that are precisely organized in space and across time. Specifically, different neural stem cell populations have varying levels of activation of mTOR, the master regulator of cell size and growth, and are variably proliferative or quiescent. We previously showed that different spatial populations of neural stem cells have differing levels of mTORC1 signaling, but the time when these signaling differences emerge, and their relationship to the cell cycle, is not known. Studies of pre- and postnatal TSC2 ablation revealed that prenatal neural stem cells are uniquely capable of generating SEGA-like tumors in the mouse. Using per-cell measurements of mTORC1 activity in murine stem cell cultures, we found that embryonic and postnatal neural stem cells have differing responses to the induction of quiescence, including further differences observed across diverse spatial populations. Complementary in situ analysis of intact embryonic mouse brain confirms these patterns. These tools are now being applied to patient-derived induced pluripotent stem cell and brain organoid models of TSC. Collectively, these data suggest that embryonic and postnatal neural stem cells are distinct populations, possibly with distinct mechanisms of regulating cell cycle entry and thus tumor susceptibility. Results from this project may reveal the developmental time window when a prenatal neural stem cell is susceptible to SEGA growth, thereby informing the development of temporally and spatially targeted preventative treatments.

62. TSC-related phenotypes in C57BL/6J and DBA/2J mice with germline Tsc1 haploinsufficiency

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Tuberous sclerosis complex (TSC) has heterogeneous neurological clinical manifestations, including epilepsy, TSC-associated neuropsychiatric disorders (TANDs), and structural brain abnormalities. The phenotypic heterogeneity across the patient population, despite a common genetic etiology, suggests the existence of genetic modifiers of disease outcomes in a patient's genetic background influencing the manner and extent of TSC1/2 mutational effects. Current mouse models of TSC can recapitulate some TSC features using conditional Tsc1 knockout (KO) alleles, but no model to date has robustly phenocopied the severe manifestations of the disease, particularly epilepsy, without unrealistic genetic dosages (homozygous loss of function) in specific cell types (e.g. neurons). This is a significant weakness in the search for genetic modifiers, given that human patients with TSC1 mutations typically inherit them from a parent carrier and are nearly always heterozygous in all cells. To address the yet unresolved genetic and phenotypic complexity seen in humans and be able to introduce controlled genetic diversity to probe the effects of genetic background on TSC-related outcomes, we created a mouse with germline heterozygous loss of Tsc1 on a pure C57BL/6J (B6) background via a backcross. Purity was confirmed by the Genome Scanning Service at the Jackson Laboratory, which uses a panel of ~150 SNPs. We then crossed female mice heterozygous for the null Tsc1 allele with DBA/2J (D2) and B6 males and evaluated the resulting F1 mice across TSC-related neurobehavioral, electrophysiological, and histological measures. TSC-D2 mice exhibited unique seizure-like discharges that were not present in littermate controls or TSC-B6s during long-term video-EEG monitoring. Furthermore, TSC-D2 mice exhibit highly stereotyped behavior not observed in littermate controls with which they were co-housed. Our novel Tsc1 conditional KO mouse and strain differences present in inbred crosses with this mouse therefore provide a mechanism to apply a systems genetics approach to TSC research.

63. Long-term safety and efficacy of add-on Cannabidiol (CBD) for treatment of seizures associated with tuberous sclerosis complex in an Open-Label extension trial (GWPCARE6)

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Introduction: In this 2nd interim analysis of an open-label extension (OLE) trial (GWPCARE6/ NCT02544763), we report safety and efficacy of add-on CBD for treatment of seizures associated with tuberous sclerosis complex (TSC).

Methods: Patients who completed a randomised controlled trial (RCT) received GW Pharmaceuticals' formulation of plant-derived highly purified CBD (100 mg/mL oral solution) in the OLE (titrated to 25 mg/kg/day, or up to 50 mg/kg/day). Primary endpoint: safety. Secondary endpoints: percent change in TSC-associated (countable focal or generalised) seizures, responder rates, and Subject/Caregiver Global Impression of Change (S/CGIC).

