EPILEPSY RESEARCH CENTRE NEWSLETTER 2020

MOSAICISM IN PARENTS INFLUENCES REPRODUCTIVE COUNSELLING

FINDING THE ANSWER TO FAME

Professor Samuel Berkovic
Neurologist

Professor Ingrid Scheffer
Paediatric Neurologist

We are delighted to bring you the latest on our research on the epilepsies and epilepsy genetics at the Epilepsy Research Centre, University of Melbourne, Austin Health. Our research focuses on all forms of epilepsy from the common epilepsies to rare, severe epilepsies. We could not do our research without you, our research participants, who now number more than 23,000 people! We thank you sincerely for working with us to understand the epilepsies as we seek to improve outcomes for people living with epilepsy. We are also indebted to our referring doctors and all our other supporters for their wonderful contributions.

We are excited to bring you updates on our projects including our recent paper in the *New England Journal of Medicine* and two large international collaborations aiming to unravel the complex genetic basis of the epilepsies. Our research starts with the contributions of all the individuals and families who are enrolled in our study. We collect detailed clinical information from individuals with epilepsy about their seizure types and obtain DNA samples for genetic analysis. The information provided by our research participants is critical to improving our understanding of the genetic causes of epilepsy.

In August we held the silver anniversary (25th) of our very successful Annual Epilepsy Research Retreat in Ballarat, Victoria. What could be more enjoyable than 3 days just discussing epilepsy? This workshop is an opportunity for our team and close collaborators to present and discuss recent research in the epilepsies and brainstorm new research ideas together.

Highlights included recent advances in our understanding of the genetics of the developmental and epileptic encephalopathies and the focal epilepsies. We also discussed new brain imaging technologies such as functional MRI performed by our imaging collaborators, and laboratory bench science studying different forms of epilepsy in models such as stem cells. We were fortunate to have two eminent international researchers participate in the retreat, Professor Jean Gotman from the Montreal Neurological Institute, Canada, and Professor Heather Mefford, our long time genetic collaborator from the University of Washington in Seattle, US.

During the past year we have welcomed several new members to our team. Dr Sylvie Picker-Minh, a paediatric neurologist from Germany, has joined us to collaborate on clinical research. Dr Haloom Rafehi, a bioinformatician who analyses the complex genetic sequences that we receive on individuals with epilepsy, is working closely with our colleague Professor Melanie Bahlo at the Walter and Eliza Hall Institute. Dr Marie Inder is assisting with grants, ethics and academic administration to keep our research program running smoothly, and Natasha Crawford has joined as an administrative assistant. We have also welcomed four new clinical research assistants to our Melbourne team – Fiona Gardiner, Rebekah Harris, Stephanie Leech and Sophie Russ-Hall. In our genetics laboratory, Josh Reid has joined as a technical assistant, so that Tim Green could do his Honours thesis. We were also delighted to have Associate Professor Annapurna Poduri, the Director of the Epilepsy Genetics Program at the Boston Children's Hospital, join us for a four month sabbatical.

25th Annual Epilepsy Research Retreat, 2019, held in Ballarat, Victoria

EPILEPSY RESEARCH CENTRE
Melbourne, Australia

EPILEPSY RESEARCH CENTRE
Austin Health
Melbourne Brain Centre
245 Burgundy Street
Heidelberg, Victoria, 3084
P: +61 3 9035 7344/7330
F: +61 3 9496 2291
E: epilepsy-austin@unimelb.edu.au
W: www.epilepsyresearch.org.au

Want to know more?
Watch the talks on Epileptic Encephalopathies at
www.genes4epilepsy.org

COVID-19

We hope you are all keeping well and managing in this time of uncertainty. We would like to assure you that although we are not physically in our offices, we continue to work hard to understand the genetic basis of epilepsy.

Most of our research is continuing with our team members working remotely, however some activities such as collection of samples and laboratory-based work performing genetic testing has been delayed.

Parental mosaicism in the developmental and epileptic encephalopathies

Developmental and epileptic encephalopathies are a large group of rare, severe epilepsies where patients typically have many different types of seizures that are difficult to control, together with slowing of their development and often loss of skills. Their EEG (brainwave tracing) has frequent epileptic activity. They often begin in infancy or childhood.

