EPILEPSY RESEARCH CENTRE NEWSLETTER 2018

Lessons learned from studying the genetics of the common epilepsies

Clinical genetic testing for focal epilepsy

SLC1A2 and *CACNA1A* are important causes of severe epilepsies

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

We are delighted to bring you the latest on our research into the epilepsies at the Epilepsy Research Centre, University of Melbourne, Austin Health. It has been a very exciting year. We have been involved in leading the first new classification of the epilepsies in 28 years for the major international epilepsy body, the International League Against Epilepsy. This classification system affects the way epilepsy is diagnosed in the clinic for every patient seen around the world. The two major papers describing (1) the framework of epilepsy classification and (2) the new terminology for seizure types can be downloaded from the ILAE website: www.ilae.org/guidelines/definition-and-classification.

In terms of genetic research, we have been leading participants in two large international collaborations aiming to unravel the genetics of the epilepsies, including the severe group of epilepsies called developmental and epileptic encephalopathies. We could not be involved in these world-leading studies without the assistance of all the individuals and families enrolled in our research. We collect detailed clinical, EEG and imaging information from individuals with epilepsy, and obtain DNA samples for genetic analysis from blood or saliva. The information provided is critical to improving our understanding of the genetic causes of epilepsy.

We held a very successful three-day Epilepsy Research Retreat at Marysville, Victoria. This annual event is an opportunity for our team and close collaborators to present and discuss recent research in the epilepsies and to brainstorm new research ideas together. Highlights included recent advances in our understanding of the genetics of the epileptic encephalopathies and a rare group called the progressive myoclonic epilepsies. We also discussed exciting new research from our colleagues using advanced magnetic resonance imaging (MRI) technologies and laboratory bench science in epilepsy model systems.

During the past year, we have welcomed new members to our team. Dr Mark Bennett, a bioinformatician who analyses the complex genetic sequence information that we receive on individuals with epilepsy, is working closely with our colleague Professor Melanie Bahlo at the Walter and Eliza Hall Institute. Dr Collin Ellis, an adult neurologist from the USA, has joined us in recent months. Five new clinical research assistants have joined the Melbourne team - Alix Macdonald, Katja Boysen, Olivia Henry, Allison Hinchcliffe and Anne Harbison. Bri Boljonis has joined our New Zealand team based in Wellington. In our genetics laboratory we have Amelia McGlade, currently on maternity leave with Matthew Coleman in her role, Kristin Rigbye as a new research assistant, Tim Green as technical assistant, and Zimeng Ye undertaking her PhD. Guillem de Valles Ibanez has come from Spain to take up a role as our bioinformatician in Wellington. Sadly, after 14 years, Jacinta McMahon who will be known to many families for her dedication to our Epileptic Encephalopathies study, has moved to a new position at St Vincent's Hospital. We thank her for her significant contribution. After two years, we also bade farewell to Dr Ken Myers, a paediatric epilepsy fellow from Canada, who has returned to take up a prestigious physician-scientist post in Montreal. We wish them all the best!

We would like to thank all our research participants (now more than 18,000 people!), the referring doctors and all other supporters for all their wonderful contributions to our research program.

The Epilepsy Research Centre and colleagues at the 2017 annual Epilepsy Research Retreat, held at Marysville, Victoria

Lessons learned from studying the genetics of the common epilepsies as part of the global epilepsy community

We have been major contributors to a large international collaboration called the "Epi4K Consortium". Researchers from around the world have come together to understand the genetic risk factors causing two of the most common epilepsies: familial genetic generalised epilepsies and familial or sporadic (no family history) focal epilepsies of unknown cause.

In January 2017, results from this large genetics study were published in the *Lancet Neurology* journal. By studying the genetic data from 1,827 individuals with epilepsy and comparing it to a collection of 3,877 individuals without epilepsy, several key findings were identified that help us to understand the "genetic architecture" of the different forms of epilepsy.

Somewhat surprisingly, the genes that cause rare, severe forms of epilepsy also play a role in causing milder, more common epilepsy syndromes. There are currently many studies underway seeking to find causes and treatments for the severe forms of epilepsy – this finding means that new treatments might also be applicable to less severe, common epilepsies.