Results: Of 201 patients who completed the RCT, 199 (99%) entered the OLE. Median (range) age: 10.7 (1.1–56.8) years. Baseline median TSC-associated seizure frequency/28 days: 57 seizures. At this analysis, 12% of patients had completed treatment, 31% had withdrawn, and 57% were ongoing. OLE median (range) treatment time: 372 (18–1127) days. Mean (SD) modal dose: 28 (9) mg/kg/day. AE incidence: 94%; serious AE incidence: 26%; 8% discontinued treatment due to AE(s). Most common AEs (≥20%): diarrhoea (45%), seizure (28%), decreased appetite (23%), pyrexia (21%), and vomiting (20%). Seventeen (9%) patients had elevated ALT/AST >3×ULN; 12 were on concomitant valproate. No patient met Hy's law criteria for severe liver injury. One death occurred due to cardiopulmonary failure and was not deemed treatment-related. Median reductions in TSC-associated seizures (12-week windows through 72 weeks): 53%–75%. Seizure reductions were 54%–80% for patients with a modal dose ≤25 mg/kg/day (n=145). ≥50%, ≥75%, and 100% responder rates were maintained up to 72 weeks, ranging 52%–63%, 29%–51%, and 6%–19%, across 12-week windows). Improvement on S/CGIC was reported by 85% and 89% of patients/caregivers at 26 and 52 weeks.

Conclusions: Add-on CBD treatment was well tolerated and produced sustained reductions in TSC-associated seizures for up to 72 weeks.

FUNDING: GW Research Ltd.

64. Economic burden associated with tuberous sclerosis complex in patients with epilepsy

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Introduction: Epilepsy is a common manifestation of tuberous sclerosis complex (TSC), but data on the associated economic burden are limited. This study quantified healthcare costs in the USA among patients with epilepsy and TSC vs. patients with epilepsy without TSC.

Methods: Claims data from the Symphony Health Solutions Database (01/04/2017– 30/06/2019) were analysed. Patients with ≥1 medical claim with an epilepsy diagnosis were identified; those with ≥1 claim with a TSC diagnosis were assigned to the TSC cohort and the remaining patients to the non-TSC cohort. The index date was defined as the first epilepsy diagnosis date during the data availability period. Eligible patients in the non-TSC cohort were randomly selected and exact matched 5:1 with patients in the TSC cohort on age, sex, and insurance plan type. Patient characteristics were evaluated during the 3 months before the index date. All-cause and epilepsy-related healthcare costs were computed over the 12-month post-index period, and differences between the matched-TSC and non-TSC cohorts were analysed.

Results: The matched-TSC and non-TSC cohorts included 2,028 and 10,140 patients, respectively. The mean age was 25.3 years, 51% were male, and 47% had Medicaid insurance. The TSC cohort incurred $14,179 (95% confidence interval: $11,805–$16,531) higher all-cause prescription drug costs ($18,836 vs. $4,657, p<0.001), mainly driven by differences in epilepsy-related drug costs ($17,017 vs. $2,378, p<0.001). Additionally, epilepsy-related medical charges were significantly higher among the TSC (vs. non-TSC) cohort ($28,445 vs. $11,607, p=0.019). All-cause medical and total healthcare charges were numerically higher in the TSC cohort versus the non-TSC cohort; the differences were not statistically significant (medical: $56,003 vs. $51,740, p=0.707; total: $74,869 vs. $56,398, p=0.078).

Conclusions: In the USA, patients with epilepsy and TSC had a greater economic burden related to their epilepsy than patients with epilepsy without TSC.

FUNDING: GW Research Ltd.

65. Epidemiology, healthcare resource use, and mortality in patients with tuberous sclerosis complex: A population-based study on German health insurance data

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Introduction: A retrospective, 10-year (2007−2016) study reported data for patients with tuberous sclerosis complex (TSC).