The causes of developmental and epileptic encephalopathies were thought to be acquired as recently as the year 2000. Now, we know the vast majority have a genetic cause and we identify a genetic abnormality (mutation) in about 50% of patients. Most patients have a dominant mutation which is where just one change in the genetic code of the DNA causes the patient's disorder. These dominant mutations usually arise newly in the patient, and are not inherited from either parent. The child's parents are told they have a low chance of having a second child with the same disorder, and doctors quote a very low recurrence risk of around 1%. Sadly, sometimes parents have a second affected child despite being given a low recurrence risk. When this occurs, it is clear that one of the unaffected parents must carry the mutation.

So, together with Professor Heather Mefford's group at the University of Washington in Seattle, we asked whether parents might carry very low levels of the mutation, such that they were normal themselves, but at risk of having more children with a severe developmental and epileptic encephalopathy. This means that the parent would be mosaic for their child's genetic change, meaning the mutation was present in just a small number of their cells and most of their cells were normal.

Our study, published in the *New England Journal of Medicine,* discovered that 8% of patients had a parent who was mosaic for their mutation, and therefore at increased risk of having a second affected child. The levels of mosaicism ranged from 1% to 30% of cells. Most of these parents did not have seizures themselves, however, a few had very mild seizures, such as febrile seizures as a young child. So if a parent has had seizures, they have an increased chance of mosaicism and should be carefully tested *before* they have a second affected child. These results provide critical reproductive information for families of patients with developmental and epileptic encephalopathies. We hope that careful testing for mosaicism will become routine in genetic clinics with time.

Defining SYNGAP1 Developmental and Epileptic Encephalopathy

In 2013 in *Nature Genetics*, we identified that mutations in *SYNGAP1* caused developmental and epileptic encephalopathies (DEEs) in 5 patients. Many patients have been subsequently diagnosed with *SYNGAP1* developmental and epileptic encephalopathy. Inspired by a wonderful family with two affected daughters and their interaction and support of other *SYNGAP1* families through Facebook, we spearheaded a large international study describing the clinical spectrum of presentations of patients with *SYNGAP1* mutations. This work was led by two students, Danique Vlaskamp, a visiting PhD and medical student from the Netherlands, and an Australian medical student, Ben Shaw.

We described 57 patients with *SYNGAP1*-DEE from many countries. They had predominantly generalised seizure types with the most common being absence seizures with eyelid myoclonia, or eyelid fluttering, which occurred in a third of patients. We identified a new seizure type in about a fifth of patients where absence seizures with eyelid myoclonia would lead to a drop attack, where the child fell to the ground often sustaining nasty injuries. About a quarter of cases had seizures triggered by eating which is a remarkable type of reflex seizure. They also had a high pain threshold and would not complain of pain with fractured bones or surgical incisions.

SYNGAP1 is a protein that plays an important role in nerve synapses, where two nerve cells interact with each other, and nerve development. It is important for our learning and memory and gene mutations were first recognized as a cause of intellectual disability. By delineating the characteristics of *SYNGAP1*-DEE, we hope that the epilepsy will be more readily diagnosed and appropriately treated, with the aim of improving long term outcome for these individuals.

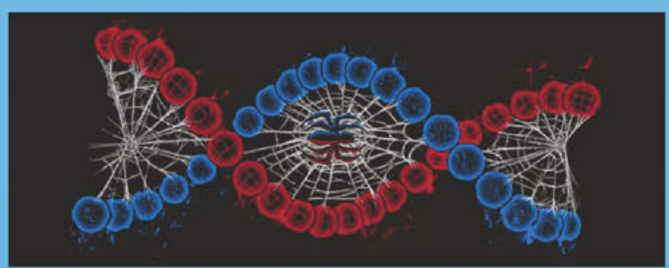
Dravet syndrome: poison exons and spider toxin

Dravet syndrome is a severe DEE in which normally developing babies begin having seizures at around 6 months of age and develop uncontrolled seizures and intellectual disability. More than 80% of patients have a causative mutation in the *SCN1A* gene, which encodes a sodium channel gateway into the cell.

