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RACP Foundation Research Awards

FINAL REPORT

Project / Program Title		Travel to Neurospecialities Centre, Belgaum, Karnataka, India
Name		Dr Kishore Raj Kumar
Award Received		2015 Bushell Travelling Fellowship in Medicine or the Allied Sciences
Report Date		13 December 2015
Chief Investigator / Supervisor		Dr Kishore Raj Kumar
Administering Institution		Kolling Institute of Medical Research
Funding Period	Start Date:	1 January 2015
	Finish Date:	31 December 2015

PROJECT SUMMARY

This project funding allowed me to travel to Belgaum, India from the 30th of March till the 12th of April 2015. I met with our international collaborator, Dr GM Wali, a Movement Disorders Neurologist. I also met with other members of the research team including a Paediatric Neurologist, Associate Professor Mahesh Kamate. We reviewed families with severe, early onset forms of a condition known as hereditary spastic paraplegia (HSP). Many of the children were from consanguineous (inbred) families which are particularly suited to genome sequencing studies. I was also invited to give two presentations to doctors and medical students associated with the local medical school (KLE University). The presentations covered the genetics of Parkinson disease and the genetics of HSP. The visit was featured in several local and national newspapers including 'The Hindu' (http://m.thehindu.com/news/belagavi-neuro-centre-ro-studyinbreedingborne-diseases/article7070646.ece/)

We selected 10 families in total after reviewing the patients' clinical phenotype and family history. DNA samples were then sent back to Australia for whole genome sequencing at the Kinghorn Centre for Clinical Genomics. We successfully identified the genetic cause in 4 out of 9 families (sequencing is still pending for 1 remaining family). We identified two families with mutations in genes known to cause HSP (DDHD2 and CYP2U1). We also identified mutations in genes not previously linked with HSP. This includes mutations in GLB1, the gene causing a metabolic disorder known as GM1 gangliosidosis. We also found mutations in a PEX16 gene linked with Zellweger spectrum disorder. Zellweger spectrum results from defects in the assembly of a cellular structure called the peroxisome, and are therefore also called a 'peroxisomal disorder'.

There are several important conclusions that can be drawn from these studies. We have shown that whole genome sequencing is an effective tool for identifying a genetic diagnosis in HSP, since it provides good coverage of the entire genetic code (the genome). We hope to now use whole genome sequencing to identify the genetic cause of HSP in patients from Australia. We also demonstrated that HSP can overlap with metabolic disorders such as GM1 gangliosidosis

and Zellweger spectrum disorder. This provides us with important insights into the mechanisms underlying HSP. One of our scientists at the Kolling Institute, Dr Gautam Wali, will now perform basic laboratory studies to explore these mechanisms further. We hope to show that peroxisomes play an important role in HSP.

I would like to sincerely thank the donors for this funding opportunity, without which the project would not have been undertaken. It has allowed me to study families with severe, infantile forms of this condition which are rarely found in Australia. I was able to personally perform a clinical assessment on these patients, which was invaluable for interpreting the results of the genetic analysis. I was able to strengthen our relationships with important research collaborators. It has also been instrumental in developing my skills in genomics/bioinformatics. Furthermore, it has helped me establish my career as a clinician-scientist and early career researcher.

PROJECT AIMS / OBJECTIVES

CONFERENCE ABSTRACT

13th International Congress of Human Genetics in 2016 (ICHG2016), Defining Genetic Causes of Hereditary Spastic Paraplegia with Whole Genome Sequencing

Kishore R Kumar*, G.M. Wali*, Mahesh Kamate*, André E Minoche, Velimir Gayevskiy, Marcel E Dinger, Tony Roscioli, Carolyn M. Sue, Mark J Cowley *These authors contributed equally

Aim: The hereditary spastic paraplegias (HSPs) are a group of inherited disorders characterised by progressive lower limb weakness and spasticity. There is a high degree of genetic heterogeneity in HSP with over 50 causative genes identified. The optimal genetic testing strategy for HSP has yet to be resolved, but whole genome sequencing (WGS) may be particularly relevant to HSP. We sought to identify a genetic diagnosis in HSP families from India using WGS.

Methods: WGS was performed in 7 consanguineous and 2 nonconsanguineous families with pure and complex forms of HSP from Belgaum, India. We identified small variants using GATK HaplotypeCaller, copy number variants using CNVnator, and structural variants using LUMPY. Using Seave, our variant filtration platform, variants were initially filtered using a panel of known HSP genes, prevalence in healthy populations, functional impact, and the likely mode of inheritance.

Results: We identified likely pathogenic variants in 4 consanguineous families. This included a frameshift homozygous deletion in CYP2U1 (c.782_785delTCTG) in one family and a homozygous donor splice site variant in DDHD2 (c.1125+1G>T) in another family. In one pedigree we identified a novel in-frame homozygous deletion in PEX16 (c.995_997delTCT), a gene known to cause a peroxisomal disorder (Zellweger spectrum disorder). Another family was compound heterozygyous for a novel splice site variant (c.553-2A>G) and a known pathogenic mutation (p.R442Q) in GLB1, the gene associated with GM1 gangliosidosis. All variants were validated using Sanger, and ancillary investigations will be performed to confirm these diagnoses.

Conclusion: We identified a probable genetic cause in 4 out of 9 families, including genes implicated in metabolic disorders such as Zellweger spectrum disorder and GM1 gangliosidosis. Interestingly, no known HSP genes were affected by structural or copy number variants. This study suggests that WGS is a useful tool for diagnosing HSP.

PUBLICATIONS / PRESENTATIONS

The study has been submitted to The 13th International Congress of Human Genetics (Kyoto, Japan, 2016, see abstract below). We will also submit the findings to a high impact journal following conclusion of further laboratory studies.