Title: Unravelling the heterogeneity of progenitor cells in the developing human retina through single cell analyses

Dr. Joseph Collin*, Dr. Darin Zerti*, Dr. Rachel Queen*, Dr. Birthe Dorgau and Prof. Majlinda Lako

Emails: joseph.collin@ncl.ac.uk; darin.zerti@ncl.ac.uk; rachel.queen@ncl.ac.uk; birthe hilgen@ncl.ac.uk; majlinda.lako@ncl.ac.uk

**these authors contributed equally*

Address: Biosciences Institute, Faculty of Medical Sciences, Newcastle University, Newcastle, UK

Phone: +44 (0)191 241 8688

Purpose: Retina is comprised of six neuronal and one glial cell type, which are derived from heterogeneous and dynamic multipotent retinal progenitor cells (RPCs) in an orderly manner. A significant part of retinal development in humans occurs *in utero*, which poses logistical issues for systematic studies of human retinogenesis. To date we have little molecular information about human RPCs competency and/or heterogeneity during human retinal development. This study aims to utilize the most recent advances in single cell sequencing to determine the spectrum of transcriptional profiles of human RPCs.

Methods: Nine samples of developing human eyes from 12-20 post-conception weeks were obtained from the Human Developmental Biology Resource. Human retina was dissected and dissociated to single cells using the neurosphere dissociation kit from Miltenyi Biotech. 10,000 cells were captured using the 10 X Chromium Single Cell 3' Library & Gel Bead Kit Genomics (version 3). Single cell libraries were sequenced to 50 k reads per cell on an Illumina NovaSeq 6000. The 10x Genomics software CellRanger was used to process the raw sequencing data and create gene expression matrices. The Seurat standard integration workflow was followed to combine the scRNA-Seq datasets and define clusters.

Results: 40,121 cells were obtained after data integration resulting in detection of 47,068 genes. Nine cell clusters representing RPCs, all key retinal cell types as well as pericytes and microglia were identified. Further subclustering and pseudotime analyses were performed for the RPCs compartment, identifying a pseudotime trajectory with 8 branches. Differential gene expression analysis during the pseudotime trajectory resulted in identification of 3578 genes, which were fitted into 8 different modules. Analysis of these gene expression modules revealed two different naïve RPCs and six primed RPCs subsets. The latter were characterised by the typical expression of retinal ganglion, horizontal and amacrine cells, photoreceptors, Muller glia and cone bipolar cells, indicating specific priming within the RPCs compartment.

Conclusion: This study indicates the usefulness of single cell sequencing analyses for understanding RPC heterogeneity and priming during retinal development and identification of very early regulators of retinal cell fate determination.