Methods: Patients with TSC were identified from the anonymised German Vilua Healthcare research database using International Classification of Disease (ICD)-10 code Q85.1. Epilepsy was identified by ≥1 epilepsy ICD code (G40*/G41*) or ≥1 antiepileptic drug (AED) prescription after TSC diagnosis. Mortality was compared with age-, sex-, and time-matched controls.

Results: In 2016, 100 patients with TSC (mean [range] age: 38 [1–86] years; male: 40%) were identified. Standardised prevalence was 7.9 per 100,000 people, and 2.2 per 100,000 for TSC with epilepsy. Other TSC manifestations and comorbidities (excluding epilepsy) were identified more frequently in patients with (vs. without) epilepsy. From 2007−2016, mean annual healthcare costs were €6,139 per patient-year (PPY), mostly attributable to medication (€2,144, 35%) and inpatient care (€1,807, 29%). Patients with (vs. without) epilepsy incurred greater total annual costs (€9,091 vs. €4,583 PPY). Mean (SD; range) annual hospitalisation rate (all TSC patients) was 0.5 (1.0; 0–9) with a length of stay (LOS) of 5.9 (18.6; 0–264) days PPY. Mean (SD; range) annual hospitalisation rate was numerically greater in patients with (vs. without) epilepsy (0.7 [1.2; 0–8] vs. 0.4 [0.8; 0–9]) PPY), as was LOS (8.4 [21.4; 0–183] vs. 4.6 [16.8; 0–264] days PPY). In TSC patients, mean (SD; range) number of different medications prescribed: 6.3 (5.1; 1–33) PPY and 19.2 (14.4; 1–83) per patient over the observed time. In TSC patients with epilepsy, 3 (2.3; 1–11) different AEDs were prescribed over the observed time. Mortality rates (vs. control): all TSC 5.08% (1.69%), p<0.001; with epilepsy 7.53% (0.98%), p<0.001; without epilepsy 3.68% (2.03%), p=0.003.

Conclusions: Healthcare costs, resource utilisation, and mortality were higher in TSC patients with epilepsy vs. those without.

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66. A novel TSC-mTORC1-GRASP55 signaling axis controls unconventional protein secretion to reshape the extracellular proteome upon stress

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Cells communicate with their environment via surface proteins and secreted factors. However, how environmental cues modify the extracellular proteome is not known. Unconventional protein secretion (UPS) is an evolutionarily conserved process, via which proteins are transported to the cell surface or into the extracellular space upon stress. The Golgi-associated protein GRASP55 facilitates unconventional secretion of distinct cargo proteins. Notably, despite the emerging importance of UPS for human disease, its regulation and biological role remain poorly understood. Here, we identify TSC-mTORC1 as the first signaling pathway controlling UPS. Mechanistically, mTORC1 phosphorylates GRASP55 directly to retain its Golgi localization, thus revealing a physiological role for mTORC1 at the Golgi. Cellular stresses or drugs that inhibit mTORC1 cause GRASP55 dephosphorylation and relocalization to secretory compartments required for UPS. In TSC-deficient cells, that show hyperactive mTORC1 even upon stress or starvation, UPS induction is also compromised. Through multiple unbiased proteomic analyses, we comprehensively characterize, for the first time, numerous cargoes that follow this unconventional secretory route to shape the cellular secretome and surfactome. Using MMP2 secretion as a proxy for UPS, we provide important insights on its regulation and physiological role. Collectively, our findings reveal the TSC-mTORC1-GRASP55 signaling hub as the integration point in stress signaling upstream of UPS, and as a key coordinator of the cellular adaptation to stress. Moreover, we identify UPS as a novel important cellular function that is dysregulated in TSC and, potentially, other mTOR-opathies.

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Background: Interleukin 6 is a pro-inflammatory cytokine, which is upregulated in numerous pathological states. The primary objective of this study was to determine the cell autonomous effects of IL-6 on TSC2-deficient cells.