Genes are made up of coding and non-coding regions of DNA (genetic material). The coding regions (exons) make up around 1-2% of our entire genetic sequence and encode the proteins that make up our body. Most diseases are caused by changes in these coding regions and so genetic testing focuses on these areas. However, together with our collaborators in Seattle and Chicago, we identified a new form of mutation, in a hidden tiny coding region (exon) in the non-coding region of *SCN1A* and found that these mutations cause Dravet syndrome. Usually, the non-coding regions of DNA are removed by the cell so that a functional protein can be made. However, we found that sometimes a mutation means that a piece of this non-coding DNA isn't removed properly, resulting in a "poison exon" and a protein that cannot function. This found an answer for the cause of the Dravet syndrome in four families, and was published in the *American Journal of Human Genetics*.

Researchers are now using this information to see if they can manipulate this part of the SCN1A protein to treat Dravet syndrome with gene therapy.

Another study into potential treatments for Dravet syndrome involves the use of spider venom, which is made up of many different compounds that can affect brain cells (neurons). Our colleagues at the Florey Institute found a type of spider venom that works specifically on SCN1A. They tested this spider venom on mice with a genetic mutation in *Scn1a*, the mouse version of the human *SCN1A* gene, and Dravet syndrome. These mice have seizures, abnormal EEGs and die prematurely. When given the spider venom, these mice had fewer seizures, improvement in their EEG, and they lived longer. The next step will be to see if this spider venom could be used to treat people with Dravet syndrome.



Size matters – The Epi25 Collaborative

The Epilepsy Research Centre is a major contributor to Epi25, which is a landmark study and the largest international genetics study of epilepsy ever performed. The Epi25 Collaborative is a partnership between the Broad Institute, a genomics research facility at Harvard University in the USA, and over 60 epilepsy research groups from around the world, spanning five continents. Epilepsy genetics research is at an exciting stage where it is now possible, with the power of a very large group of patients, to understand the more complex genetic components of epilepsy. This scale of research is only possible by working together.

Epi25 is analysing DNA samples from more than 25,000 patients with epilepsy. The samples undergo whole exome sequencing (WES) at the Broad Institute. WES involves sequencing the 1-2% of our genome (DNA code) that is the code for making the proteins that make up our body.

By analysing this information, and comparing it with WES information from people who do not have epilepsy, we will identify new genes associated with epilepsy. We have contributed more than 1,500 DNA samples from patients with genetic generalized epilepsies, focal epilepsies and developmental and epileptic encephalopathies. We also are the centre that coordinates the collection of key clinical information on each patient from all 60 contributing research groups so we can interpret the genetic results.

We hope that by understanding the genetic basis of the epilepsies, we will understand why each person has epilepsy. This is fundamental in working towards 'precision medicine' - where the biological basis of each patient's epilepsy leads to a targeted treatment.

For further information

Please do not hesitate to contact us at any time if you have questions about our research. Thank you again for your participation and support.

In order to assist us with keeping in touch with you, if you change your address we would be very grateful if you could advise us of your new contact details (see attached sheet). If you do not wish to receive future editions of this newsletter, please fill in the check box on the attached contact sheet along with your name and address and return it to us, or email us at epilepsy-austin@unimelb.edu.au.

We continue to be at the forefront of Epilepsy Genetics research. We have two websites that may be of interest. ***www.epilepsyresearch.org.au*** *provides a range of information about the Epilepsy Research Centre, our research projects and also information for epilepsy patients interested in seeking treatment through the Comprehensive Epilepsy Program at Austin Health. Past issues of our newsletters and links to other useful sites can also be found.*

Our new website ***www.genes4epilepsy.org*** *has information about developmental and epileptic encephalopathies. This includes the talks from our last genetic epilepsies conference where there are many practical tips for looking after children or adults with these diseases. We are always keen to recruit new patients if they are interested.*

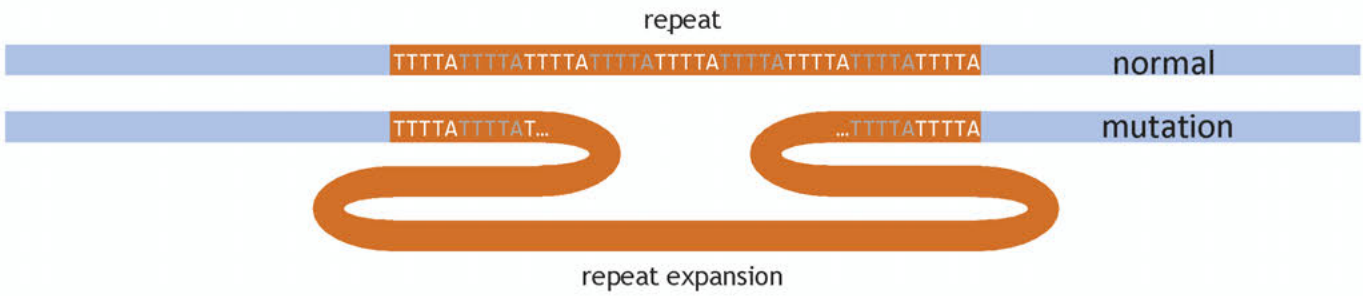
Familial Adult Myoclonic Epilepsy (FAME) and Repeat Expansions

