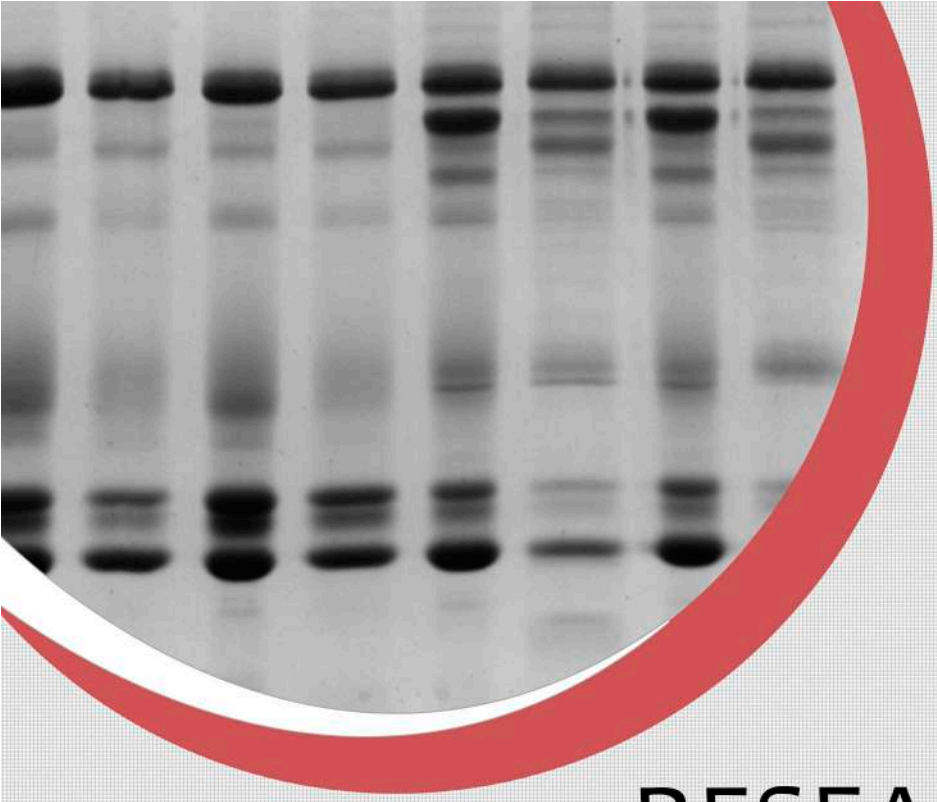

RESEARCH IN OPHTHALMIC SCIENCES

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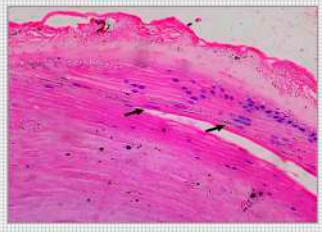

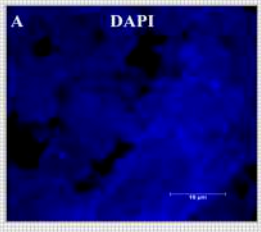

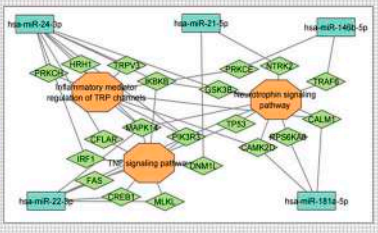

Annual Report 2024 - 2025

ARAVIND EYE CARE SYSTEM

Aravind Medical Research Foundation

Aravind Medical Research Foundation is recognised as Scientific and Industrial Research Organization (SIRO) by the Department of Scientific and Industrial Research (DSIR), Government of India

MISSION

To eliminate needless blindness by providing evidence through research and evolving methods to translate existing evidence and knowledge into effective action.

RESEARCH IN OPHTHALMIC SCIENCES

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Annual Report 2024 - 2025

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Aravind Medical Research Foundation,
Madurai

FOREWORD

I am happy to note that a large number of new areas of research are initiated during this period at AMRF. These proposals have been submitted for funding and also got approvals from granting agencies. Project on the Glaucoma Topic "Adult stem cell derived exosomes: a cell-free therapy for glaucoma" is aimed to show the role of a subcellular component namely extracellular nano vesicles in controlling the disease process. This will lead to appropriate interventional method development.

Another major project submitted during this year on the topic of Glaucoma also got approved. In this projects many investigators from AMRF jointly will explore the possibility of identifying the change in glaucomatous eye and from the data generated they will develop a model to predict the disease onset, well ahead of the development of clinical symptoms.

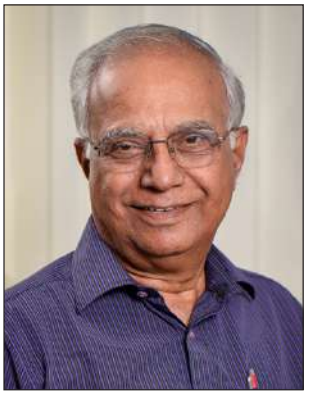
These and other projects undertaken this coming year will allow AMRF to come up with new research fundings and possible solutions to eye health problems.

Dr. P. Namperumalsamy
President, AMRF

INTRODUCTION

Several major research projects were submitted in different areas of eye health and disease in the past year. Among these the projects on glaucoma will allow us to study the mechanisms and interventional strategies to combat this deadly disease. Projects on microbiome and its role in ocular fungal infection and bacterial infection is another major project funded.

As in previous year awareness programmes and workshops were also conducted. Students and faculty also participated in National and International conferences. The support of the Government funding agencies and donors allowed us to achieve our objectives. We hope to continue our effort to eliminate blindness through research in the coming years. I take this opportunity to thank all our donors for supporting AMRF financially



Prof. K. Dharmalingam
Director - Research

CONTENTS

MOLECULAR GENETICS

The genetic variability in retinal dystrophies and optic neuropathies enables the use of next-generation sequencing (NGS) technologies to identify the genetic contributors to disease pathogenesis. Even though it is incurable, this irresistible approach offers accurate molecular diagnosis, thereby facilitating access to precise novel therapeutic interventions and enabling more personalized genetic counselling, to optimize disease management strategies.

Currently, our lab is focused on characterizing the genetic factors underlying rare neural degenerative disorders in the human visual system, such as Leber's hereditary optic neuropathy (LHON), and Leber's congenital amaurosis (LCA). Additionally, our endeavour aims to construct a gene registry of the visual system specific to the South Indian ethnicity.

Investigation of nuclear genes involvement in a Mitochondrial Disorder: Leber's Hereditary Optic Neuropathy

Investigators : Dr. P. Sundaresan
Dr. S. Mahesh Kumar
Funding Agency : SERB – CRG
(CRG/2022/000926)

Introduction

LHON was considered as a maternally inherited mitochondrial genetic disorder which is caused by mutations of the mitochondrial DNA (mtDNA). Recent studies revealed there is a subclass in LHON inherit in autosomal recessive pattern (arLHON), due to the homozygous mutations occurring in the nuclear genes DNAJC30 that are essential for the Oxidative Phosphorylation (OXPHOS) system located in the inner mitochondrial membrane. Although gene therapy and mitochondrial transplantation have improved visual abnormalities in LHON, still delayed representation fails to restore RGC regeneration pose a major challenge in LHON. Mitochondria is an intracellular cytoplasmic organelle, also called as power house of the cell, generates adenosine triphosphate (ATP) via electron transport chain (ETC). It encompasses five complexes each are assembled by the multimeric protein subunits encoded by both mitochondrial (13 proteins) and nuclear DNA (70 proteins) and ATP is produced through oxidative-reduction reaction. Additionally, 1136 nuclear genes known to be interacted with mitochondria, including: OXPHOS subunits, assembly factors, proteins involved in mtDNA maintenance and expression, and proteins involved in the biosynthesis of nonprotein components of the respiratory chain. LHON is exclusively associated with electron transport complex I dysfunction causing bioenergetic failure. The defects in any of these mito-nuclear genes causing impairment in the mitochondrial dynamics could leads to hereditary optic neuropathy disorders. Therefore, the current study aims to investigate the

molecular cause of LHON in patients lacking mtDNA mutations by analysing their mito-nuclear genes through NGS.

Results

The whole mtDNA sequencing result showed about 70% of probands had mutations in their mitochondrial genome. Based on these findings, the probands were further subdivided into three groups. Probands in Group I had primary mtDNA mutations (n=9, 30%). These mutations were encompassed in m.3460G>A/Mt-ND1 (n=2), m.11778G> A/Mt-ND4 (n=3), m.14484T>C/Mt-ND6 (n=3) and a rare primary mutation (m.4171C > A) in MT-ND1. Additionally, three individuals had co-occurrence of more than one mitochondrial variant, specifically m.4216T>C/MT-ND1, m.12033A>G/MT-ND4 and m.13708G>A/MT-ND5. It is worth noting that WES analysis from this group did not reveal any pathogenic variants linked to LHON. Group II probands harbored secondary mtDNA mutations (n=12, 40%). Among them, six individuals carried a single mitochondrial variant: m.13708G>A in MTND5, m.9139G>A in ATP6, m.4454T>C in MT-TM, m.11544T>A in MT-ND4, m.7859G>A in MT-CO2, and m.4216T>C in MT-ND1. In addition, one proband exhibited 9 bp deletion in NC-7 gene. Moreover, five probands had two mitochondrial variants such as (m.4842A>G/MT-ND2 and m.12308A> G/MT-TL2); (m.8950G>A/MT-ATP6 and m.11696G>A/MT-ND4); (m.4216T>C/MT-ND1 and m.7444G>A/MT-CO1); (m.13708G>A/MT-ND5 and m.15927G> A/MT-TT); (m.4216T>C/MT-ND1 and m.8573G>A/MT-ATP6). Furthermore, WES results indicated 50 % of the probands in group II had mutations in nuclear genes NDUFS2, NDUFS7, OPA1, MTFMT, PDSS1, and MYOC. Notably, mutations in NDUFS2, NDUFS7, and MTFMT were strongly associated with mitochondrial complex I deficiency nuclear types (Figure 1).

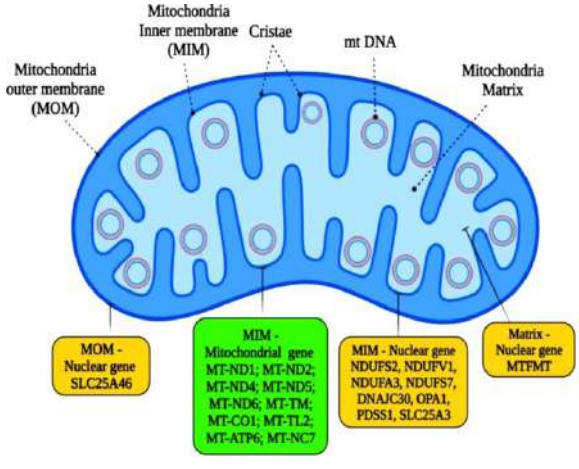


Figure 1: Mito-nuclear genes linked to LHON

Conclusion

The present work sheds light on the significance of mitonuclear genetic involvement in LHON disease pathogenesis. Furthermore, this study also emphasizes the importance of comprehensive genetic evaluations, including both mtDNA and nuclear gene analyses, in the diagnosis and management of LHON. In addition, the mutations found in nuclear genes such as NDUFS2, OPA1, NDUFS7, MTFMT, PDSS1, NDUFV1, NDUFA3, DNAJC30, SLC25A46, SLC25A3 and MYOC extend our understanding of the genetic landscape of LHON.

Decoding the unknown genetic etiology to ameliorate the molecular diagnosis of Leber's Congenital Amaurosis

Investigators : Dr. P. Sundaresan
Dr. S. Senthil Kumari
Dr. Rupa Anjanamurthy

Junior Research Fellow : S. Shiva Sankari

Funding Agency : Indian Council of Medical Research - Grant-in-aid Scheme (R.11015/10/2023-GIA/HR)

Introduction

Leber's Congenital Amaurosis (LCA), one of the most severe and earliest forms of IRD, accounts for approximately 5% of all IRD cases, and a leading cause of congenital blindness in children. It is classified as a rare disorder, with an estimated prevalence ranging from 1 in 30,000 - 81,000 live births. Despite its rarity, the incidence of LCA is quite often in the communities practicing consanguineous and endogamous marriages. With the advent of gene therapy as a promising treatment option, achieving an accurate molecular diagnosis has become increasingly critical for effective clinical management and therapeutic intervention. Therefore. this study aimed to reveal the causative mutations in LCA patients, and to develop an ethnic specific gene panel for molecular diagnosis.

Results

Whole exome sequencing successfully identified causative mutations in 37 of 51 patients, achieving a detection rate of 72.5%. Twenty-six patients possessed a mutation in fourteen LCA candidate genes, including CRB1 (n=5), GUCY2D (n=3), NMNAT1 (n=3), RDH12 (n=2), AIPL1 (n=2), LCA5 (n=2), CRX (n=2), RPE65, ALMS1, TULP1, KCNJ13, PRPH2, IFT140 and RPGRIP1. Additionally, eleven patients had mutations in genes associated with other retinal and syndromic diseases, including

ARHGEF18, PRPF31, RPGR, RP1L1, MERTK, FSCN2, GRM6, AHI1, and GDF3 (Figure 2). Nine novel LCA gene mutations were identified in IFT140 (c.G2426C), RPGRIP1 (c.C2861G), RDH12 (c.C481T), GUCY2D (c.G169A), KCNJ13 (c.T740C), RPE65 (c.A523G), AIPL1 (c.G844T), and PRPH2 (c.T995C). The sanger validation analysis for each proband reveals homozygous peaks, confirming their affected status and the probands parents and siblings display heterozygous peaks, indicating their carrier status, in alignment with the autosomal recessive inheritance pattern observed in these families. Notably, we observed a novel association of LCA phenotype with AHI1 known to cause Joubert syndrome.

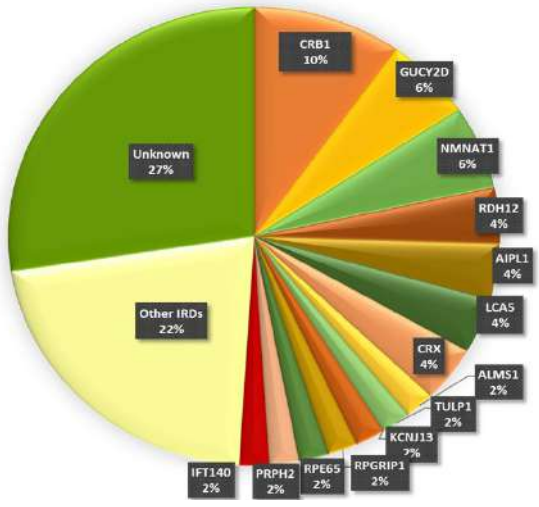


Figure 2: Mutation spectrum of 51 LCA patients

Conclusion

This study provides an insight on the genetic profile and mutation spectrum of candidate genes of LCA. Molecular findings have also helped in accurate diagnosis, better patient management, and genetic counselling.

GENETICS OF OCULAR TUMORS

Ocular tumors pose a significant global health challenge, affecting individuals across all age groups. Delayed/ advanced-stage presentation, differential diagnosis and chemoresistance remains a key obstacle in effective disease management. Hence reliable diagnostic and prognostic markers are essential to improve the overall treatment outcome. Our research focuses on unravelling the molecular mechanisms underlying various ocular malignancies, including retinoblastoma and lymphoma. By integrating cellular and molecular approaches, we aim to identify novel therapeutic and prognostic targets. We developed a cost-effective genetic testing methods for retinoblastoma, now implemented in routine clinical care. Our multi-omic analysis reveals dysregulation of oncogenic signalling pathways, providing insights for newer, effective and targeted treatment of ocular lymphoma. Additionally, we have developed an in vitro drug resistance model and patient-derived spheroids, facilitating the translation of laboratory findings into clinical applications.

Genetic Testing of Retinoblastoma

Investigators : Dr. A. Vanniarajan
Dr. Usha Kim
Genetic Analyst : K. Murugan
Funding Agency : Aravind Eye Care System, Madurai

Introduction

Retinoblastoma (RB) is a paediatric childhood tumour with good disease prognosis. If it is diagnosed in time, the vision and even globe can be salvaged. The major gene involved in the retinoblastoma is RB1 gene which serves as a cell cycle regulator. A diverse spectrum of mutations are reported in retinoblastoma. Our step wise method of analysis include Sanger Sequencing, Multiplex Ligation dependent Probe Amplification and Next Generation Sequencing as needed.

Results

During the last year, RB1 genetic testing was carried out in 65 patients including 44 unilateral and 21 bilateral RB. Of the 65 patients, 14 were older patients who were visiting the clinic for follow-up and rest of them were paediatric new patients. Germline mutations were identified in 18 patients. Somatic mutations were identified in 3 tumours that were available for the genetic analysis. Absence of the mutations in the blood samples of other patients implied the low risk of RB in the future generation.

Two patients were found to have indels with and without frameshift. One patient with bilateral RB was treated in 2013 and did not have any recurrence till 2024. When the patient came for follow-up last year, new tumor lesions were noted and was under treatment. The patient harboured an in-frame deletion (figure 1). In another patient, there are two bases

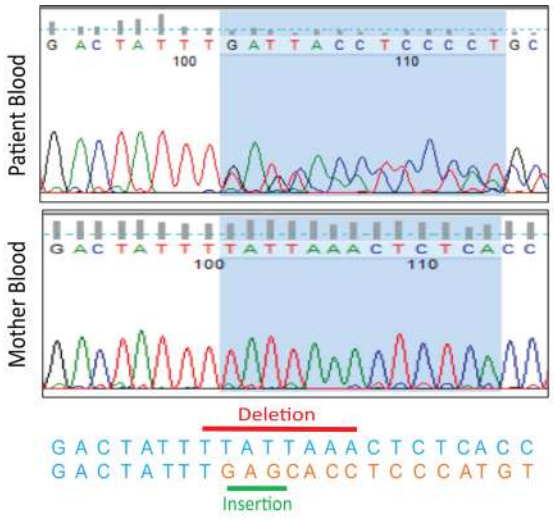


Figure 1: Indel identified in a RB patient with tumor recurrence

"GC" replaced with "AA". This replacement of bases caused the frameshift and truncation of the RB1 protein.

Conclusion

Genetic analysis of retinoblastoma patients identified new variants of RB1 that are distinct in our cohort. Genetic counselling was provided to the family members of the patients that helped in disease management.

Investigation of GD2 synthase as a potential marker for retinoblastoma poor prognosis

Investigators : Dr. A. Vanniarajan
Dr. Usha Kim, Dr. Shanthi
Research Scholar : R. Sethu Nagarajan
Funding : VISTA – Aravind Medical Research Foundation, ICMR – SRF (Fellowship)

Introduction

Retinoblastoma is a pediatric ocular malignancy with a high prevalence of advanced-stage presentation in developing countries, often resulting in CNS metastasis. Standard diagnostic tools, such as imaging techniques and CSF cytology, have limitations in detecting minimal tumor dissemination. GD2, a class of sphingolipids, has been proposed as a promising therapeutic target in several cancers. This study evaluates GD2 synthase expression in RB tumors and CSF samples to assess its association with poor prognosis in RB.

Results

Expression of GD2 synthase was significantly higher in RB tumors compared to neural retina controls, indicating the upregulation of GD2 synthase in RB tumors. Tumor dissemination was evaluated by assessing the expression of GD2 synthase in

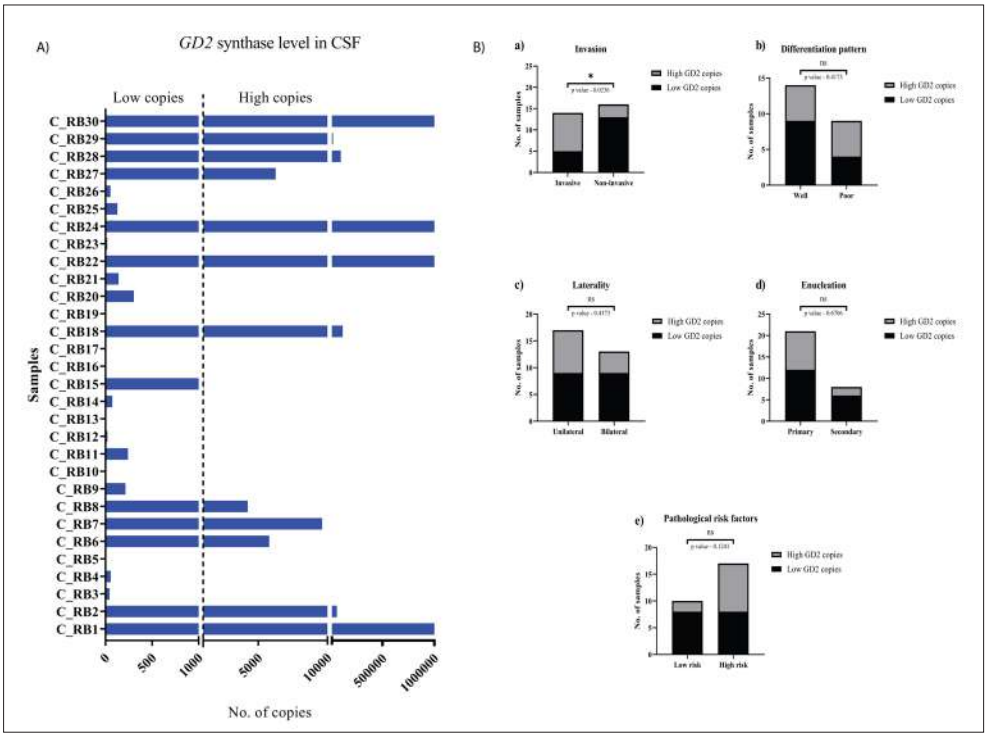


Figure 2. Expression of GD2 synthase and clinical significance (A) Absolute quantification of GD2 synthase expression in RB patient CSF samples (B) Clinicopathological correlation with GD2 synthase copies

CSF samples (n=30) of RB patients. Based on the absolute quantification, patients with more than 1000 copies were classified as high copies of GD2 synthase. Patients with high copies of GD2 synthase showed invasive features like retrolaminar or optic nerve head tumor infiltrations (Figure 2).

Conclusions

The present study has demonstrated the increased expression of GD2 synthase in RB patient samples and correlated it with high-risk clinical features. Hence GD2 synthase can be used as a potential marker for poor prognosis.

Elucidating the role of cancer stem cells in chemoresistant retinoblastoma and their therapeutic implications

Investigators : Dr. A.Vanniarajan, Dr. Usha Kim, Dr. Shanthi
Research Scholar : R. Sethu Nagarajan
Funding : ICMR – SRF (Fellowship)

Introduction

Chemoresistance remains a major challenge in the treatment of retinoblastoma. Several factors contribute to the development of chemoresistance in which cancer stem cells (CSCs) being a key contributor. This study focuses on investigating the role of CSCs and their regulatory pathways in chemoresistant RB using patient-derived cultures.

Results

Ten RB samples (1 vitreous and 9 tumors) were successfully cultured and characterized. The transcript analysis of stemness markers in primary cultures revealed differential expression of pluripotent markers such as Nanog, SOX2, OCT4, KLF4 and MKI67 across different samples. Samples with overexpression of Nanog and SOX2 showed reduced expression of the proliferative marker MKI67. The sphere formation assay showed that two samples (PC15, PC16) with enhanced sphere-forming ability when compared to others. These two patients were clinically segregated as high-risk RB (Figure 3).

Conclusion

Transcript-level analysis revealed the differential expression of stemness markers and the sphere forming assay confirmed the stemness with high proliferation.

Detection of larger chromosomal rearrangements in ocular lymphoma

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Dr. R. Shanthi
Research Scholar : K. Saraswathi
Funding Agency : Aravind Eye Foundation; Lady Tata Memorial Trust (Fellowship)

Purpose

Ocular Adnexal B Cell Lymphoma (OABL) is genetically diverse, with prognosis varying significantly across disease subtypes. This underscores the crucial need to establish precise predictions of disease subtypes, which serve as key prognostic indicators. In B-cell lymphomas, each subtype is characterized by a hallmark chromosomal translocation, predominantly involving the rearrangement of oncogenes with the immunoglobulin heavy chain (IGH) locus at 14q32.

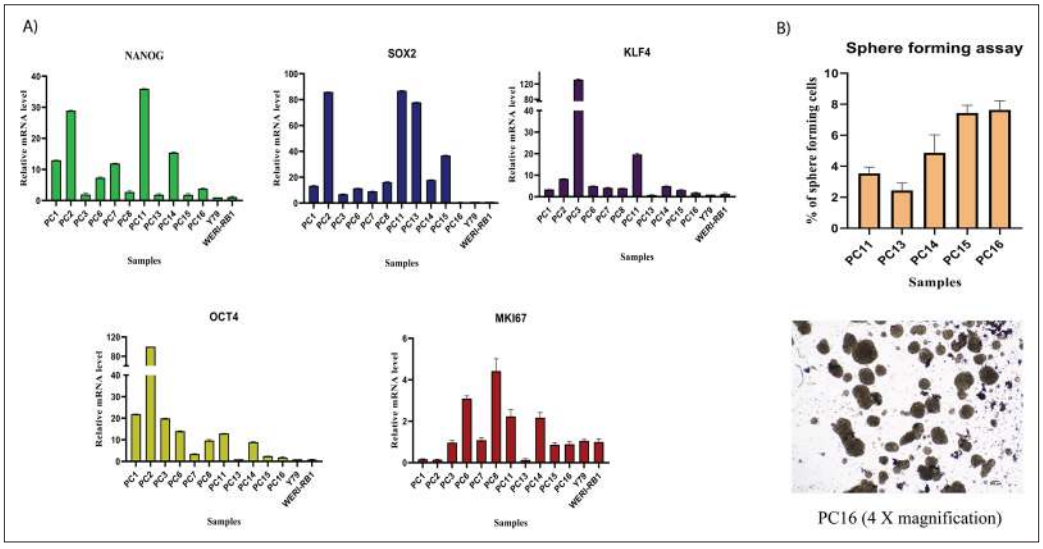


Figure 3. Characterization of cancer stem cells in chemoresistance (A) Transcript level expression of stemness and proliferative marker in RB primary cells (B) Sphere forming ability of RB primary cells

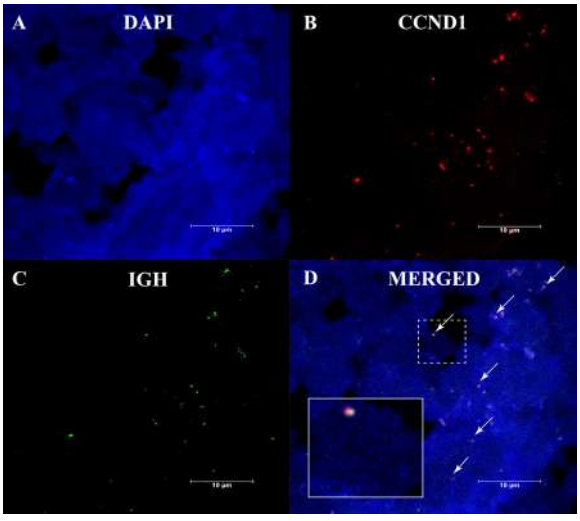


Figure 4: FISH analysis revealed IGH (green)/CCND1(orange) fusion represented by yellow signals (arrowheads showing merged fusion signals)

To detect chromosomal translocations in formalin-fixed, paraffin-embedded (FFPE) tumor sections, Fluorescence in situ Hybridization (FISH) analysis was performed.

Results

IGH/CCND1 dual colour fusion translocation probe was utilized to detect the t(11;14)(q13;q32) translocation. This translocation is a hallmark of Mantle Cell Lymphoma (MCL), characterized by the juxtaposition of the IGH gene's transcriptional enhancer on chromosome 14q32 with the proto-oncogene CCND1 on chromosome 11q13, resulting in cyclin D1 overexpression. FISH analysis revealed the presence of the IGH/CCND1 translocation in two FFPE tumor sections (positive control-REC1 cell line) (Figure 4). In both cases, histopathological and molecular findings substantiated the diagnosis of MCL.

Conclusion

FISH analysis identifies chromosomal translocations in two FFPE sections, consistent with histological and molecular findings. It can be used in diagnosis correlating with clinical and histological findings for disease prgnostication.

Altered molecular pathways in ocular lymphoma identified by whole exome sequencing

Investigators : Dr. A. Vanniarajan, Dr. Usha Kim, Dr. R. Shanthi
Research Scholar : K. Saraswathi
Funding Agency : Aravind Eye Foundation; Lady Tata Memorial Trust (Fellowship)

Purpose

Ocular Adnexal B Cell Lymphoma (OABL) is the most prevalent primary orbital malignancy in adults, with disease recurrence posing a major challenge in treatment. Identifying molecular drivers linked to lymphoma pathology is crucial for developing effective prognostic markers. Chromosomal translocations and alterations in cell-signalling pathways were frequently reported in lymphomas. The present study aims to uncover the molecular factors associated with OABL recurrence using whole exome sequencing (WES).

Results

Nineteen histologically confirmed OABL tumor samples were analysed, comprising 11 males (58%) and 8 females (42%). The mean age at diagnosis was 61.10 ± 5.83 years, ranging from 30 to 85 years. Among the 19 OABL patients, 95% (n=18) presented with unilateral disease, while 5% (n=1) exhibited bilateral involvement. The predominant symptoms at presentation were proptosis and swelling. The tumor lesions were localized in all cases, and all instances were identified as primary OABL. Mutations in TNFAIP3, MYD88, CD79B, EP300, KMT2D, IGLL5, ATM, BIRC6, ROS1, TMEM37, ERBB2 and TBL1XR1 genes were identified in more than one tumor samples. These genes were clustered in the BCR / NFκB, chromatin modulation, cell cycle, apoptosis, and immune surveillance pathways (Figure 5).

Conclusion

Exome analysis revealed potential pathogenic variants in OABL samples. Furthermore, correlation with expression analysis and clinical features will reveal potential prognostic targets.