Results: We began our study by demonstrating that IL-6 is significantly upregulated in vitro and in vivo in preclinical models of TSC and in LAM patient plasma. The expression of IL-6 in vitro was both TSC2 and mTORC1 dependent. Using pharmacological and genetic approaches we determined that IL-6 inhibition represses proliferation ~60% (p<0.0001) and migration ~50% (p<0.0001) of TSC2-deficient cells. IL-6 knockout decreased basal oxidative phosphorylation and glycolysis by ~50% (p<;0.05). Using metabolomics we discovered that IL-6 plays a role in regulating de novo serine synthesis to support proliferation. We confirmed this finding by rescuing the proliferation of IL-6 knockout cells with overexpression of PSAT1, an essential enzyme in serine metabolism. We next used Tsc2+/- mice to investigate the therapeutic potential of IL-6 inhibition. Treatment with an aIL-6 antibody (200ug / 3 times a week) for one month reduced renal tumor burden by ~30% (p=0.03) and proliferation by ~30% (p=0.008) in 9 month old Tsc2+/- mice. In a second cohort of mice, Tsc2+/- mice were treated with aIL-6 antibody (200ug), rapamycin (1mg/kg) or combination three times a week for 1 month and harvested two months after the final injection. The mean microscopic tumor burden was reduced by 40% by aIL-6 antibody (p<0.01), 60% by rapamycin (p<0.0001), and 70% by the combination (p<0.0001) compared to the IgG treated controls. These data suggest that IL-6 targeted therapies may improve the therapeutic response of mTORC1 inhibitors.

Conclusions: IL-6 contributes to the extensive metabolic reprogramming observed in TSC lesions and is a potential therapeutic target for patients with TSC or LAM.

67. Histamine signaling and metabolism identify biomarkers and therapies for lymphangioleiomyomatosis

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Lymphangioleiomyomatosis (LAM), a rare multisystem disease affecting about one-third of TSC women, is treated with allosteric inhibitors of mTOR. However, this therapy has variable tolerability and some patients show progressive decline of lung function despite treatment. LAM diagnosis and monitoring can also be challenging due to the heterogeneity of symptoms and insufficiency of non-invasive tests. Here, we identify monoamine-derived biomarkers through a targeted metabolomic analysis that provide preclinical evidence for novel therapeutic approaches. The major histamine-derived metabolite methylimidazoleacetic acid (MIAA) is relatively more abundant in LAM plasma compared with healthy and other cystic lung diseases samples, and MIAA values are independent of VEGF-D measures. This metabolite is the result of altered metabolism of monoamines. Higher levels of histamine are associated with poorer lung function and greater disease burden. Combined molecular and cellular analyses, and causes of disease heterogeneity.

68. Heterogeneity and cancer-related features in lymphangioleiomyomatosis cells and tissue

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Lymphangioleiomyomatosis (LAM) is a rare, low-grade metastasizing disease characterized by cystic lung destruction. LAM predominantly affects women and can occur sporadically or in association with tuberous sclerosis complex (TSC). LAM exhibits extensive heterogeneity at the molecular, cellular, and tissue levels. However, the molecular similarities and differences among LAM cells and tissue, and their connection to cancer features are not fully understood. Using complementary gene and protein LAM signatures, and single-cell and bulk tissue transcriptome profiles, we show sources of disease heterogeneity, and how they correspond to cancer molecular portraits. Subsets of the neoplastic LAM cells involved in disease pathogenesis may differ with respect to hormone-, metabolism-, proliferation-, and stemness-related gene expression profiles. The RUNX1 and IRF1 transcription factors regulate LAM cell signatures, and both regulators are expressed in LAM lung lesions, with differences between spindle-like and epithelioid LAM cells. The cancer single-cell transcriptome profiles most similar to those of LAM cells include a breast cancer mesenchymal cell model and lines derived from pleural mesotheliomas. Heterogeneity is also found in LAM lung tissue, where it is influenced by interplay with the immune system. Variable expression of the multifunctional innate immunity protein LCN2 is linked to disease heterogeneity. This protein is found to be more abundant in LAM plasma than in plasma from healthy women. By analyzing LAM and cancer molecular portraits, this study proposes LAM molecular and cellular features, master regulators, cancer similarities, and causes of disease heterogeneity.