Very rare variations in genes are contributing to these common forms of epilepsy. This means that the variants seen in one person or family with epilepsy are not likely to be the same variants seen in an unrelated person or family. This type of information helps us work out how to interpret the complex genetic information we obtain through our research testing when we are trying to identify new epilepsy genes.

In almost 10% of individuals with epilepsy who had a family history of focal epilepsy of unknown cause, one of five known epilepsy genes *(DEPDC5, LGI1, PCDH19, SCN1A* or *GRIN2A)* plays an important role in causing their epilepsy.

DEPDC5 is a key epilepsy gene that we identified with collaborators a few years ago. The Epi4K study confirmed that only *DEPDC5* abnormalities that stop the protein (gene product) from being made are relevant to epilepsy.

Following from the success of the Epi4K study, researchers and clinicians from around the world are coming together to generate genetic data from 25,000 patients; this is the Epi25 Collaborative Consortium, which will run from 2015-19.

Emily, a participant in our research studies, enjoys a walk along the beach

Epilepsy in Israel: a study of 211 families

Studying families with epilepsy led to our discovery of the first epilepsy gene in 1995. Twenty-two years later, family studies continue to be a successful strategy for gene discovery; the larger the family, the better! It is a strategy we have not only successfully implemented in Australia, but also in Israel. We did this to see if discoveries in our Australian families could be replicated in different ethnic groups and to determine if new genes could be found. Over a 12-year period, we studied 211 families from the ethnically diverse Israeli population with our Israeli and Palestinian collaborators.

We classified each family into one of six broad epilepsy groups: generalized, focal, fever-related, special, mixed and unclassified. The largest group comprised 61 families with generalized epilepsy; this finding is consistent with generalized epilepsy having the highest heritability amongst common epilepsy syndromes. To date, this has not yet translated to major gene discoveries in these families with the majority remaining unsolved, similar to findings in our population.

Overall 23% of the 211 families did have a gene discovered and, somewhat unexpectedly, just four genes accounted for half of all the solved families. The sodium-channel gene *SCN1A* accounted for the most findings, known to cause many different types of epilepsy, meaning this is arguably the most important epilepsy gene. This was followed by *SLC2A1* which encodes GLUT1, the protein responsible for transporting glucose across the blood-brain barrier.

Next was potassium-channel gene *KCNQ2*, which causes an inherited form of benign neonatal epilepsy. This is different to the severe epileptic encephalopathy phenotype that can also be caused by *KCNQ2*. *PRRT2*, the gene for benign familial infantile epilepsy, was the fourth most common gene. We discovered *PRRT2* in epilepsy with genetic scientists in 2012, and families from this Australian-Israeli collaboration contributed to our original gene discovery.

The magnitude and duration of this project allowed us to identify previously unrecognized clinical and genetic patterns and we expect this well-characterized cohort will continue to contribute to even more gene discoveries in the future.

Fatal brain swelling in Dravet syndrome

Dravet syndrome is a severe form of epilepsy in which normally developing babies begin having seizures at around 6 months of age and go on to develop uncontrolled seizures and intellectual disability. Sadly, 17% of children with Dravet syndrome will die before their 20th birthday and little is known about what can be done to prevent their early death.

Following the untimely death of Molly with marked brain swelling, we reviewed all of our patients with Dravet syndrome who had died and found that five children had come to hospital having prolonged seizures with fever, then developed extremely severe brain swelling (oedema) that led to their death. This unusual cause of death has been published in the journal *Pediatrics* in 2017. We are now working on ways to identify the swelling much earlier when children come to hospital, so that it can be treated, potentially saving lives.

A Pathway Brain Disease

Hypothalamic hamartoma (HH) is a rare, benign brain tumour associated with drug-resistant epilepsy. Patients develop laughing attacks, known as gelastic seizures, often in the newborn period. If left untreated, this can evolve into a severe epileptic encephalopathy with refractory generalized seizures. Surgery to remove these tumours is the only effective treatment, leading to seizure freedom in over half the patients. Little is known about the genes causing HH tumours.

