Single-cell Transcriptome Data Analysis of Age-related Macular Degeneration Using Weighted Correlation Network Analysis

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1. Introduction

Age-related macular degeneration (AMD) is a leading cause of blindness characterised by disruption of the photoreceptors within the macula (central part of the retina), resulting in loss of clarity of vision. (Voigt *et al.*, 2022).

The early, intermediate and late stages have been established to describe the disease progression of AMD. Although AMD is multifactorial disease, that involves dysfunction and degeneration of the macula. There is increasing evidence that changes in the choroid play a critical role in the pathogenesis of AMD (Farazdaghi and Ebrahimi, 2019). The choroid is a vascular layer that provides oxygen and nutrients to the outer retina, including the macula as shown in Fig. 1.

AIM

This study seeks to elucidate highly interconnected genes present in the choroid cells that is associated this complex disease in the early phase.

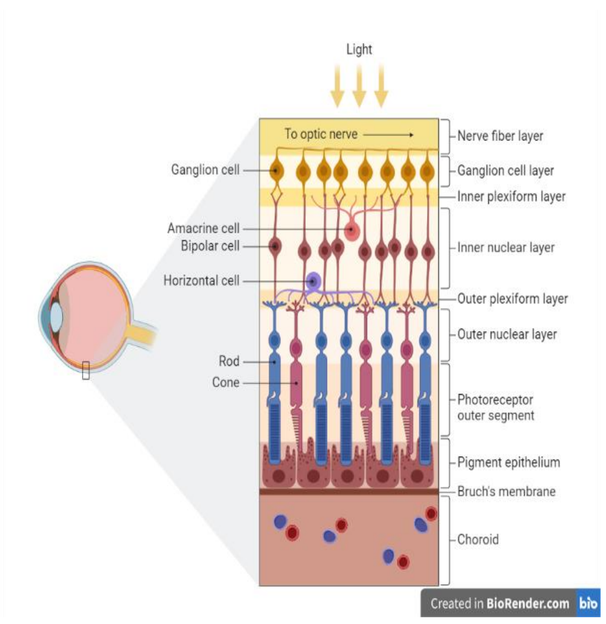

Fig. 1| The structure of the posterior eye *(Created with BioRender.com).*

2. Methods

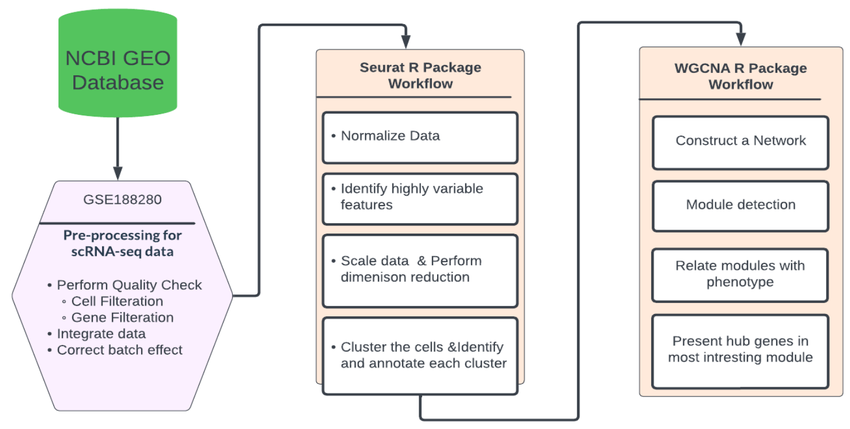

Fig. 2| *Schematic illustration of the study design.*

Single-cell transcriptomics data sourced from choroid tissue of the eyes were obtained from a study with accession number GSE188280 in the NCBI GEO database. GSE188280 contained 24,390 and 9,295 cells from human donors with early AMD and normal eyes respectively. The workflow of this study involves analysing the scRNA data with the Seurat R package to cluster and identify the cell types. The assay data of the identified fibroblast cells type were inputted into the WGCNA R package to construct a co-expression network and identify hub genes as shown in Fig. 2