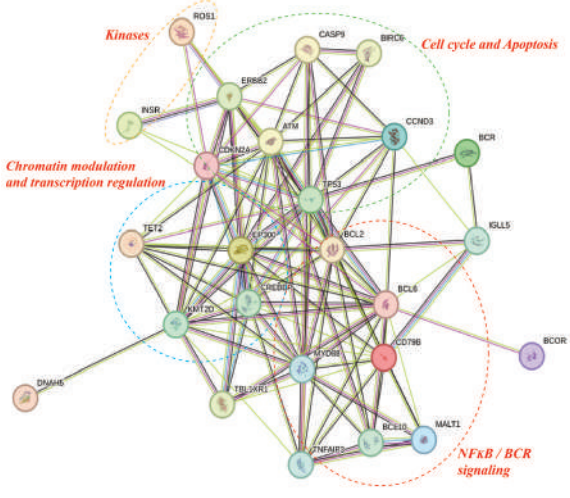


Figure 5. STRING analysis of somatic pathogenic genes reveals a closely interconnected network

IMMUNOLOGY AND STEM CELL BIOLOGY

Adult tissue resident stem cells play a significant role in the maintenance of tissue homeostasis throughout life and have emerged as a major cellular source for cell-based therapies in the field of regenerative medicine. The focus of research in this department is to understand the basic biology of adult ocular stem cells in its native condition and to develop better cell-based therapies for several ocular diseases/ conditions. Last year molecular studies were carried out to identify the (i) trabecular meshwork stem cell derived exosomal cargos responsible for tissue regeneration; (ii) factors regulating retinal pigment epithelial stem cells and (iii) factors regulating the maintenance of lens epithelial homeostasis and changes in cataractous condition.

Characterization of adult human lens epithelial stem cells in the maintenance of tissue homeostasis throughout life and their functional status in cataractous lens

Investigator : Dr. Madhu Shekhar
Co-Investigators : Dr. Gowri Priya Chidambaranathan
Dr. Haripriya Aravind
Research Scholar : P. Saranya
Funding agency : Science and Engineering Research Board

Introduction including Background

The crystalline lens is entirely derived from a single cell type –the anterior lens epithelial cells. The epithelial cells are known to differentiate into lens fibres throughout life. The objective of this study was to identify and characterize the lens epithelial stem cells (LESCs), their role in maintaining tissue homeostasis and in the development of age related cataract. Having confirmed the presence of stem cells in the central zone of human anterior lens epithelium and their absence in cataractous lens, the current study assessed the regenerative potential of the adult human lens by culturing it (for 5, 10, 15, and 21 days) with and without the neural retina. The histological changes of the cultured lens at each duration were analyzed by Haematoxylin and Eosin staining and immunostaining for the stem cell marker SOX2, proliferating cell marker Ki-67, epithelial cell marker E-cadherin, and late elongation marker γ-crystallin.

Results

Characterization of adult human lenses cultured with and without neural retina:

In lens cultured without neural retina, FGF induced the epithelial cell proliferation, migration towards the posterior capsule, and encircled it after 21 days of culture. In contrast, in lens cultured with neural retina, the epithelial cells differentiated into lens fibres and orientated along with the fibre compartment (Figure 1). Thus, the neural retina played a significant role in ceasing cell migration while promoting fiber differentiation.

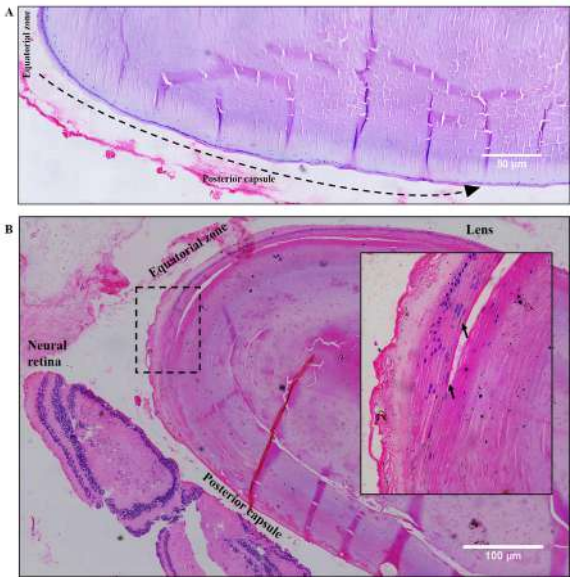


Figure 1: Histology of human lenses cultured with and without neural retina for 21 days. H&E stained paraffin sections of human lenses cultured without neural retina for 21 days showed epithelial cells encircing in the posterior capsule (dotted arrow) (A). The lenses cultured with neural retina exhibited lens epithelial-to-fibre differentiation at the equatorial zone (B). Higher magnification of highlighted equatorial zone given as an inset (arrow in the inset indicates fibre differentiation).

differentiation only at the equatorial zone (Figure 1; Table 1). The above findings indicated the importance of neural retinal signalling in preserving the lens epithelial homeostasis.

Conclusion:

The current study demonstrated the maintenance of lens tissue homeostasis by neural retina - the induction of fibre differentiation at the equatorial zone and maintenance of the epithelial cells in the anterior region.

Decline in YAP nuclear expression in lens epithelium of cataractous donor compared to healthy donor lens

Investigator : Dr. Gowri Priya Chidambaranathan
Co-Investigator : Dr. Madhu Shekhar
Research Scholars: Thushmitha P, Saranya P
Funding agency : ICMR fellowship

Table 1: Expression level of SOX2, Ki-67, γ crystallin, E-cadherin markers in lenses cultured with and without neural retina

S. No.	Markers	Cultured lenses							
		Lens without Neural Retina				Lens with Neural retina			
	Culture days	5	10	15	21	5	10	15	21
1	SOX2	+++	+	+	+/-	+++	+	+	+/-
2	Ki-67	+++	++	++	++	+++	++	++	++
3	γ crystallin	+++	+++	+++	+++	-	-	-	-
4	E-Cadherin	-	-	-	-	++	++	++	++

+++ indicates the higher expression; ++ indicates the moderate expression; + indicates the minimal expression; - indicates the negative expression.

Characterization of the lenses cultured with and without neural retina revealed the higher nuclear expression of SOX2 (lens-84.8 ± 9%; lens with neural retina-80.3 ± 4.5%) and Ki67 (lens-89.6±10.4%; lens with neural retina-90.7±4.4%) after 5 days of culture, which reduced after 10 days. The expression of γ crystallin, was observed consistently across all epithelial cells during the culture period, suggesting the initiation of lens fiber differentiation in lens cultured without neural retina. Lens cultured with neural retina revealed the expression of γ crystallin exclusively in the equatorial zone. Furthermore, the cytoplasmic expression of E-cadherin only in lens cultured with neural retina indicated that cells retained their epithelial characteristics, and fiber

Introduction including Background

YAP (Yes-associated protein) is a transcriptional co-activator protein that regulate target gene expression via binding interactions with TEAD (TEA/ATTS domain) transcription factors. Nuclear localization of YAP has been reported to be associated with stemness in various tissues including human eyes. Moreover conditional knockout of YAP in mice lead to the development of cataract, suggesting YAP as a promising target for cataract therapy. Hence this study aims to analyze the expression of YAP along with alpha-SMA and/or SOX2 in whole mounts of the anterior human lens epithelium of normal and cataractous donor lens by immunostaining.

Results:

Expression of YAP and SOX2 in normal lens epithelium

The expression of nuclear YAP and SOX2 was identified only in the central and equatorial zones of normal lens epithelium (Figure 1).

Expression of YAP in cataractous lens epithelium

In cataractous lens epithelium, YAP expression was observed in both the nucleus and cytoplasm in the central zone (Figure 2). However, the expression was cytoplasmic in the cells of the equatorial zone. In both normal and cataractous lenses, nuclear YAP expression was observed more in central zone of

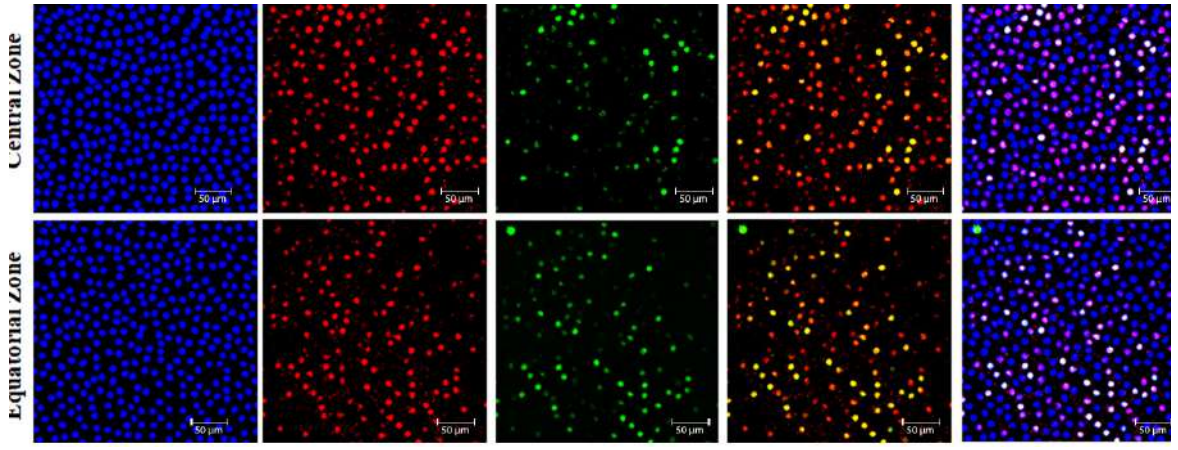


Figure 1: Representative confocal microscopic images revealed nuclear YAP and SOX2 expression in both the central and equatorial regions of normal lens epithelium

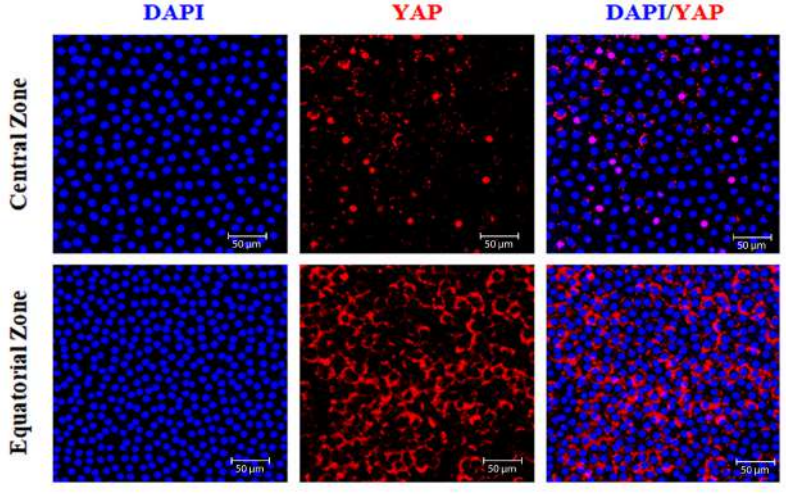


Figure 2: Representative confocal microscopic images revealed the nuclear and cytoplasmic YAP expression in the central zone while it was cytoplasmic in the equatorial zone in cataractous lens epithelium.

Quantification of YAP+ Cells, YAP+ + SOX2+ Cells

Table 1: Percentage of YAP+ Cells, YAP+ and SOX2+ Cells in normal and cataractous conditions.

Zone	Normal (n=3)		Cataract (n=4)
	YAP^+ Cells (%)	YAP^+ and $SOX2^+$ Cells (%)	YAP^+ Cells (%)
Central zone	21.89 ± 12.53	7.25 ± 3.63	2.92 ± 3.86
Equatorial zone	16.28 ± 12.36	9.15 ± 6.09	0.96 ± 1.79

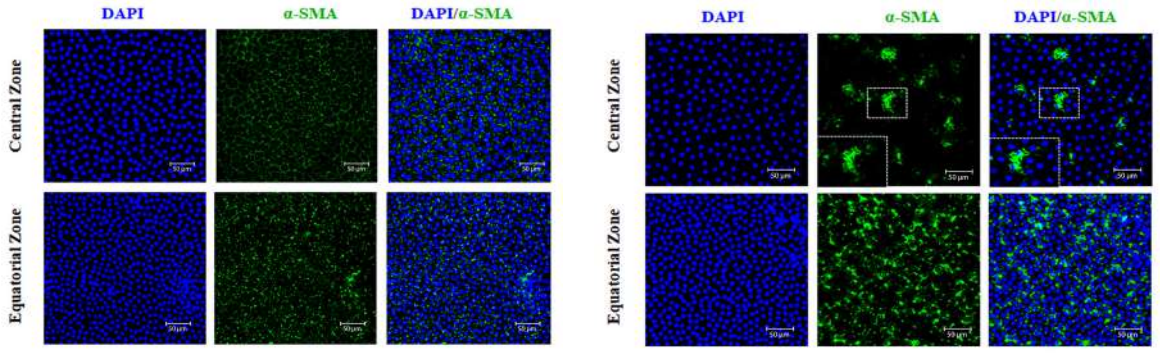


Figure 3: Representative confocal images showing α-SMA *expression in central and equatorial zone cells of normal (A) and cataractous (B) lenses.*

lens epithelium when compared to equatorial zone. Moreover in cataractous condition a reduction in the nuclear YAP expression was observed in both central and equatorial zones (Table 1). While cells double positive for SOX2 and YAP were identified in normal lens, such cells were absent in cataractous lens epithelium. This confirms the probable role of stem cells in the development of cataract.

Expression of α-SMA in

α-SMA is an epithelial to mesenchymal transition marker (EMT) and it was observed in both normal and cataractous lens. It showed higher expression and complex patterns including stress fibre-like structures in the cataractous human lens epithelium (Figure 3).

This indicated that α-SMA is present in normal human lens epithelium but its complex patterns might be associated with cataract development.

Quantification of α-SMA+ Cells

The expression of α-SMA was higher in cataractous lens compared to normal lens (Table 2)

Table 2: Percentage of α-SMA+ Cells were analysed in both normal and cataractous conditions.

Zone	Normal (n=3)	Cataract (n=4)
	α-SMA$^+$ Cells (%)	α-SMA$^+$ Cells (%)
Central zone	0.22 ± 0.38	5.39 ± 8.91
Equatorial zone	2.61 ± 1.04	11.75 ± 16.55

Conclusion

The absence of SOX2 positive cells, reduction in nuclear YAP positive cells, and increased α-SMA expression in cataractous lens provided additional proof for a probable role of stem cells in the development of cataract.

Role of trabecular meshwork stem cell-derived extracellular vesicular miRNAs in human trabecular meshwork regeneration

Investigators : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Dr. S. R. Krishnadas, Prof. K. Dharmalingam
Research Scholar : R. Iswarya
Funding agency : Sun Pharma, CSIR-UGC Senior Research Fellowship

Introduction including Background

Previous studies from this laboratory have shown that TMSC-derived small EVs (sEV) mitigated oxidative stress-induced cell death by reducing intracellular reactive oxygen species (ROS) and enhanced TM cell migration, proliferation, and survival. The aim of this study was to identify the molecular cargo responsible for the increased functional efficacy of TMSC sEV. The protein profiles of the TM and TMSC sEV were deciphered by mass spectrometry and NanoString analysis was used to identify the miRNA cargo.

Results

Protein Profiling of TM and TMSC sEV by mass spectrometry

Mass spectrometry analysis of TM and TMSC sEV proteins identified 2848 proteins in TM and 2802 proteins in TMSC. Comparison of these data sets with the ExoCarta revealed the identification of the top 93 proteins out of the top 100 in ExoCarta, which include the predominantly reported EV markers- CD9, CD63, CD81, Alix, TSG101, Syntenin, and HSP70 (Figure 1).

To determine whether sEV retains a molecular signature of the parental/source cell, we examined the TM and stem cell markers in the corresponding sEV. Interestingly, the MSC markers CD90 (THY-

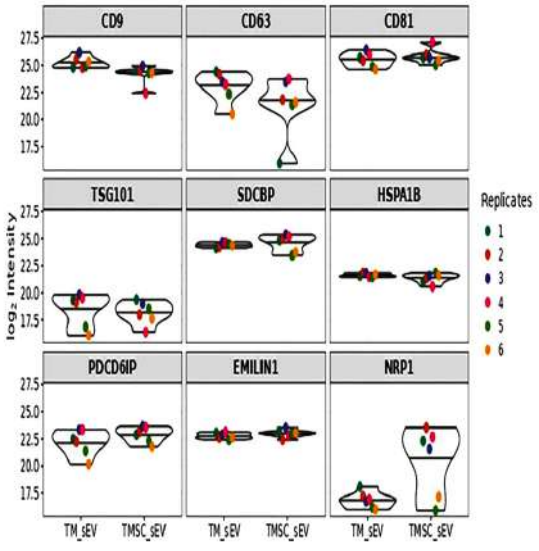


Figure 1: Violin plot representing the expression of common EV markers in TM and TMSC sEV. Each dot represents a replicate

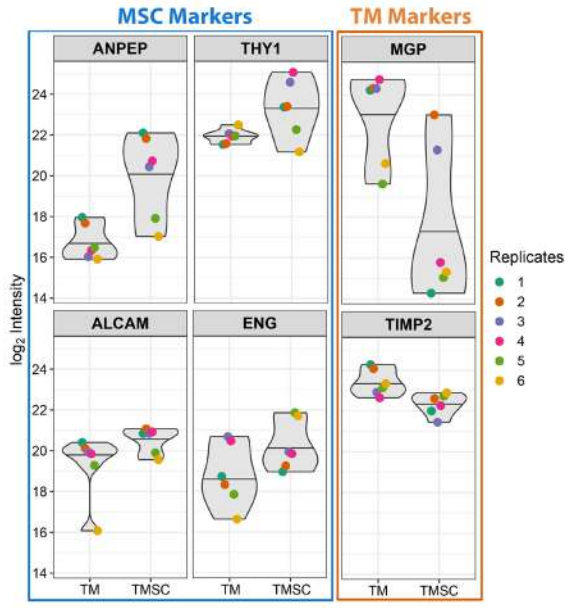


Figure 2: Expression of MSC and TM markers in the sEV. Each dot represents a replicate

1- 1.38 fold), CD13 (ANPEP-2.99 fold), CD166 (ALCAM-1.44 fold), and CD105 (ENG-1.55 fold) were upregulated in TMSC sEV compared to TM sEV (Figure 2). In contrast, differentiated TM cell markers Matrix Gla Protein (MGP-5.46 fold), and tissue inhibitors of metalloproteinase (TIMP-1.08 fold) were up-regulated in TM sEV compared to TMSC sEV (Figure :

Pathway Analysis

Pathway analysis revealed the presence of regulatory proteins associated with integrin-mediated signaling, PI3-AKT, MAPK pathway, TGF beta signaling, BMP signaling, HIF signaling, mTOR signaling, and Wnt signaling pathways (Figure 3).

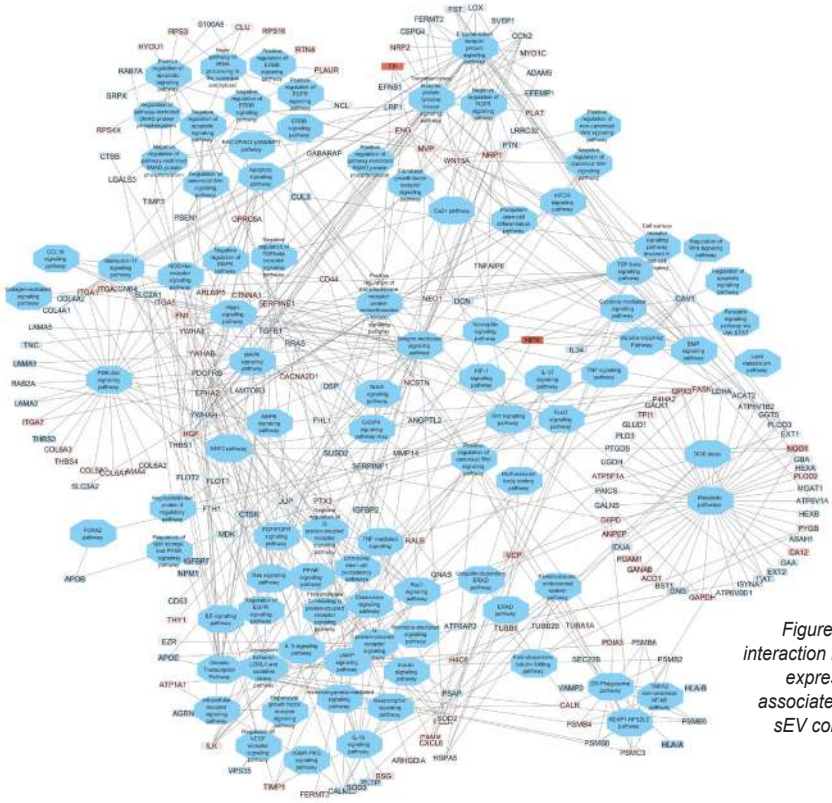


Figure 3: Protein-protein interaction network of differentially expressed proteins and associated pathways in TMSC sEV compared to TM sEV.

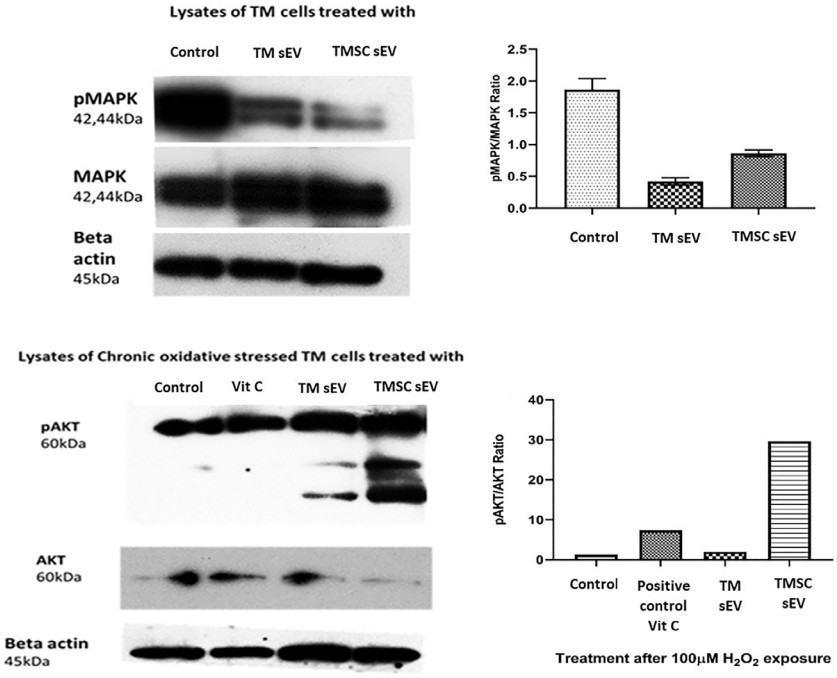


Figure 4: Validation by western blotting: A) Effect of sEV treatment in the MAPK pathway of TM cells. B) Effect of sEV treatment in the P13-AKT pathway of TM cells under chronic oxidative stress. Band intensity and phosphorylated to total protein ratio were calculated using image J software.

Validation by Western blotting:

In the mass spectrometry data, a higher number of proteins involved in PI3-AKT and MAPK signaling pathways were identified. Since these pathways have also been already reported to be associated with TM and in the therapeutic aspects of glaucoma, the expression level of this pathway protein in sEV-treated TM cells was examined by western blotting. The band intensity quantification revealed a reduction in the levels of pMAPK/MAPK ratio indicating the down-regulation of the MAPK pathway upon TMSC sEV treatment compared to the TM sEV and control (Figure 4). Further, as activation of the PI3-AKT signaling pathway is known to be responsible for the TM cell survival under oxidative stress, chronic oxidative stressed TM cells were treated with TM/TMSC sEV. Analysis of the pAKT/AKT ratio after western blotting revealed that the TMSC sEV treatment enhanced the phosphorylation of AKT, thereby activating the pathway in TM cells under chronic oxidative stress. Interestingly, with TMSC sEV phosphorylation in the isoforms of AKT was higher which was not observed in control.

miRNA Cargo analysis

RNA from the TMSC and TM sEV were isolated and miRNA profiling was carried out on NanoString nCounter SPRINT. Heat map analysis of the miRNA data from all three donors (Figure 5) indicated distinct clustering of miRNAs from TMSC and TM sEV.

Further analysis indicated that 95 of the miRNAs were significantly altered between TMSC and TM sEV. The top ten upregulated and down regulated miRNAs are given as bar graph (Figure 6). Further analysis is essential to identify the role of these upregulated and downregulated miRNAs.

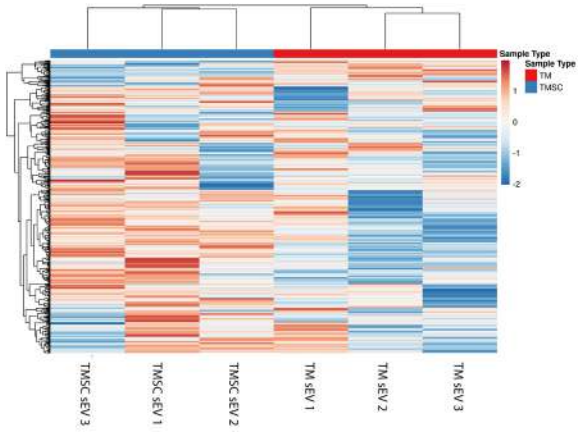


Figure 5: Heat map analysis illustration of TM and TMSC sEV miRNA cargo

Conclusion

Mass spectrometry analysis, followed by validation, confirmed that TMSC-derived sEV are capable of modulating key signaling pathways, including the MAPK and PI3K-AKT pathways which are crucial for the TM cell proliferation and survival under oxidative stress conditions. Further analysis of identified miRNAs are being carried out to elucidate their targets and the molecular mechanism associated with the increased functional efficacy of TMSC-sEV.

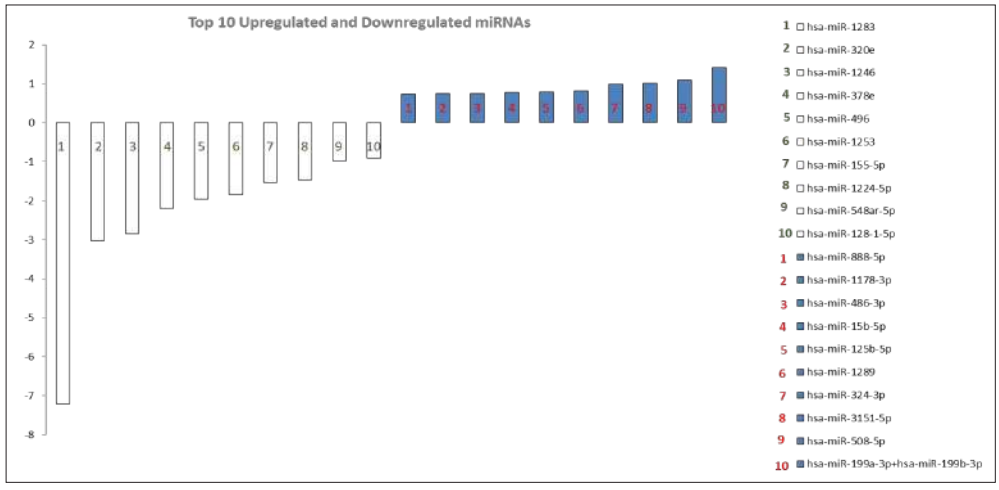


Figure 6: The top ten upregulated and downregulated miRNA in TMSC sEV in comparison with TM sEV.

Molecular regulators associated with the maintenance of human trabecular meshwork stem cells in relation to their reduction in ageing and glaucoma

Investigators : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Dr. S. R. Krishnadas
Dr. D. Bharanidharan
Research Scholar : Sneha Nair
Funding Agency : Science and Engineering Research Board

Introduction including Background

Previous studies from this laboratory confirmed the presence of stem cells in the anterior non-filtering region (NF) of the meshwork, with a significant reduction in their content with ageing, and a drastic reduction in glaucomatous condition. This study aims to investigate the molecular regulators involved in the maintenance of TMSCs and their progressive reduction with aging and glaucoma. By identifying key signaling pathways, transcription factors, and epigenetic regulators that govern TMSC self-renewal, proliferation, and differentiation, this study seeks to uncover potential therapeutic targets for glaucoma treatment. MiRNA profiling of the filtering (F) and non-filtering (NF) regions of three donor tissues was carried out last year to identify the miRNAs regulating TMSCs using Nanostring nCounter SPRINT.

Results

MiRNA profiling of filtering and non-filtering TM

Analysis of miRNA data using nSolver identified 26 significantly up regulated miRNAs and three down regulated miRNAs in the NF region of TM with fold change >±1.2, p-value < 0.05. Among them, the top 10 significantly up-regulated miRNAs were- hsa-miR-564, hsa-miR-1296-5p, has-miR-1255a, hsa-miR-519d-3p, hsa-miR-548k, hsa-miR-3168, hsa-miR-5010-3p, hsa-miR-887-5p, hsa-miR-649, hsa-miR-210-5p and the three down-regulated were miRNAs- hsa-miR-376a-3p, hsa-miR-22 3p, hsa-miR-145-5p. Based on literature on stem cells, seven up regulated and three down-regulated miRNAs were selected for RT-PCR analysis which confirmed the differential expression of the selected miRNAs in the NF region.

Analysis of the expression of the differentially expressed miRNAs in TM of different age groups

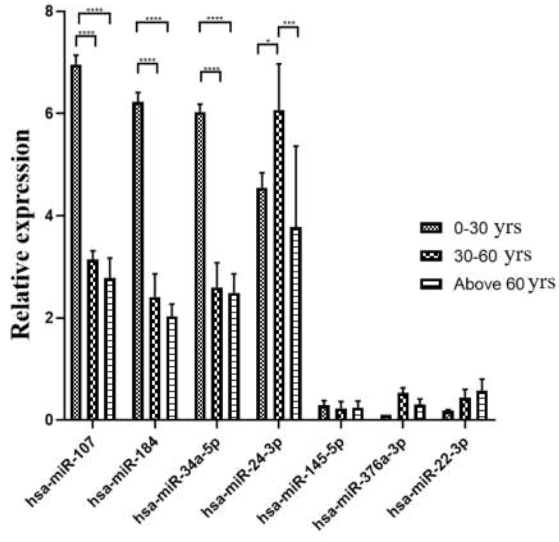


Figure 1: Validation of miRNA expression across different age groups using real-time PCR. The data was expressed as mean ± SD and relative fold change of expression (RQ) was calculated by 2-ΔΔCT method after normalization with U6 control. Statistical significance was determined using two way ANOVA, where p<0.05 was considered significant. Asterisks Indicate statistical significance: p<0.000001 (****), p<0.00001(***).

