Accelerating therapeutic discoveries for Retinitis pigmentosa.

Aims

We sought to establish inherited retinitis dystrophy (IRD) -specific cellular models to understand the mechanisms underlying photoreceptor degeneration and blindness. This will enable the identification of novel therapeutic targets.

Aim 1: Generation of a world-leading collaborative resource of induced Pluripotent Stem Cells (iPSCs) ***from patients with IRD.***

Aim 2: Differentiation of iPSCs into retinal-specific cells and phenotype screening.

Aim 3: Screening of therapeutic compounds to correct retinal dysfunction.

This project will result in the establishment of a human cellular model of IRD, focusing on using iPSCs to produce IRD specific cells, namely retinal pigmented epithelium (RPE) and photoreceptors for disease modelling, drug screening and potentially cell replacement therapy.

The ultimate outcome is the prevention of blindness and restoration of sight to people affected with IRD. Our direct access to well-characterised patients places Australian-researchers with a firm and internationally unique, competitive advantage.

Progress

We are in the unique position of having large, well-established pedigrees with robust genotype-phenotype data from previous work conducted by Professor David Mackey and colleagues. In addition, we collaborate with the Inherited Retinal Disease (IRD) who have established a national register of clinical and DNA data from people with retinal dystrophies.

With these two participant cohorts, referrals from ophthalmologists and general publicity we have established the largest repository in the world for iPSCs from patients with IRD and other inherited retinal dystrophies. At present we have 139 participants with an IRD as well as over 200 healthy controls who have provided a skin biopsy for this research.

As indicated in the initial application, we secured philanthropic funding to purchase the Tecan Freedom EVO 200, a platform to ensure our team have a greater throughput of samples (faster turnaround time) and better standardisation of analyses. The system is now working well. In fact, we've made more iPSC lines in the last few months than we have over the preceding couple of years.

Gene therapy (delivering copies of a whole new normal gene to replace the defective copies causing vision loss) in human studies is rapidly evolving. CRISPR/CAS9 (Clustered regularly interspaced short palindromic repeats) is different and could potentially revolutionize modern

medicine. CRISPR is a gene "cut-and-paste" technology. It works like a molecular scissors to cut out the mutated portion of the gene and inserts a healthy piece of DNA.

More recently our group (Hung and colleagues) assessed the feasibility of utilizing the adeno-associated virus 2 (AAV2) to deliver CRISPR/Cas for gene modification of retinal cells in vivo. Using a transgenic fluorescent mouse model, we have definitive evidence for CRISPR/Cas-mediated gene editing of Thy1-expressing retinal cells. [1]

By demonstrating that CRISPR/Cas can also cause substantial gene modification activity when introduced by a viral delivery method in the retina, we are closer to translating gene editing technology for therapeutic purposes. Importantly, we found that AAV2-delivered SpCas9 was not retinotoxic over a 5-week treatment period. An important limitation of our work is the fact that off-target effects were not directly quantified. As such it will be important to continue to develop CRISPR/Cas systems that could be tightly regulated and thereby be able to create an optimal window for gene modification while reducing the chance for potential off-target activity.

Dr Hung and colleagues published a review on the current progress and recent breakthroughs in CRISPR/Cas9. It outlined some of the technical issues that must be addressed before gene correction, be it *in vivo* or *in vitro,* is integrated into ophthalmic care.[2] As this work transitions from pre-clinical laboratory work to mainstream therapeutics, there is a need to establish firm guidelines and understand the public perception of it's application.

Tristan McCaughey, a Monash Medical Student completed a research year (2015) within our unit and examined the social and ethical aspects of CRISPR/Cas9 technology. Three of his publications were published in prestigious journals (*Cell Stem Cell and Cell Tissue Bank*) this year.

Part of Tristan's work sought to investigate global perceptions of human genome editing applications and explored the factors associated with these views. We developed an online survey about attitudes to the application of genome engineering in different contexts, translated it into a range of international languages, and recruited study respondents via social media (Facebook, Twitter, Google, and WeChat). We analyzed responses from 12,562 people across 185 countries.