3. Results

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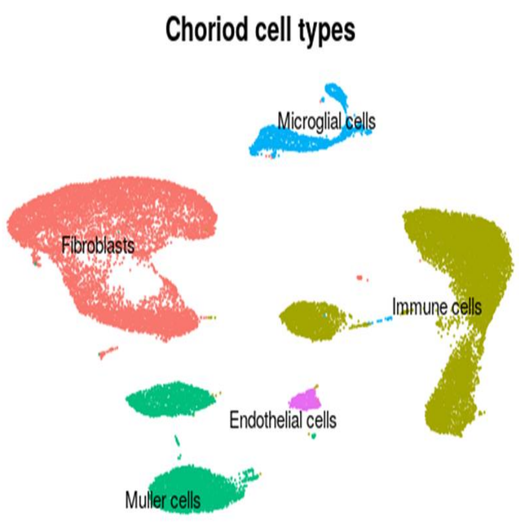

Fig. 3| *UMAP showing the cell types in the choroid tissue.*

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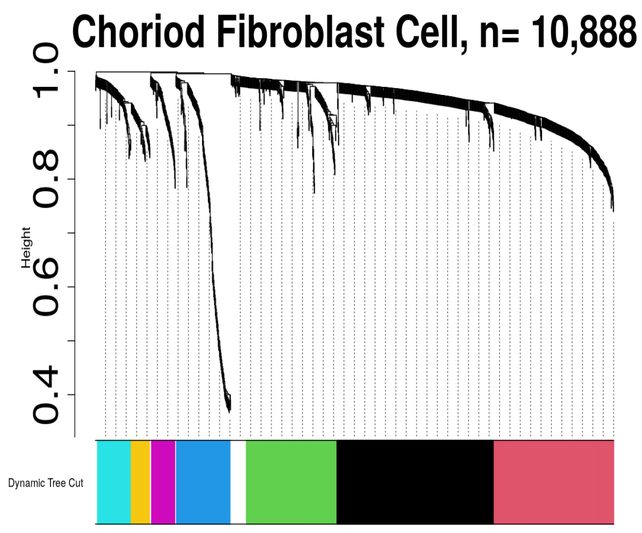

Fig. 4| *Dendrogram showing different co-expression modules obtained from a correlated network analysis of fibroblast cell types in the choroid. The dendrogram leaf represents each gene while the colour at the bottom represents the co-expression module assignment.*

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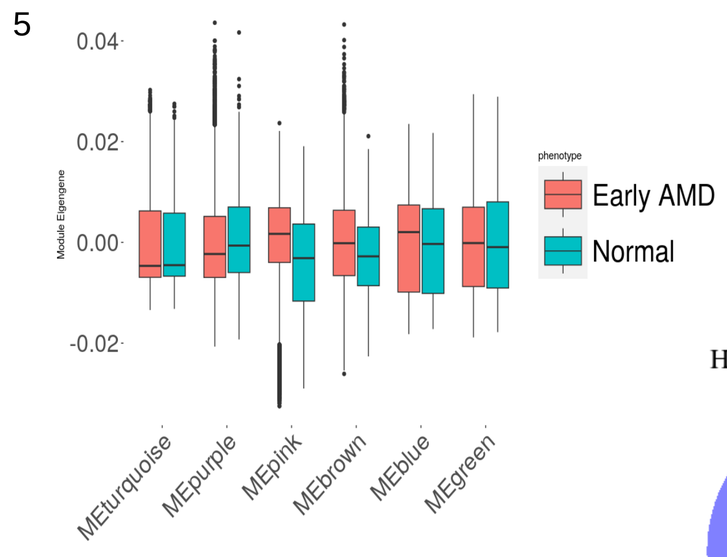

