The Zebrafish:
An Emerging Research Model for Human Disease

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Author Introduction: I am an ambitious final year medical student aiming to pursue a clinical-academic career in a neuroscience-related specialty (neurosurgery/ophthalmology). I achieved first-class honours in my intercalated degree in Neuroscience at the University of Cambridge, for which I was awarded an academic scholarship. I was given a national platform to present my laboratory-based project at the BAPIO Annual Conference 2019 in London and was awarded first prize in the Research and Innovation Oral Presentation category. I have also had the privilege of being granted a travel fellowship to Nagpur, where I undertook an inspiring neurosurgical internship under the supervision of Dr Lokendra Singh. Motivated by these experiences, my longer-term ambitions include translational research, medical education, and global health, with the ultimate aim of distributing the best medical care and education to underserved populations.

What are zebrafish?

Zebrafish (Danio rerio) – so-called due to their distinctive horizontal blue stripes – are small tropical freshwater fish of the minnow family, which are native to the Ganges River and its tributaries in northern India but now are more widely distributed. Adult male and female zebrafish exhibit distinct morphological features and can reach up to 4-5 cm in length, with a lifespan of around 3 years.

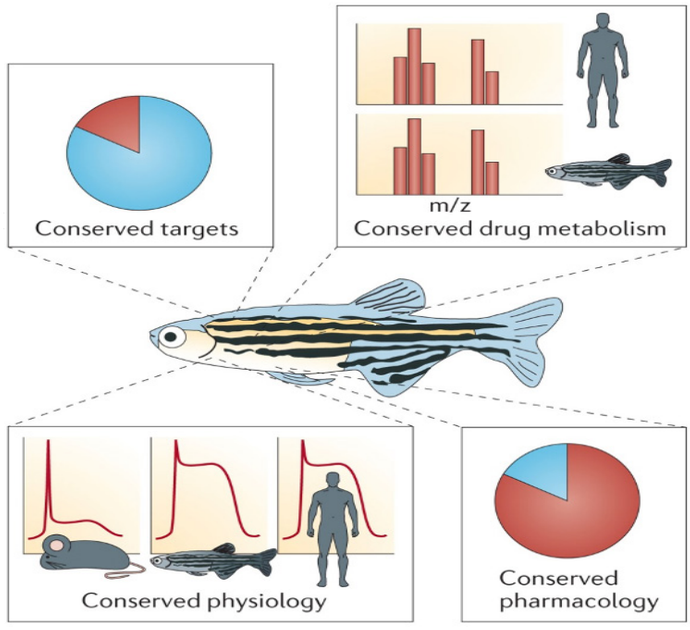
Figure 1 - Adult male and female zebrafish (figure from Teame et al., 2019)

The applicability of zebrafish in medical research

Laboratory research requires appropriate experimental models. At the most basic level, in vitro studies can be carried out (e.g. on cell cultures) and these are useful to generate working scientific hypotheses regarding disease processes. They must, however, be tested and validated in a whole organism before they can be feasibly translated to humans. Rodent models have traditionally been the most popular, but their usage is limited by their relatively slow development and the consequently slow disease onset and progression. As such, high-throughput (large scale) genetic and chemical screening studies are not possible.

The zebrafish, on the other hand, is a promising tool for translational research. Compared to other vertebrates, zebrafish exhibit short generation time (the time between their being born and giving birth is only 3 months), high fecundity (they have a high reproductive potential, being able to spawn at intervals of 2-3 days and laying hundreds of eggs per gestation) and rapid external development (the embryos develop to adult-form entirely outside of the parents, allowing each stage of development to be investigated). Additionally, the transparency of zebrafish embryos also allows fluorescent genes to be inserted into them and functions to be visualised in vivo. These advantages are combined with their considerable similarity to humans as approximately 82% of disease-causing human genes have at least one zebrafish equivalent. When considered together, these features allow the zebrafish to bridge the research gap between cellular and rodent models as a useful, efficient and cost-effective in vivo modelling system for large-scale screens.

There is, however, a limit to which diseases can be studied in zebrafish. This includes cases where the human disease lacks appropriate zebrafish equivalents in terms of genes or organs (e.g. the prostate, mammary glands and lungs are all absent in zebrafish).



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Examples of significant medical research using zebrafish

As zebrafish demonstrate similarities with humans across many physiological and organ systems, with significant evolutionary conservation, there are diverse examples of research breakthroughs that have been made using zebrafish as an experimental tool. In cancer research, for instance, zebrafish models of melanoma (a type of skin cancer) have enabled the identification of melanoma oncogenes (e.g. SETDB1) that drive tumour formation (Ceol et al., 2011). In cardiology, the zebrafish has been used to model cardiovascular development, heart

