Summary of second year report

Identification and functional characterization of the missing ABCA4 variants in Stargardt disease

Principal Investigator: Prof Frans Cremers
Research Institution: Radboud UMC, The Netherlands
Start date and duration: July 2017, three years


Prof Cremers

Background and aims of the project

Mutations in the *ABCA4* gene are a frequent cause of inherited retinal dystrophy and affect the majority of people with Stargardt disease (STGD1). In general, Stargardt disease follows a recessive inheritance pattern, meaning that both of an individual's copies of the *ABCA4* gene need to be faulty for the condition to develop. However, in a significant number of STGD1 cases, one or even both copies of the *ABCA4* gene don't contain any mutations in the sections that code for the building block 'ingredients' of the ABCA4 protein. New therapies currently in development for STGD1 may not be appropriate in these cases.

The vast majority of the *ABCA4* gene actually consists of non-coding regions, known as introns. These sections are 'edited out' during protein construction by a process known as splicing. However, mutations within introns can still have a significant influence on how the coding regions are interpreted by the cell's protein-building machinery, often resulting in a faulty protein.

Prof Cremers' project aims to develop a cost effective method of sequencing the entire *ABCA4* gene, including the introns, and use this to look for intronic mutations in the genetic material from 1,000 worldwide STGD1 cases which have either no or only one mutation in the protein coding sections of *ABCA4*. His team will also complete development of a test to establish how intron mutations impact on the editing of the *ABCA4* gene and use other new tests to investigate how the intronic variations they find affect the editing process.

The development of improved methods to identify the functional effects of non-coding mutations would also improve the identification of such variants in other human IRD genes.

Progress to date

Prof Cremers has gathered around 1,000 STGD1 genetic samples from twenty international collaborators. These all came from cases where the disease could not be explained by mutations in the protein-coding regions of *ABCA4*.

Prof Cremers' team developed an ultra-cheap, but nonetheless high quality, method for examining the sequence of the entire *ABCA4* gene, including all of the introns, which costs around ten times less than other techniques (around €40 per sample). Their method employs up to 4,000 small fragments of DNA, known as smMIPs, which seek out and bind to regions of interest in the gene.

The researchers have now used the smMIPs to examine the entire *ABCA4* sequence of 1,054 samples. Prof Cremers has also completed design of a test, known as a splice assay, to investigate how *ABCA4* intron variations influence the editing of the genetic code during protein construction.

By employing these two techniques, the team has found fourteen known and thirteen new disease-causing variants deep within the non-coding sections of ABCA4 in 117 samples. They constitute 11% of all tested cases and 26% of STGD1 cases with two casual *ABCA4* variants. This work has contributed to two publications, with a further large paper currently being written.

The development of the splice assay itself has led to a high impact paper in the journal Genome Research. This method was then used to test many 'hidden mutations' that cause the aberrant inclusion of an extra section of code, known as a pseudoexon. They interrupt translation into correct *ABCA4* protein. These results were gathered in collaboration with Dr. Gavin Arno and Prof. Andrew Webster (London) and led to a paper in Genetics in Medicine. For a few mutations, pseudoexons could not be shown using their standard test system but could be identified in retinal-like cells that were generated from STGD1 patient-derived stem cells. Using a molecular "patch", known as an antisense oligonucleotide, the researchers were able to block the occurrence of these pseudoexons in the stem cell-based disease model, providing promise that this could be employed in future approaches to treatment.

Prof Cremers intends to make the smMIPs available to other research and diagnostic groups and train them in their use, so that others can benefit from this development. In addition, he accepts unlimited additional STGD1 samples from all over the world for free genetic testing in a research context in the next two years. His group plans a new project to use the same technology to sequence 75 other genes for mutations in thousands of STGD-like and other maculopathy cases.

This is a summary of the second year project report submitted to Retina UK by Professor Frans Cremers, July 2019.

Report prepared by:
Kate Arkell, Research Development Manager
kate.arkell@RetinaUK.org.uk, 01280 821334