69. Drug inhibition of reduction-oxidation factor 1-apurinic/apyrimidinic endonuclease 1 restores the hypoxic-driven disease state of tuberous sclerosis complex

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At present, mTOR inhibitors (principally rapamycin and its analogues) are first line therapy for TSC patients and are effective at reducing associated tumour volume and delaying disease progression. However, mTOR inhibitors effect is cytostatic in nature and crucially they do not ameliorate symptoms for all patients. This suggests that loss of TSC1/TSC2 results in mTORC1 independent signalling that contributes to TSC pathology. Within both patient-derived and murine TSC2-deficient cells, we have shown expression of the redox-sensing protein reduction-oxidation factor 1-apurinic/apyrimidinic endonuclease 1 (REF-1) is elevated, as is the activity of hypoxic responsive transcription factors which REF-1 transactivates. Namely HIF-1α, STAT3 and NF-κB. We hypothesise that within TSC tumours, loss of TSC1/TSC2 results in the hyperactivity of this REF-1/HIF-1α/STAT3/ NF-κB signalling nexus through a multitude of inputs that are not dependent on mTORC1 signalling. We further postulate that the hypoxic gradients within tissues principally affected within TSC (namely kidneys, brain, skin, and lungs) further elevate the activity of this signalling nexus which in turn drives TSC pathology. Within TSC2-deficient cells, we establish that the dysregulated transcriptional activity of HIF-1α, STAT3, NF-κB can be normalised through treatment with REF-1 inhibitors, with mTOR inhibitor treatment having a lesser or no effect on activity.

Furthermore, Ref-1 inhibition is able to decrease protein expression of the HIF-1α targets VEGFA and BNIP3. Through a multitude of tissue culture assays, namely tumour spheroid growth, anchorage independent growth, cell migration, cell invasion and vasculature mimicry, we demonstrate that REF-1 inhibitors are able to normalise these measures of tumorigenicity. The present work not only reveals REF-1 as mediator of aberrant hypoxic signalling within TSC, but also that REF-1 inhibitors may be of therapeutic benefit, potentially able to treat patients that are not responsive to current mTOR inhibitors.

70. ABSTRACT WITHDRAWN

71. Regulation of de novo serine synthesis by Interleukin 6 in tuberous sclerosis complex (TSC)

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Background: Interleukin 6 is a pro-inflammatory cytokine, which is upregulated in numerous pathological states. The primary objective of this study was to determine the cell autonomous effects of IL-6 on TSC2-deficient cells.

Results: We began our study by demonstrating that IL-6 is significantly upregulated in vitro and in vivo in preclinical models of TSC and in LAM patient plasma. The expression of IL-6 in vitro was both TSC2 and mTORC1 dependent. Using pharmacological and genetic approaches we determined that IL-6 inhibition represses proliferation ~60% (p<0.0001) and migration ~50% (p<0.0001) of TSC2-deficient cells. IL-6 knockout decreased basal oxidative phosphorylation and glycolysis by ~50% (p<;0.05). Using metabolomics we discovered that IL-6 plays a role in regulating de novo serine synthesis to support proliferation. We confirmed this finding by rescuing the proliferation of IL-6 knockout cells with overexpression of PSAT1, an essential enzyme in serine metabolism. We next used Tsc2+/- mice to investigate the therapeutic potential of IL-6 inhibition. Treatment with an aIL-6 antibody (200ug / 3 times a week) for one month reduced renal tumor burden by ~30% (p=0.03) and proliferation by ~30% (p=0.008) in 9 month old Tsc2+/- mice. In a second cohort of mice, Tsc2+/- mice were treated with aIL-6 antibody (200ug), rapamycin (1mg/kg) or combination three times a week for 1 month and harvested two months after the final injection. The mean microscopic tumor burden was reduced by 40% by aIL-6 antibody (p<0.01), 60% by rapamycin (p<0.0001), and 70% by the combination (p<0.0001) compared to the IgG treated controls. These data suggest that IL-6 targeted therapies may improve the therapeutic response of mTORC1 inhibitors.