Familial adult myoclonic epilepsy (FAME) is a rare, and usually mild, type of genetic generalised epilepsy. Individuals in FAME families develop a prominent tremor in adolescence or early adulthood, and some also have generalised tonic-clonic seizures in adult life. In 2012, we reported a very large New Zealand and Australian family with FAME which included 55 affected family members over six generations. We have collaborated with many international groups in the search for the elusive genetic cause of FAME over many years. Just recently, we have been involved in finding the cause – a very unusual type of genetic mutation called a 'repeat expansion'.

Repeat expansions are a type of genetic variation, that have proven extremely challenging to detect. They occur as 'normal' repeated sequences of the genetic code of our DNA but, in disease, the number of copies of the repeated sequence increases to a much larger number than normal. Repeat expansions cause a range of neurological disorders, such as Huntington's disease, fragile X syndrome and ataxia (difficulties with balance).

In FAME, there are large expansions of two different repeat sequences of five bases, "TTTTA" and "TTTCA". Interestingly, the "TTTTA" and "TTTCA" repeat expansions occur in different genes in different families with FAME, suggesting that the actual gene is not important but, rather, the repeat expansion itself causes the epilepsy.

Using a new strategy for analysing DNA sequencing data, developed with researchers at The Walter and Eliza Hall Institute and Murdoch Children's Research Institute, we are able to search for repeat expansions that previously required specialised laboratory tests to detect. Together with colleagues at the University of Adelaide, these new search tools have enabled us to identify repeat expansions and provide genetic diagnoses for many individuals with FAME and understand its molecular basis. Recently, we joined forces with international collaborators in writing two major papers in *Nature Communications* on repeat expansions causing FAME.



Ethical Considerations

The conduct of our research is over-seen by Human Research Ethics Committees. Study participants are asked to allow the indefinite use of their DNA sample for our research. People who were enrolled as children are asked to give their own consent when they reach 18 years of age provided we are able to contact them. If you have any concerns about this, please contact us so we can discuss this with you. If you have recently turned 18 and have not heard from us, please complete the change of address form or email us at epilepsy-austin@unimelb.edu.au to check we have your current details. Participants are free to withdraw from the study at any time.

If we obtain a positive result on your sample or in your family, we will send you a letter stating that we have obtained a result. If you would like further information about this, we will be happy to provide it after you contact us to discuss the result.

All information collected for our research is strictly confidential and is not used for any purpose other than for research to understand epilepsy and related conditions. In particular we do not share any of your information with other members of your family, including any results. Information will be shared with parents of children in the study if they are under 18 years of age. Some information may be shared with collaborating scientists to identify or better understand epilepsy genes.

Donations

We are always in need of support to take our research forward. Donations can be made via credit card, direct bank transfer and cheque.

- Credit card donations can be made online at http://alumni.online.unimelb.edu.au/epilepsyresearch
- **Cheques** should be made payable to the ***University of Melbourne.***
- Complete your contact details below and return this slip with your cheque to:

  Epilepsy Research Centre, Lvl 2 Melbourne Brain Centre
  245 Burgundy St, Heidelberg VIC 3084
- For details on **direct bank transfer**, please contact Natasha Crawford on 9035 7230 or epilepsy-austin@unimelb.edu.au.

We greatly appreciate all the assistance we receive from our supporters.

Please find enclosed a cheque for my tax-deductible donation of $ .....

Name ..... Phone .....

Address .....

Update from New Zealand

Our team based at the University of Otago, Wellington, continues to study children and families with epilepsy from around New Zealand, with more than 2000 participants recruited to date. We have welcomed two new PhD students, pharmacologist Shayma Ali and geneticist Dr Gemma Poke, whose projects work 'across the ditch'.