Functional enrichment analysis of validated miRNAs

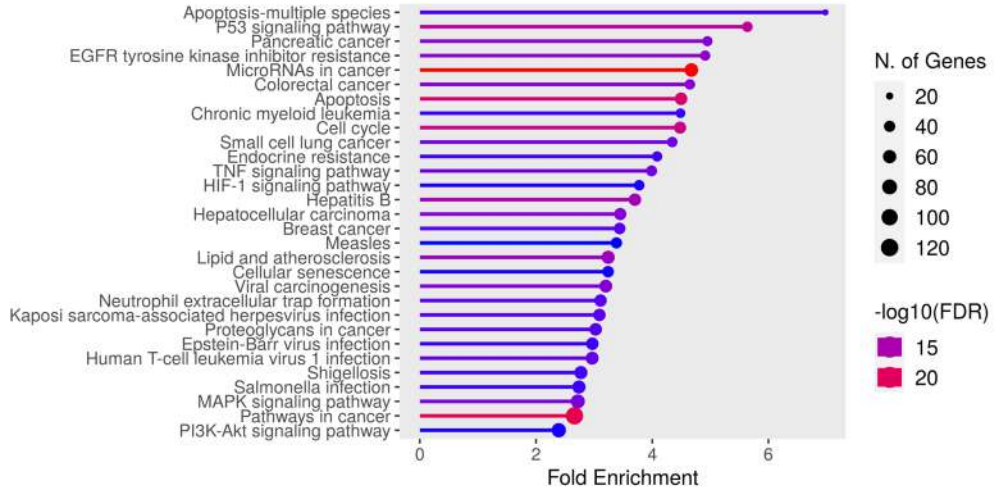


Figure 2a: KEGG pathway analysis of significantly up-regulated miRNAs in NF region compared to F TM

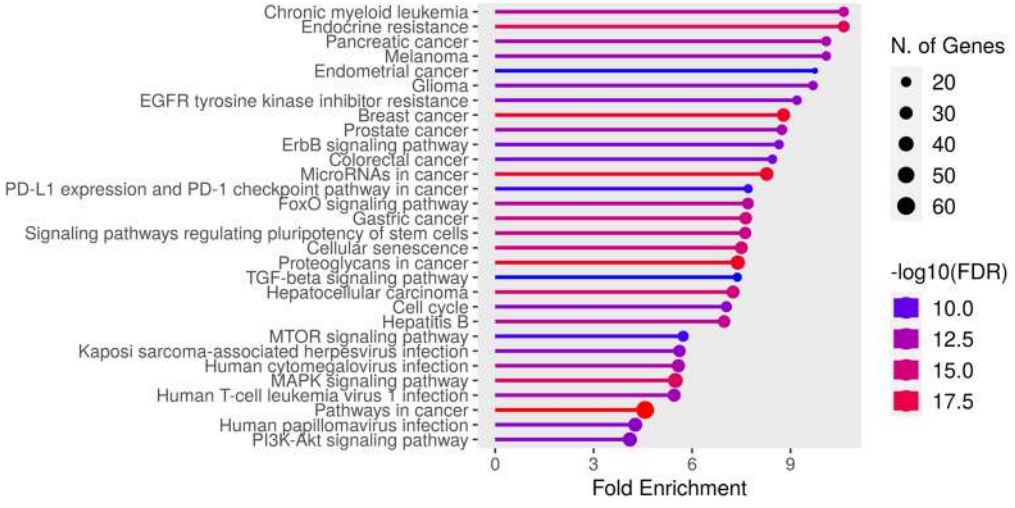


Figure 2b: KEGG pathway analysis of significantly down-regulated miRNAs in NF region compared to F TM

revealed that the up-regulated miRNAs – hsa-miR-107, hsa-miR-184 and hsa-miR-24-3p in the NF region to reduce with ageing. This was in concordance with the previous finding from this laboratory that there was an age-related reduction in TMSCs residing in the NF region (Figure 1).

KEGG pathways analysis identified MAPK signalling pathway and PI3K-Akt signalling pathway as the most enriched pathways targeted by differentially expressed miRNAs in both up-regulated and down-regulated miRNAs (Figure 3). MAPK1, MEK, Ras, Raf, ERK, PI3K and AKT were associated with multiple miRNAs. Hypoxia signalling - HIFs (HIF 1α and HIF 1β) play important roles in modelling cellular metabolism in both stem cells and niches to regulate stem cell biology, which represent an additional dimension that allows stem cells to maintain an undifferentiated status and multilineage differentiation potential.

Conclusion

MiRNAs specific to the NF region of TM, where the stem cells are located were identified. The probable association of these miRNAs in the maintenance of TMSCs through down regulation of the specific targets involved in MAPK, PI3-AKT and HIF signalling requires further validation.

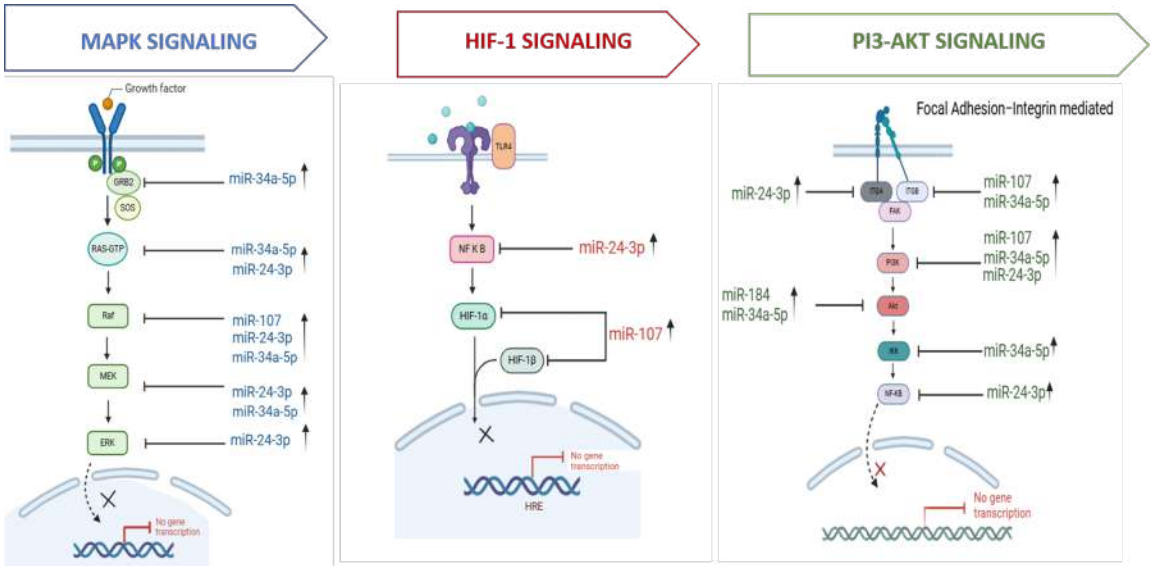


Figure 3: Postulated mechanism of regulation of MAPK, HIF and P13-AKT signalling by the validated miRNAs.

Molecular characterization of human retinal pigment epithelial stem cells and their role in age related macular degeneration

Investigators : Dr. Gowri Priya Chidambaranathan
Co-Investigators : Dr. D. Bharanidharan
Dr. Kim Ramasamy
Dr Siddharth Narendran
Research Scholars: Waseema A and Kanthimathi R
Funding Agency : Indian Council of Medical Research

Introduction including Background:

Retinal Pigment Epithelium (RPE) is a pigmented monolayer beneath the photoreceptors of the human eye. It is important for the functioning of photoreceptors and its degeneration leads to age-related macular degeneration, a leading cause of blindness in the elderly population. Previous studies from this laboratory identified the presence of stem cells (SCs) in the peripheral region of human RPE based on their functional characteristics and demonstrated a reduction in their number with ageing.

However, specific biomarkers to confirm the exact location of these RPESCs in native tissues are still not known. Hence, this study aimed to identify putative SC markers by meta-data analysis and confirmed its expression at mRNA level in native human RPE.

Results

Identification of stem cell-associated genes expressed by human RPE:

Through a literature survey, three research articles describing the genes expressed by various clusters of cells in human RPE, identified by single-cell RNA sequencing technology, were selected for metadata analysis. The retrieved genes were analyzed for their association with stemness through a PubMed search. Upon analysis, (i) a set of 81 genes, listed in atleast any two datasets, and (ii) a set of 12 genes, common in all three datasets, were identified to be associated with stemness.

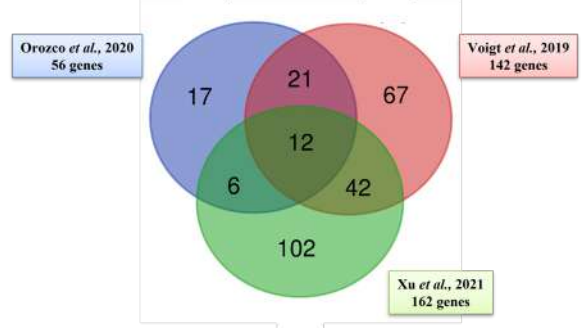


Figure 1: Venn diagram representing the number of stem cell - associated genes identified in three publicly available single cell RNA datasets of human RPE. Twelve genes were common in the three datasets.

Table 1: List of SC-associated genes identified to be common in all 3 single-cell RNA sequencing datasets of human RPE and their function.

GENE	FUNCTION	REFERENCE
MET	Maintenance of stemness property	Gu et al., 2021 (PMID: 34795238)
BMP7	Induce differentiation of SC	Tóth et al., 2021 (PMID: 34201124)
WWC1	Maintenance of stemness property	Mussell et al., 2018 (PMID: 30042827)
ARHGAP18	Maintenance of stemness property	Xu et al., 2019 (PMID: 31130822)
NEAT1	Maintenance of stemness property	Yang et al., 2017 (PMID: 27878295)
RDH10	Maintenance of stemness property	Murakami et al., 2022 (PMID: 35634847)
SPOCK1	Promotes proliferation	Luo et al., 2022 (PMID: 35360859)
RBM47	Promotes proliferation	Li et al., 2020 (PMID: 32218862)
NFIB	Maintenance of stemness property	Choi et al., 2023 (PMID: 37633334)
FTH1	Self-renewal of SC	Kanojia et al., 2012 (PMID: 22997041)
SH3KBP1	Self-renewal of SC	Song et al., 2021 (PMID: 33643898)
PTGDS	Self-renewal of SC	Zhang et al., 2018 (PMID: 29604141)

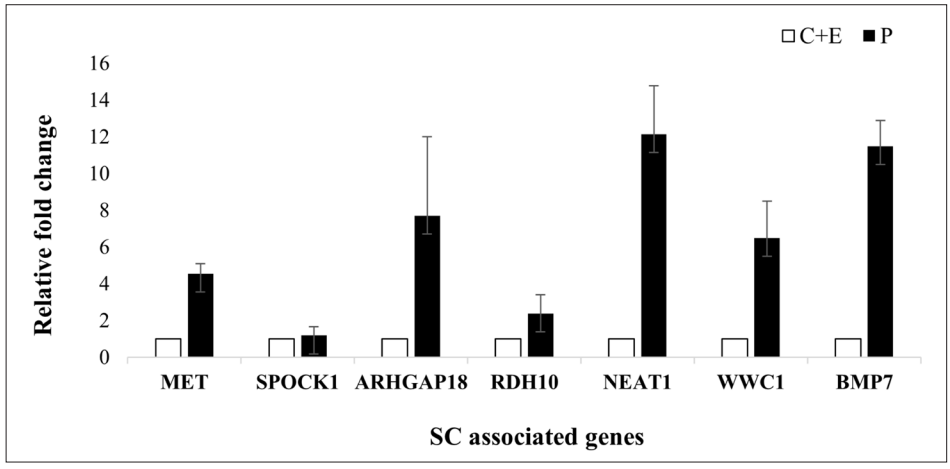


Figure 2: Graphical representation of the relative qualification of the expression of SC-associated genes in the peripheral, compared to equatorial and central RPE. Error bar represents standard error mean.

To validate the above findings, the expression of SC-associated genes common to all three datasets was examined in different regions of native human RPE by qRT-PCR. Among the 12 genes analysed, the expression of 7 genes, including MET, SPOCK1, ARHGAP18, RDH10, NEAT1, WWC1, and BMP7 was observed in native RPE. In addition, their expression was higher in the peripheral region compared to the central and equatorial regions, supporting our previous findings on the presence of RPESCs in the periphery. NEAT1, BMP7, and ARHGAP18 were identified as the top three genes with the highest fold change in expression and can be considered as putative biomarkers for RPESCs.

Conclusion

The study identified and validated the higher expression of putative RPESC-specific markers in the peripheral RPE at mRNA level. Further studies at protein level are required to validate the utility of these genes as biomarkers.

PROTEOMICS

The Proteomics Department investigates major ocular diseases contributing significantly to vision loss in the Indian population, including diabetic retinopathy, fungal keratitis, keratoconus, pterygium, and glaucoma. We employ a multi-omics approach to explore disease mechanisms, identify biomarkers, and develop therapeutic interventions. Our research integrates proteomics, transcriptomics, whole-genome sequencing, genome-wide methylation analysis, and metabolomics, alongside advanced cell and molecular biology techniques. The proteomics facility houses two mass spectrometers for both gel-based and non-gel-based proteome analysis of ocular tissues, tears, blood, and cells. Additionally, we study the role of extracellular vesicles (EVs) in ocular diseases, utilizing ultracentrifugation and NanoParticle Tracking Analysis (NS300). The insights gained from our research are translated into diagnostic and therapeutic strategies to enhance disease management and improve patient outcomes.

Stress induced alterations in the Extracellular Vesicles of Aspergillus flavus.

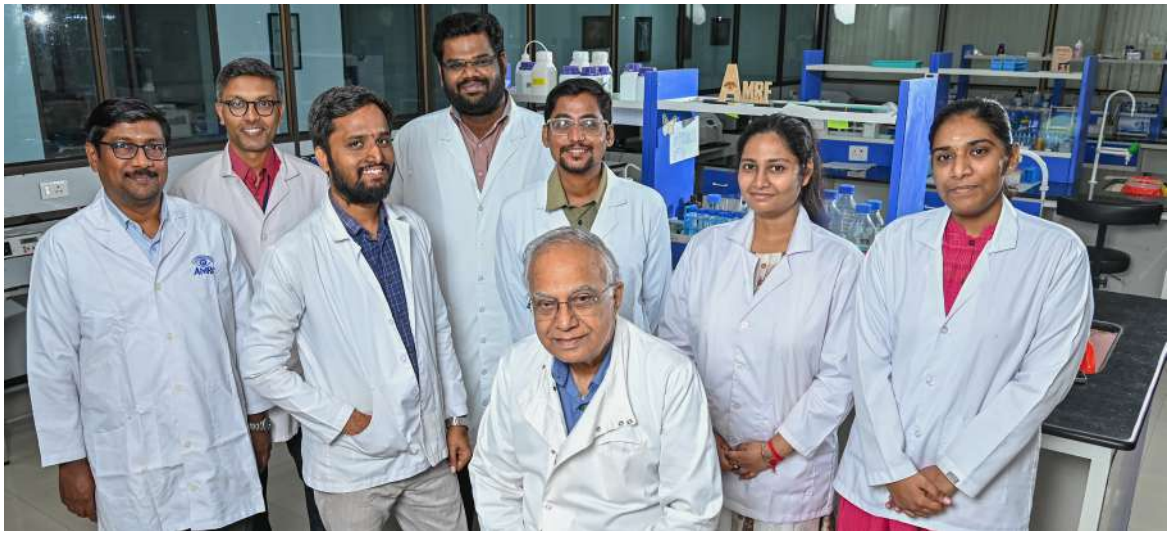
Investigators : Prof. K. Dharmalingam
Dr. Venkatesh Prajna
Dr. Lalitha Prajna
Research fellow : Hariharan Gnanam

Introduction and Background

Extracellular Vesicles (EVs) are non-replicating, lipid bi-layer structures produced by all organisms including bacteria and fungi. These vesicles carry protein, RNA and DNA cargo whose composition and function reflect the cells producing these vesicles. In this study we optimised isolation methods and characterised the proteome of the EVs from *A.flavus* clinical isolates. We explored whether the fungal cells exposed to stress produce EVs carrying the specific stress factors from treated cells, and if yes, whether these vesicles transfer the stress factors to unexposed neibouring cells including the host cells. Congo red (CR) has been historically used for histological examination as well to inhibit the growth of yeast and filamentous fungi. CR presumably binds to β-1,3-glucans in the fungal cell wall, disrupting the organization of the cell wall structure and also cell wall polysaccharide synthesis. This study aims at characterizing the EVs produced by *A. flavus* exposed to CR. We examined the EVs of *A. flavus* saprophyte and Ocular isolates from keratitis patients treated with CR. Clinical isolate MTCC 13369 (isolated from patient who responded to antifungal treatment) and MTCC 13368 (isolated from a patient who did not responded to antifungal treatment and underwent TPK) were used.

Results

All three isolates showed a significant decrease in growth measured by colony morphology at different concentrations up to 300 µg/ml of CR (Fig.1.A). The



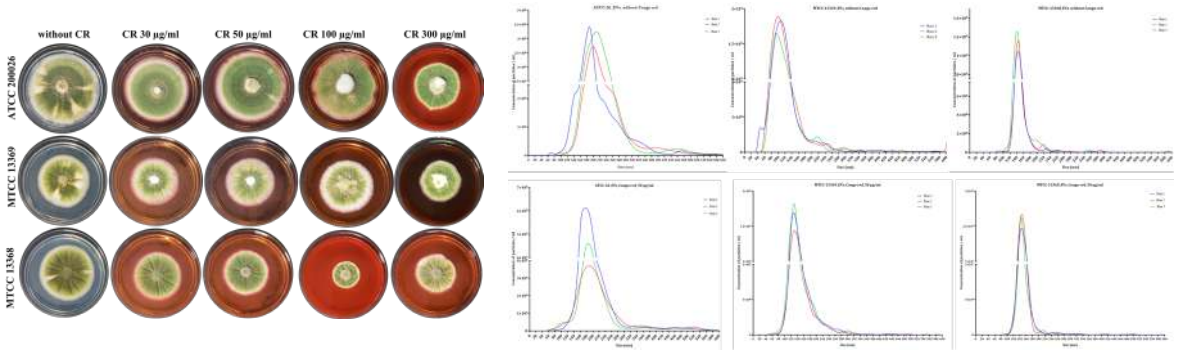


Fig.1. A. Aspergillus flavus colony morphology on Congo red containing plates. B. Particle size distribution: NTA data.

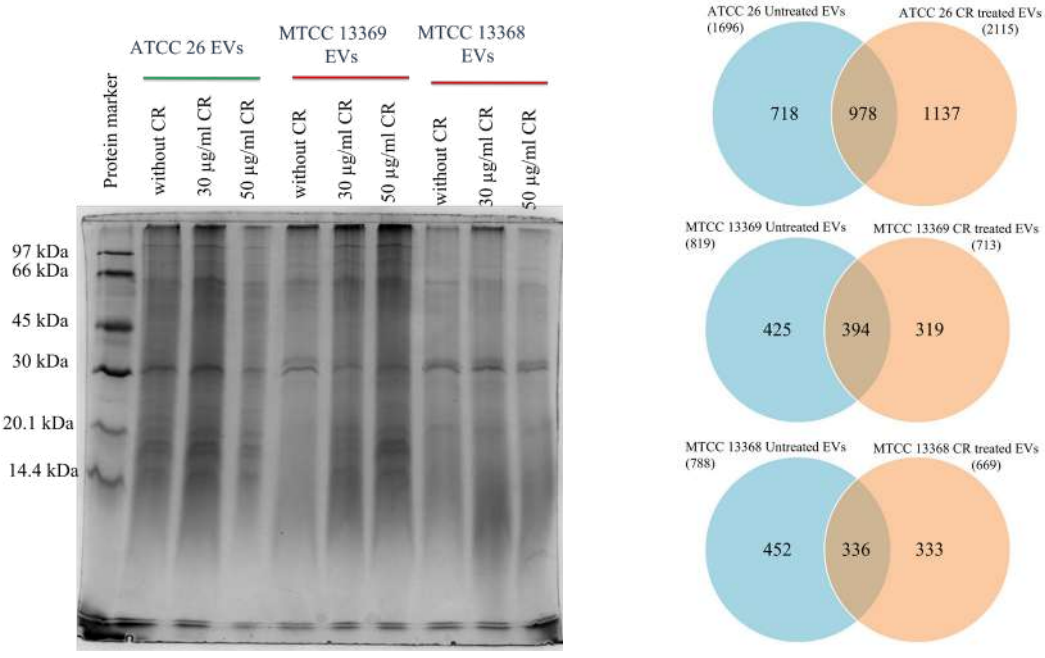


Fig.2. A. Protein profile of Fungal EVs. B. Venn diagram of EV protein cargo .

clinical isolates are more sensitive, based on the colony diameter when treated with even 30µg/ml of CR. Spores were inoculated on CR containing plates and incubated for 48 hrs and the secretome was extracted by flooding the plates with extraction buffer. EVs were isolated from the secretome. The EVs size distribution was analysed compare Nanoparticle Tracking Analysis. The average sizes of the EVs isolated from saprophyte are almost twice the size of clinical isolates Congo red treated altered the size of the EVs only marginally. (Fig.1.B).

Total EV proteins profile of treated and untreated EVs is shown in Fig 2A. EVs proteins were identified using for mass spectrometry are shown in Fig.2.B. The gel profile and the mass spectrometry showed distinct proteins in the EVs from treated cultures compared to untreated cultures.

Gene ontology was performed using FunrichV3.1.3 software (Fig.3.). ATCC26 derived EVs have a higher percentage of expression in cell wall organisation and polysaccharide catabolic processes, whereas CR-treated ATCC26 showed significant decrease in the percentage of expression of these two processes. In the case of clinical isolate 13369, the responder culture, the Congo red treatment led to a decrease in cell wall organisation and polysaccharide catabolic processes in the EV cargo. The difference in the sensitivity of the two

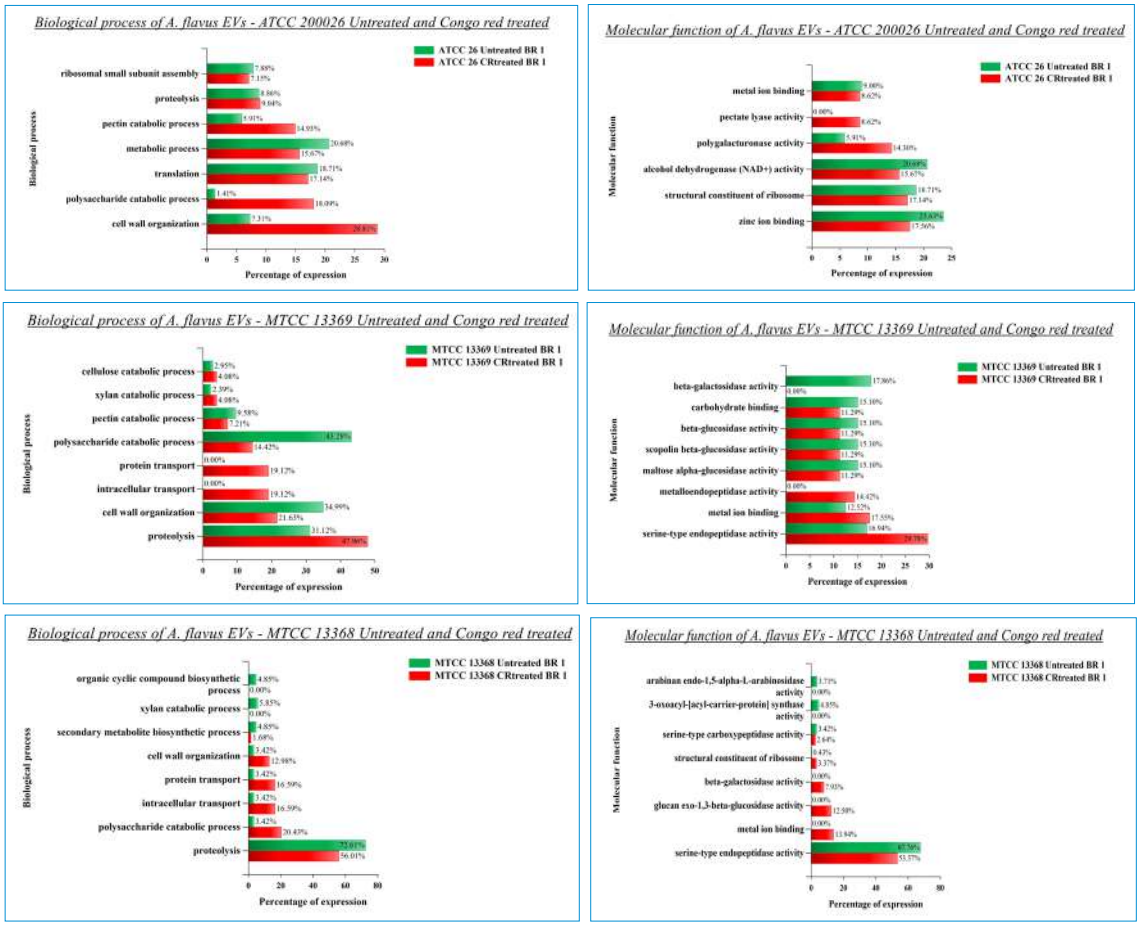


Fig.3. Gene enrichment Analysis of A. flavus Extracellular vesicles

isolates and their correlation with the altered cell wall structure are under investigation. Interestingly the cell wall organisation and polysaccharide catabolic process are up regulated in Congo red treated non responder cultures as in the case of saprophytes.

Isolation and Characterisation of Extracellular Vesicles from Human tear film

Investigators : Prof. K. Dharmalingam
Research Fellow : Karthik Alagarsamy

Background and Introduction

Study of EVs from human tear remains a challenge due to the low volume of tear available from a single individual. Traditional methods of EV isolation using ultracentrifugation (UC) are feasible only when tear from multiple patients are pooled. Titanium dioxide (TiO_2) microparticles offer a selective and efficient approach for EV isolation by exploiting their affinity for phosphate groups on the EV surface. We developed a method of isolation using TiO_2 and the isolated vesicles are characterised with respect to the size, concentration, and protein composition. Our results show that this method could be used for the isolation of EVs from small volumes of tear

Results and Discussion

Nanoparticle Tracking Analysis (NTA):

The size distribution and number particles of isolated EVs were analysed with nanoparticle tracking Analysis. The average of duplicate experiments was done. NTA data indicated that the average diameter of the of control tear EVs was approximately 150 nm and the tear EV from the keratitis patient tear were 200 nm. The average particle concentration of EVs isolated from control, and keratitis patient tear were approximated 1-2 X108 per ml of tear.

Protein profile of EVs

Protein profile of the tear film from control and keratitis patient tear are shown in fig1a. Compared to the tear film the amount of protein found in the EV cargo was much less (Fig.1b).

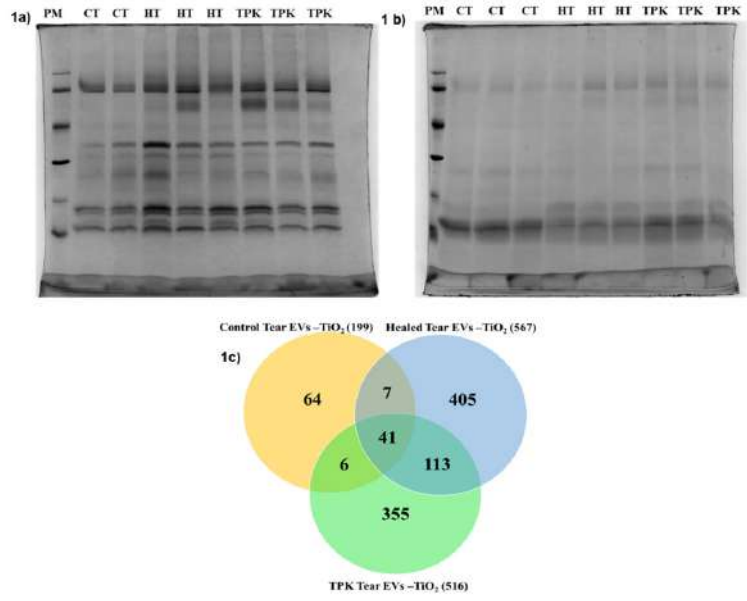


Fig.1. a) The 1D gel electrophoresis of tear protein from control tear (CT), Keratitis patient tear Healed Tear (HT) and Keratitis patient tear from Non responder tear (TPK), b) The 1D gel electrophoresis of Tear EV protein profile prepared by TiO_2 method from control tear (CT), Keratitis patient tear Healed Tear (HT) and (TPK), c) Number of protein identified through In gel digestion (Shotgun) from Tear EVs prepared by TiO_2 method.

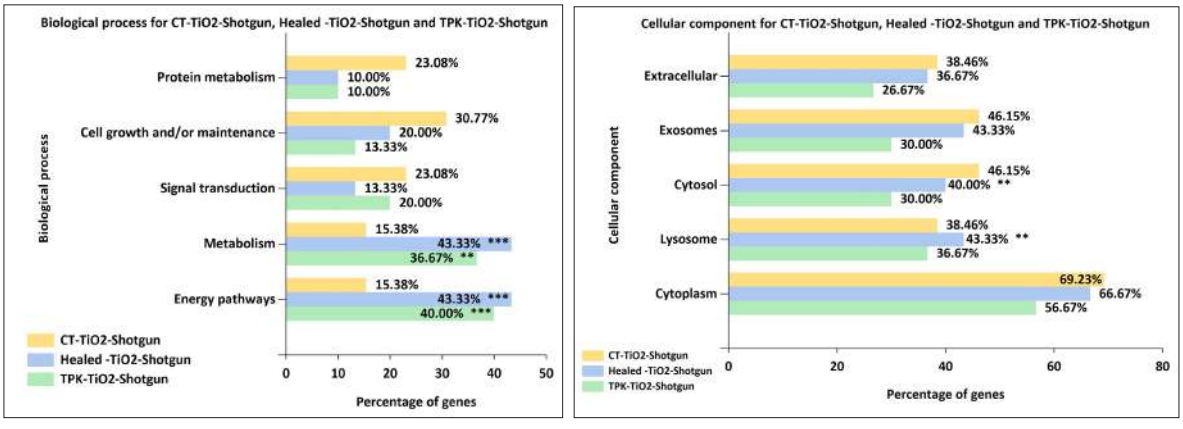


Fig.2. Fun rich Analysis of tear EV form control tear (CT), Keratitis patient tear Healed Tear (HT) and (TPK), isolated by TiO_2method. Top 5 significant categories are listed in biological processes (2a) and cellular components (2b)

Protein analysis of Tear EVs isolated by TiO_2 Method

More than 500 proteins were identified using from the EVs prepared from the tear films. Data in fig.1c shows the distribution of proteins from the three groups. Each of these groups has distinct protein profile and about 40% of the proteins are shared among the three groups. The identified proteins were further analysed with gene ontology using FunRich_3.1.3 software, which reveals that biological process, cellular component of the samples. Biological process data showed that most of the genes were enriched in metabolism, protein metabolism, and energy pathway and cell communication (Fig 2).