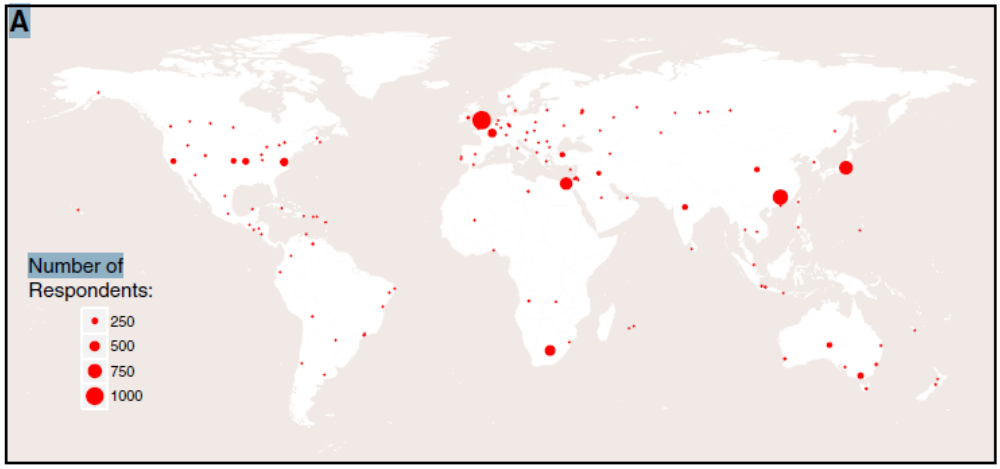

Figure 1. Demographic Profile of Survey Participants

(A) Worldwide distribution of study participants, based on their IP address details and geolocate details. Geolocate data for a total of 1,362 participants were not available.

Overall, there was support among our survey respondents for the use of gene editing in children and adults to cure life threatening and debilitating diseases. There was substantially less support among our respondents for the use of gene editing technology for non-health related purposes. (Figure 2) Participants who agreed with gene editing for non-health related purposes (2,402/8,961, 26.8%) were questioned about the specific non health- related traits they would modify with this technology. Intelligence had the highest acceptance at 68.0%, followed by strength or sporting ability (58.4%) and appearance (51.3%). [3]

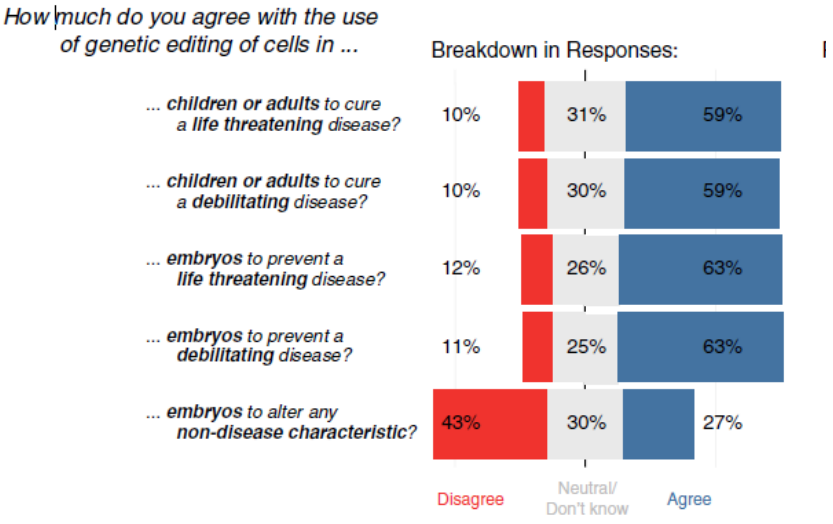

Figure 2. Breakdown of Responses for Survey Items Used to Gauge Participant's Agreement for Various Applications of Human Gene Editing

The rapid uptake of CRISPR/Cas-based gene editing is forcing the scientific community to confront complex ethical issues quickly, and in our view, it is imperative that public opinion is considered to ensure that ongoing progress is supported and well received by broader society.

During Tristan's year and with colleagues, we developed an interactive tool utilizing multimedia to support understanding and improve the consent process among iPSC research participants.

iPSC website available at: http://ipscdb.org

Overall, the interactive consent group had an equal or better understanding across all comprehension assessments when compared to those in the video animation or standard consent group. We believe the interactive consent sets a new standard for informed consent in iPSC research and, if utilized by researchers globally, will ensure that participants undertaking iPSC research are sufficiently informed, avoiding research impeding controversy in the future.[4]

Conclusions

Our group has been able to demonstrate major breakthroughs in research to gain a much better insight into inherited retinal dystrophies that can develop into future novel therapies.

Support from Retina Australia was greatly appreciated and gave CERA/UTAS the resources to recruit and develop the number of iPSC. Preliminary work was used to apply for NHRMC funding. We are pleased to inform Retina Australia that A/Prof Alex Hewitt received a Research Excellence Award as the top ranked NHMRC Practitioner Fellowship applicant to continue the iPSC and emerging gene-editing techniques.

References

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