Ocular-eyelid coloboma and facial clefts- a genetic study to identify universal clefting genes Dr Mariya Moosajee

BIPOSA Research Award - Report Year 1

Aim 1: To recruit patients and families with ocular-eyelid coloboma and facial clefts to identify novel universal clefting genes through whole exome sequencing of parent offspring trios

Five families have been recruited; each patient has an oblique facial cleft, eyelid coloboma and an associated ocular coloboma +/- microphthalmia +/- anophthalmia. There was no family history, pregnancy history and both parents are unaffected. Three parent offspring trios have undergone whole exome sequencing. Bioinformatic analysis using Ingenuity Variant Analysis and an interactive filter cascade, reviewed manually by the Integrative Genomics Viewer, has identified a list of potentially pathogenic *de novo* variants. A single, shared causal variant was not identified in all three probands. A *de novo* missense mutation (p.A19V) in the first exon of *protein tyrosine phosphatase, receptor type R* (*PTPRR*) has been identified in one proband. *PTPRR* belongs to the protein tyrosine phosphatase (PTP) family, which consist of key factors in a variety of cellular processes including cell growth, differentiation, metabolism, cell cycle, cell–cell communications, cell migration, gene transcription, ion channels, immune response and survival. Chromosome 12q deletions, encompassing *PTPRR,* have been associated with cleft lip and palate. Microarray and quantitative RT-PCR analysis of foetal (24 weeks gestation) versus adult eyes suggests that *PTPRR* is differentially expressed with a >3-fold increase in foetal retina/retinal pigment epithelium (RPE) and choroid, and a contrasting 1.5 fold increase in adult scleral tissue. A single nucleotide polymorphism (rs3803036) in *PTPRR* is nominally associated with high myopia. This evidence suggests that this gene may play a role in oculo-facial development. *De novo* variants of interest have also been identified in the other two families for further functional analysis e.g. *C3,* which encodes complement component 3 in the lectin complement pathway; other complement proteins have been associated with disorders that exhibit a spectrum of developmental features including cleft lip and/or palate. Future work: Recessively inherited and compound heterozygote variants will be filtered to eliminate systematic false positives. Potential candidate genes will be investigated in aim 3.

Aim 2: To examine the transcriptome of genes expressed at the site of the developing optic fissure in zebrafish using laser capture microdissection and RNA-sequencing

The extraction of RNA from laser-captured zebrafish optic fissures has proved technically challenging. Troubleshooting has included modification of tissue processing, embedding and sectioning, slide treatment, laser capture technique and RNA-extraction (Fig 1A-C). Despite optimizing each of these steps in a methodical workflow, extracting minimum RNA yields for RNA-Seq was unsuccessful. The cDNA library obtained was 50 pM, however the chosen sequencing company require at least 500 pM. We are now trialling a second method of RNA extraction to complete this aim, which involves microdissection of fixed whole embryonic retinas with the direct excision of relevant optic fissure tissue with two small cuts (black lines, Fig 1D). Using this technique, we are planning on pooling tissue from 10 zebrafish eyes per timepoint with 4 biological replicates at each developmental stage. Higher yields of RNA should generate suitable concentrations of cDNA libraries for RNA-seq. The immediate focus will be optimization of this technique to collect these samples.

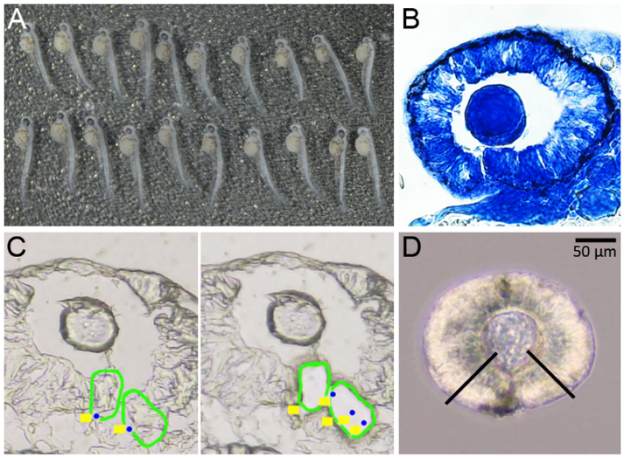

Fig 1. Technical troubleshooting for RNA-seq sample collection. (A) Multiple staged zebrafish embryos orientated in cryoprotective medium prior to freezing for cryosectioning. (B) Stained cryosection of the zebrafish eye at 48 hours post fertilisation (hpf) showing the presence of the optic fissure. (C) Laser capture microdissection of the optic fissure (green line highlights the pathway of the laser prior to and after microdissection). (D) Whole dissected eye without the lens at 48 hpf, scale bar 50 µm.

Aim 3: Study of candidate genes identified in aims 1 and 2 using morpholino antisense gene knockdown and *in situ* hybridisation to improve our understanding of the molecular events controlling tissue fusion

We have designed a morpholino for *PTPRR* to generate a zebrafish knock-down model to assess the phenotype. If craniofacial and ocular malformations are seen, we will undertake *in-situ* hybridisation to identify its expression patterns. We also plan to perform an *in vitro* splicing assay will test the splicing efficiency of wild-type versus mutant human *PTPRR* transcript, where alterations in the balance between different isoforms of normal gene products as a result of aberrant splicing, may be an important mechanism for pathogenesis of this disease. Other candidates will be examined including *C3*. If informative, these results will be published in the next year.