Multiple isoforms Human Tear lysozyme are localised in the Extracellular Vesicle

Investigators : Prof. K. Dharmalingam
Dr. N. Venkatesh Prajna
Dr. Lalitha Prajna
Research fellow : Karthik Alagarsamy

Background of the Study

Lysozyme is an enzyme that plays a key role in the body's immune defence by breaking down bacterial cell walls. Lysozyme also exhibits antibacterial, antiviral, antitumor and immune modulatory activities. It is found in various body fluids, including tears, saliva, seminal fluid, milk and mucus, where it helps protect against infections. Many disorders can be diagnosed with the help of the lysozyme content in bodily fluids. Lysozyme levels in tears are

used as a biomarker for eye health, with reduced levels observed in conditions like conjunctivitis, keratoconjunctivitis sicca (dry eye syndrome), and corneal ulcers. In this study we have shown the existence of three isoforms of lysozyme in the tear. We also demonstrated the lysozyme are localised in the tear EVs.

Result

The profile of neat tears of control and the tear sample from keratitis patients is show in fig.1a. Compared to control the amount of high abundant proteins was increased in the patient tear samples. Protein profiles show that the protein cargos of the EVs resemble the tear film proteins. (Fig.1a). Our previous data show that the band migrating as 14.4 kDa protein is lysozyme.

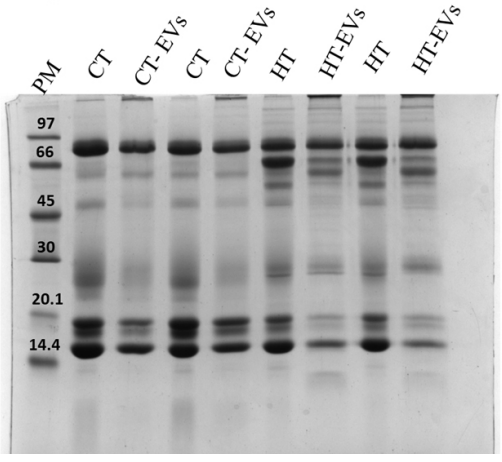


Fig.1a) D profile of neat and EVs of Tear sample-CBB stained

Western blot analysis was used to identify the lysozyme proteins from the tear film and EVs from the tear. HL-60 cell extract was used as control. (Fig.1b). EVs specific marker CD -63, CD9 confirmed the identity of the EVs. As reported previously CD-63 showed multiple bands and CD-9 another EV marker has a single protein of 24kDa. (Fig.1c).

We measured the enzymatic activity of lysozyme using Lysozyme Assay Kit . Lysozyme concentration (Unit /ml) was calculated based on the standard graph from the Lysozyme Assay Kit. We used detergent triton X-100 to lyse the EVs for the measurement of lysozyme activity. The units of lysozyme are shown in the table. Interestingly the EVs from control tear showed much more activity than the EVs from keratitis patient tear,192 units /ml vs 82 units /ml) (Fig.2).

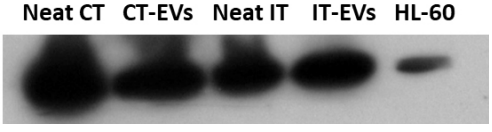


Fig.1b) Blotting image of neat and EVs Lysozome of Tear Sample (5µg)

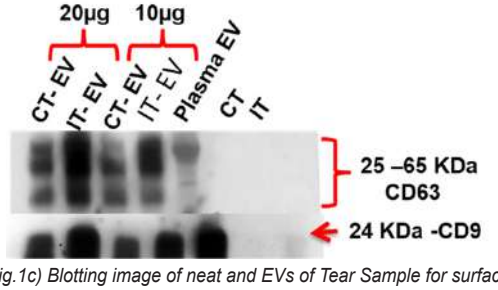
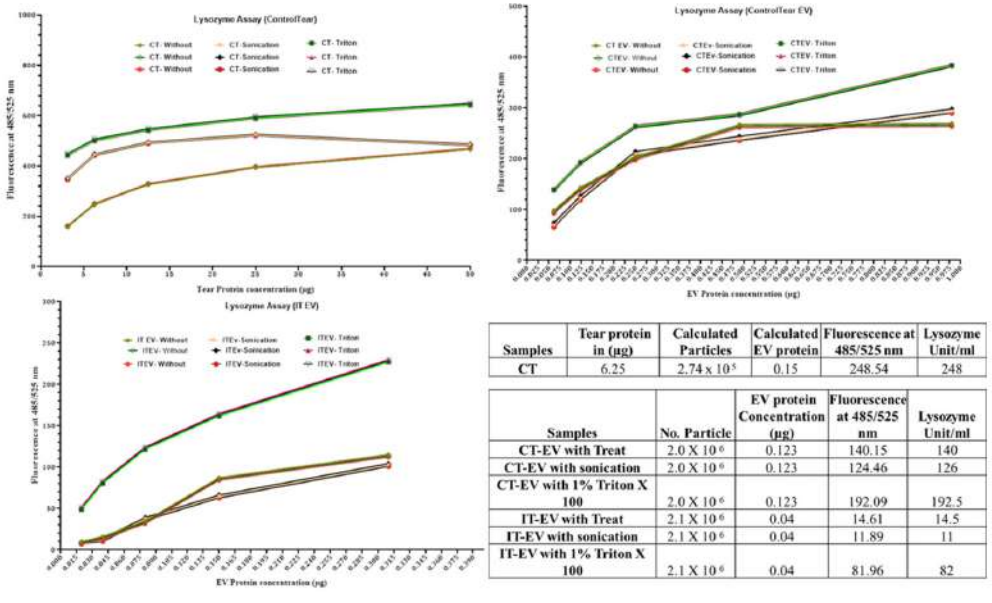


Fig.1c) Blotting image of neat and EVs of Tear Sample for surface markers



Samples	Tear protein in (µg)	Calculated Particles	Calculated EV protein	Fluorescence at 485/525 nm	Lysozyme Unit/ml
CT	6.25	2.74 x 10^5	0.15	248.54	248

Samples	No. Particle	EV protein Concentration (µg)	Fluorescence at 485/525 nm	Lysozyme Unit/ml
CT-EV with Treat	2.0 X 10^6	0.123	140.15	140
CT-EV with sonication	2.0 X 10^6	0.123	124.46	126
CT-EV with 1% Triton X 100	2.0 X 10^6	0.123	192.09	192.5
IT-EV with Treat	2.1 X 10^6	0.04	14.61	14.5
IT-EV with sonication	2.1 X 10^6	0.04	11.89	11
IT-EV with 1% Triton X 100	2.1 X 10^6	0.04	81.96	82

Fig:2) Detection of Lysozyme activity using EnZchek Lysozyme assay kit from neat tear, EVs of Tear and lysed EV samples

Identification of Retinol Binding Protein 3 (RBP3) from vitreous and plasma extracellular vesicles as a predictive biomarker for diabetic retinopathy.

Investigators details: Dr. Daipayan Banerjee,
Dr. Bhavani S, Dr. Sahana K,
Dr. Prithviraj Udaya,
Aadhithiya T. Gr,
Dr. K. Naresh Babu, Dr. R. Kim,
Prof. K. Dharmalingam

Funding : Sun Pharmaceuticals

Introduction including background

Diabetic retinopathy (DR) is a severe microvascular complication of diabetes, progressing asymptomatically in its early stages and often leading to irreversible blindness. With the global diabetic population projected to reach 643 million by 2030, there is an urgent need for reliable predictive molecular signatures of vision-threatening DR (VTDR). Most reported circulatory biomarkers lack evidence of direct involvement in DR pathogenesis, underscoring the need for DR-specific factors that reflect retinal angiogenic pathophysiology. In this study, we utilized extracellular vesicles (EVs), lipid-encased nanovesicles known for their stability in biofluids, to explore the altered protein cargo of vitreous humor-derived small EVs (VH-SEVs) from patients with proliferative DR (PDR), an advanced stage of DR. Shotgun mass spectrometry identified retinol-binding protein 3 (RBP3), a photoreceptor-derived retinoid transporter with protective roles in DR, within VH-SEVs. VH-SEV-associated RBP3 levels were significantly reduced in PDR patients compared to those with macular hole (MH), as confirmed by immunoblotting and ELISA. Additionally, we detected RBP3 in plasma SEVs using immunoblotting and ELISA, revealing a decreasing trend in SEV-RBP3 levels across DR groups, with progressively lower levels in patients with non-proliferative DR (NPDR), and PDR. Notably, plasma SEV-RBP3 levels were significantly lower in diabetic patients with PDR compared to those without retinopathy. In conclusion, this study identifies RBP3, a DR-relevant retinal protein, within circulatory SEVs, highlighting its potential as a biomarker for VTDR and paving the way for its clinical applications.

New results

To validate RBP3 in plasma SEVs, SEVs isolated from plasma samples of patients with Type II diabetes mellitus without retinopathy (DM), healthy individuals without systemic illness (No-DM), as well as patients with non-proliferative diabetic retinopathy (NPDR) and PDR. Plasma SEVs were validated using exosomal markers (ALIX, CD9, CD63, and TSG101) (Figure 1A) and characterized via Nanoparticle Tracking Analysis (NTA) (Figure 1B). NTA analysis revealed no significant differences in the mean diameter or concentration of EVs among the different

Figure 1

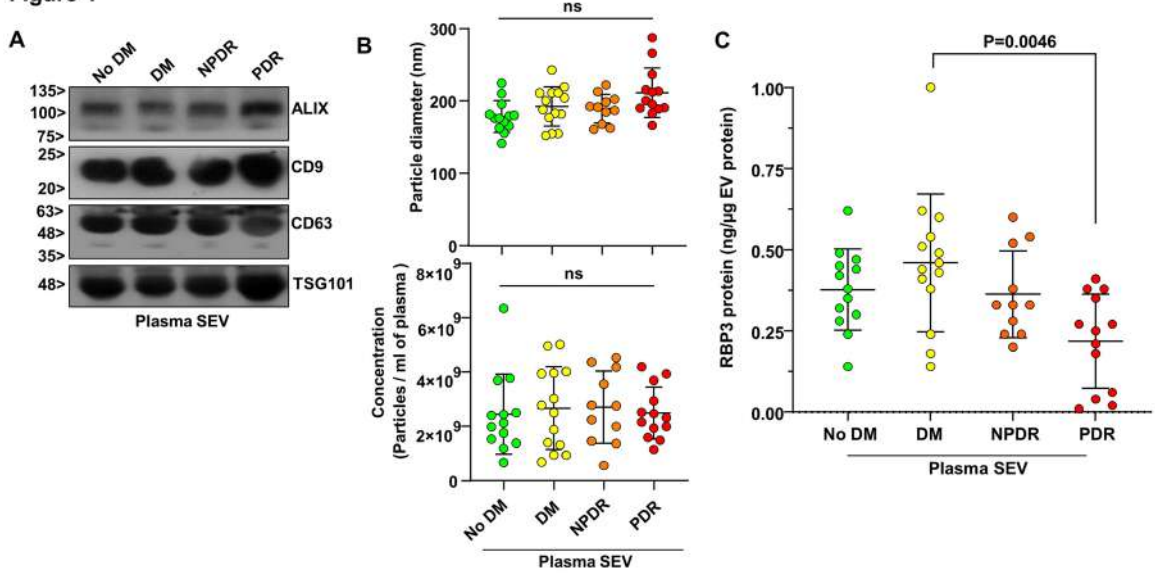


Figure 1. RBP3 is detected in plasma SEVs and is reduced in patients with PDR. (A) Immunoblot for SEV markers (ALIX, CD9, CD63, TSG101) in plasma SEVs from donors without DM (No DM) and patients with DM, NPDR and PDR. (B) Graph showing the diameter and concentration of plasma SEVs measured using NTA. Each data point represents an individual patient, with an average of 15 independent readings per patient. (C) Quantification of plasma SEV RBP3 levels using ELISA in individual patients. Data are presented as mean ± SD. Sample sizes: No DM (n = 13), DM (n = 14), NPDR (n = 11), and PDR (n = 13). Statistical analysis was performed using a Kruskal-Wallis test with Dunn's multiple comparisons test. n.s. indicates no significant difference.

groups. The ELISA established the presence of RBP3 in plasma SEVs and revealed a progressive decreasing trend in SEV-RBP3 levels across DR stages, with lower levels observed in patients with non-proliferative DR (NPDR) and further reduction in those with proliferative DR (PDR). Importantly, plasma SEV-RBP3 levels were significantly lower in diabetic patients with PDR compared to those without retinopathy (Figure 1C).

Conclusion

- Shotgun mass spectrometry identified Retinol Binding Protein 3 (RBP3), a photoreceptor-derived retinoid transporter with protective roles in diabetic retinopathy (DR), within vitreous humor-derived small extracellular vesicles (VH-SEVs).
- Mass spectrometry findings were validated through immunoblotting and ELISA, confirming significantly lower levels of VH-SEV-associated RBP3 in patients with proliferative diabetic retinopathy (PDR) compared to a control group with macular hole (MH).
- RBP3 was also detected in plasma SEVs, with immunoblotting and ELISA revealing a progressive decrease in SEV-RBP3 levels across diabetic and DR stages, showing the lowest levels in PDR patients.
- The study identified a DR-relevant retinal protein RBP3 within circulatory SEVs and underscores that factors challenging to detect in bulk biofluids can potentially be identified within lipid-encased extracellular vesicles.

Proteomic analysis of plasma small extracellular vesicles (SEVs) in patients with diabetic retinopathy (DR) in Indian population.

Investigators details: Dr. Daipayan Banerjee, Dr. Bhavani S, Dr. Sahana K, Dr. Prithviraj Udaya, Aadhithiya T. Gr, Dr. K. Naresh Babu, Dr. R. Kim, Prof. K. Dharmalingam

Funding : Sun Pharmaceuticals.

Introduction including background

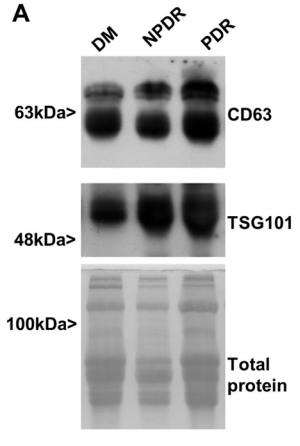
With a broader goal of identifying potential biomarkers and expanding the current repertoire of known biomarkers for a multi-analyte panel for DR prediction in Indian population, this study was aimed to evaluate the proteomic landscape of plasma derived small extracellular vesicles (plasma SEVs).

Results

Plasma SEVs were isolated patients with Type II diabetes mellitus without retinopathy (DM) as well as patients with non-proliferative diabetic retinopathy (NPDR) and PDR (N=4 in each group) using ultracentrifugation, characterized using immunoblotting, Nanoparticle Tracking Analysis and Transmission Electron Microscopy (Figure 2A, B and C).

The plasma SEV protein samples were processed for mass spectrometry as previously described by our group (Shait Mohammed et al., 2019). Peptides detected through mass spectrometry were identified using Proteome Discoverer software (version 1.4) (Table 1).

Figure 2



B

Group	Average concentration (µg/µl)	Average Particles per ml of plasma
DM	3.26±1.08	3.88E+09
NPDR	1.09±0.24	3.00E+09
PDR	4.79±1.13	3.12E+09

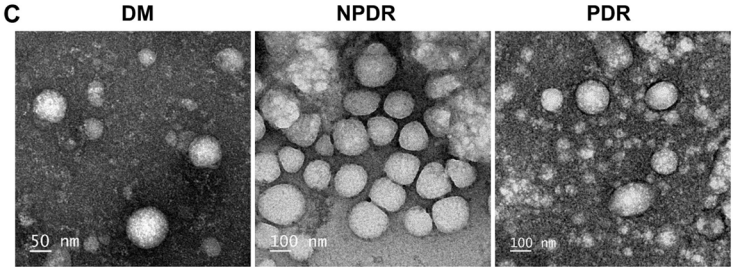


Figure 2: Characterization of plasma SEVs from patients with DM, NPDR, PDR. A. Western blot of exosomal proteins CD63 and TGS101. B. Plasma SEV concentration from NTA and protein concentration using BCA assay C. Transmission Electron Microscopy of plasma SEVs.

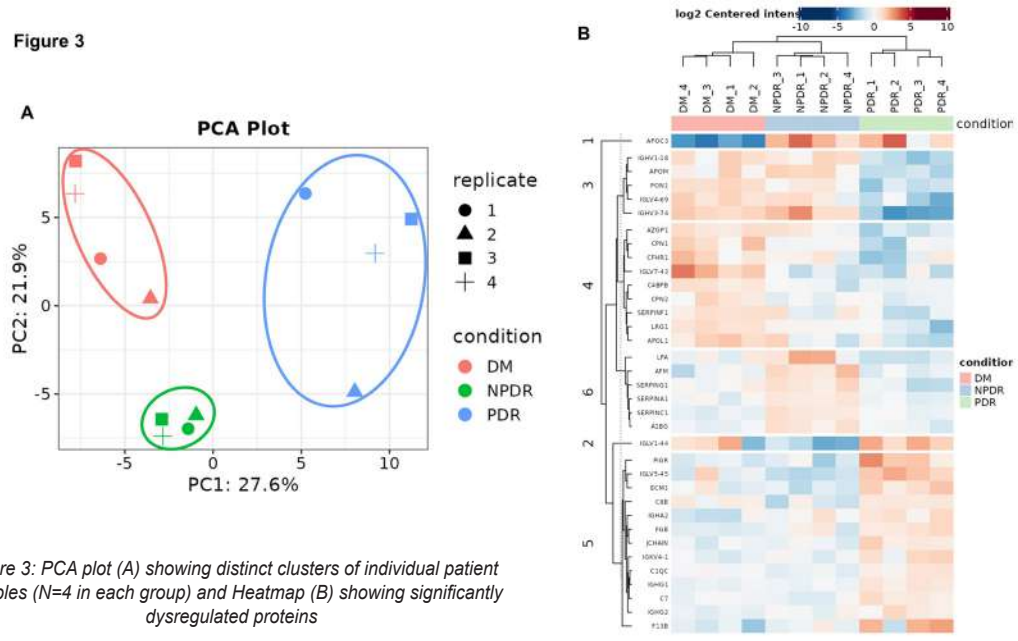


Figure 3: PCA plot (A) showing distinct clusters of individual patient samples (N=4 in each group) and Heatmap (B) showing significantly dysregulated proteins

Table 1: Number of plasma SEV proteins identified by LC-MS

Group	Patient 1	Patient 2	Patient 3	Patient 4
DM	1171	1108	1167	1068
NPDR	1005	1083	1044	1183
PDR	1096	1041	1157	1130

The raw mass spectrometry data was also processed with MaxQuant (version 2.6.3.0), followed by downstream analysis using LFQ-Analyst to generate PCA plot, heatmaps and volcano plots (Figure 3).

The PCA plot (Figure 3A) demonstrates that individual patient samples within each group form distinct clusters, indicating high intra-group similarity. Furthermore, the heatmap (Figure 3B) highlights the significantly dysregulated proteins across the DM, NPDR, and PDR groups. The volcano plot demonstrates the top upregulated and downregulated proteins in pairwise comparison between groups (Figure 4).

Conclusion

This study has identified several dysregulated plasma SEV proteins in patients with DR compared to DM patients without retinopathy. Future studies will entail validation of some of these probable markers in individual patients across DR groups.

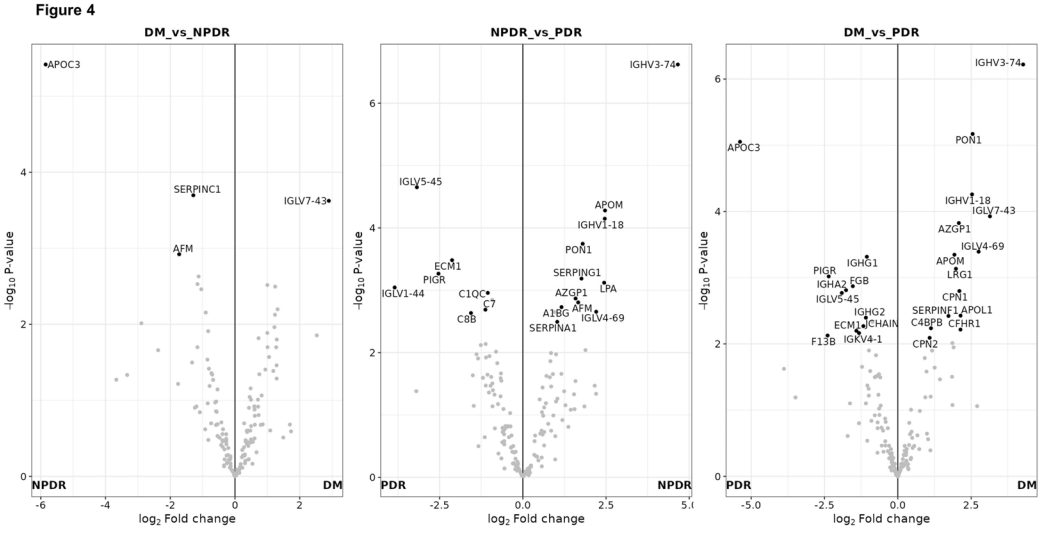


Figure 4: Volcano plot showing the top upregulated and downregulated proteins in pairwise comparison between groups (DM versus NPDR, NPDR versus PDR and DM versus PDR).

Decipher the role of epigenetic modifications in regulating gene expression in pterygium pathogenesis.

Investigators : Dr. Daipayan Banerjee, Mathan L, Dr. Tejaswi Prasad, Dr. N. Venkatesh Prajna, Prof. K. Dharmalingam

Funding : SERB-SRG

Introduction including background

Pterygium is a highly prevalent ocular surface disease, particularly in equatorial regions, with no pharmaceutical intervention available and surgical excision remaining the only treatment option. Ultraviolet (UV) radiation from sunlight is widely recognized as the primary cause of pterygium. While chronic UV exposure induces epigenetic changes in the skin contributing to skin cancer, comprehensive studies on epigenetic alterations in pterygium remain unpublished, and causal relationships have yet to be established. This study aimed to investigate genome-wide methylation changes in pterygium using the Illumina Infinium Epic v2.0 Methylation array. We identified 1,052 hypermethylated CpGs (499 genes) and 687 hypomethylated CpGs (340 genes) in pterygium tissue compared to control conjunctival tissue from patients undergoing cataract surgery ($\Delta\beta>|0.1|$, $P<0.05$). Hypomethylated genes were mainly associated with PI3K-Akt and MAPK pathways, while hypermethylated genes were enriched in pathways related to oxidative stress, autophagy, DNA repair, and Wnt signaling inhibition. Comparing these findings with transcriptomic datasets revealed 28 hypermethylated genes with downregulated transcripts and 74 hypomethylated genes with upregulated transcripts. qPCR validation confirmed upregulation of hypomethylated genes (MMP2, FBLN5, ZEB1) and downregulation of hypermethylated genes (SAMSN1, CBX4) at the transcript level. These findings suggest that dysregulated DNA methylation may contribute to pterygium pathogenesis by upregulating genes involved in cell proliferation, survival, angiogenesis, fibrosis, and extracellular matrix remodeling, while silencing genes associated with oxidative stress response, autophagy, and DNA damage repair. These insights into the global methylation landscape of pterygium open avenues for detailed functional analysis, potentially guiding targeted therapeutic strategies.

New results

Validation of Differentially Methylated Genes in Pterygium patients

To explore the relationship between DNA methylation and gene expression in pterygium, as well as to validate the differentially methylated genes identified in this study, we compared the 4,113 differentially methylated genes ($|\Delta\beta| > 0.05$, $p < 0.05$) with 1,342 differentially expressed genes (DEGs) identified through mRNA sequencing by our group. The mRNA sequencing was conducted on total RNA extracted from pterygium tissues from patients undergoing pterygium excision surgery (n=8) and a control group consisting of conjunctival tissues from patients undergoing cataract surgery (n=5) using the Illumina HiSeq 4000 platform (NCBI SRA Accession#PRJNA1154413) (unpublished). This comparison revealed 28 hypermethylated genes that downregulated at the transcript level and 74 hypomethylated genes that were upregulated at the transcript level (Figure 5A). Among these genes with concordant changes in methylation and

Figure 5

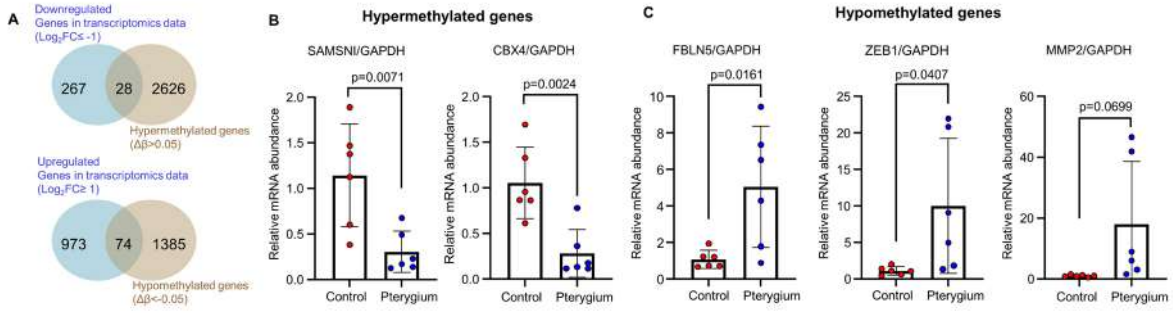


*Figure 5. Overlap between Differentially Methylated Genes (DMGs) and Differentially Expressed Genes (DEGs), and Validation. A. Venn diagram illustrating the overlap between hypermethylated genes that downregulated at the transcript level and hypomethylated genes that are upregulated at the transcript level. B. Validation of genes with concordant changes in methylation and mRNA expression: mRNA abundance of selected genes, as assessed by qPCR, in conjunctival tissue of pterygium patients (n = 6 eyes) and age-matched control eyes (n = 6 eyes). *P <0.05, two-tailed t-test. Data are shown as geometric means ± SD with individual points plotted.*

expression, five were selected for further validation using quantitative PCR: three upregulated genes (MMP2, FBLN5, ZEB1) and two downregulated genes (SAMSN1, CBX4). These genes were chosen for their relevance to key biological processes implicated in pterygium, such as extracellular matrix remodeling, fibrosis, and inflammation. Notably, MMP2 and FBLN5 are involved in matrix degradation and elasticity, while ZEB1 is a regulator of epithelial-mesenchymal transition (EMT), that plays a key role in pterygium pathogenesis. Conversely, SAMSN1 and CBX4 are associated with tumorigenesis and chromatin remodeling21,22. Consistent with their known roles, hypermethylated genes (SAMSN1, CBX4) showed downregulation at the transcript level. (Figure 5B), while the hypomethylated genes (MMP2, FBLN5, ZEB1) exhibited upregulation at the transcript level (Figure 5C). These findings support the potential involvement of these genes in the molecular mechanisms underlying pterygium pathogenesis.

Conclusion of the project

- First study on genome-wide methylation analysis in pterygium using Illumina Infinium Epic v2.0 Methylation array.
- Identified 1,052 hypermethylated CpGs (499 genes) and 687 hypomethylated CpGs (340 genes) in pterygium.
- Hypomethylation was identified in PI3K-Akt and MAPK pathway genes, while hypermethylation was noted in genes linked to oxidative stress, autophagy, DNA repair, and Wnt signaling inhibition.
- Comparison with the transcriptomics dataset identified genes with concordant changes in methylation and expression levels.
- qPCR validation confirmed the upregulation of selected hypomethylated genes and the downregulation of hypermethylated genes.

Understanding the mechanism of action of a novel chemical cross-linker designed to treat keratoconus

Investigators : Dr.O.G.Ramprasad, Prof. K. Dharmalingam and Dr. Venkatesh Prajna
Research fellow : Adhithya Subramanian
Clinical fellow : Dr. Pooja Deepak Andhare
Funding : ICMR

Introduction including background

Corneal collagen crosslinking involving UV light and riboflavin solution is a standard and approved method to treat keratoconus (KC), a progressive, bilateral corneal ectatic disorder affecting the young population usually in their second and third decades of life. The novel, PBS soluble, eye-drop based chemical cross-linker (CXL) developed along with the University of Liverpool, consists of EDCI/NHS [1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide/N-hydroxysuccinimide] and a suberic acid spacer. It has the potential to be developed as an alternative form of treatment for mild to moderate keratoconus. It can cause corneal cross-linking without removing the corneal epithelium, or the use of UV-A irradiation, therefore avoiding the pain associated with the conventional crosslinking treatment of keratoconus and the risk of infection. AMRF and Aurolab have established the proof-of concept in human corneas, wherein, treatment of the diseased keratoconus cornea for 15 minutes at 37°C with the novel chemical cross-linker is able to increase the stiffness of the weak keratoconus cornea by cross-linking collagen molecules.

The cross-linker also does not cause cytotoxicity or morphological changes to the corneal cell layers. The details about the investigation are available in the previous progress reports as well as in the publication. One of the major factors involved in KC progression is the destruction of extracellular matrix proteins by the proteases produced by the corneal epithelial cells and fibroblasts. A group of zinc-peptidases under metzincin superfamily called matrix metalloproteases (MMPs) are highly expressed in KC. A fine balance in the expression of MMPs is essential in maintaining the integrity and transparency of the cornea and for its proper healing, and an imbalance in this tightly regulated process may result in the progressive weakening of the cornea.