Conclusions: IL-6 contributes to the extensive metabolic reprogramming observed in TSC lesions and is a potential therapeutic target for patients with TSC or LAM.

Early Career Researcher awards list, sponsored by Noema Pharma

Noema Pharma have sponsored six Early Career Researchers Awards to assist attendance at the conference. We would like to congratulate the six awardees, who came through a tough competition for support. They are:

Dr Katarzyna Klonowska

Brigham and Women's Hospital and Harvard Medical School, Boston, USA

90% TSC1/TSC2 mosaicism detection rate in Tuberous Sclerosis Complex patients without mutation identified in commercial labs

Dr Stacey Bissell

School of Psychology, University of Birmingham, UK

Exploring Sleep in Neurodevelopmental disorders through Online and Remote Evaluation (e-SNORE): Pilot and feasibility study in tuberous sclerosis complex

Dr Angela Peron

Human Pathology and Medical Genetics, San Paolo Hospital, Milan, Italy

Subependymal Giant Cell Astrocytoma in adult patients with Tuberous Sclerosis: incidence rate, timing and causes of new diagnoses in adulthood

Dr Pranetha Baskaran

King's College London, London, UK

Unkempt is a novel downstream regulator of mTOR signalling in mammalian neurogenesis

Dr Daniel Ebrahimi-Fakhari

Department of General Pediatrics, University Children's Hospital Muenster, Munich, Germany

Prenatal Sirolimus Treatment for Intrauterine Rhabdomyomas in Tuberous Sclerosis

Ms Jessica Martin

King's College London, London, UK

Investigating the impact of the pandemic on wellbeing in families of children with rare disorders: the CoIN Study

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Cannabinoids in the management of TSC-associated seizures

Friday 18 June, 18:05–19:00 BST

Time (BST)	Title/Presenter
18:05	**Welcome and introductions** *Prof. Finbar O'Callaghan, Chair*
18:10	**What is cannabidiol (CBD)?** *Dr Chandni Hindocha*
18:20	**Scientific insights into CBD and TSC** *Prof. Andrew Tee*
18:30	**TSC-associated epilepsy and GW CBD** *Prof. Finbar O'Callaghan*
18:45	**Q&A / Meeting close** *All*

For more information/resources
please visit our virtual booth

Job code: VV-MED-19957
Date of preparation: May 2021

This session is organised and funded by GW Pharmaceuticals and is intended for an HCP or prescriber audience only.

Prescribing information will be available at this session and from the GW virtual booth on the congress website.

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About the Tuberous Sclerosis Association

The Tuberous Sclerosis Association (TSA), founded in 1977, is the only UK charity solely focused on improving the lives of people affected by the rare genetic disorder Tuberous Sclerosis Complex (TSC). The TSA aims to provide help for today and a cure for tomorrow by:

- Providing direction or a listening ear through our support and information services for the TSC community, including through our UK-wide TSA Support Line
- Organising events and opportunities across the UK and virtually for those affected by TSC, allowing the TSC community to come together and feel less alone
- Funding internationally-significant research into the causes, diagnosis, management and treatment of TSC that has the greatest impact on those affected by the condition
- Campaigning on behalf of the TSC community to ensure that the TSC community has consistent and meaningful access to social support and healthcare provision

Through the TSA, individuals and families affected by TSC should never feel alone.

