# Progress Report

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Title of Project: **Use of resected epilepsy surgery tissue to identify genetic causes of cortical malformations**

*Summary: (approximately 1,000 words)*
We wish to thank the Brain Foundation for their generous support of this ongoing research project led by A/Professors Richard Leventer and Paul Lockhart which seeks to understand the cause of drug-resistant epilepsy in children caused by the brain malformations focal cortical dysplasia and tuberous sclerosis complex. The research is conducted with the close collaboration of the Royal Children's Hospital epilepsy surgery team led by Dr Simon Harvey and Miss Wirginia Maixner. At the Royal Children's Hospital, a child is newly diagnosed with a brain malformation on a weekly basis. The cause of brain malformations, how they develop and the best methods of treatment remain poorly understood. Epileptic seizures are commonly observed in children with brain malformations. In some cases, the seizures are not able to be controlled using medication. An effective treatment method in these cases involves removal of the malformed brain from where the seizures are coming. In late 2015 The Brain Foundation generously provided funding which would allow our research team to study the brain tissue that is removed during surgery using new and cutting edge genomic technologies. Using new genetic technologies such as whole exome sequencing and gene panels on these patient samples would provide an excellent opportunity to identify the alterations that occur in the genes of children with brain malformations.

Since the commencement of the funding we have been able to obtain biological samples from 30 patients undergoing epilepsy surgery through the Royal Children's Hospital Epilepsy Program. These are comprised of 25 patients with focal cortical dysplasia (both type I and type II) and five patients with tuberous sclerosis complex. From each patient we have obtained blood and brain tissue samples, and obtained consent for genetic research studies. For patients with tuberous sclerosis complex we have obtained specimens from the base of the lesion (tuber pit) as well as the side of the lesion (tuber rim) and surrounding normal tissue when available. The tissue samples have been fresh frozen at -80C at the time of surgery in the operating theatre and then taken to our laboratory for DNA extraction.

Our initial plan was to sequence these samples using whole exome sequencing. We have modified our plan somewhat in that we will now be using a customised gene

panel developed in house in our laboratory at the Murdoch Children's Research Institute. The gene panel will allow us to rapidly screen known and candidate genes for cortical dysplasia and tuberous sclerosis (MTOR pathway genes), rather than all 20,000 genes present in the human genome. We have used advanced bioinformatics analysis to identify 460 genes that are known or predicted to function in the MTOR and related brain development pathways. This includes all known genes associated with brain malformations to date. By using genomic technologies similar to whole exome sequencing, we will be able to use this gene panel to rapidly screen these genes. This approach has several advantages over standard whole exome sequencing, including being faster and being able to detect mosaic mutations with greater sensitivity. Mosaic mutations are mutations that are present in some cells of the body but not all. It has become evident recently that some brain malformations such as cortical dysplasia may result from low level mosaicism only in the affected brain tissue, and means other than standard whole exome sequencing are required for their detection. We have now validated the gene panel technology with a number of positive and negative controls and are ready to begin running through DNA samples extracted as part of the project funded by the Brain Foundation. The costs of using the gene panel approach as opposed to standard whole exome sequencing are similar, but we believe that we will be able to obtain a greater yield from the gene panel approach due to its ability to detect mosaic mutations from brain tissue with greater sensitivity than an exome, thus improving the chances of a positive outcome from the study. We anticipate that our aim of analysing 15 paired blood/fresh frozen brain samples and five paired normal brain/tuber samples will be completed by midway through 2017, and a final report to the Brain Foundation will be provided on project completion.

*What these research outcomes mean*

Findings from this study will be both clinically significant and significant to understanding the biological basis of brain malformations and therefore normal human brain development. Identification of the genetic basis of brain malformations will directly translate into improved clinical care by allowing clinicians to provide families with a more accurate understanding of their child's condition ("why did this happen"), the mechanism of the underlying cause of the malformation ("how did this happen) and the risk for future pregnancies ("will this happen again"). Ultimately it hoped that by understanding these epilepsy- causing malformations better we might be able to provide improved treatments in the form of more focussed surgical approaches and pharmaceutical interventions such as MTOR inhibitors.