Our study, published in the leading journal *American Journal of Human Genetics*, involved a comprehensive search for genetic abnormalities found in the tumour but not in the blood (known as somatic mutations) of 38 patients with hamartoma tumours. Nowadays, tumour tissue is often destroyed during surgery rather than removed, making it an especially precious and rare resource. Paired hamartoma tumour tissue and blood samples underwent whole exome sequencing (WES) analysis, where 1% of the genome is sequenced, followed by chromosomal microarray and targeted resequencing.

Somatic mutations were identified in 14 patients in genes involving regulation of the sonic-hedgehog (Shh) pathway. This included multiple loss-of-function mutations in the newly implicated Shh gene *PRKACA*, and known HH gene *GLI3*. A further 15 genes involved in the Shh pathway were identified, including several genes (e.g. *CREBBP*, *GLI2*, and *SMO*) which were implicated in HH due to their presence in large chromosomal abnormalities, of which some were found in tumour tissue from more than one patient.

Taken together, our data implicate disruption of the Shh pathway in at least 37% of patients with HH and gelastic seizures, supporting the idea that this disorder is a developmental 'pathway' brain disease. This phenomenon is reminiscent of the remarkable contribution of another pathway called mTOR, in which mutations involving many genes lead to malformations of cortical development.

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 alumni.unimelb.edu.au/epilepsyresearch
- **Cheques** should be made payable to the ***University of Melbourne*** **with a note advising that your donation is directed to us at the Epilepsy Research Centre.**
- 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 Bronwyn Grinton on 9035 7013 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 .....

SLC1A2 and *CACNA1A* are important causes of severe epilepsies called developmental and epileptic encephalopathies

This year, together with Dr Heather Mefford's group at the University of Washington in Seattle, we discovered that the genes *SLC1A2*, *CACNA1A* and *GABRB3* are important causes of the severe epilepsies beginning in infancy and childhood called the developmental and epileptic encephalopathies. These patients often have many different types of seizures that are difficult to control, can slow their development or lead to loss of skills, together with frequent epileptic activity on their EEG (brainwave tracing).

As part of Epi4K and other studies, numerous genes have been identified as potentially causing these encephalopathies, but more proof was needed to confirm which were truly causative. We tested some of these genes in patients in our study with many different types of developmental and epileptic encephalopathy. We found a genetic cause for 17 participants across 7 different genes: *SLC1A2, CACNA1A, GABRB3, ALG13, DNM1, GNAO1,* and *IQSEC2*.

These 7 genes have various functions in the human body and demonstrate how diverse the causes of these severe diseases are. We tested participants with the same epilepsy syndromes as in the Epi4K study but also tested participants with different syndromes, and still found the same genetic causes. This is important for researchers as it suggests they should look beyond a specific syndrome when trying to understand the importance of a gene and that each gene has a spectrum of presentations.

We also found that in two families where siblings shared the same disorder, their mutations had been passed down from parents who were unaffected, providing critical reproductive information for families.

By uncovering the genes that cause developmental and epileptic encephalopathies and describing the clinical details of their disorders, we hope to enable earlier diagnosis and assist in the management of children and adults with these diseases.

The 5th Australian and New Zealand Dravet and Genetic Epileptic Encephalopathies Family Conference 2017. Attended by over 300 people in person and online. Videos of the talks are available to view at www.genes4epilepsy.org

Ethical Considerations

The conduct of our research is overseen 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 study 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 it, 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.

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. Our website, ***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. If you would like to contact us with any specific queries about our research, please do so via email at* ***epilepsy-austin@unimelb.edu.au****.*

Update from our New Zealand team

In 2016 our Epilepsy Research Group at the University of Otago, Wellington, were delighted to host three All Blacks--Dane Cross, Damian McKenzie and Aaron Smith--and a group of rugby-mad children with epilepsy. While fun was on the agenda, this was a great opportunity to shine a light on epilepsy research in NZ through Kiwis' love of the All Blacks. This occasion was organised by Cure Kids, a New Zealand-based charity that generously funds vital medical research for diseases that affect children.