The New Zealand team has received fantastic support from Cure Kids, a New Zealand charity that funds vital medical research for diseases that affect children. To celebrate Chinese New Year, the Rotary Club of Auckland Harbourside Inc hosted a charity gala ball to fundraise for Cure Kids and our group. More than 450 people attended the night filled with authentic Chinese cuisine and exciting performances, including a traditional Chinese Lion dance. The proceeds from the evening will support our team with a focus on identifying new genes for developmental and epileptic encephalopathies. We thank all participants, referrers and funders for their support.

Genome-Wide Association Study discovers 11 new epilepsy genes

In contrast to whole genome sequencing (WES), which looks for rare genetic variation, another approach to solving the genetics of the epilepsies focuses on common variation. A genome-wide association study (GWAS) searches the genome (all the genetic code) for small common genetic variations, called single nucleotide polymorphisms or SNPs (pronounced "snips"), that occur more frequently in people with a particular disease (cases) than in people without the disease (controls).

The largest and most powerful genome-wide association study (GWAS) of epilepsy was undertaken by more than 150 researchers from multiple centres in Australia, the UK, Europe, USA, Brazil, and Hong Kong. The DNA of more than 15,000 people with epilepsy was compared to the DNA of 30,000 healthy controls. As a result, the number of known genetic associations for epilepsy was tripled and importantly 11 new genes were implicated and published in *Nature Communications.*

These genes encode proteins with many different functions in the human body, including regulating signals between brain cells and controlling expression of proteins in the brain. Interestingly, these proteins are the target of most of our current anti-epileptic drugs. We also identified an additional 166 drugs that target these proteins and are therefore promising new candidates for epilepsy therapy.

Plans for the next even larger GWAS are underway and we expect to double the number of patients with epilepsy to ~30,000. This will greatly increase our power to find more epilepsy genes. This work is led by Professor Sam Berkovic in his role as Chair of the International League Against Epilepsy Consortium on Complex Epilepsies and has been a wonderful example of all corners of the world working together to improve the lives of people with epilepsy.

Genetic Epilepsy Team Australia (GETA) Conference 2019

GETA is a group of amazing Australian parents whose children have rare, severe genetic epilepsies. Despite their children having different genetic diseases, they have banded together to support each other and promote research. On 30 May 2020, they held their fourth annual conference online, showcasing ground-breaking research. Their conferences can be watched online at your leisure. If you would like to learn more about GETA or join the group, please see this link: https://www.geneticepilepsyteam.com.au/

GETA: GET A Team, GET A Target, GET A Cure

The 2019 annual Genetic Epilepsy Team Australia Conference attendees, held in Parkville, Melbourne

Our team

Prof Sam Berkovic
03 9035 7330

Prof Ingrid Scheffer
03 9035 7344

Dr Saul Mullen
03 9035 7137

Dr Sylvie Picker-Minh
03 9035 7084

Amy Schneider
03 9035 7349

Brigid Regan
03 9035 7012

Fiona Gardiner
03 9035 7331

Rebekah Harris
03 9035 7336

Stephanie Leech
03 9035 4847

Sophie Russ-Hall
03 9035 7299

Bronwyn Grinton
03 9035 7013

Kate Esnault
03 9035 7355

Dr Marie Inder
03 9035 7228

Natasha Crawford
03 9035 7230

Lisa Johnson
03 9035 7330
PA: Sam Berkovic

Natalie Bryant
03 9035 7344
EA: Ingrid Scheffer

A/Prof Michael Hildebrand
03 9035 7330

Karen Oliver

Dr Mark Bennett

Dr Haloom Rafehi

John Damiano

Tim Green

Zimeng Ye

Josh Reid

Prof Lynette Sadleir
+64 4 918 6138
ext 6138

Dr Guillem de Valles Ibanez
+64 4 918 6138

Chontelle King
+64 4 918 6147

Shayma Ali
+64 4 918 6553

Gemma Poke
+64 4 385 5999
ext 5180

Meghan Evans
+64 4 918 6166

Thank you

We would like to thank everyone who has contributed to our research by participating in the research studies, referring patients and families, or making donations to support our research. We have been especially delighted when the families who have participated in our studies have sent donations. This reinforces that our families, as well as the researchers, value our work.