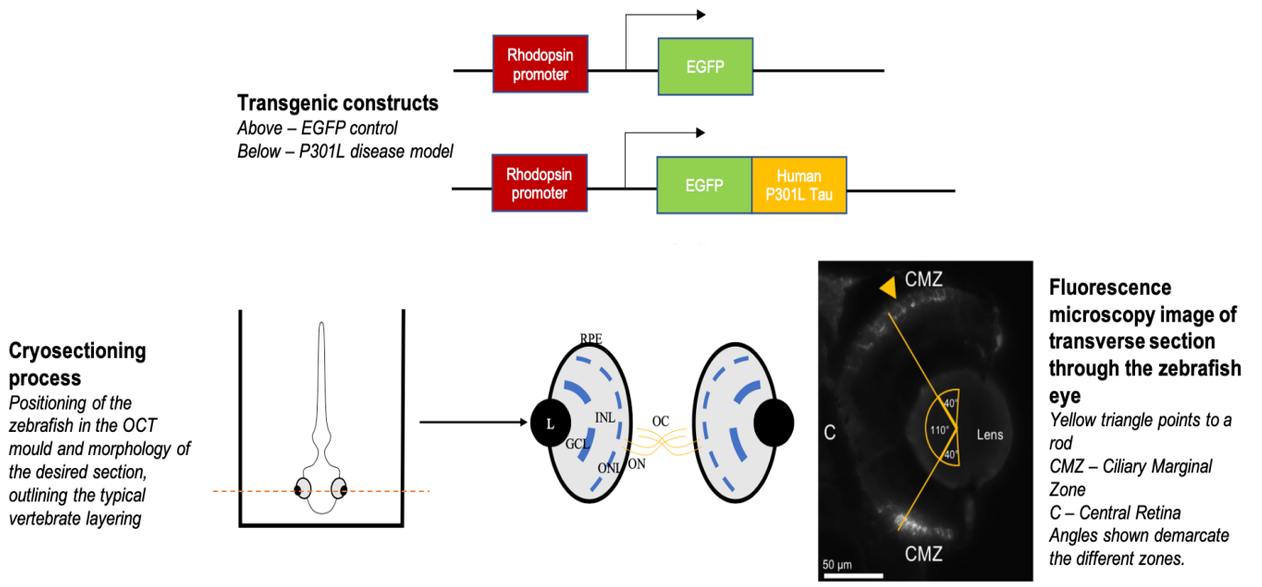
failure, and congenital heart disease (Asnani & Peterson, 2014). Similarly, In immunology, innate and adaptive immunity, as well as the genetic controls of inflammation and the identification of novel immunomodulatory drugs is being investigated with zebrafish (Lee-Estevez et al., 2018). In ophthalmology, the zebrafish has aided our understanding of retinal development and has furthered the development of stem cell treatments for diseases of retinal degeneration (e.g. macular degeneration, glaucoma and diabetes) (Raymond et al., 2006). Furthermore, endocrinologists have used zebrafish to expand our understanding of diabete through the development of glucose tolerance tests and the study of genes that indicate a predisposition to obesity (e.g. AgRP) (Zang et al., 2017). Zebrafish models have also increased our understanding of diseases of bone formation (e.g. osteogenesis imperfecta) and degeneration (e.g. osteoporosis) (Carnovali et al., 2019). Another important discovery has been made in neuromuscular disease, where genetic knockouts of the dystrophin gene in zebrafish have closely mimicked the severity and progression of the human disease -Duchenne muscular dystrophy - where dystrophin gene mutations are present (Bassett et al., 2003). This wide range of processes conserved by evolution enables the zebrafish to serve as a highly applicable tool across the spectrum of human diseases, as the range of examples given highlights.

My research with zebrafish

Neurodegenerative diseases are growing in prevalence in our ageing global population. Abnormalities in the tau protein underlie an entire class of neurodegenerative diseases known 'tauopathies', an example of which is Frontotemporal Dementia with Parkinsonism linked to chromosome 17 (FTDP-17). This is a rare and aggressive genetic neurodegenerative disease which is commonly caused by the P301L mutation in the gene for tau protein. It has an early disease-onset, rapid disease progression, and no disease-modifying treatment available at present.

In order to improve our understanding and treatment of this neurodegenerative disease process, we harnessed the advantages of the zebrafish as an effective translational research model. For our experiments, we developed two transgenic lines of zebrafish: a disease model (containing the mutant human tau gene) and a control (containing the normal human tau gene). Crucially, we genetically programmed the activity of these human tau genes to be limited to the rod photoreceptors, which were simultaneously engineered to fluoresce when alive. This allowed us to characterise disease progression in the retina (as a surrogate for the CNS) - measured by reduced rod fluorescence - without risking early lethality. We also investigated other disease markers (e.g. hyperphosphorylation and protein aggregate formation) to assess whether our model showed similarities with the human condition. In normal physiology, tau protein aggregates are cleared by a cellular housekeeping process known as autophagy, so we investigated whether promoting this process had therapeutic potential. In short, we concluded that our zebrafish model showed rapid neurodegeneration whilst faithfully resembling the distinguishing aspects of the human disease and that autophagy induction is ultimately a promising treatment approach that warrants further study.

Figure 3 - Methodology underlying the P301L zebrafish characterisation experiments, detailing the transgenic constructs, cryosectioning process, and the fluorescence microscopy quantitative analysis of the rod photoreceptors within the larval zebrafish retina.



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