Report for Save Sight Society New Zealand June 2016

**Novel Retina Disease Genes: Investigation and functional characterisation using exome sequencing and zebrafish tools**

Grant awarded in 2014 for research January-December 2015

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We have identified a NZ family with an early-onset retinal dystrophy with a candidate mutation in the *TITIN* gene. Our project aimed to characterise the role of TITIN in the retina using zebrafish as a model. We have established the optokinetic response assay (OKR) in our laboratory using a specifically designed instrument, which evaluates the ability of zebrafish embryos to see based on a response to a moving stimulus. We have successfully used the OKR assay on zebrafish with the *TITIN* genes temporarily silenced during early development using morpholinos. At 4-5 days post-fertilisation (dpf), these morphant embryos have decreased vision compared to controls (Figure 1). When either the *ttna* or *ttnb* genes were knocked down with morpholinos, the morphant zebrafish showed reduced (responding to larger stimuli on the instrument) or no vision. Morphant zebrafish also present with smaller eye size (Figure 2A) and a poorly differentiated retina (Figure 2B). We have also established immunohistochemistry assays to investigate zebrafish retinal morphology (Figure 3). Combined, our preliminary findings support a role for *TITIN* in retinal disease.

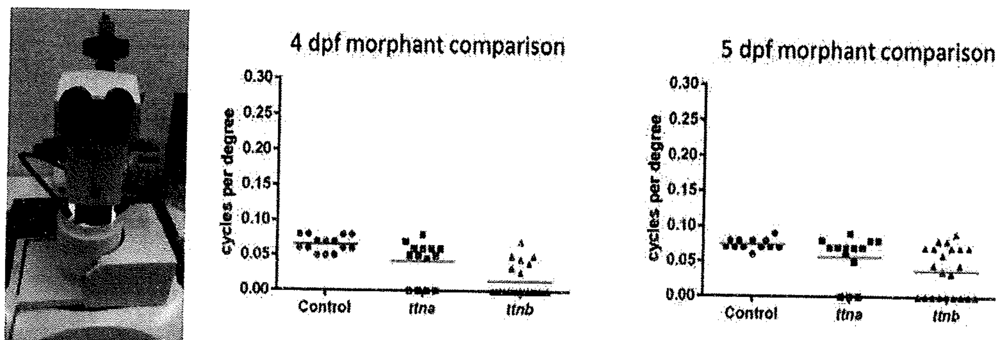


**Figure 1.** OKR instrument and readings from individual morphant zebrafish at 4-5 dpf. A decrease in (or absence of) vision, indicated by smaller cycles per degree values, is seen in the *ttna* and *ttnb* morphants.

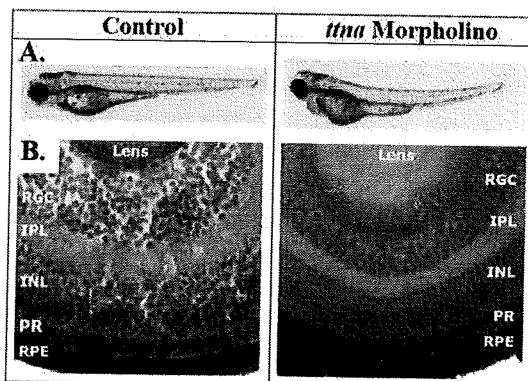


**Figure 2.** 4 dpf zebrafish injected with control and *ttna* morpholinos. A) *ttna* morphants presented with smaller eye size and heart odema. B) H&E staining revealed the morphant retinas are poorly differentiated.

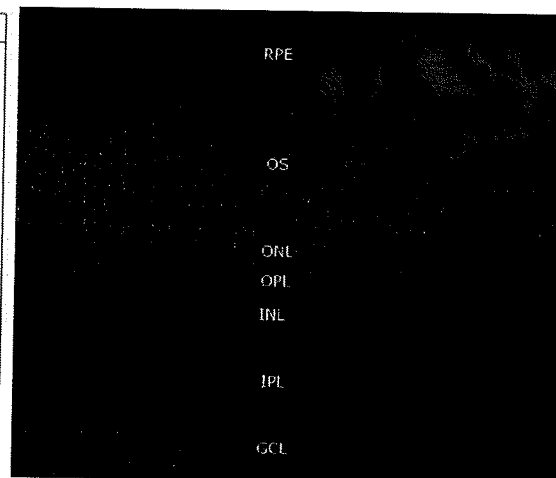


**Figure 3.** zpr-1 and zpr-3 antibodies (staining in pink) used to assess morphology in the retina.

The preliminary data generated as part of this project has provided the basis for a successful external research funding from the Auckland Medical Research Foundation to interrogate the role of *TITIN* in the retina further. We have established immunohistochemistry assays in the zebrafish, which will enable us to determine the exact location of the TITIN protein in the retina, where we propose it may be important in the ciliary body of the photoreceptors. We have optimised laser capture microscopy (LCM) techniques to collect RNA from retinal tissue (both zebrafish and human retina) to examine the isoforms of *TTN* present in this tissue. Due to the size of the *TTN* transcripts, Q-PCR cannot be performed to analyse expression. Because of this constraint, we sought additional funding to use RNA-seq to investigate *TTN* isoforms in the retina. We have successfully obtained a $15,000 grant from the Maurice and Phyllis Paykel Trust which will enable us to perform RNA-seq using a NimbleGen SeqCap RNA Developer Kit (Roche) specifically designed to include all *TTN* exons from both the human and zebrafish transcripts. This grant covers consumables, but additional funds were used from this Save Sight Society grant to cover the costs of a technician to perform the labour-intensive collection of RNA from human and zebrafish tissue using LCM. We felt that this aspect of the project required progression in a timelier manner than exome sequencing of additional retinal dystrophy patients.

We are progressing with creating zebrafish lines which carry stable mutations in *TITIN*, using CRISPR-Cas9 genome engineering, to better characterise the effect of *TITIN* loss/insufficiency in the adult fish. We have designed CRISPR-Cas9 assays to delete the majority of the large exon containing our mutation (~17kb), as well as gRNAs to target the regions proximal to the mutation site.

In summary, the seed funding for this project has enabled:

1. Establishment and optimisation of all techniques for this project
2. Functional evidence of reduced visual function in zebrafish morphants
3. Further research funding to complete all aspects of this project