Investigation of Cystic Kidney Disease Genes Using Whole Exome Sequence

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Aims

- To identify new genetic defects (mutations) that cause cystic kidney diseases and related ciliopathies within consanguineous families
- Improve diagnostic tests, which will help in delivering the right treatment.
- Expand the knowledge about genetic kidney disease.

Introduction

Nephronophthisis (NPHP) and associated ciliopathies are autosomal recessive inherited kidney diseases (Figure 1) that lead to kidney failure in children and adults [1].
Mutations in a number of different genes are known to cause the disease. In this project, a number of affected families' genes were investigated in order to identify the unknown mutation that underlies the kidney disease.
Different in-silico (computer) and laboratory techniques were used to investigate and confirm the mutations in these families.

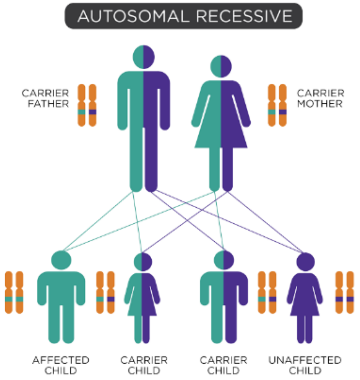

Figure 1: Autosomal recessive inheritance is when two copies are required for the disease to occur; one faulty copy from each parent is required for the disease [2]

Methods & Results

Following consent, DNA samples were collected from patients and then were sequenced using "Whole Exome Sequence (WES)".
The data from the patients and their families were then investigated using in-silico techniques, including using "Ingenuity Variant Analysis". The variants in the patient were then compared with the rest of the family in order to confirm the disease causing mutation.
A number of variants were selected for further investigations and confirmation by Sanger sequencing and segregation analysis. Polymerase Chain Reaction (PCR) then DNA purification were carried in order to prepare samples for Sanger sequencing.
The gene mutations which were investigated are shown in Table 1.

Gene variant	Chrom.	Region	Family	Normal gene function	Mutation type
ZNF785	16	Exonic	JS15	Involved in gene expression and might be involved in transcriptional regulation	Frameshift
PRPF40B	12	3'UTR,Exonic	JS15	Encodes a WW-domain containing protein	Missense
SHPRH	6	Exonic	JS15	Ubiquitously expressed protein that contains motifs characteristics of several DNA repair proteins, transcription factors, and helicases	Missense
WDR19	4	Exonic	F3	Encodes a member of the WD repeat protein family.	Missense

Table 1: Shows some of genetic variants that were investigated, in two families (JS15 and F3) , the predicted gene function [3], and the type of sequence variant.

Discussion

The in-silico analysis that was carried helped to decide which genetic variants are most likely to be disease causing by determining the read depth, how common and dangerous the variant/ mutation was. All the chosen mutations had high read depth and a SIFT score that was between (0.05-0.00) (high damaging probability). In addition, all of them were in exonic regions. These are more likely to cause a disease as they affect the proteins produced and are easier to be confirmed later.
Family F3 was genetically "solved" by the identification of a mutation found in WDR19, a known cause of NPHP. For the JS15 family, all the variants that were investigated were not proven to be disease causing (lack of segregation or spurious WES calling).
Further sequencing of additional variants in JS15 is underway to understand the underlying genetic change in this family and how this leads to kidney disease.

Conclusions

- In family F3 the mutation in WDR19 was confirmed to cause NPHP. In family JS15 variants in PRPF40B, SHPRH and ZNF785 are proven to be spurious.
- Understanding how mutations in WDR19 play a role in kidney disease, will allow new mechanism of disease to be identified.
- Novel causes of NPHP remain to be found in family JS15.

References:

1 NEPHRONOPHTHISIS 1 [Internet].; [cited 22nd October 2015]. Available from: http://www.omim.org/entry/256100
2. Understanding the genetics of CSID [Internet].; [cited 22nd October 2015]. Available from: http://csidcares.org/about/genetics/
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