The effect of the novel chemical cross-linker in the expression of select matrix metalloproteases (MMPs)- MMP-1, 2, 3 and 9 under in vitro conditions were analyzed in depth which has led to the publication of the manuscript (Gopalakrishnan et al., 2025).

Results

CXL inhibits the activity and the secretion of MMPs from HCE cells and KC fibroblast cultures but upregulates the activity of a natural cross-linking enzyme LOX

Previously we reported that the activity of MMPs- 2 and 9 are reduced in the epithelial and stromal layers of the KC cornea after treatment with the CXL. Now, we have analyzed the effect of CXL on the secretion of MMPs from human corneal epithelial cell line (HCE) and fibroblasts derived from KC cornea in

vitro. Induction of inflammatory conditions in HCE cells akin to keratoconus disease condition was stimulated in HCE cells treated with 10 ng/ml TNF-α

We analysed the activity of secreted and cytosolic MMPs- 2 and 9 before and after CXL treatment in HCE cells by gelatin zymography (Figure-1 A). HCE conditioned media indicated

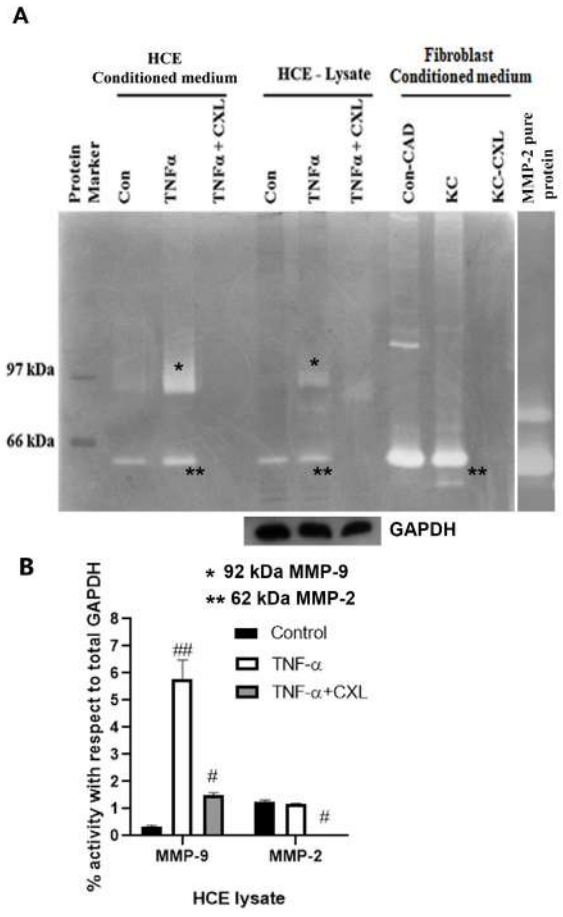


Figure 1: Analysis of MMP-9 and MMP-2 activity in human corneal epithelial cells and fibroblasts

(A) Representative gelatin zymogram image (10% SDS PAGE) for secreted MMP-9 and MMP-2 activity in human corneal epithelial cell line and fibroblasts. Bands represent MMP-9 and 2 from conditioned medium and lysates of control, 10 ng TNF-α treated and 10 ng TNF-α + 2 mM CXL treated HCE cells. Under fibroblast conditioned medium, the lanes represent samples from control cadaver, KC and 2 mM CXL treated KC fibroblasts. 30 ng MMP-2 pure protein is loaded as a positive control. GAPDH represents the western blot for the internal control for HCE cell lysate samples from the portion of the gel cut below the 50 kDa mark. (B) MMP-9 and MMP-2 activity in HCE cell lysates in control, 10 ng TNF-α treated and 10 ng TNF-α + 2 mM CXL treated conditions represented relative to the total GAPDH levels in those samples. # represents P < 0.05 for CXL treated vs. 10 ng TNF-α treated conditions for MMP-2 and MMP-9 activity. ## represents P < 0.05 for 10 ng TNF-α vs. control conditions for MMP-9 activity. n=3 biological replicates for all the experiments.

increased MMP-9 activity (92 kDa) after TNF-α treatment and no activity post-CXL treatment. In HCE cell lysates faint MMP-9 activity was visible with TNF-α treatment which decreased after CXL treatment. In HCE lysates, the CXL inhibited the activity of MMP-9 and MMP-2 inspite of the levels of GAPDH remaining largely unaffected across the control, 10 ng TNF-α treated and CXL treated conditions (Figure-1 B). Active MMP-2 at 62 kDa in HCE conditioned medium and lysates were absent after CXL treatment. The zymography results of KC fibroblast conditioned media further ascertain the statement that MMP-9 activity is not prominent in them. The strong activity of MMP-2 at 62 kDa (Figure-1 A) and two other bands around 120 kDa reduced after CXL treatment. This was despite the presence of other secreted proteins in the CXL-treated KC fibroblast conditioned medium.

Secretion of MMPs-1, 2 and 3 from KC fibroblasts highly declined after CXL treatment (Figure-2 A-C). In KC fibroblasts, secreted MMP-1 and MMP-2 levels were more than the control, while there was no significant difference in secreted MMP-3 levels. The presence of MMP-9 was negligible to be detected in the conditioned media of KC fibroblasts. While the MMPs were inhibited after CXL treatment, a natural cross-linking enzyme known as lysyl oxidase (LOX) was upregulated in CXL treated HCE cells (Figure-2 D). Taking the above results together, we infer that the CXL effectively reduced the secretion and activity of the MMPs while increasing the activity of LOX.

Conclusions

We have deciphered the reduction in the activity and secretion of select metalloproteases as one of the mechanisms by which the chemical cross-linker can act on the corneal tissue to halt the degradation of the matrix in the keratoconus cornea. In the in vitro cellular systems, a decrease in the activity and secretion of MMP9 from HCE cells and of MMPs-1, 2 and 3 from KC fibroblasts was observed. Additionally, the CXL cross-links structural proteins in the KC corneal epithelium and stroma to bring about the stiffening of the cornea. Thus, the CXL is now proven to have clinically relevant effects on stiffening the cornea by the inactivation of MMPs.

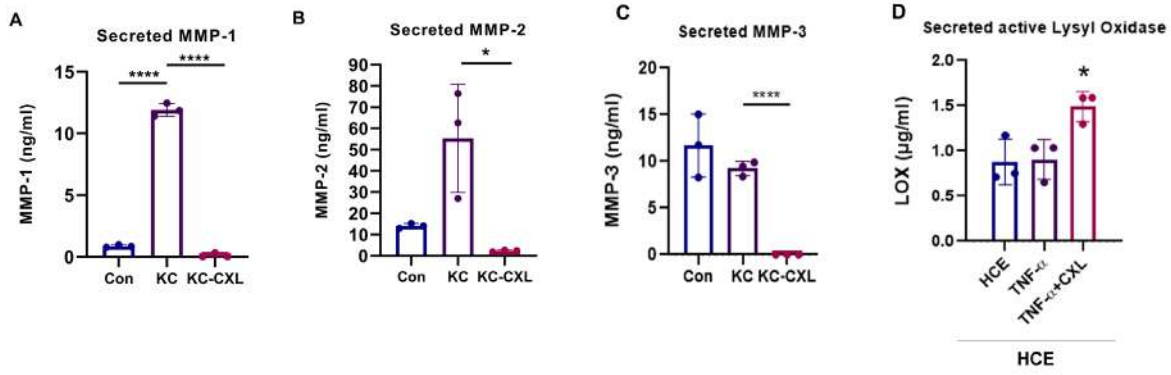


Figure 2: Quantification of secreted MMPs 1, 2 and 3 and secreted active lysyl oxidase

Levels of secreted MMP-1 (A), MMP-2 (B) and MMP-3 (C) from cadaver fibroblasts (control), KC fibroblasts before and after 2 mM cross-linker treatment (KC-CXL). Data in mean ± SD (P < 0.05; **** P < 0.0001). n=3 biological replicates for all the experiments. (D) represents secreted active lysyl oxidase in HCE cultures before and after cross-linker treatment. * P<0.05 for Con. Vs. CXL. All data in mean ± SD. n=3 biological replicates for all the experiments.*

Analysis of tear MMPs from Down's syndrome patients to assess the predisposition of Keratoconus among Down's syndrome patients

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Dr. Meenakshi (AEH, Tirunelveli).
Research Personnel: Agnes Angel

Introduction

Children born with Down Syndrome (DS) have many developmental issues related to vision. There is an increased incidence (10-300 times) of Keratoconus (KC) with DS in other ethnicities (Soheila et al., 2020). However, the factors causing the occurrence of KC with DS is not known in Indian population. The proposed reasons include habitual eye rubbing, hypothyroidism, and allergic conjunctivitis (Stephenson et al., 2022). Even in absence of these risk factors, KC exists in DS due to the unidentified molecular factors between them. Hence, there exists a need for identification of specific molecular markers responsible for the manifestation of KC in DS patients, which helps in early screening and prevents visual morbidity.

Previous reports analyzed the upregulation of matrix metalloproteases in the tears and corneal tissues of patients affected with keratoconus. Gelatinases MMP-2 and 9 were largely studied in KC and in those studies, mRNA and protein levels of MMP-9 were found upregulated in epithelium and tear respectively (Shetty et al., 2015). However, there are no reports of the levels of MMPs 2 and 9 in the tears of DS patients. In order to find the molecules involved in the predisposition of KC in DS patients, we planned to analyse the levels of MMPs 2 and 9 in the tear samples of DS patients. In this report, we have anlayzed the tear levels of MMP-9 in Down's syndrome patients.

Results

The MMP-9 secretion in tears of Down's patients was measured by ELISA and vary from 0 to 9.95 ng/ml. Patients 8, 9 and 10 show increased MMP-9 values (Figure-1). Secreted MMP-9 from cultured KC epithelial cells served as a positive control. Previous report from the lab measured the MMP-9 values from KC corneal epithelium and they ranged from 15-30ng/ml (Gopalakrishnan et al., 2025). The clinical correlation of the Down's syndrome patients with the corneal tomography data revealed that two of the

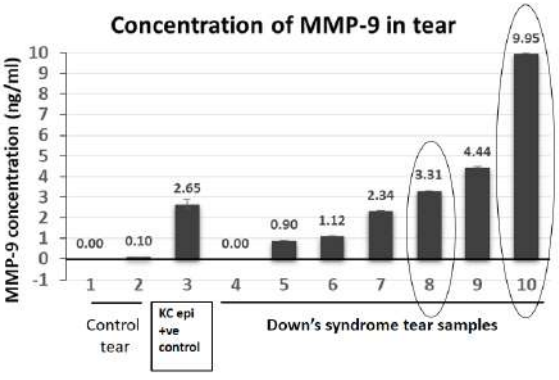


Figure 1: Concentration of MMP-9 in tear samples of Down's syndrome and control tears. Secreted MMP-9 from keratoconus epithelial cells served as a positive control.

Table 1: Correlation of tear MMP-9 values with the corneal tomography data of few Down's patients:

Down's sample number	MMP-9 levels (ng/ml)	RE	RE Pachymetry (µm)	LE	LE Pachymetry (µm)	Diagnosis
		K max (D)		K max (D)		
10	9.95	46.3	494			KC suspect
8	3.31	47.9	462	49.5	474	KC suspect
7	2.34	48.7	531	48.8	535	No KC

"Kmax" refers to the maximum keratometry, which is a measurement of the steepest corneal curvature at a single point on the cornea, essentially indicating the most pronounced cone-shaped bulge in the cornea and often used as a key parameter to diagnose and monitor the progression of KC.

"Pachymetry," is a measurement of the corneal thickness, and in the case of Keratoconus, it indicates the abnormally thin areas of the cornea that are characteristic of the disease. RE-right eye; LE-left eye.

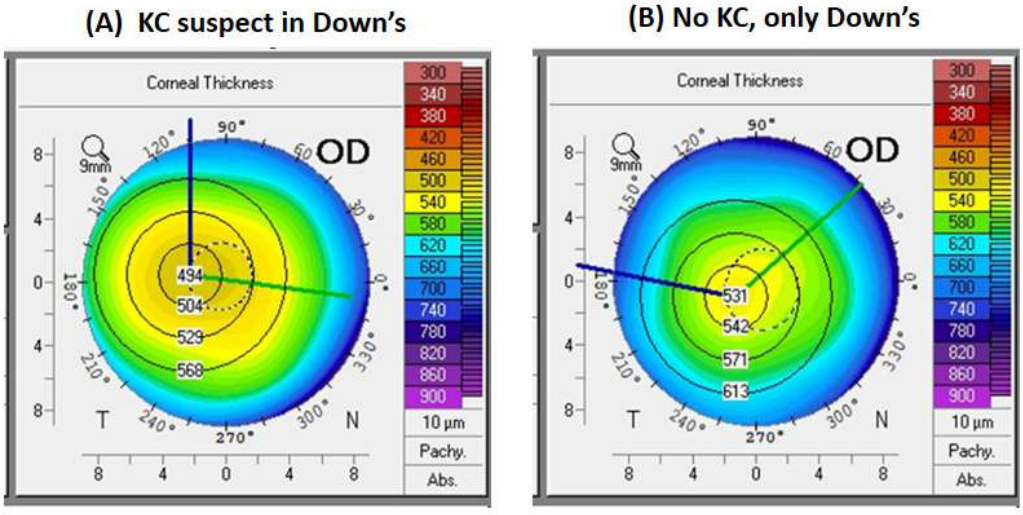


Figure 2: (A) Pachymetry image of the KC suspect in Down's syndrome patient (number 10); (B) pachymetry image of a No KC but only with Down's syndrome patient.

Down's patients with higher tear MMP-9 values were identified as KC suspects (patients 8 and 10, circled in Figure-1; table 1), while another patient with lesser MMP-9 value was not identified as a KC suspect. A patient is termed KC suspect if the Kmax values are higher and pachymetry values are lower. The pachymetry images for the KC suspect patient 10 and the Down's patient alone without KC are shown in Figure-2. While the KC suspect has a central corneal thickness of 494 µm, the Down's patient alone without KC has a central corneal thickness of 531µm.

Conclusion

From the results obtained for secreted MMP-9 in tear samples of Down syndrome patients, atleast two patients with high MMP-9 values show the possibility of having keratoconus. They also have a low central corneal thickness. The above preliminary observations indicate a correlation of tear MMP-9 levels in Down's patients with progression of KC, which needs to be confirmed in more samples.

Work in progress:

Measurement of MMP-2 levels in the tears of Down's patients.

OCULAR PHARMACOLOGY

In glaucoma, elevated IOP is the only modifiable risk factor. The medical management of glaucoma mainly relies on the topical application of anti-glaucoma medications which act either by reducing the aqueous humor formation (Adrenergic agonist, Beta blockers and carbonic anhydrase inhibitors etc.) or by increasing the uveoscleral outflow (prostaglandin derivatives). Only the recently approved Rho kinase inhibitors (Ripasudil and Netarsidil) reduce elevated IOP by acting on the affected tissue, the trabecular meshwork. The combination of vaso-dialatory and IOP lowering properties of rho kinase inhibitors proved beneficial action on glaucoma patients' care. Despite of the effective IOP control, some proportion of glaucoma patients' progress to optic neuro-degeneration. Therefore, there is always a need to search for a new compounds with improved efficacy.

Ocular Pharmacology team has been working on understanding the molecular mechanism(s) involved in the pathogenesis of glaucoma (primary open angle-glaucoma and steroid glaucoma) and to develop newer therapeutics for its management.

Title of the Project: Evaluation of IOP lowering property and Anti-fibrotic property of Relaxin on TGFβ2-induced Elevated IOP Ex vivo model of glaucoma using Human Organ Cultured Anterior Segment (HOCAS)

Investigators	: Dr. S. Senthilkumari
Co-Investigator	: Dr. R. Sharmila
Junior Research Fellow	: Ms. Lakshmi Priya
Funding Agency	: Science & Engineering Research Board - Core Research Grant [2023-26]

Introduction

Relaxin is a heterodimeric peptide hormone with known vaso-dilatory and anti-fibrotic activity. This hormone was first identified by Frederick Hisaw in guinea pig model of pregnancy and parturition. In our previous study in identifying the differentially expressed genes between steroid responsive and non-responsive human cadaveric eyes in perfusion culture, we found that relaxin 1 gene was significantly down-regulated in steroid responder eyes as compared to non-responder eyes and its expression was evident in 7d group as compared to 16h steroid-treated HTM cells. However, the role of relaxin and its non-allelic gene variants in human aqueous outflow facility and IOP regulation is not clearly understood.

Considering the distribution of relaxin and its receptors in human aqueous outflow pathway tissue, it is hypothesized that relaxin 2 may have an active role in the regulation of outflow facility and IOP in human eye. Currently, the establishment of TGFβ2-

induced elevated IOP ex vivo model of glaucoma has been established successfully. Further the demonstration of IOP lowering property of Relaxin2 on elevated IOP induced by TGF β2 in an ex vivo model is in progress. We believe that relaxin 2 with proven IOP lowering and anti-fibrotic properties will be more beneficial in the management of glaucoma.

Establishment of TGFβ2-induced Elevated IOP ex vivo model of glaucoma using HOCAS

Human Donor Eyes: Post-mortem human eyes were obtained from the Rotary Aravind International Eye Bank, Aravind Eye Hospital, Madurai. All eyes were examined under the dissecting microscope for gross ocular pathological changes, and eyes without such changes were used for the experiments. The presence or absence of glaucomatous changes in the study eyes was confirmed by histo-pathological analysis of the posterior segments. The study protocol was approved by the standing Human Ethics committee of our institute [RES2023022BAS]

HOCAS was established using paired human donor eyes as described previously by us and others (Ashwinbalaji et al., 2019; Haribalaganesh et al., 2022). After baseline (BL) equilibration (~2-3 days), anterior segments were exchanged with 5 ml of hrTGFβ2 (3ng/mL) at 200µl/minute as described by Gottenka et al., 2004 whereas, the contralateral anterior segment received the plain medium. Then the flow rate is resumed to 2.5µl/min and continued for 7d. AH outflow facility (OF) (µl per minute/mm Hg)

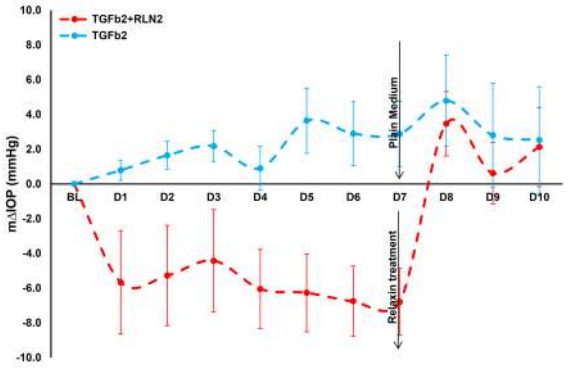
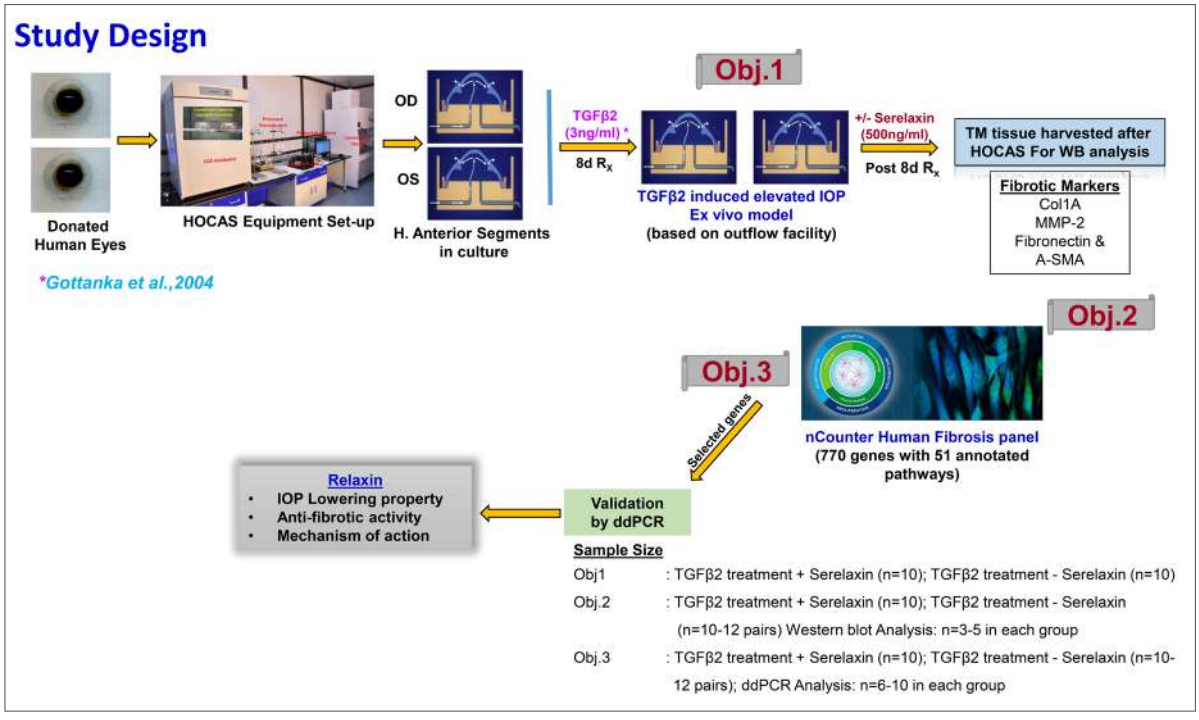


Figure showing the Effect of TGFβ2 on IOP. The mean ± SEM of ΔIOP of plain medium (vehicle control), TGFβ2-treated eyes were plotted over time. The basal IOP on day 0 (before TGFβ2 treatment)was set at 0 mmHg. The outflow facility was decreased by 25% after treatment with TGFβ2 as compared to vehicle-treated eyes.

was calculated as the ratio between the inflow rate (µl/ minute) and the measured IOP (mm Hg). The OF was calculated every hour as the average of 6 values recorded every 10 minutes, beginning 4h before the drug infusion and continuing for the duration of the culture. The average OF value between 3–4h before hrTGFβ2 infusion was taken as baseline. After treatment, anterior segments were fixed by perfusion with 7 ml of 4% paraformaldehyde for assessing the morphology of aqueous outflow tissues.

Out of 20 eyes used for the experiment, X/20 received TGFβ2 treatment and the remaining received plain medium as vehicle control. The over



percentage of change in outflow facility was found to be 25% which is comparable to the previously published findings (Gottenka et al., 2004).

Effect of Relaxin (RLN2) on Elevated IOP induced by TGFβ2 Treatment

In an another experiment, 6 eyes received TGFβ2 Treatment for 7d and then 3/6 eyes received RLN2 (500ng/ml) in one eye and the other reeived plain medium as a vehicle control. The presence of RLN2 increases the outflow facility by 2.3 fold as compared to vehicle treated eyes.

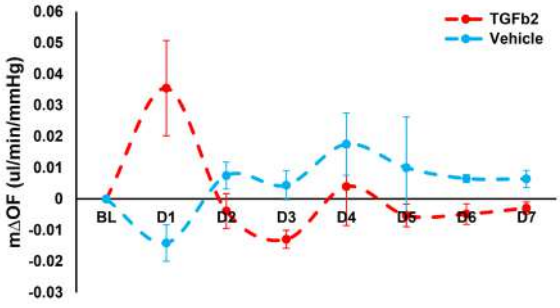


Figure showing the effect of RLN2 on elevated IOP induced by TGFβ2 Treatment

Conclusion

TGFβ2 treatment caused 25% reduction in outflow facility and the presence of human relaxin (RLN2) enhanced the outflow facility by 2.2 fold as compared to vehicle treated eyes. Further studies are underway to analyse the mechanism by which relaxin reduces the fibrosis induced by TGFβ2 treatment using nCounter Fibrosis assay.

Prospective Study Analyzing the Role of Cytokine- Mediated IOP reduction in POAG/ OHTN Patients Receiving Therapeutic Ultrasound for Glaucoma (TUG) Treatment

Investigators

- Dr. Sharmila Rajendrababu
- Dr. Senthilkumari Srinivasan
- Prof. K. Dharmalingam

Collaborators

- Dr. Donald Schwartz, President and Founder, EyeSonix, Los Angeles, USA
- Mr. Eric Schultz, CEO, CTO, EyeSonix, Los Angeles, USA

Project Assistant: Fazil H.

Funding agency: EyeSonix, USA

Introduction

Glaucoma accounts for 5.5% of total blindness caused in India is expected to affect 27.8 million more people of the Asian population by 2040. In consideration of alternative treatments for glaucoma and OHT, therapeutic ultrasound for glaucoma (TUG) which utilizes a low power, low-frequency, focused ultrasound device can be used in controlling the IOP. Earlier studies have reported the beneficial effects of TUG on triggering cascade of cytokines which in turn leads to the reduce in IOP through mild inflammatory effect casing effective drainage of aqueous humor through TM. Therefore, the present study is proposed to investigate the mechanism & levels of cytokines involved (IL-1α, IL-1β, IL-6, TNFα, MMP-3, MMP-9 and TGFβ) in reducing IOP after TUG treatment in glaucoma patients undergoing cataract surgery (Phacoemulsification). This study will provide the insight into the key role of cytokines in reducing IOP.

Table showing the characteristics of Patients Enrolled in this Study.

Study ID	Age	Gender	Diagnosis	Systemic Diseases	Study Eye	Baseline IOP	Post TUG IOP
TUG-CYT-01	55	Female	(BE) POAG, IMC	-	RE	23	17
TUG-CYT-02	70	Male	(BE) OHT, IMC	Diabetes, Hypertension	RE	22	19
TUG-CYT-03	63	Female	(BE) POAG, (LE) Total Cataract	Diabetes, Hypertension	LE	27	12
TUG-CYT-04	57	Female	(BE) OHT, IMC	Diabetes	LE	24	17

The inclusion criteria for this study includes patients with POAG or OHT with significant cataract with age > 18 years, IOP more than 21mm Hg and less than 30mmHg, not on any anti-glaucoma medications (AGM), disc showing features of mild to moderate glaucoma (<0.7 CDR) and VFI not worse than 60 % will included in this study after giving their written informed consent. The study protocol was carried in accordance to the tenets of Declaration of Helsinki. Plasma, Aq. humor and tear samples were collected and stored at -80° until the cytokine assay by Multiplex cytokine analysis.

Based on the above inclusion criteria as of now, 4 patients were recruited and the samples were collected and stored.

Recruitment of study patients and sample collection process are underway. This study is expected to provide the insight into the key role of cytokines in reducing IOP and also to overcome several barriers associated with AGM like the side effects, poor compliance, cost, and adherence to therapy.

A Retrospective Study Reporting the Incidence, Risk Factors and Magnitude of Intraocular Pressure (IOP) Rise in Patients Receiving Intravitreal Steroids for Retinal Vascular Diseases in a Tertiary Eye care Hospital of South India

Investigators : Senthilkumari Srinivasan
Sharmila Rajendran
Iswarya Mani

Background

A significant side-effect of intravitreal steroid use is associated with the development of raised intraocular pressure (IOP) termed as steroid-induced ocular hypertension (SI-OHT) and glaucoma (SIG) which may be severe. There are several studies in which elevated IOP has been documented following intravitreal steroid use and the estimated incidence of SI-OHT after intravitreal steroid injection ranged from 11% to 79%. In India, the reported incidence of SI-OHT following intravitreal triamcinolone acetonide (IVTA) was 27-50% and 17-26% with dexamathasone implant. TA responders had more common early and severe IOP rises than among DEX administrations and found that myopia is a risk for Dex-OHT. Such information is not available for our patient cohorts. Therefore, the present study was conducted to investigate the incidence of SI-OHT, magnitude, time course of IOP elevation and the risks factors following intravitreal steroids among our patient cohorts in a tertiary eye care set-up. This study may serve as a baseline data for our future study of identifying the genetic risk factors associated with SI-OHT in our patient cohorts.

Results

This retrospective observational study included the data that was collected from January 2018 to December 2023. Patients aged 18 and older diagnosed with Diabetic Macular Edema (DME), Retinal Vein Occlusion (RVO), Central Retinal Vein Occlusion (CRVO), or Branch Retinal Vein Occlusion (BRVO) who were treated with intravitreal Tricort or Ozurdex injections at a tertiary eye care center. Only patients who had three consecutive visits following the intravitreal injection were included in the study. Patients with less than one month of follow-up after the injection were excluded.

The total number of patients was 1,178, of which 761 patients dropped out of the study due to irregular follow-up, and finally, 417 patients were included in the study. A total of 417 unilateral patients who received Tricort and Ozurdex during the study period were reviewed. Tricort was administered to 284 patients (68.1%), while 133 patients (31.9%) received Ozurdex.

The baseline characteristics of the patients who received intravitreal steroid injections are presented in Table 1. The average ages of the patients receiving Tricort and Ozurdex IV injections were 59.02±8.29 and 57.03±10.08 years, respectively. In the Tricort group, 56.3% of the patients were male, compared to nearly 71% in the Ozurdex group. Among the Tricort patients, 67.3% received a single injection, while this figure was 72.2% for the Ozurdex group.

Patients with branch retinal vein occlusion (BRVO) comprised 37% of the Tricort group and 42.8% in the Ozurdex group. The distribution of patients with central retinal vein occlusion (CRVO) was similar in both groups, with 39.8% in the Tricort group and 31.6% in the Ozurdex group. Additionally, the percentage of patients requiring anti-glaucoma medications at the final visit was 15.5% in the Tricort group and 21.1% in the Ozurdex group.

Diabetes was the most common systemic illness reported for both steroid injections, affecting 34.6% of patients in the Tricort group and 33.5% in the Ozurdex group, followed by hypertension at 30.9% and 31.7%, respectively. The mean intraocular pressure (IOP) at baseline was similar in both groups, and more than 80% of the patients in both the Tricort and Ozurdex injection groups were classified as steroid non-responders.

