



RACP Foundation Research Awards

FINAL REPORT

Project / Program Title		Molecular Pathogenesis of Inherited Kidney Disease
Name		Dr Amali Mallawaarachchi
Award Received		2018 Jacquot Research Entry Scholarships in Nephrology
Report Date		21 May 2019
Chief Investigator / Supervisor		Prof John Shine
Administering Institution		Garvan Institute of Medical Research
Funding Period	Start Date:	31 March 2018
	Finish Date:	31 March 2019

PROJECT SUMMARY

Autosomal Dominant Polycystic Kidney Disease (ADPKD) is a common genetic condition that affects about 1 in 1000 Australians. The disease causes cysts to form in the kidney, that eventually take over the normal kidney tissue and lead to kidney failure. Patients with kidney failure then require dialysis or a kidney transplant to survive. Even though ADPKD is so common, genetic testing for the condition is challenging. This is because the genes that are involved in ADPKD are difficult to sequence by standard laboratory techniques. In the initial part of my project, we have successfully trialled a new genetic sequencing technique, Whole Genome Sequencing (WGS), to diagnose ADPKD. We have demonstrated that WGS is highly effective in diagnosing ADPKD. The test allows all mutation types to be detected at once and allows broader analysis for patients who don't have an immediate diagnosis found. These findings have been translated into a new genetic test for ADPKD that has been established in Australia for the first time.

My project also investigated the reason why cysts develop in the kidney. It is theorised that the kidney cysts are caused when a person has two mistakes in their PKD1 or PKD2 gene in the kidney cell, one that they inherit and one mistake that is acquired in the kidney cell alone (called a somatic mutation). We performed experiments deeply sequencing kidney tissue to identify these acquired or somatic mutations. We found that some cells did have acquired mutations, but this requires further study to be certain. We are continuing to investigate this as understanding the reason cysts develop is a crucial step in developing an effective treatment.

PROJECT AIMS / OBJECTIVES

To validate WGS as a clinical test for ADPKD -

This has been achieved by sequencing a set of blinded samples via collaboration with Mayo Clinic. The manuscript related to these findings is currently under review at 'Kidney International'.

To investigate the efficacy of clinical WGS for the diagnosis of ADPKD This has been achieved by analysis of the first 85 samples to have gone through clinical WGS testing for ADPKD. The manuscript related to these findings is currently under review at 'Kidney International'.

To assess variant pathogenicity in ADPKD - This project has been completed through analysis of population and disease datasets. The findings of this study have been published in 'Genetics in Medicine'.

To demonstrate the two-hit hypothesis in ADPKD –

Tissue from affected ADPKD kidneys has been collected and deep sequencing was performed on this tissue. Somatic mutations were identified in a proportion of cells

SIGNIFICANCE AND OUTCOMES

Accessible and reliable genetic testing is valuable for optimal management of patients with polycystic kidney disease as this information can be used for clarifying diagnosis, family planning, living-related kidney donor selection and estimating prognosis. The initial findings of our project have been translated into the first accredited genetic test for ADPKD in Australia and the first WGS-based ADPKD test in the world. This test has improved access to genetic diagnosis for Australian families with ADPKD.

We analysed the data from the first year of diagnostic WGS testing for ADPKD. Eighty-five patients underwent testing and a diagnosis was made in over 70%. Importantly it was identified that there was a high diagnostic rate in patients with atypical clinical features of polycystic kidney disease, who would not have had a diagnosis made without genetic testing. This highlights the value that is added with genomic testing.

Our analysis of population and disease variant databases has improved our understanding of variant pathogenicity and penetrance in ADPKD. Better understanding of uncertain variants is crucial for improving genetic diagnosis. Our study showed that there were more disease-causing variants in the control population databases in ADPKD-related genes than would be expected based on our current estimates of disease prevalence. This suggests that AD PKO is more prevalent than previously understood or that there is more variability of disease-penetrance than previously thought.

The final aspect of the project was in investigating pathogenesis of cyst formation. Our study of somatic mutation in cysts identified somatic variants in a proportion of cysts. This promising finding requires further validation.

PUBLICATIONS / PRESENTATIONS

Publications:

Mallawaarachchi A, et al, 'Whole genome sequencing overcomes pseudogene homology to diagnose Autosomal Dominant Polycystic Kidney Disease', Eur J Hum Genet. 24:1581-1590 (2016).

Mallawaarachchi A, et al , 'Population data improves variant interpretation in Autosomal Dominant Polycystic Kidney Disease', Genetics in Medicine, 2018 Oct 29. doi: 10.1038/s41436-018-0324-x.

Mallawaarachchi A, et al, 'Clinical Whole Genome Sequencing enables diagnostic certainty in Polycystic Kidney Disease', Kidney International, 2019, under review.

Abstracts:

Mallawaarachchi A et al, 'Extended Analysis of an Australian cohort of Autosomal Dominant Polycystic Kidney Disease patients by WGS', Abstract, Nephrology 21, Suppl. 2:58-149 (2016)

Mallawaarachchi A, Hort Y, Cowley M et al, 'Whole Genome Sequencing as a molecular diagnostic method for Autosomal Dominant Polycystic Kidney Disease - overcoming the challenges of pseudogene homology and high GC content', Oral Presentation, Human Genetics Society of Australasia, Annual Scientific Meeting, 2016.

Mallawaarachchi AL Hort Y, Senum Set al, 'Validating Whole Genome Sequencing (WGS) as a Diagnostic Technique for Autosomal Dominant Polycystic Kidney Disease (ADPKD)', Poster Presentation, American Society of Nephrology, Annual Scientific Meeting, 2017.

Invited Presentations:

Diagnostic Genomics in ADPKD, 2018 KidGen Renal Genetics Symposium

Genetics of Polycystic Kidney Disease, 2017 Patient Education Seminar PKO Foundation of Australia

ACKNOWLEDGEMENTS

Mallawaarachchi A, et al, 'Population data improves variant interpretation in Autosomal Dominant Polycystic Kidney Disease', Genetics in Medicine, 2018, Oct 29. doi: 10.1038/s41436-018-0324-x.

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