Report for Save Sight Society New Zealand April 2015

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Characterisation of the genetic basis of recurrent corneal erosion corneal dystrophy using zebrafish model.

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Corneal dystrophies are a genetically heterogeneous group of disorders. A New Zealand (NZ) family with a unique autosomal dominant recurrent corneal erosion dystrophy is previously described (Vincent et al, Mol. Vis., 2009). We aimed to identify the underlying genetic cause in this family and to characterise the disease mechanism utilising zebrafish.

20 members of a 3-generation NZ family were phenotyped and DNA samples collected. Candidate corneal dystrophy genes were excluded as published previously. Genome-wide scanning using a SNP microarray and subsequent exome sequencing of two affected individuals was undertaken. Bioinformatic analysis, protein prediction, and splice site software were used to identify putative pathogenic variants within the linkage region. Known genetic variants were excluded using public databases of human variation, and changes validated with Sanger sequencing, as well as 3 additional families with a similar phenotype (NZ, Tasmania and UK). Expression was analysed in human cornea with immunohistochemistry (IHC) and RT-PCR, and in zebrafish with whole-mount *in situ* hybridisation. Morpholinos were used to transiently knockdown gene expression during zebrafish development, and CRISPR-Cas9 is being utilised to both create knockout lines and to introduce identified genetic variants.

A genome-wide scan of the NZ family identified only one significant peak, on chromosome 10. Exome sequencing and bioinformatics identified candidate variants in three genes (*COL17A1*, *DNAJC9* and *FRMPD2*) in this region. Segregation was confirmed for *COL17A1* (p.Gly1052Gly,c.3156C>T, new splice donor site leading to deletion of 17 amino acids) and *DNAJC9* (p.Asp112Asn, c.334G>A) in the NZ family. The *COL17A1* variant is present in additional NZ, Tasmanian and UK families. IHC on human cornea indicates *COL17A1* in the epithelium and *DNAJC9* in the Bowman layer. Both proteins are similarly expressed in zebrafish cornea (see poster attached). Zebrafish lacking *Col17a1a* and *Dnajc9* during development demonstrate phenotypic abnormalities (see poster).

The *COL17A1* splice variant causing deletion of 17 amino acids is likely to be the causative mutation in our recurrent corneal erosion families; however, *DNAJC9* cannot be excluded as a modifier gene. A zebrafish model of this dystrophy is being developed using the CRISPR/Cas9 genome-engineering methodology.

A manuscript describing our findings is in preparation. Our results were selected for a best paper presentation at RANZCO (abstract attached), and are being presented at the ARVO Annual Meeting on 4th May 2015 (poster attached).