Parameters	Tricort n=284 /patients n(%)	Ozurdex n=133 patients n(%)	P-value
Number of injections: 1 2 3	 191 patients (67.3) 91 patients (32.0) 2 patients (0.7)	 96 patients (72.2) 35 patients (26.3) 2(1.5)	 0.3114 0.2353 0.4350
Injections(Months): Time interval between first and last injections Mean±SD (Min-Max)	 4.35±4.54 (11 days – 40 months)	 5.04±4.65 (1 month - 35 months)	 0.0046^{R}
Age(in years): Mean ± SD Range	 59.02 ± 8.29 (37-88)	 57.03±10.08 (27-82)	 0.0348T
Gender: Male Female	 160(56.3) 124(43.7)	 94(70.7) 39(29.3)	 0.0064 0.0035
Eye(s): Right eye Left eye	 142(50.0) 142(50.0)	 74(55.6) 59(44.4)	 0.2828 0.2828
Etiology: BRVO CRVO DME	 105(37.0) 66(23.2) 113(39.8)	 57(42.8) 34(25.6) 42(31.6)	 0.2505 0.6044 0.1059
Need for AGM at final visit: No Yes	 240(84.5) 44(15.5)	 105(78.9) 28(21.1)	 0.1615 0.1615
Systemic illness*: Asthma Cardiac Diabetes Hyperlipidaemia Hypertension Non-Ocular surgery Renal Thyroid Others	 14(2.3) 43(7.1) 209(34.6) 71(11.8) 187(30.9) 31(5.1) 15(2.5) 15(2.5) 19(3.1)	 4(1.4) 24(8.5) 94(33.5) 34(12.1) 89(31.7) 9(3.2) 12(4.3) 6(2.1) 9(3.2)	 0.3802 0.4567 0.7370 0.8827 0.8314 0.1983 0.1502 0.7514 0.9639
Baseline IOP: Mean±SD (Min-Max)	 16.73±2.88 (8-21)	 16.89±3.51 (10-42)	 0.9244R
Steroid responsiveness: Steroid Non-Responder(NR) Steroid Responder (SR)	 243(85.6) 41(14.4)	 109(82.0) 24(18.0)	 0.2900

*%-Column Percentage; *-some patient had more than one systemic illness; BRVO- Branch Retinal Vein Occlusion; CRVO- Central Retinal Vein Occlusion; DME-Diabetic Macular Edema; NR-Increase in the IOP at final visit is less than 5 mmHg; SR- More than 5 mmHg increase in the IOP at final visit; R-Wilcoxon rank sum test; P-Proportion Test; Bolded P-value is significant (P<0.05).*

Table 2: Logistic regression analysis for SR and NR in both injections

Variable	Outcome variable(Steroid responder/Steroid non responder)							
	Tricort				Ozurdex			
	Univariate		Multivariate		Univariate		Multivariate	
	OR (95% CI)	P-value	OR(95% CI)	P-value	OR (95% CI)	P-value	OR (95% CI	P-value
Age (years)	0.96 (0.93-1.01)	0.085	0.96 (0.92-1.01)	0.098	0.97 (0.92-1.01)	0.143	0.97 (0.91-1.04)	0.382
No. of Injections: 1 2 3	Ref 1.10 1	0.781 NC	Ref 1.71 (0.75- 3.89) 1	0.199 NC	Ref 0.96 (0.35-2.68) 4.65 (0.28-78.03)	0.940 0.286	Ref 2.44 (0.58-10.29) 12.63 (0.53-299.21)	Ref 2.44 (0.58-10.29) 12.63 (0.53-299.21)
Eye: Right eye Left eye	Ref 0.75 (0.39-1.46) 0.400	0.400	Ref 0.67(0.31-1.44)	0.303	Ref 0.71 (0.29-1.76)	0.456	Ref 0.91(0.28-2.98	0.873
Gender: Female Male	Ref 1.59 (0.79-3.19)	0.187	Ref 1.72 (0.78-3.79)	0.180	Ref 2.36 (0.75-7.44)	0.141	Ref 2.48 (0.57-10.88	0.228
IOP at baseline	0.87 (0.78-0.98)	0.018	0.78 (0.68-0.89)	<0.001	1.02 (0.90-1.15)	0.771	0.84 (0.69-1.02)	0.080
Need for AGM at final visit No Yes	Ref 8.69 (4.13 18.28)	<0.001	Ref 14.73 (5.99-36.21)	<0.001	Ref 21.64 (7.36-63.62)	<0.001	Ref 44.63 (10.93-182.15)	<0.001

OR-Odds Ratio; Ref-Reference; Bolded faces are significant (P<0.05); NC-Not computed

Both univariate and multivariate logistic regression analyses was conducted using the Steroid Responder (SR) and non-responder (NR) as the outcome variables and the results are given in Table 2. The independent variables included age, the number of injections, the affected eye, the patients' gender, baseline intraocular pressure (IOP), and the need for anti-glaucoma medications at the final visit. This analysis was performed separately for the Tricort and Ozurdex groups. For the Tricort group, it was found that patients with a higher baseline IOP had a significantly lower chance of achieving a SR ($p < 0.05$) in both the univariate and multivariate models. Additionally, the need for anti-glaucoma medications at the final visit significantly influenced ocular hypertension (OHT) in both the Tricort and Ozurdex groups ($p < 0.01$).

The various parameters were compared between the SR (Steroid Responder) and NR (non-responder) groups in both the Tricort and Ozurdex treatments. The mean change in intraocular pressure (IOP) was significantly higher in the SR group for the Tricort injections (13.51 ± 10.52) compared to Ozurdex (9.5 ± 3.91). Additionally, the need for anti-glaucoma medication (AGM) at the final visit was 48.8% for the SR group in the Tricort injection group, while it was 71% in the Ozurdex group ($p < 0.01$). The hazard ratio was calculated for various independent variables concerning the significance of ocular hypertension (OHT). It was found that patients with hypertension had a lower risk of increased IOP in the Tricort group. In contrast, patients with conditions such as Tuberculosis (TB), stroke, and other drug allergies experienced a fourfold greater increase in IOP values in the Ozurdex group (Table 3).

In Table 4, we examined the relationship between systemic parameters and steroid responsiveness. We found that patients with renal diseases comprised nearly 9% of the steroid-responsive (SR) group, compared to only 3% in the non-responsive (NR) group for Ozurdex. This difference was statistically significant, with a p-value of less than 0.05. No other systemic illnesses showed a significant association with ocular hypertension (OHT).

Table 3: Characteristic features of eyes among SR and NR in steroid-treated groups

Parameters	Tricort NR N=243 Eyes	Tricort SR N=41 Eyes	P-value	Ozurdex NR N=109 Eyes	Ozurdex SR N=24 Eyes	P-value
Mean change in IOP, mm HG (Min-Max)	-0.06±2.81 (-7 to 5)	13.51±10.52 (6-64)	<0.0001	0.17±2.78 (-10 to 5)	9.5±3.91 (6-22)	<0.0001
Number of Injections: 1 2 3	 164 (67.5) 77 (31.7) 2 (0.8)	 27 (65.9) 14 (34.2)	0.895F	 79 (72.5) 29 (26.6) 1 (0.9)	 17(70.8) 6(25.0) 1(4.2)	0.468F
Age, years: <=40 41-60 >=60	 3 (1.2) 121 (49.8) 119 (48.9)	 2 (4.9) 23 (56.1) 16 (39.0)	0.136F	 4 (3.7) 55 (50.5) 50 (45.9)	 2 (8.3) 14 (58.3) 8 (33.3)	0.295F
Gender: Male Female	 133 (54.7) 110 (45.3)	 27 (65.9) 14 (34.2)	0.184C	 74 (67.9) 35 (32.1)	 20 (83.3) 4 (16.7	0.132C
Disease etiology: BRVO CRVO DME	 91 (37.5) 55 (22.6) 97 (39.9)	 14 (34.2) 11 (26.8) 16 (39.0)	0.829C	 53 (48.6) 25 (22.9) 31 (28.4)	 4 (16.7) 9 (37.5) 11 (45.8)	0.017C
Need for AGM at final visit: No Yes	 219(90.1) 24(9.9)	 21 (51.2) 20 (48.8)	<0.001C	 98(89.9) 11(10.1	 7 (29.2) 17 (70.8)	<0.001C
Time, magnitude, and management of IOP spike among SR		Tricort SR N=41 Eyes			Ozurdex SR N=24 Eyes	P-value[P]
Time of IOP rise: <3 months 3 months-6 months 6 months-1 year >1 year	NA	 19 (46.3) 19 (46.3) 2 (4.9) 1 (2.4)		NA	 15 (62.5) 5 (20.8) 2 (8.3) 2 (8.3)	 0.208 1 0.039 7 0.575 9 0.274 4
The magnitude of IOP rise: 6-15 mm Hg (Moderate responders) >15 mm Hg (Severe Responders)		 27 (65.9) 14 (34.2)			 22 (91.7) 2 (8.3)	 22 (91.7) 2 (8.3)
Parameters		HR (95% CI)	P-Value		HR (95% CI)	P-Value
Cox-proportional hazardous analysis						
Age		0.99 (0.95-1.03)	0.517		0.97 (0.93 1.01)	0.139
Gender-Female		0.58 (0.29-1.13)	0.108		0.44 (0.12-1.59)	0.213
Asthma		NC	NC		0.63 (0.04-10.63)	0.751
Cardiac		0.71 (0.26-1.97)	0.511		1.13 (0.35-3.62)	0.836
Diabetes		1.21 (0.56-2.62)	0.632		1.77 (0.54-5.83)	0.348
Hyperlipidaemia		0.96 (0.43-2.16)	0.928		0.58 (0.16-2.09)	0.407
Hypertension		0.49 (0.26-0.94)	0.031		1.13 (0.41-3.11)	0.814

Non-Ocular surgery		0.94 (0.29-2.99)	0.919		0.43 (0.05-3.78)	0.444
Renal disease		1.36 (0.39-4.67)	0.621		3.47 (0.99-12.12)	0.052
Thyroid		1.06 (0.23-4.80)	0.941		0.83 (0.10-6.70)	0.861
Others		2.48 (0.88-7.01)	0.087		4.33 (1.17-15.99)	0.028

%-Column Percentage; C-Chi square Test; F- Fishers Exact Test; R-Wilcoxon rank sum test; P-Proportion Test; SR-Steroid Responder; NR-Non-Steroid Responder; HR-Hazard Ratio; Bolded P-values are significant (P<0.05); NC-Not Computed (present only in one case)

Table 4: Systemic parameter association with steroid responsiveness group

Systemic illness	Tricort NR N=243 patients N (%)	Tricort SR N=41 patients N (%)	P-value[P]	Ozurdex NR N=109 patients N (%)	Ozurdex SR N=24 patients N (%)	P-value[P]
Asthma	14(2.7)	0	-	3(1.3)	1(1.8)	0.7829
Cardiac	38(7.3)	5(6.0)	0.6761	19(8.4)	5(9.1)	0.8707
Diabetes	177(34.0)	32(38.6)	0.4152	74(32.7)	20(36.4)	0.6098
Hyperlipidaemia	62(11.9)	9(10.8)	0.7813	31(13.7)	3(5.5)	0.0920
Hypertension	164(31.5)	23(27.7)	0.4906	74(32.7)	15(27.3)	0.4341
Non-Ocular surgery	27(5.2)	4(4.8)	0.8893	8(3.5)	1(1.8)	0.5155
Renal disease	12(2.3)	3(3.6)	0.4759	7(3.1)	5(9.1)	0.0487
Thyroid	13(2.5)	2(2.4)	0.9629	5(2.2)	1(1.8)	0.8561
Other systemic illness@	14(2.7)	5(6.0)	0.1057	5(2.2)	4(7.3)	0.0559
Total	521(100)	83(100)		226(100)	55(100)	

**-Some patient had more than one systemic illness; %-Column percentage; SR-Steroid Responder; NR-Non-Steroid Responder; P-Proportion Test; Bolded P-values are significant (P<0.05); @-TB, Stroke, other drug allergies*

Conclusion

The present study found the incidence of SI-OHT in both groups was found to be 14.4% [TA group] and 18% [DEX intravitreal insert group] at some point during the follow-up of 4.3 ± 4.5 months and 5 ± 4.6 months respectively. Age and gender were not associated with SI-OHT. It is found that Diabetes as a co-morbid condition and DME was higher among the TA group (40%) whereas BRVO was higher in DEX group (43%). Renal disease was significantly associated with SR in DEX group.

BIOINFORMATICS

The primary goal is to develop and provide bioinformatics methods to analyze the omics data generated by next-generation sequencing methods. This includes i) developing data analysis pipelines for whole genome/ exome genome data analysis to identify eye-disease-specific pathogenic variants ii) transcriptomic analysis to detect the altered gene expression and transcripts and its role in the disease pathogenesis, iii) ocular microbiome analysis in fungal keratitis

Updation of eyeVarP, A computational framework for identifying eye-disease pathogenic variants from whole Exome/ Genome sequencing.

Investigator : Dr. D. Bharanidharan
Project student : Yadhukrishnan R

Background

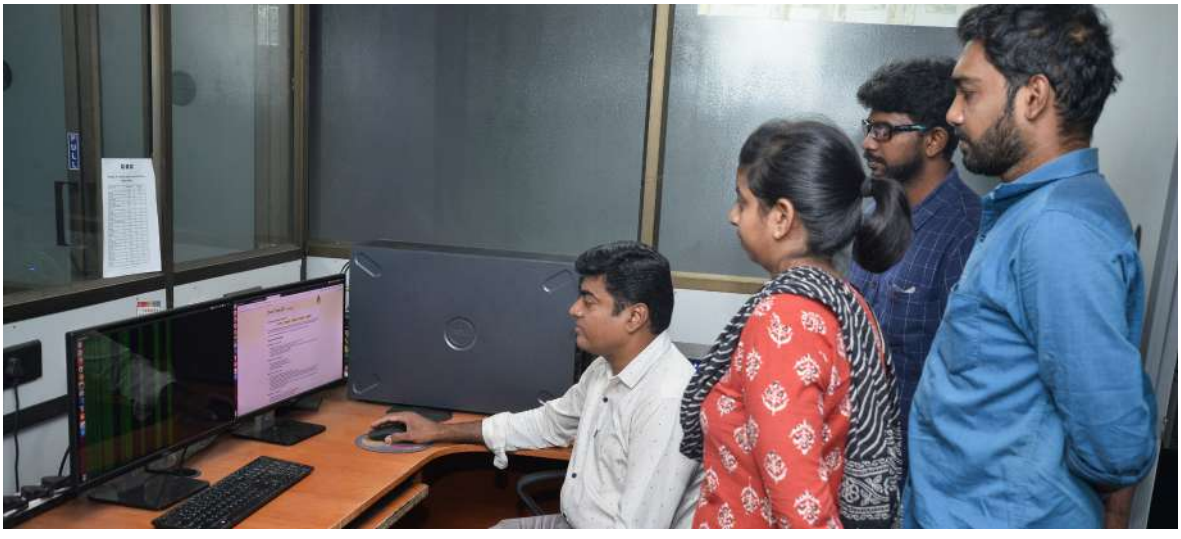
eyeVarP represents a state-of-the-art computational framework for identifying eye disease-specific pathogenic variants from whole exome/genome sequencing data. This machine learning-based command-line tool integrates variant features, Gene Ontology, phenotype ontology, and gene expression profiles with a variant pathogenicity prediction tool (VarP). Comparative analyses demonstrate eyeVarP's superior precision in prioritising ocular disease-associated pathogenic variants within the top 10 candidates, outperforming pan-disease-specific computational tools. Moreover, VarP exhibits enhanced predictive capability compared to 12 existing pathogenicity prediction algorithms, achieving 95% classification accuracy in distinguishing pathogenic from benign missense and insertion-deletion variants in benchmarking analyses.

The tools used in the eyeVarP pipeline were recently updated to the latest version for enhanced performance, accuracy, and reliability.

Table 1: an updated version of the tools in the pipeline with the release date

Tool	From	To	Release date
SRA Toolkit	2.3.5	3.2.0	14-01-2025
Fastqc	0.11.5	0.12.0	1-03-2023
Fastp	0.21.1	0.23.4	30-05-2023
BWA	0.7.12	0.7.18	15-04-2024
Samtools	1.7	1.21	12-09-2024

To evaluate the performance of the updated eyeVarP pipeline, whole exome sequencing data from four families affected by primary open-angle glaucoma were utilised (Figure 1). These data were obtained from the Sequence Read Archive database, accessible under the accession identifier PRJNA394051.



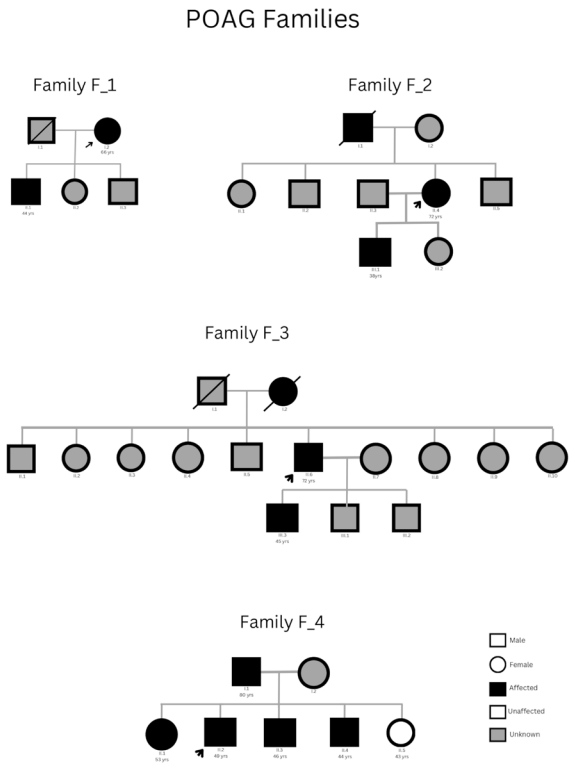


Figure 1. Pedigrees of the four families used for exome sequencing. Squares represent males, and circles represent females. Filled symbols indicate affected individuals, while unfilled symbols denote unaffected individuals. A diagonal line through a symbol signifies a deceased person. An arrow pointing to a symbol identifies the proband. Double horizontal lines connecting a couple indicate a consanguineous union.

Results and Discussion:

The eyeVarP pipeline effectively prioritised variants potentially associated with primary open-angle glaucoma. A subsequent literature review corroborated the relevance of these prioritised variants to the observed phenotype. Specifically, the pipeline identified variants in genes such as FREM2, COL11A1, RP1L1, and RAPSN, all previously implicated in ocular pathologies.

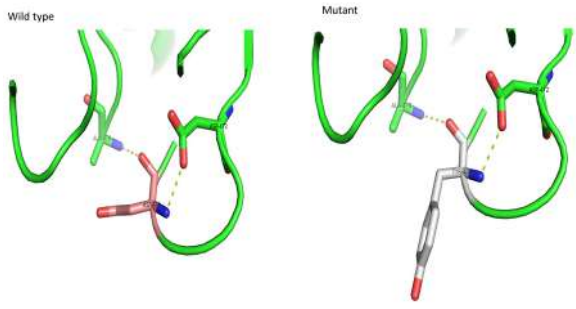


Figure 2: D475Y mutation visualised using FREM2 predicted protein structure. Wild-type (Left) and Mutant (Right) proteins were visualised, focusing on the amino acid and its interaction with neighbouring amino acids.

FREM2 mutations can lead to Fraser syndrome, characterised by cryptophthalmos, which may result in glaucoma(D. Das et al., 2022). A study on congenital anterior segment anomalies linked COL11A1 to glaucoma(Hussain et al., 2025). RP1L1 mutations have been associated with primary angle-closure glaucoma (Liu et al., 2020). RAPSN has been identified as a shared gene between Alzheimer's disease and glaucoma pathology, suggesting a potential genetic link between these neurodegenerative conditions (Zheng et al., 2022).

The FREM2 gene was selected from the prioritised genes to visualise mutation-induced structural changes. The protein structure was predicted using AlphaFold 3, and mutagenesis was performed using PyMOL (Figure 2). The updated eyeVarP pipeline demonstrates improved performance in identifying eye disease-specific pathogenic variants. Its application to primary open-angle glaucoma cases has yielded promising results, highlighting genes with known associations to glaucoma and related conditions. Ongoing development and dockerization will further enhance the tool's accessibility and effectiveness for researchers and clinicians in ocular genetics.

Table 2: Identified eveVarP variants with their scores.

Family	Gene	Mutation	Mutation type	Inheritance	Mutation ID	VarP	eyeVarP
F1	FREM2	c.G1423T:p.D475Y	Non synonymous SNV	Heterozygous dominant		55.15	0.96
F2	COL11A1	c.C3437T:p.P1146L	Non synonymous SNV	Heterozygous dominant	rs76447 8054	71.2	0.96
F3	RP1L1	c.G6298C:p.G2100R	Non synonymous SNV	Heterozygous dominant		44.15	0.96
F4	RAPSN	c.T824G:p.I275S	Non synonymous SNV	Heterozygous dominant	rs11316 5683	67.2	0.75

2. ML-Based Tool to Predict Alternative splicing specific to retinoblastoma

Investigators : Dr. D. Bharanidharan
Dr. A. Vanniarajan, Dr. Usha Kim
Funding : ICMR-SRF
Research Scholar : Mohamed Hameed Aslam A

Background

Retinoblastoma (RB) is an aggressive ocular cancer primarily affecting young children, with an incidence rate of approximately 1 in 15,000 live births. While RB1 gene mutations are the primary cause, recent research has uncovered additional genetic and molecular factors contributing to its development and progression. Emerging evidence suggests that alternative splicing plays a crucial role in retinoblastoma pathogenesis beyond RB1 mutations. Deep intronic mutations within the RB1 gene have been found to disrupt normal splicing mechanisms, leading to the production of dysfunctional pRB proteins. Our study employs machine learning techniques to systematically identify and analyse alternative splicing events and the resulting alternative splicing specific to retinoblastoma. Compared to traditional methods, this approach allows for a more comprehensive and unbiased examination of the splicing landscape in retinoblastoma cells.

Results and discussion

Using publicly available data from retinoblastoma (RB) and fetal retina samples, as illustrated in Figure 1, we conducted a comprehensive analysis and identified 163,484 alternative splicing events. Skipped exon (SE) events were the most prevalent splicing pattern among these. To identify RB-specific DAS events, we first compared all tumour-associated alternative splicing (AS) events with RB-specific AS events using the Boruta algorithm-based

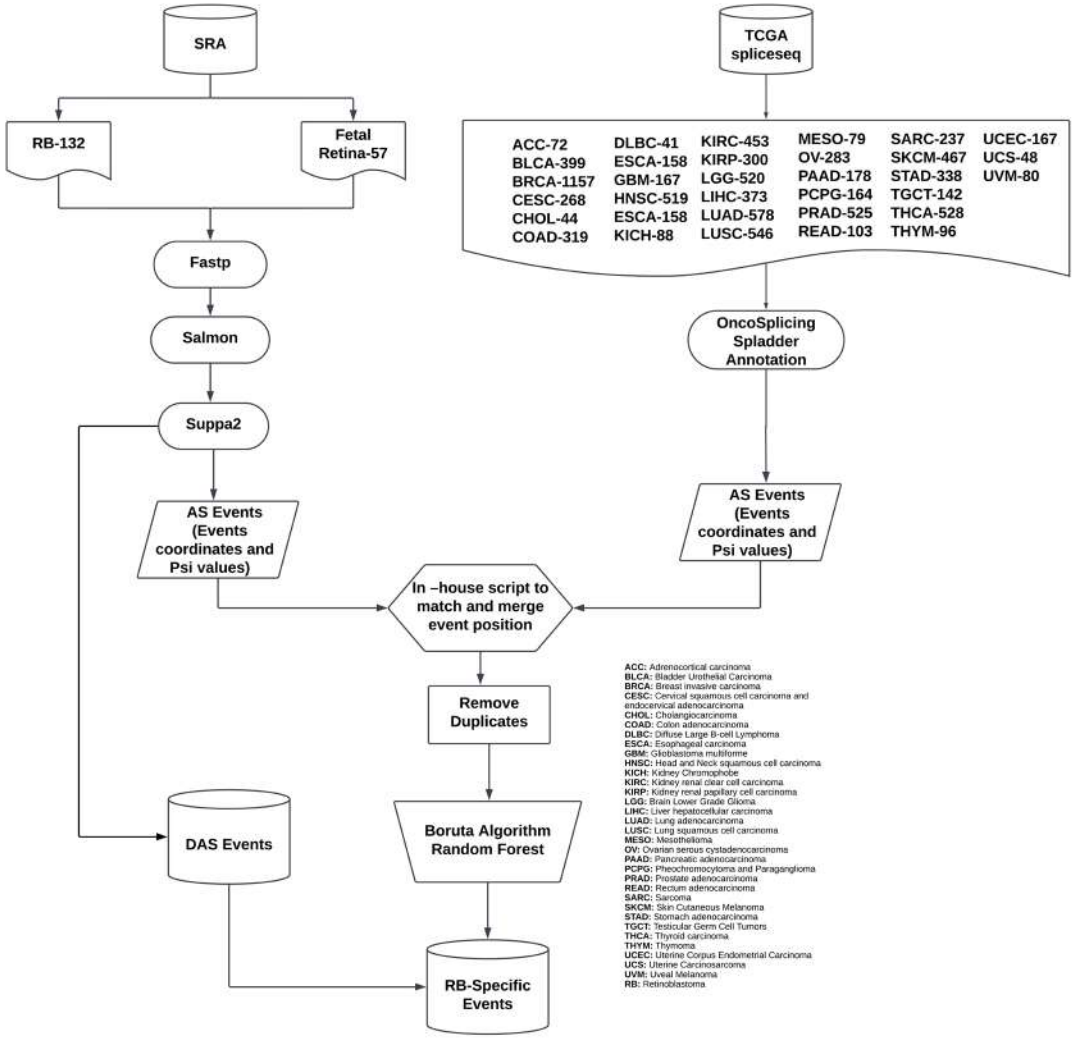


Figure 1: Workflow of this Study

classification program. This analysis identified 383 RB-specific events. Subsequently, a Venn diagram comparison of these DAS events with RB datasets refined the list to 108 unique RB-specific DAS events. Functional enrichment analysis of these splicing alterations revealed significant associations with pathways and processes critical to RB tumorigenesis, providing insights into the molecular mechanisms underlying this pediatric cancer (Figure 2).

The functional network analysis of the protein-protein interaction network in retinoblastoma (RB) reveals three primary functional clusters: DNA repair and cell cycle regulation, metabolic pathway integration, and RNA processing mechanisms (Figure 3). The first cluster highlights CDK5RAP3's interaction with MDM2/MDM4 family proteins and SDCBP's connections to DNA repair components, while the second cluster emphasises MTRR's involvement in oxidative phosphorylation and metabolic pathways. The third cluster focuses on RNA processing, involving spliceosome components and transcriptional regulation via MYC targets. This analysis underscores the critical roles of MTRR, CDK5RAP3, and SDCBP in RB pathogenesis, linking them to cellular energy metabolism, p53 regulation, cell cycle control, and genomic stability. The intersection of these splicing events with key oncogenic pathways suggests potential therapeutic targets for disrupting RB-specific tumorigenic mechanisms.

The three significant RB-specific Differential Alternative Splicing (DAS) events—MTRR_A3,

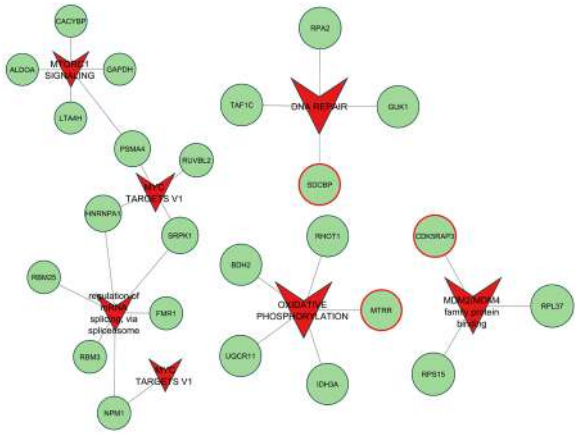


Figure 3: Functional Network Analysis of Enriched Pathways. This network illustrates the functional interactions of enriched pathways associated with RB-specific DAS events. Key pathways, represented by red triangles, Green nodes represent proteins or genes linked to these pathways, while red-outlined nodes highlight RB-specific DAS gene.

CDK5RAP3_RI.6, and SDCBP_SE.2—are intricately linked to retinoblastoma (RB) biology pathways. MTRR encodes methionine synthase reductase, a key enzyme in folate metabolism and DNA repair, and its aberrant splicing in RB may contribute to genomic instability and altered energy metabolism(Peres et al., 2016; Wu et al., 2022; Zhang et al., 2012) , exacerbating the effects of RB1 mutations. CDK5RAP3 functions as a tumour suppressor by regulating DNA damage repair, cell cycle checkpoints, and apoptosis. Still, its splicing alterations could impair these functions, leading to

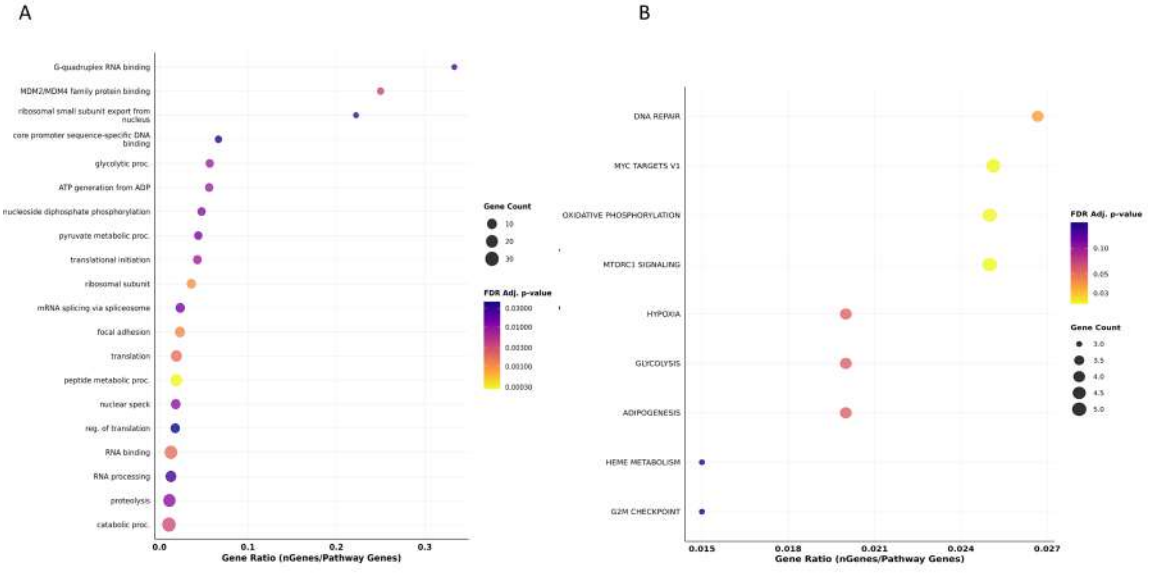


Figure 2: Functional enrichment analysis for RB-specific DAS events (A) Gene Ontology (GO) terms enriched for RB-specific DAS events, highlighting key biological processes, molecular functions, and cellular components associated with splicing dysregulation in retinoblastoma. (B) Enriched pathways from the HALLMARK gene set in MsigDB, showcasing critical signaling and regulatory pathways implicated in RB pathogenesis.