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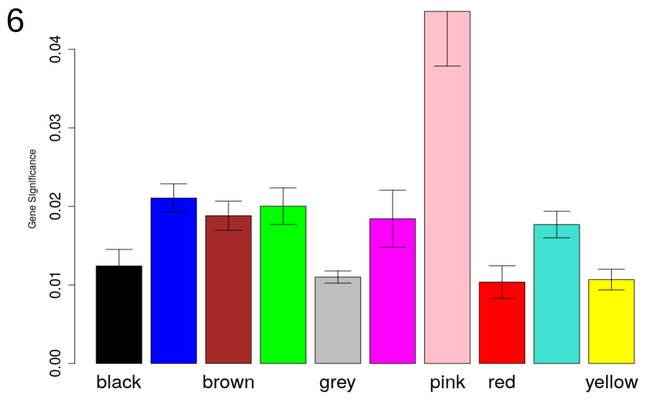

Fig. 6| Bar plot showing the significance of module correlation with early AMD and normal eye.

Fig. 5| Boxplot that shows the correlation between early AMD and normal eyes module eigengenes within each module

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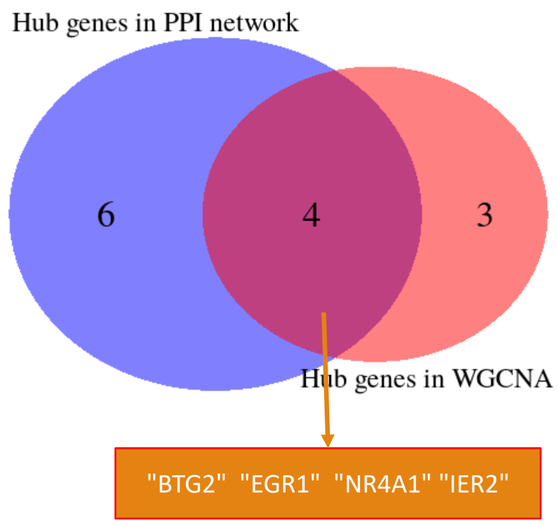

Fig. 7| Validation of Hub genes of pink module with Hub genes from PPI network of DEGs.

4. Discussion

In this study, we employed the Seurat tool to identify different cell types (Fig. 3) in scRNA sequence data sourced from the choroid of human donors with early AMD and normal eyes. WGCNA was used to identify biologically relevant transcripts significantly altered in choroidal fibroblast cells of early AMD (Fig. 4). Module-traits relationships revealed pink (r= -0.098, p-value < 2.2e-16) and magenta (r=0.03, p-value = 0.001712) as interesting modules. Furthermore, pink module was identified as the key module (clusters of highly interconnected genes) for having the highest module significance (r = 0.045, p-value = 1.4e-54) with early AMD as shown in Fig. 6. The pink module comprises of 60 gene. Among them, 7 hub genes were identified based on the set thresholds as module membership > median and Gene Significance > 0.05. To validate the "true" hub gene, we compared these hub genes with hub genes obtained from PPI network of 34 differentially expressed genes (DEGs) in normal and early AMD in the fibroblast cells. Fig. 7 shows the common genes seen are "BTG2" "EGR1" "NR4A1" and "IER2" . "NR4A1" is druggable target, small molecule binder and high quality ligand.

5. Conclusions

Our study used the WGCNA to construct a gene co-expression network and to identify the key modules and hub genes associated with early AMD. These hub genes differentiated early AMD from Normal eye and can serve as therapeutic targets for the treatment of the early phase of AMD.

6. References

Langfelder, P. and Horvath, S. (2008) 'WGCNA: an R package for weighted correlation network analysis', *BMC Bioinformatics*, 9(1), p. 559.

Voigt, A.P. *et al.* (2022) 'Choroidal endothelial and macrophage gene expression in atrophic and neovascular macular degeneration.', *Human molecular genetics*, 31(14), pp. 2406–2423.

Zauhar, R. *et al.* (2022) 'As in Real Estate, Location Matters: Cellular Expression of Complement Varies Between Macular and Peripheral Regions of the Retina and Supporting Tissues.', *Frontiers in immunology*, 13, p. 895519.