Utilising patient-specific retinal organoids *in vitro* to investigate the effects of an SNRNP200 mutation causing early onset Retinitis Pigmentosa.

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Retinitis pigmentosa (RP) is a large group of slowly degenerative disease of the retina caused by mutations in over 100 different genes. The diversity of underlying causes of RP is reflected in the varied onset and progression of this disease. The common end result in RP is the loss of the light-sensitive photoreceptor cells of the retina leading to varying degrees of vision loss. How these genetic mutations or faults lead to photoreceptor cell loss remains unknown for most forms of RP. This lack of understanding in the disease process is still the greatest barrier for discovery of new therapies. It is, therefore, imperative that the mechanisms underlying vision loss are investigated thoroughly.

Facilitated by the award of $40,000 from Retina Australia, we were able to investigate how mutations in the SNRNP200 gene mutation (linked to RP type 33) cause photoreceptor cell degeneration. A 9-year-old girl with severe early-onset RP was examined at the Lions Eye Institute. Genetic analysis showed that she had two distinct mutations in the *SNRNP200* gene, one inherited from her mother and one inherited from her father. These mutations have never been reported elsewhere and they were considered to be detrimental to the gene based on computer prediction software. In order to validate this prediction, we obtained skin cells from her to analyse the effects of mutation on cell function. A special type of stem cell (see below) was made from her skin cells so that it could be grown into the retina for us to study the retinal disease in the laboratory.

Stem cells have great potential for research as they can be used to make any of the cell types or organs found in the body. A major breakthrough in 2007 enabled researchers to produce a new type of stem cell, called induced pluripotent stem cells (iPSCs), from the skin, which avoid the need to use human embryonic stem cells. As iPSCs contain all of the genetic information of the individual from which they were made, scientist can make any type of human tissues from iPSCs to study the development and disease process in that particular individual.

In our study, we used iPSCs derived from this patient to make a complex 3-dimensional miniature retina (also called 'retinal organoids'). The retinal organoids that we can make (entirely in the laboratory) are comparable to that which grows during normal human development. Retinal organoids are useful for the study of disease process, especially in those diseases that manifest early in life. By growing patient-specific retinal organoids in the laboratory, we are able to examine the development of the disease in real-time and begin to understand how these 2 mutations in the *SNRNP200* gene leads to photoreceptor cell loss.

The genetic information carried by the *SNRNP200* gene is used to produce a protein. The SNRNP200 protein works with many other proteins to coordinate essential steps in RNA processing called "splicing". Disruptions in splicing mechanisms are implicated in various forms of human disease not limited to the eye. Exactly how this and many other patient's *SNRNP200* mutations causes visual loss is unknown. There are no studies to date that examine the effect of this gene mutation in human retinal cells. Our aim was to investigate the effects of these *SNRNP200* mutations on human retinal tissue derived from patient's own skin, with a focus on the structure and function of photoreceptor cells.

In our study, we were able to successfully grow retinal organoids from our RP33 patient's skin cells for nine months. Retinal organoids were also grown from skin cells taken from a healthy individual, in order to compare the development of healthy retinal tissue to that of RP33 patient tissue. We were able to see photoreceptor cells by 3 months of development using a technique called immunohistochemistry (for looking at the specific cell types in detail). However, the structure and form of these cells were more immature when compared with photoreceptor cells in retinal organoid grown from the healthy subject. Furthermore, uncharacteristic 'holes' could be seen in the cell body of several of these photoreceptors. Similarly, spaces or gaps could also be observed within developing neural retinal tissue made from this patient iPSCs, which is distinctly uncharacteristic. By 4 months, developing photoreceptors in both the RP33 patient and the healthy subject retinal organoids had grouped together to form an outer layer within the retinal tissue, which is a normal process during human retinal development. Some of the photoreceptors in the RP33 retinal organoid appeared to have started to mature by this stage, with outer segments starting to develop. However, uncharacteristic holes could still be observed in the RP33 patient retinal organoids. Further investigation is required to characterise the cause of these gaps within the developing retinal tissue and cell bodies.

By 6 months, the RP33 retinal organoids started to look more disorganised, with photoreceptor cells forming clusters instead of remaining as a layered structure. This was also evident within patient retinal organoids grown in the laboratory for 9 months, which is approximately equivalent to 40 weeks of gestation. Remember these retinal organoid are equivalent to the developing retina in a fetus.

We used another technique called transmission electron microscopy to look at the ultrastructure of cells at incredibly high magnification. When we used this technique on our RP33 patient retinal organoids, we found that photoreceptors were starting to develop outer segments and these were comparable with controls. However, we also discovered large vacuole-like shapes within retinal cells in the patient organoids, which is in contrast to the structural and cellular integrity seen in control organoid samples.

In order to study any differences in how each cell uses the genes in the DNA during retinal development, we used the technique of single cell transcript sequencing on our RP33 patient and healthy subjects retinal organoids at 0, 3, 5 and 9 months of development (in collaboration with Prof. Abha Chopra at Murdoch University in Perth). This data was analysed using a complicated bioinformatics approach to identify whether differences could be observed between control and patient samples. Preliminary analysis of this data indicates altered immune gene expression in patient tissue, and we are awaiting the data from our final experimental time point to complete the analysis of this dataset in full.

In summary, the award from Retina Australia has allowed us to successfully create retinal organoids from our RP33 patient and a healthy subject and support their development in the laboratory for 9 months. We were able to use highly specialised techniques to pick up subtle differences between these retinal organoids. For the first time, we have found that cells harbouring mutations in the *SNRNP200* gene lead to altered retinal development. Further work is ongoing to tease out the exact processes that leads to this altered retinal development.