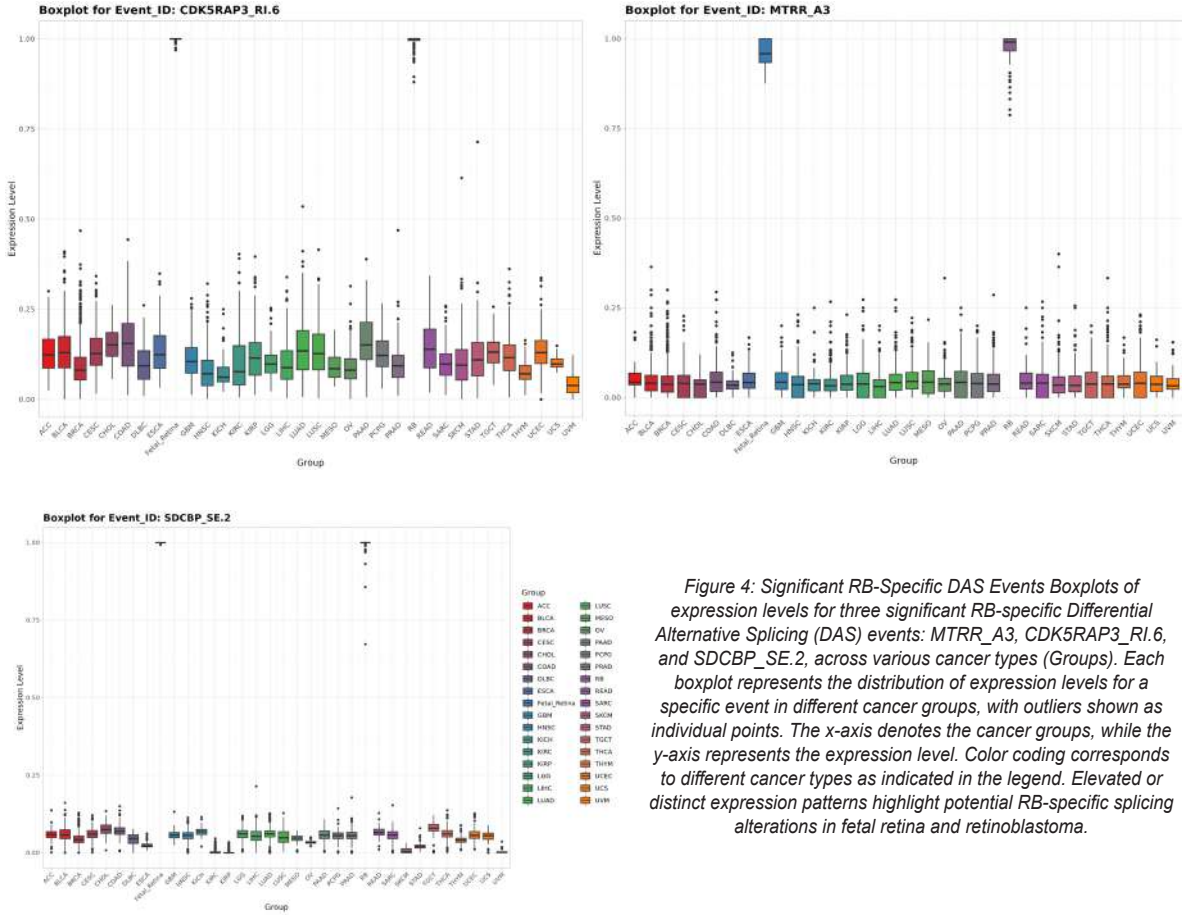


Figure 4: Significant RB-Specific DAS Events Boxplots of expression levels for three significant RB-specific Differential Alternative Splicing (DAS) events: MTRR_A3, CDK5RAP3_RI.6, and SDCBP_SE.2, across various cancer types (Groups). Each boxplot represents the distribution of expression levels for a specific event in different cancer groups, with outliers shown as individual points. The x-axis denotes the cancer groups, while the y-axis represents the expression level. Color coding corresponds to different cancer types as indicated in the legend. Elevated or distinct expression patterns highlight potential RB-specific splicing alterations in fetal retina and retinoblastoma.

unchecked proliferation, reduced apoptosis, and chromosomal instability in RB cells(El Marabti & Younis, 2018; Minguillón et al., 2022; Sheng et al., 2021). Similarly, SDCBP (syndecan-binding protein) is involved in promoting cancer cell migration, invasion, and metastasis through pathways like PI3K/AKT and epithelial-mesenchymal transition (EMT) (S. K. Das et al., 2020) ; it's alternative splicing in RB may enhance oncogenic potential by facilitating tumour dissemination and resistance to therapy. These splicing events collectively highlight the complexity of alternative splicing in RB and its impact on key oncogenic pathways, underscoring the need for further experimental validation and exploration of RNA-based therapeutic strategies to target these alterations for clinical benefit.

OCULAR MICROBIOLOGY

The main focus is the diagnosis and prognosis of ocular infections and understanding their molecular mechanisms. The lab develops new molecular methods or identify molecular biomarkers such as miRNAs for the diagnosis of no growth or unidentified organisms in addition to routine diagnosis using microbiological, biochemical and molecular methods. The department uses genomic and transcriptomic approaches, and invitro cell culture and exvivo model system to understand the pathogenesis of ocular pathogens. .

Role of Human Corneal miRNAs in the Onset and Severity of Fungal Keratitis

Investigators : Dr. D. Bharanidharan (PI),
Dr. K. Dharmalingam,
Dr. Venkatesh Prajna,
Dr. Lalitha Prajna
Research Scholar : Shreya Dinesh
Funding : ICMR

Background

Fungal keratitis, primarily caused by either *Aspergillus flavus* or *Fusarium solani*, is a severe corneal inflammatory disease and is more common in India. We aim to identify the human corneal miRNAs that can help detect the onset and severity of the disease early since more than 40% of patients are refractory to antifungal treatment. We have identified potential miRNAs based on their fold change, abundance, and functional significance in fungal keratitis pathogenesis. We conducted real-time qPCR analyses to validate further the miRNAs' role in the onset and severity of the disease using at least 30 samples in each group. We performed ROC curve analysis to identify them as prognostic or diagnostic biomarkers.

Results and Conclusion

We selected 17 miRNAs based on their fold change, abundance, functional relevance and differential expression in disease onset and progression. The selected miRNAs were validated in patient samples from *Fusarium sp*. and *Aspergillus flavus* keratitis and categorized into six groups: Control, responder presentation, responder review, non-responder presentation, non-responder review, and poor outcome. Through qPCR validation of 17 miRNAs in 30 samples from each group, we identified five potential miRNA biomarkers with significant relative expression and concordant fold change with NGS data (Figure 1.1). Among these, hsa-miR-142-5p is recognised as a keratitis-specific putative biomarker, while hsa-miR-184 is specific to fungal keratitis.

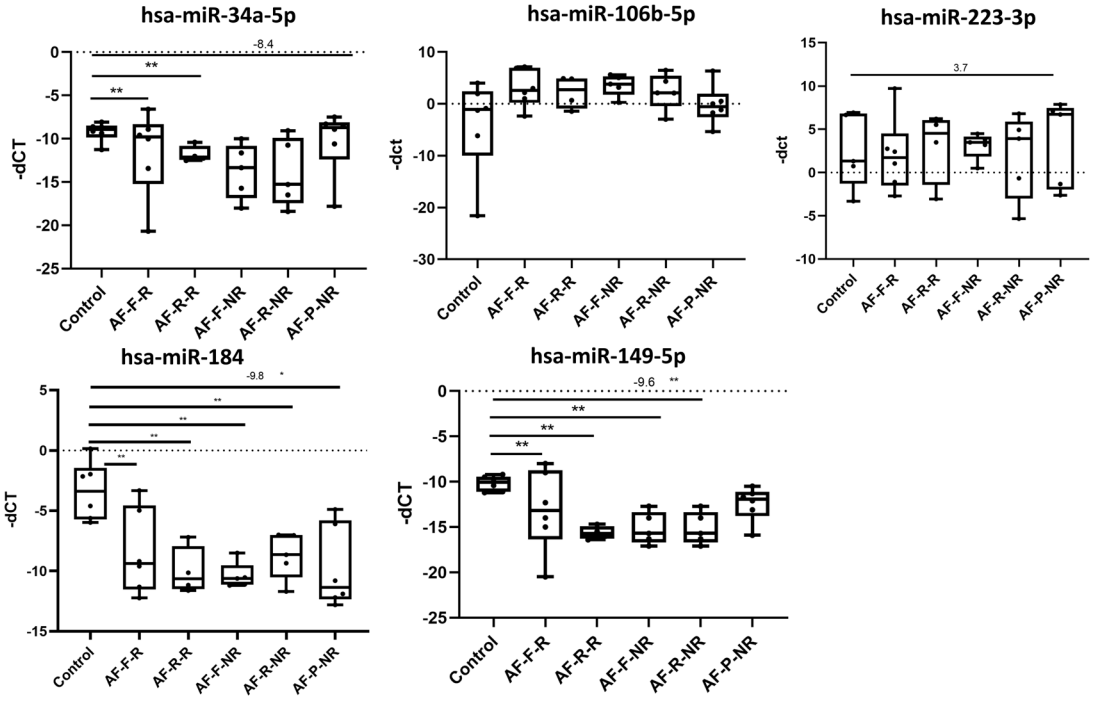


Figure 1.1: qPCR validation of putative miRNA biomarkers in Aspergillus flavus. Keratitis.

CC- Cadaver Cornea,AF- Aspergillus flavus, F-R - At First Presentation Responder, R-R - At Review Responder. F-NR - At First Presentation. Non-Responder, R-NR - At Review Non-Responder, P-NR- Poor Outcome Non-Responder.

*The relative expression of miRNAs were plotted in presentation and review cases of responder and non-responder swabs and tissues compared to cadaver controls. Median values are shown by horizontal lines using the Mann-Whitney test. *P<0.05.*

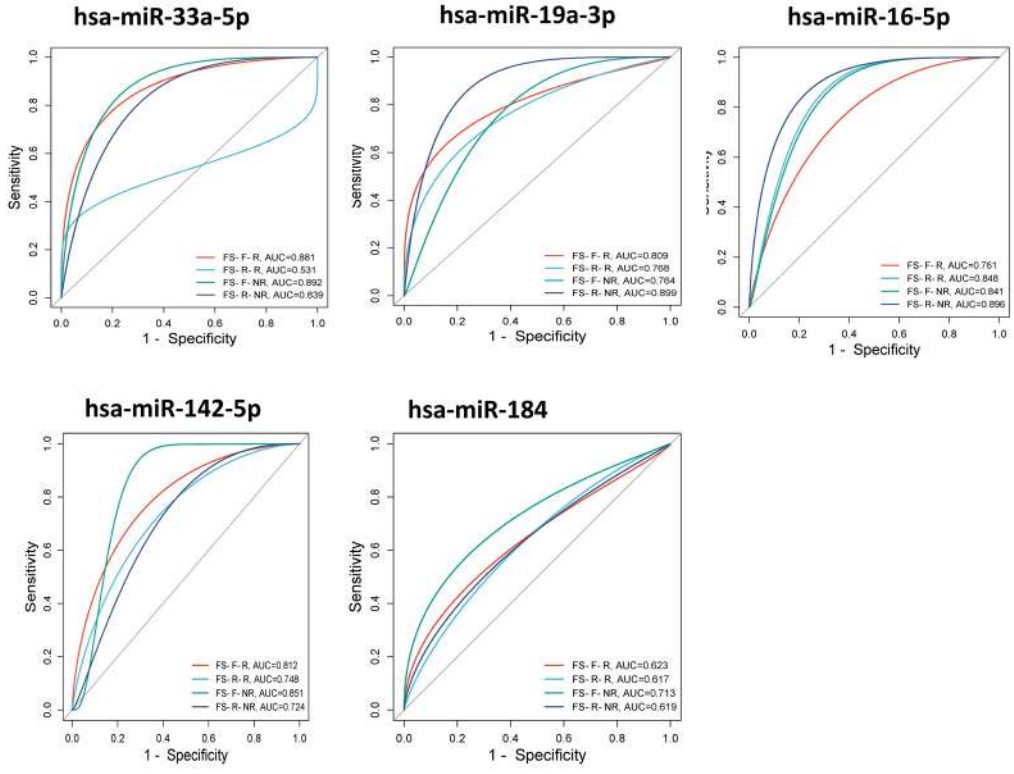


Figure 1.2: ROC curve for miRNA biomarkers in Fusarium sp. Keratitis.

The graphs were plotted with sensitivity (true-positive rate) vs. 1-specificity (false-positive rate) over all possible ΔCT values

Diagnostic value of miRNAs

To assess the statistical relevance of the biomarkers, an ROC curve was constructed using ΔCt, and the AUC (Area Under the ROC Curve) was determined. In *Fusarium sp*. keratitis, The AUC values obtained for all five hsa-miR-142-5p, hsa-miR-184, hsa-miR-33a-5p, hsa-miR-19a-3p, and hsa-miR-16-5p were significant at 95% confidence in all the presentation and review groups, suggesting the potential diagnostic markers (Figure 1.2). In the case of Aspergillus flavus keratitis, we plotted ROC for has-miR-184, has-miR-149-5p and has-miR-34a-5p (Figure 1.3), which showed significant differences among groups in the qPCR analysis. All three miRNAs were identified as potential diagnostic markers for *Aspergillus flavus* keratitis (Figure 1.3). However, we require more sample volume to support these findings.

Prognostic value of miRNAs

In *Fusarium* keratitis, hsa-miR-33a-5p, hsa-miR-19a-3p and hsa-miR-16-5p were identified as potential prognostic miRNAs to predict the severity. The ROC curve, as shown in figure 1.4.4, the severity can better be predicted at the time of review, specifically by hsa-miR-33a-5p and hsa-miR-19a-3p. we could not perform ROC in *Aspergillus flavus* keratitis due to the small sample volume.

This study identified unique miRNA profiles in fungal keratitis depending on the infecting fungus (*Aspergillus flavus* or *Fusarium sp*.). These findings hold promise for developing miRNA-based strategies for earlier diagnosis (has-miR-184, hsa-miR-149-5p, hsa-miR-142-5p), targeted treatments based on fungal type (species-specific miRNAs, such as has-miR-33a-5p, has-miR-19a-3p and has-miR-16-5p for *Fusarium sp*. and hsa-miR-34a-5p for *Aspergillus flavus* keratitis), and potentially predicting disease severity (hsa-miR-33a-5p and hsa-miR-19a-3p for *Fusarium sp*. keratitis).

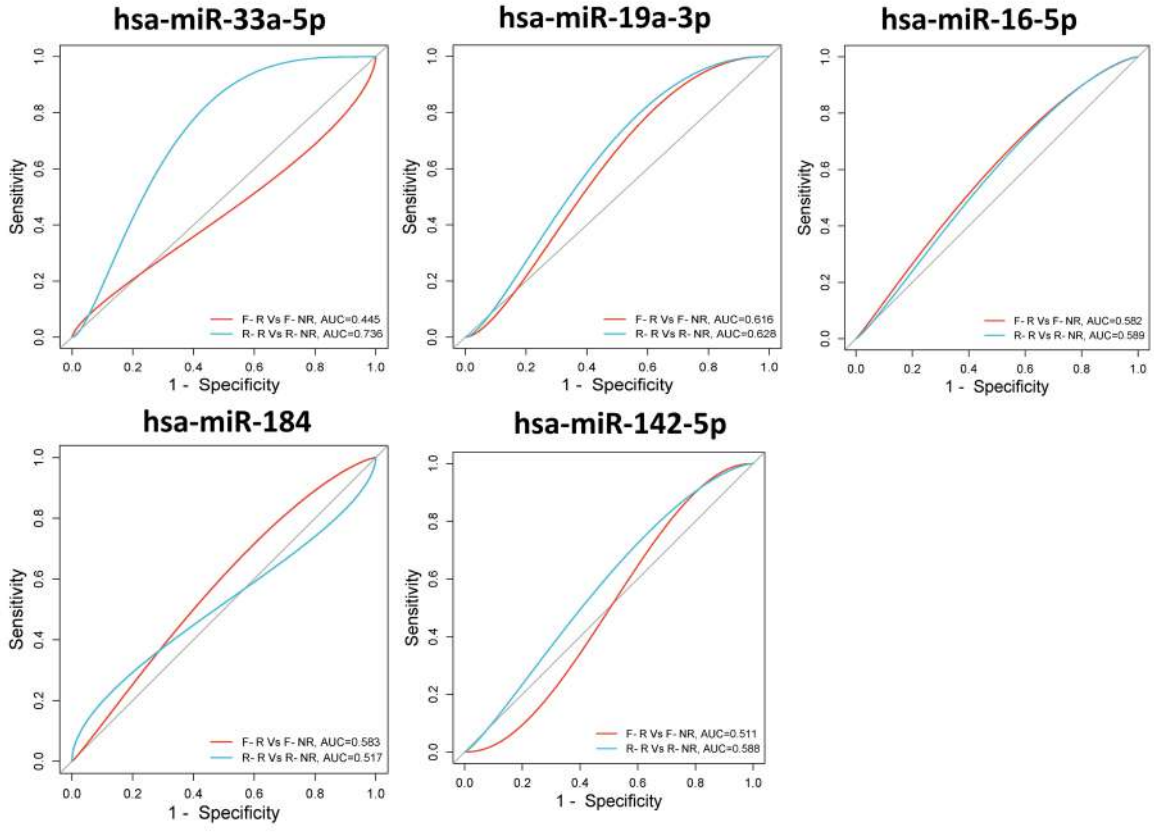


Figure 1.4: ROC curve for prognosis miRNA biomarkers in Fusarium sp. Keratitis.
The graphs were plotted with sensitivity (true-positive rate) vs. 1-specificity (false-positive rate) over all possible ΔCT values

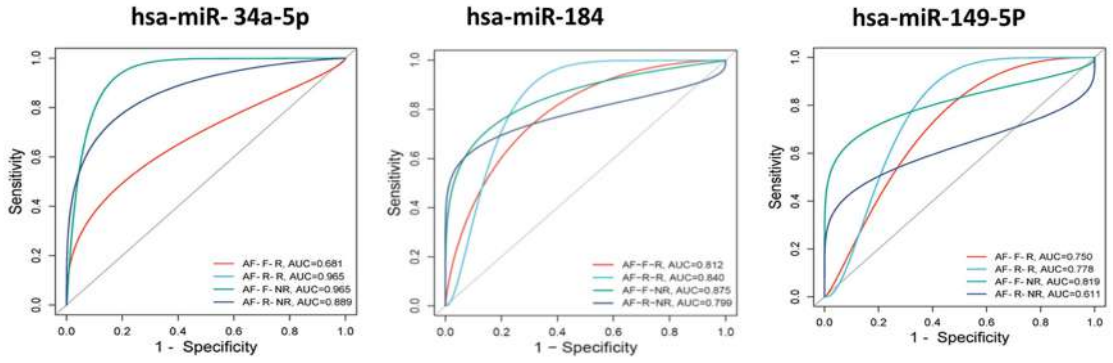


Figure 1.3: ROC curve for miRNA biomarkers in Aspergillus flavus Keratitis.
The graphs were plotted with sensitivity (true-positive rate) vs. 1-specificity (false-positive rate) over all possible ΔCT values

Expression Profiling of Human Corneal miRNAs and their role in Pseudomonas aeruginosa Keratitis

Investigators : Dr. D. Bharanidharan (PI),
Dr. Venkatesh Prajna,
Dr. Lalitha Prajna
Research Scholar : R. Praveenkumar
Funding : DBT-JRF

In this project, we aim to profile the expression of miRNAs in patients with *Pseudomonas aeruginosa* (PA) keratitis and study their role in disease progression. Earlier, we identified a comprehensive profile of human corneal miRNAs in PA keratitis patients using small RNA sequencing. Further, we analyzed the differential expression of miRNAs in PA keratitis corneal tissues compared to cadaver controls and their implications for disease progression through bioinformatic functional analysis. Following this, we profiled miRNAs from corneal swabs taken from the ulcers of patients who were prospectively known to have a good outcome after treatment. Samples were collected at presentation (before treatment) and at review (5-7 days after treatment). By comparing the expression of miRNAs in each outcome group, we identified miRNAs associated with disease severity. These miRNAs will be studied for their role in the pathogenesis of PA keratitis through in vitro experiments in the future.

Results and Conclusions

MiRNAs were profiled from cadaver corneas (n=3), poor outcome corneas (n=3), corneal swabs at presentation (n=4) from good outcome patients, and corneal swabs at review (n=4) from good outcome patients using small-RNA NGS sequencing. Differential expression analysis was performed among the profiled miRNAs in the three groups as follows: i) control vs. poor outcome, ii) control vs. good outcome at presentation, and iii) control vs. good outcome at review. The DE analysis was conducted using the edgeR platform with filtering criteria: nominal p-value < 0.05, fold change > ±1.5, and LogCPM> 3. A total of 112 miRNAs were found to be differentially regulated in poor-outcome patients, of which 54 were upregulated, and 68 were downregulated (Table 2.1). Additionally, 259 and 271 miRNAs were found to be differentially expressed in good outcome patients at presentation and review, respectively.

Table 2.1: List of top differentially expressed miRNAs in poor outcome patients compared to control (1-5 – top downregulated; 6-10 – top upregulated).

S.No.	Dysregulated miRNAs	logFC	logCPM	P value
1	hsa-miR-184	-2.91	18.43	0.01
2	hsa-let-7f-5p	-2.28	16.10	0.01
3	hsa-let-7a-5p	-2.22	16.07	0.01
4	hsa-miR-199a-3p	-1.63	15.00	0.03
5	hsa-miR-27b-3p	-2.59	14.61	0.00
6	hsa-miR-21-5p	3.37	16.34	0.00
7	hsa-miR-146a-5p	4.08	13.44	0.00
8	hsa-miR-146b-5p	4.04	12.79	0.00
9	hsa-miR-223-3p	4.90	10.79	0.00
10	hsa-miR-142-5p	3.76	10.36	0.00

i) MiRNAs associated with disease severity:

Comparing the expression of DE miRNAs in each disease group, i.e) control, poor outcome patients with good outcome patient at presentation and review, the miRNAs associated with disease severity were shortlisted. Seven miRNAs namely,

Fig 2.1a)

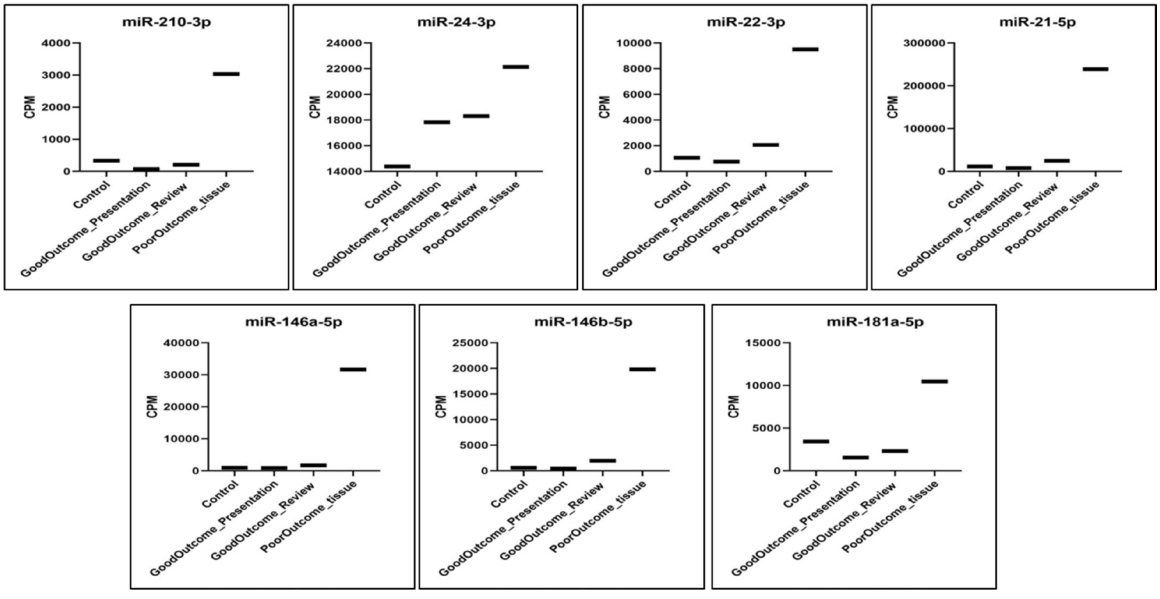


Fig 2.1b)

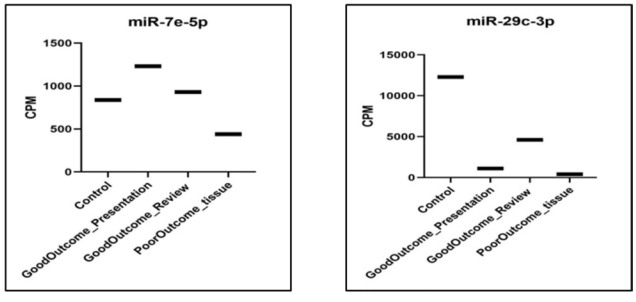


Figure 2.1a & 2.1b: Expression of selected miRNAs either upregulated (top) or down-regulated (bottom) during disease severity is compared by their CPM values in control, good outcome – presentation, good outcome – review and poor outcome patient samples.

miR-210-3p, miR-24-3p, miR-22-3p, miR-21-5p, miR-146a-5p, miR-146b-5p, miR-181a-5p were found to be upregulated during disease severity. While two miRNAs,(miR-7e-5p and miR-29c-3p) were downregulated during disease severity (Figure 2.1a and 2.1b).

ii) Target prediction and Functional network analysis of disease severity associated miRNAs:

To decipher the role of the above-selected miRNAs in PA keratitis pathogenesis, they were subjected to target prediction and functional network analysis. Target prediction was achieved using the bioinformatics tool miRDB, and the predicted targets were subjected to pathway analysis using DAVID. The top significant KEGG pathways enriched upon pathway analysis have been tabulated below (Table 2.2). Among the enriched pathways, the TNF-signaling pathway, the Neurotrophin signaling pathway and the Inflammatory mediator regulation of TRP channels were selected for further studies due to their relevance in corneal ulcer pathology.

TNF-signaling pathway is a master regulator of inflammation, primarily acting through its ability to trigger cell death when activated excessively in response to infection (Phuong et al., 2021). In our network (Figure 2.2), TNF signaling was shown to be regulated by the miRNAs: miR-24-3p through the targets CFLAR, IRF1 and MAPK14; miR-22-3p through the targets PIK3R3, MAPK14, FAS, CREB1 and MLKL; and miR-21-5p through DNM1L. Neurotrophin signaling pathway includes the cascade of nerve growth factors and its downstream effectors which ultimately brings about cell survival and proliferation. Multiple studies have previously described the role of nerve growth factors in the nourishment and homeostasis of corneal epithelium (Compagnoni et al., 2022).

Any dysregulation in this pathway may cause poor prognosis by affecting the corneal epithelial cells' survival and proliferation, leading to delayed wound healing. Notably, this pathway was shown to have a role in fungal keratitis in our previous

study (Boomiraj et al., 2015), thus suggesting its involvement in corneal pathology. From our network (Figure 2.2), five miRNAs miR-181a-5p, miR-22-3p, miR-21-5p, miR-24-3p and miR-146b-5p were found to regulate neurotrophin signaling. The role of these selected miRNAs in regulating their respective predicted pathways and their functional relevance in PA keratitis will be studied further.

Table 2.2: List of enriched KEGG pathways for the predicted targets of the miRNAs upregulated during disease severity.

KEGG pathway	Count	P value
hsa04931:Insulin resistance	13	3E-04
hsa04010:MAPK signalling pathway	22	0.001
hsa04360:Axon guidance	15	0.004
hsa04310:Wnt signalling pathway	14	0.006
hsa05152:Tuberculosis	13	0.020
hsa04722:Neurotrophinsignaling pathway	10	0.021
hsa04151:PI3K-Akt signaling pathway	21	0.022
hsa04015:Rap1 signalling pathway	14	0.029
hsa05222:Small cell lung cancer	8	0.039
hsa04148:Efferocytosis	11	0.040
hsa04150:mTORsignaling pathway	11	0.043
hsa04910:Insulinsignaling pathway	10	0.046
hsa04668:TNF signalling pathway	9	0.049
hsa04750:Inflammatory mediator regulation of TRP channels	8	0.050

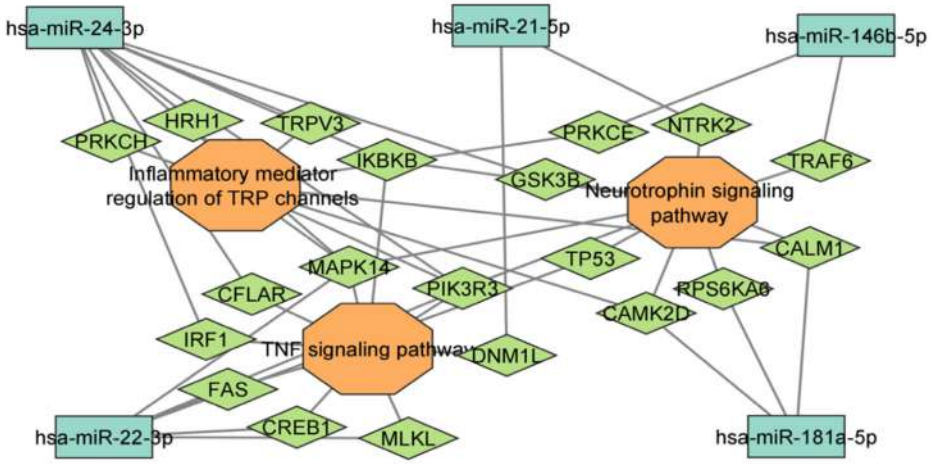


Figure 2.2: Functional network constructed between the selected KEGG pathways (Octagon box), the miRNAs associated with disease severity (Rectangle box) and their predicted target genes (Diamond box)

Investigation of ocular immuno-inflammatory signatures in uveitis patients for prognostication and risk stratification

Investigators : Dr. S.R. Rathinam
Dr. Swagata Ghosh
Funding : MEND Study

Introduction including background

Public health problem: Ocular inflammatory and infectious diseases inflict 14.31% of all patients with ocular conditions in the United States annually. Uveitis, a heterogenous collection of intraocular inflammation involving the uveal tissue (Iris, ciliary body and choroid), represents a predominant type. Uveitis accounts for 10-20% of blindness in the developed world and 25% in developing countries.