Our NZ team are fortunate to receive funding support from Cure Kids and The Ted and Mollie Carr Endowment Trust & Estate of Ernest Hyam Davis. This funding has allowed us to expand, with an additional research assistant to focus on involving individuals from Auckland in our epilepsy genetic studies. We have also appointed a postdoctoral fellow in bioinformatics who is analysing the vast amount of genetic data generated from our patients through specific projects. We also received a 3-year project grant from the Health Research Council of New Zealand in 2015. This funding supports our whole genome sequencing to find genes in families in which multiple people have epilepsy, as well as children with developmental and epileptic encephalopathies. We thank all NZ participants, referrers and funders for their support of this important epilepsy genetics research program.

Sudden Unexpected Death in Epilepsy (SUDEP)

Rarely and tragically, some people with epilepsy die unexpectedly. This is known as Sudden Unexpected Death in Epilepsy (SUDEP). The causes of SUDEP are not well understood. Recent studies have suggested that a problem with breathing immediately after a seizure or a problem with regulating the beating of the heart, also known as cardiac arrhythmia, immediately after a seizure may lead to SUDEP. Together with cardiologist, Prof Chris Semsarian, and geneticist, Dr Richard Bagnall from the Centenary Institute in Sydney, we are conducting an ongoing study of SUDEP and looking for factors that might increase a person's chance of dying of SUDEP.

One part of this study was recently published in the prestigious journal, *Annals of Neurology*. We analyzed DNA samples from 61 patients who had died of SUDEP to search for changes in their genes that might have contributed to their death. Whole exome sequencing (WES) involves sequencing 1% of all the genes, the part that codes for proteins in the body. We performed WES on the samples and analysed genes responsible for respiratory control, known to cause cardiac arrhythmia or epilepsy.

We found a proportion of patients with changes in cardiac arrhythmia genes and some with changes in known epilepsy genes. Some of the cardiac arrhythmia gene changes were significant and thought to contribute to the cause of death. The significance of other gene changes seen in our SUDEP cohort is difficult to interpret because our cohort is too small to draw meaningful conclusions. We are therefore working hard to set up collaborations with other scientists to add strength to our results.

This project forms part of our continuing studies investigating the causes of SUDEP. We hope to gain a better understanding of the causes of SUDEP, so that we can work towards prevention. If you are interested in learning more about this study, please contact our team at epilepsy-austin@unimelb.edu.au.

Clinical genetic testing for focal epilepsy

Focal epilepsies are common, occurring when seizures come from one part of the brain. In the past, they were thought to be caused by an acquired problem in the brain, such as a serious head injury, stroke or tumour. Our research has shown that focal epilepsy can also be genetic and we have discovered several genes that cause focal epilepsy.

Together with researchers from the Royal Melbourne Hospital, the Royal Children's Hospital, and the Melbourne Genomics Health Alliance, we asked whether genetic testing was of value in the routine care of adults and children with focal epilepsy in whom there was a clue to a genetic cause. The clue was that they had to have at least one second degree relative with epilepsy or febrile seizures. Of 40 people with focal epilepsy who had a normal brain MRI scan, we found that 5 (12.5%) had a genetic mutation causing focal epilepsy.

In one 22-year-old man whose seizures had not responded to several antiepileptic medications, finding the causative mutation in a sodium-channel gene called *SCN1A* led to a change in management. The genetic finding meant that the anti-epileptic drug carbamazepine was withdrawn, as it can worsen seizures in people with *SCN1A* epilepsies, a decision that led to complete seizure control.

In summary, genetic testing for focal epilepsy is clinically useful. It can provide an explanation for the occurrence of seizures in a person in whom standard investigations have failed to find a cause. It can also assist clinicians with providing better clinical care, including selecting the best treatment. This study was published in the journal *Epilepsy Research*.

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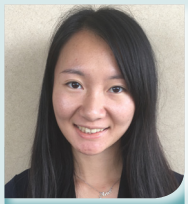

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Thank you

We would like to thank everyone who has contributed to our research by participating in our research studies, referring patients and families, or making donations to support our research. We have been especially thankful to the families who have participated in our studies and have made donations. This encourages and drives us to keep working hard to make a positive impact on the lives of our families.