The TSA's position in research

The TSA is proud of our long-standing history of being a world-leader in funding important research into causes, diagnosis, management and treatment for TSC. From improving diagnosis of TSC to trialling new strategies and medicines for managing the condition, funding new research has always been a key priority for us.

The TSA's research strategy

The TSA's current research strategy is focused on co-funding projects alongside other groups. By doing this, we encourage groups who might not otherwise fund TSC research alone to pledge money by working with us. Often, this will mean that more funds are made available overall to continue to drive research into TSC.

Our co-funding approach also means that we can make important links and partnerships with research groups worldwide, whilst minimising the cost impact compared to if the TSA funded research alone.

This ensures that the TSA continues to be a world-leader in TSC research, ultimately benefiting the TSC community in the UK and globally.

Current and upcoming TSA-funded research

Current and upcoming TSA-funded research (co-funded and otherwise) includes:

- Early Development in Tuberous Sclerosis - The EDiTS study
- The TANDem Project
- A model system of cell invasion in TSC-LAM
- Better understanding the accessibility of mental health services for children and young people with TSC
- Identifying and creating more specific medicines for TSC
- Understanding the limits of everolimus and rapamycin
- Characterising the role of exosomes in TSC

Thank you to our Board of Trustees and staff

The International TSC Research Conference 2021, and the wide-reaching work of the TSA, would not be possible without our committed Board of Trustees and determined staff:

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Rare – A Journey of Self-Acceptance

By Zoë Bull

Growing up, Zoë didn't want to have anything to do with her rare condition, Tuberous Sclerosis Complex (TSC), she just wanted to be the same as everyone else. But when she suffered a life-threatening kidney bleed aged twenty, and multiple lung collapses a few years later, Zoë discovered she had a second rare disease called LAM.

Through informative research and deep introspection, Zoë has written about her experiences with TSC and LAM, and how she has learnt to accept them in a way she never had imagined.

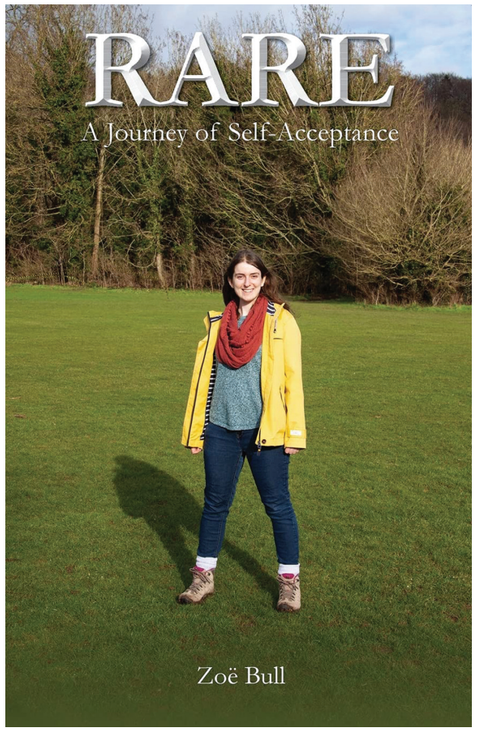
"This book captures the reality of a life touched by TSC and will be an encouragement to those who read it. Zoë has achieved so much, in spite of all that was stacked against her.

"I have read and written several scientific papers and book chapters on TSC in my professional capacity, however, Zoë's book has given me a deeper insight into TSC, from a different angle."

Dr Sam Amin, Paediatric Neurologist with a special interest in TSC

'Zoë's honesty and curiosity about her experience of living with two rare genetic conditions make for compelling reading."

Louise Fish, Chief Executive, Tuberous Sclerosis Association



Copies of Zoë's book can be purchased from: **Amazon.co.uk; Blackwells.co.uk; Bookdepositary.com; Hive.co.uk; and Waterstones.com**.

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Abstracts Due August 16, 2021

To learn more and register your interest visit tscalliance.org/researchconference.

Questions? Email Zoë Fuchs at zfuchs@tscalliance.org.

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