Unmet Clinical Need

The diagnosis and medical therapy of uveal inflammatory diseases rely heavily on clinical, microbiological and biochemical parameters. Although the pathogenesis and treatment outcome of these diseases depend significantly on patients' immuno-inflammatory status, ocular immunological parameters are not currently used in clinics for risk stratification and prognostication.

Proposed Solution

Patient and disease specific host immuno-inflammatory parameters-based stratification, prognostication and treatment planning in uveitis. Immuno-inflammatory parameters including stimulatory, inhibitory and regulatory factors will be correlated with the clinical parameters during course of the disease and treatment.

Background and Hypothesis

Uncontrolled host immune-inflammatory responses remain a key factor contributing to morbidity and vision loss in uveitis, regardless of its infectious, non-infectious, or idiopathic origin. While alterations in inflammatory mediators, such as cytokines and chemokines, have been extensively studied in intraocular fluids, translating this knowledge to clinical practice remains challenging, primarily due to enormous individual variability and the lack of established normal ranges. Here we attempt two new approaches in postulating a clinically implementable immune monitoring strategy: (i) analyzing the relationships between immunological parameters, rather than their absolute concentrations, focusing on the correlations between stimulatory, inhibitory, and regulatory proteins that maintain immune balance and how they alter with disease; and (ii) assessing these markers in tear fluid, a non-invasive and accessible sample for disease monitoring. Our selected panel of 15 pro- and anti-inflammatory proteins includes the Macrophage Migration Inhibitory Factor (MIF), a dichotomous immune regulator that can promote or suppress inflammation depending on the physiological context and the type of perturbation. Early studies on MIF status in patient sera and ocular fluid implied an immunostimulatory role of MIF in non-infectious uveitis, however, MIF's association with immune dampening factors such as IL-10 and TGFβ has not been explored in infectious uveitis or in other regionally manifested forms of the disease. Here we hypothesized that the balance of the pro and anti-inflammatory factors and their association with MIF, alters under diseased condition and differs between different uveal inflammatory diseases.

Results and Conclusions

As an initial step to test our hypothesis, we analyzed tear samples from treatment-naïve patients with non-infectious uveitis, comparing them to samples from healthy controls and patients with ocular surface inflammations such as scleritis and episcleritis, using two multiplex immune assay panels covering a total of 15 analytes, comprised of 12 pro-inflammatory (IL-1b, IL-6, IL-8, IL-17A, TNFα, IFNγ, MPO, NGAL, MMP9, TIMP1) and 3 anti-inflammatory factors (IL-10, IL-13, TGFβ).

i) Panel 1 (Low concentration analytes): IL-1b, IL-2, IL-6, IL-8, IL-10, IL-17A, 1L-13, TNFα, IFNγ, TGFβ
ii) Panel 2 (High concentration analytes): MPO, NGAL, MIF, MMP9, TIMP1

Tear samples were collected using Schirmer's tear test strips and eluted in 300 µL of elution buffer (1x phosphate-buffered saline). From each sample, 25 µL was assayed. A total of 66 patient samples were analyzed, comprising 27 from contralateral eyes and 39 from affected eyes. Additionally, tear samples from 20 healthy individuals served as controls. The results of our preliminary experiment show:

A. MIF, IL-1β and IFN-γ are significantly increased in the tear samples of the uveitis patients compared to the healthy controls (Fig 1A, 1F & 1M).
B. IL-6 is significantly increased in the tear samples of both the uveitis and scleritis patients as compared to the healthy controls (Fig 1G). Additionally, patients affected with scleritis and uveitis at the same time, show a further increase in IL-6 levels, although not statistically significant as compared to the healthy controls (Fig 1G).
C. The anti-inflammatory IL-10 shows a significant reduction in concentration in the tear samples from the uveitis patients as compared to the healthy controls (Fig 1J).

D. Several tear molecular factors show significant correlation with tear MIF levels (Figure 2). We observed moderate but statistically significant correlation between MIF and IL-1β or TGFβ-1 specifically in the disease group but not in the healthy controls or disease contralateral group. In contrary, MIF is found to be moderately but significantly associating with IL-2, MMP9 or MPO specifically in the healthy controls which disappears in the disease or the contralateral eye group (Figure 2). In case of TIMP1 and NGAL, MIF shows correlation with each of them irrespective of the sample groups (Figure 2).

Thus, our initial findings are supportive of the idea that altered tear immunological factors might associate with an intra-ocular inflammatory disease like uveitis. We also observed that tear MIF levels correlate with different tear immunological factors differently among disease group, contralateral eye group and healthy controls. These data provide preliminary evidence to examine a large number of patients to identify disease specific MIF-related immunological signatures potentially useful in disease monitoring purposes.

Figure 1

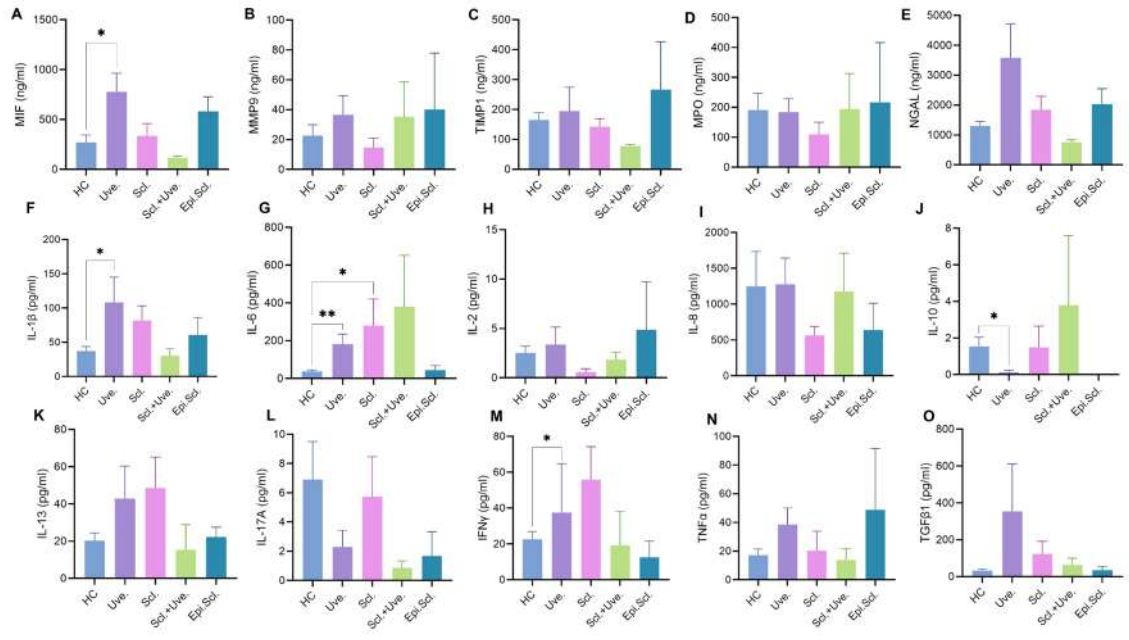


*Figure 1: Levels of tear immunological factors in each of the disease groups and healthy controls. HC-Healthy control (n=20 eyes); Uve- Uveitis (n=24 eyes); Scl- Scleritis (n=9 eyes); Scl.+Uve- eyes with both scleritis and uveitis (n=3 eyes); Epi.Scl- Episcleritis (n=3 eyes). *P<0.05; **P<0.01; Mann-Whitney test.*

	Healthy Controls			Disease group			Contra-lateral unaffected eye		
	r	P value	n	r	P value	n	r	P value	n
IL-1b	0.044	0.852	20	0.519	0.001	39	0.367	0.060	27
IL-2	0.545	0.013	20	0.007	0.967	39	0.022	0.914	27
IL-6	0.368	0.110	20	-0.117	0.478	39	0.265	0.181	27
IL-8	0.349	0.132	20	0.081	0.622	39	0.094	0.640	27
IL-10	-0.28	0.232	20	-0.091	0.581	39	-0.039	0.849	27
IL-13	0.116	0.627	20	0.249	0.126	39	0.209	0.295	27
IL-17A	0.173	0.466	20	-0.221	0.177	39	0.226	0.256	27
TNFa	-0.144	0.545	20	0.299	0.065	39	0.335	0.087	27
IFNg	-0.107	0.654	20	-0.051	0.760	39	-0.043	0.833	27
TGFb1	0.094	0.692	20	0.416	0.009	39	0.261	0.188	27
MMP9	0.536	0.015	20	0.202	0.218	39	0.315	0.109	27
TIMP1	0.666	0.001	20	0.465	0.003	39	0.392	0.043	27
MPO	0.571	0.009	20	0.247	0.129	39	0.253	0.203	27
NGAL	0.623	0.003	20	0.817	<0.0001	39	0.781	<0.0001	27

Figure 2: Spearman Rank Correlation test between tear MIF and each of the other tear immunological factors in healthy controls, disease group and contralateral unaffected eyes. Significant correlations are highlighted in yellow.

REGIONAL RESEARCH CENTRES (RRC)

AMRF applies fundamental science to clinical problems to eliminate the needless blindness specifically focusing on Indian patients. In this endeavor, the scientists of AMRF have strong interactions with clinician scientists from Pondicherry, Coimbatore, Tirunelveli, Chennai and Madurai. One of the successful projects in this area is Identification of families with myoclilin mutations causing JOAG in multiple members of the same family. The examination of the EMR data of families with clinical JOAG in multiple members has found 10% mutation in families. Similar approaches are being carried out starting from the clinician scientists in other areas such as Viral keratitis, Keratoconus. We also explore constantly other areas of collaborative research focusing mainly on clinically relevant problems.

RRC, AEH - MADURAI, PONDICHERRY, TIRUNELVELI & COIMBATORE

Screening of Family Members of Juvenile Open Angle Glaucoma (JOAG) Patients for Myocilin Gene Mutations

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: Dr. Ganesh V. Raman AEH, Coimbatore
: Prof. K. Dharmalingam, Dr. A. Vanniarajan Dr. D. Bharanidharan, AMRF

Research Personnel : V. Saravanan Dr. T M Viswanathan, Ms. Sridevi

Funding : AMRF, AEF, Sun Pharma

Background

Myocilin mutations are significantly associated with the severe form of Juvenile-onset Open-Angle Glaucoma (JOAG). Early detection of myocilin mutations is crucial in preventing vision loss in high-risk groups. While population-level screening for myocilin mutations is cost-intensive, selective screening of JOAG patients and their family members is cost effective and essential for early detection and preventive measures. The study aimed to screen JOAG patients seeking treatment at Aravind Eye Hospitals in Madurai, Pondicherry, Tirunelveli, and Coimbatore for Myocilin gene and selectively screen all the family members for myocilin mutations.

Results

AEH, Madurai

Based on the electronic medical record data, 218 JOAG patients who visited the hospital in the last two years were called and identified 51 probands with a family history of glaucoma. Blood samples

Table1: Myocilin gene mutations identified in eight families

Sr.No.	Families	Genomic Position (NC_000001.11)	cDNA position (NM_000261.2)	Protein change (NP_000252.1)	Known/ Novel
1	J89 family	g.171636341C>T	c.1099G>A	G367R	Known
2	J85 family	g.171636000G>C	c.1440C>G	N480K	Known
3	J51 family	g.171636347A>C	c.1093T>G	Y365S	Novel
4	J59 family	g.171636352G>A	c.1088C>T	A363G	Novel
5	J144 family	g.171636352G>A	c.1088C>T	A363G	Novel
6	J126 family	g.171636353C>T	c.1087G>A	A363T	Known
7	J149 family	g.171636310G>A	c.1130C>T	T377M	Known
8	J196 family	g.171636352G>A	c.1088C>T	A363G	Novel

Figure 1: Location of myocilin mutation positive JOAG families

were collected, isolated DNA, and stored for further experiments. For the myocilin gene mutational screening, exon 3 was first screened; later, if they turned negative, the other two exons of the myocilin gene were screened. The location of mutations identified in marked in Figure 1, Four families were identified with known pathogenic myocilin mutations and four families with novel myocilin mutations, as shown in Table 1.

Known pathogenic myocilin mutations G367R, N480K, A363T and T377M were identified in four families. In addition, one novel heterozygous mutation, Y365S, and one novel homozygous mutation, A363, were detected in one and three families, respectively (Table 1), which requires further screening of family members and experimental studies to confirm their pathogenicity.

AEH, Pondicherry

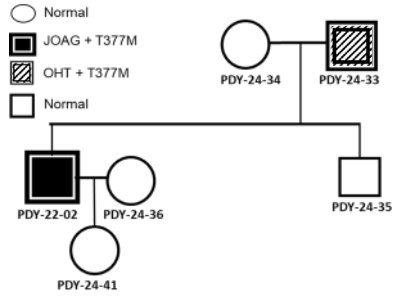
During the study period, 36 patients were recruited. Blood samples were collected and DNA was isolated from 22 samples in RRC at Pondicherry and sent to AMRF. For rest of the samples, DNA was isolated at AMRF. Out of 36 samples, 25 showed good-quality DNA and used for Myocilin gene screening. Out of the five families, myocilin mutation T377M was detected in one family (Figure 2).

AEH, Coimbatore

So far, 23 patients have been recruited for the study. DNA was extracted from all 23 samples, and good-quality DNA was used for Myocilin gene screening. Out of seven families, Myocilin mutation P254L was identified in one family (Figure 3). Three family members with POAG/JOAG were screened and a pathogenic heterozygous mutation P254L was detected in all of them. P254L has been reported as a pathogenic mutation in glaucoma patients (Li et al., 2021; Nakahara et al., 2022).

AEH, Tirunelveli

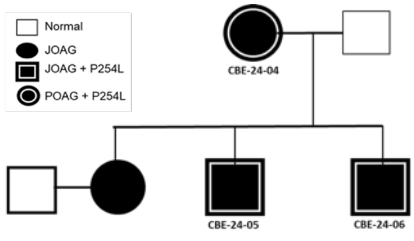
Earlier, Myocilin gene mutation N480K was detected in 20 members of Kadaladi family, including eight JOAG patients, seven POAG patients, three JOAG



Details of Family Members Screened for Myocilin Mutation

S.No	Sample ID	Age / Sex	Diagnosis	Myocilin Mutation
1	PDY-22-02	29/M	JOAG	T377M
2	PDY-24-33	66/M	OHT	T377M
3	PDY-24-34	53/F	Normal	No Change
4	PDY-24-35	27/M	Normal	No Change
5	PDY-24-36	29/F	Normal	No Change
6	PDY-24-41	2/F	Normal	Low Yield

Figure 2: Pedigree of the family PDY-22- 02 and myocilin mutation screening results



Details of Family Members Screened for Myocilin Mutation

SL NO	Study No	AGE/ SEX	M.R.No	Diagnosis	Myocilin Mutation
4	CBE-24-04	46/F	P3004219	POAG	P254L
5	CBE-24-05	21/M	P1923158	JOAG	P254L
6	CBE-24-06	19/M	P1923159	JOAG	P254L

Figure 3: Pedigree of the family CBE-24-J04 and myocilin mutation screening results

suspects, and two unaffected. Among the unaffected, one was under five and another was 25. The earliest onset of the disease was noted at ten years in the family. The mutation's penetrance was 95% after age ten. Additionally, normal individuals carrying Myocilin N480K are being followed up.

We have established a new research facility in February 2025. With this, the retrospective analysis, further recruitment of JOAG patients, and details of family history have commenced.

Conclusion

Preliminary findings revealed multiple families with known and novel Myocilin mutations, underscoring the benefits of proactive genetic screening for JOAG management. The approach is sound, and numerous Myocilin mutations were identified across Tamil Nadu. Four of the mutations have been shown to be pathogenic; consequently, the families will be informed about the implications and counselled accordingly.

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RRC, AEH - MADURAI, TIRUNELVELI & PONDICHERRY

Genetic biomarkers for preclinical prediction for the onset of Keratoconus (KC) among Down's syndrome (DS) patients from Southern India

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Prof. K. Dharmalingam, AMRF

Research Personnel : Ms. Agnes Angel

Introduction

Down's syndrome (DS) is a chromosomal disorder affecting both mental and physical developmental abnormalities in children. Among the various ophthalmic problems, Keratoconus (KC) is an early and common manifestation in DS patients. There are no reports of genetic profiles for the manifestation of KC in DS in the Indian population. Early prediction of specific gene loci in DS patients would identify the patients prone to KC, and thereby regular follow-up and early intervention with CXL would improve their visual quality and quality of living in the future.

Results

Using tomographic parameters, a cross-sectional observational study was done at Aravind Eye Hospital, Tirunelveli. A total of 65 individuals were enrolled from special needs schools and relevant non-profit organizations. The study participants were divided into five groups: (1) KC, (2) KC suspect, (3) FFKC, (4) Advanced KC and (5) No KC. We have observed that 20.8% of the DS individuals had KC, and the incidence was higher in females (92.8%).

The mean age of KC patients was 13.46 years, compared to the non-KC group (29 years). All patients diagnosed with KC had a history of vigorous eye rubbing and blepharoconjunctivitis.

In the analysis of tomographic indices, Z K max, ART max, BAD-D, Mean CT, ARC, PRC and HOA were noted to be statistically significant for the diagnosis of KC. In ROC analysis, the Z K max and HOA were found to be the best predictors for diagnosing KC from non-KC among the DS population. The visual acuity measured between the groups was found to be statistically significant.

From the tear samples collected from the patients, the levels of MMP9 was measured by ELISA. Out of the 7 DS patients, two showed higher MMP levels and it was ranging up to 9.95 ng/ml.

RRC ARAVIND - MADURAI & PONDICHERRY

Analysis of tear protein profile in Herpes simplex immune stromal keratitis

Investigators : Dr. Josephine Christy,
Dr. Aditi Parab
AEH, Pondicherry:
: Prof. K. Dharmalingam
Dr. A. Vanniarajan, AMRF

Research Personnel : Karthik, Dr. Viswanathan

Introduction

Diagnosing viral keratitis traditionally relies on patient history and clinical signs, with HSV keratitis lacking a standardized diagnostic method. Virus isolation,

while a gold standard, is time-consuming, insensitive, and requires specialized labs. Newer methods like real-time PCR for HSV DNA and ELISA for HSV-specific IgA antibodies remain promising. Additionally, tear film proteomics is emerging as a diagnostic tool, revealing protein patterns linked to ocular surface health. This study on tear protein profiles in HSV keratitis aims to understand the disease process and to identify biomarkers for different forms of the disease.

Results

Tear samples were collected from 21 individuals including 2 healthy controls and 19 patients. Among the 19 patients, 8 had recurrent dendritic keratitis, 5 had recurrent immune stromal keratitis, 4 had recurrent endothelitis and 1 each had keratouveitis and recurrent stromal keratitis. The protein profiles reveal distinct bands corresponding to different proteins present in the tear samples (Figure 4). Some protein bands (indicated by blue arrow) showed different intensities in the infected eyes (Lanes 3, 5, 7) compared to fellow eyes (Lanes 4, 6, 8). These differences will be further followed up with Mass spectrometric analysis.

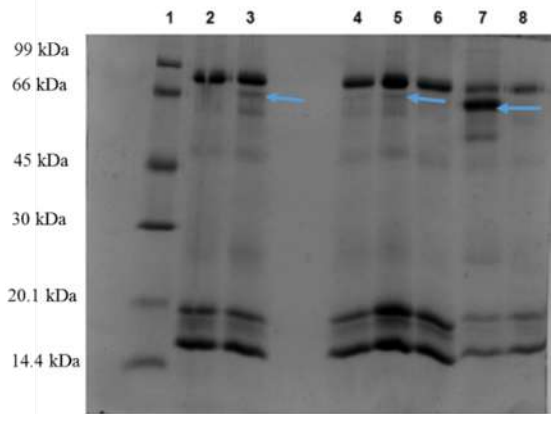


Figure 4: Protein profiles of tear samples from patients with viral keratitis

Conclusion

Distinct protein profile was observed in infected eyes compared to the fellow eyes. Mass spectrometric analysis of the peptides will provide further insights in the disease process.

RRC, AEH - THENI & AMRF PROJECT

Evaluation of defect in Serine biosynthesis and PHGDH gene variant in South Indian Macular Telangiectasia Type 2 patients

Investigators : Dr. D. Hema
AEH, Theni
: Prof. K. Dharmalingam,
Dr. A. Vanniarajan, AMRF

Introduction

Macular telangiectasia type 2 (MacTel) is an idiopathic, bilateral, degenerative condition characterized by perifoveal telangiectatic vessels and neurosensory retinal atrophy. Serine deficiency was found in MacTel patients due to the accumulation of deoxysphingolipids. Serine biosynthesis defect was known to cause MacTel due to haploinsufficiency of PHGDH. This proposal is aimed at evaluating serine and other amino acid levels in plasma of MacTel patients and correlating the PHGDH gene variant G228W in patients with decreased Serine levels.

Results

In a pilot study, blood samples were collected from 5 MacTel and 2 Control patients. Plasma was separated from the blood and processed for the amino acid quantification. The amino acid levels were measured by metabolomic analysis. Asparagine, proline and glycine showed significant down regulation in Mactel group compared to control group, however, serine levels were similar as controls.

Conclusion

Variations in the levels of amino acids were identified in the plasma samples of MacTel patients. Further analysis with larger samples and genetic analysis will find out the molecular changes involved in MacTel.

RRC, AEH - COIMBATORE

AEGIS Objective I: Advancing CRISPR-Based Fungal Diagnostics

Investigators : Dr. Siddharth Narendran, Dr. Saravanan VR, Dr. Sree Lakshmi, Dr. Divya, Dr. Ram RamMohan, Dr. Lalitha Prajna, Dr. Venkatesh Prajna, Dr.Daipayan Banerjee

Project students : Hanith Raj, Balaji, Deepa, Dharshini

Background

Fungal infections of the eye, such as Fungal Keratitis (FK) and Fungal Endophthalmitis (FE), pose significant diagnostic challenges, particularly in resource-limited settings. FK predominantly affects the rural agrarian population, compelling patients to travel on average 160km to reach tertiary care facilities for essential diagnostic services. Conversely, Fungal Endophthalmitis presents a different set of challenges; approximately 80% of endophthalmitis cases result in culture-negative outcomes using current gold standard methods. Recent next-generation sequencing (NGS) data from India suggests a startling revelation that 65% of these culture-negative samples could indeed be fungal. The pressing need for a more accessible and accurate diagnostic tool led to the development of a CRISPR-based diagnostic approach under the AEGIS core to tackle these infections efficiently.

Results

1. Development of the RID-MyC Assay: Successfully developed a rapid and accurate CRISPR/Cas12a-based molecular diagnostic assay, termed Rapid Identification of Mycoses using CRISPR (RID-MyC), specifically designed to detect fungal nucleic acids.
2. Validation in Fungal Keratitis (FK): The RID-MyC assay was validated in a clinical setting for FK diagnosis, demonstrating a high sensitivity of 93.27% (95% CI, 86.62%–97.25%) and a specificity of 89.47% (95% CI, 66.86%–98.70%). The median time to diagnosis was notably quick at 50 minutes, ranging from 35 to 124 minutes.
3. Validation in Fungal Endophthalmitis (FE): In cases of FE, the RID-MyC assay showed a sensitivity of 90.48% (95% CI, 69.62%–98.83%) and a specificity of 96.43% (95% CI, 91.11%–99.02%), affirming its reliability and effectiveness in diagnosing more severe fungal eye infections.
4. Clinical Utility in Smear and Culture Negative FK: The RID-MyC assay has proven particularly useful in diagnosing FK in situations where traditional smear and culture methods are inadequate, such as in patients who have undergone prior antifungal therapy or in cases of polymicrobial infections.
5. Simplification of the Diagnostic Process: Efforts to streamline the diagnostic procedure led to the development of the ST-RID-MyC (Single Tube Rapid Identification of Mycoses using CRISPR) assay. This one-tube RPA-coupled CRISPR/Cas12a-based assay has significantly reduced the mean time to diagnosis to just 6 minutes, compared to 32 minutes with the traditional RID-MyC assay, with statistical significance ($p<0.001$).

Future Directions

1. Lyophilization and Commercialization: The next step for the RID-MyC assay involves the lyophilization of assay components to enhance stability and shelf-life, facilitating easier storage and transport. This development is pivotal as it paves the way for the commercialization of the assay, making it accessible as a standardized diagnostic tool in both rural and urban healthcare settings.
2. Diagnostic Randomized Controlled Trial: To further validate the efficacy and impact of the RID-MyC assay, a diagnostic randomized controlled trial is planned. This study will explore the role of RID-MyC in clinical decision-making and therapeutic outcomes in culture-negative fungal endophthalmitis, aiming to establish the assay as a critical tool in managing these challenging cases.
3. Further Simplification of RID-MyC: Efforts are underway to simplify the RID-MyC process further by developing an asymmetrical RPA-free version of the assay. This adaptation aims to reduce the complexity and time required for the diagnostic process, making it even more feasible for point-of-care use without specialized laboratory equipment.
4. Development of a Point-of-Care Device: The ultimate goal is to develop a portable, user-friendly point-of-care device for the RID-MyC assay, specifically tailored for the diagnosis of fungal keratitis. This device would significantly reduce diagnostic delays and allow for immediate treatment initiation, which is crucial in preventing sight-threatening complications from fungal infections.

Publications

1. Deivarajan HR et al. Development and Clinical Evaluation of a CRISPR/Cas12a-Based Nucleic Acid Detection Platform for the Diagnosis of Keratomycoses, Ophthalmology Science, Volume 4, Issue 5, 2024,
2. Deivarajan HR et al. Clinical Utility of CRISPR-Based RID-MyC Assay in Smear and Culture-Negative Fungal Keratitis: A Case Series. Cornea. 2025 Feb 3.
3. Deivarajan HR et al. Clinical Evaluation of a Novel CRISPR-Cas12a-Based RID-MyC Assay for the Diagnosis of Fungal Endophthalmitis Article in Press

Funding

1. VELUX STIFTUNG Foundation – INR 206 Lakhs (2021-2025)
2. HABISTAT Foundation – INR 20 Lakhs (2024-2025)

AEGIS Objective II: Aravind Registry for Inherited Diseases of the Eye (ARIDE)

Investigators : Dr. Siddharth Narendran, Dr. Rodney J Morris, Dr. Anju Jose, Dr. Abhishek, Dr. Sree Lakshmi, Dr. Prema

Project students : Jeyashree, Dharani.

Background

The Aravind Registry for Inherited Diseases of the Eye (ARIDE) represents a crucial initiative by the Aravind Eye Care System, designed to establish a comprehensive DNA biobank along with detailed phenotyping and pedigree analysis. This registry is focused on identifying cohort-specific genetic variants associated with inherited eye diseases, which have a notably high prevalence in India.

Results

1. Participant Recruitment: The ARIDE registry has successfully enrolled 626 participants, including 434 probands and 192 family members. It features extensive data collection encompassing demographics, symptoms, dietary and birth history, medical and women's health history, family lineage, and clinical diagnoses.
2. Whole Exome Sequencing (WES): To date, ARIDE has analyzed whole exome sequencing data for 108 patients, identifying 187 genetic variants across 90 genes in patients with Retinitis Pigmentosa. Out of these, 24 variants have been classified as pathogenic, 36 as likely pathogenic, and 128 as variants of uncertain significance(VUS).
3. Biobank Establishment: ARIDE has developed a structured biobank that meticulously stores genomic DNA extracted from blood and buccal samples, ensuring high-quality genetic material is available for current and future research.
4. Pre-Test Genetic Counselling: A comprehensive pre-test genetic counseling service has been integrated within the registry framework to educate patients and families about the hereditary nature of their conditions and the preventive measures available to mitigate the risk of disease transmission to future generations.

Future Directions

Enhancing Ophthalmologist Partnerships: One of the immediate future directions for ARIDE involves strengthening partnerships with ophthalmologists across the Aravind Eye Care System and beyond. The goal is to build a more holistic ecosystem that can effectively handle the complexities of inherited eye diseases.

Expanding Genetic Testing and the Registry: ARIDE plans to significantly expand its genetic testing capabilities and extend the registry's reach. This expansion will include enrolling more participants to deepen the genetic data pool and extend the impact of the registry across a broader spectrum of inherited eye diseases.

Funding

1. GKD Charity Trust – INR 101 Lakhs (2023-2026)

ADORE Objective I: Gender Disparity in Cataract Prevalence

Investigators : Dr. Siddharth Narendran, Dr. Rekha, Dr. Daipayan Banerjee, Dr. Jaishree, Dr. Ruhel Chacko

Project students : Elakkiya Nandhini, Akshaya.

Background

Cataract remains a leading cause of blindness globally, with notable disparities in the prevalence and management between genders. Initial observations suggest that women are disproportionately affected by cataract-related blindness. This disparity is influenced by a combination of socioeconomic, cultural, and biological factors that hinder women's access to timely and adequate eye care services.

Results:

1. Cataract Surgery Disparity: An analysis of 3.9 million cataract surgeries highlighted that women not only undergo cataract surgery at a younger

average age compared to men (mean ± SD; 60.44 ± 9.87 years vs. 62.56 ± 10.15 years; p < 0.001), but also face this issue earlier in life, especially noticeable in the 40–49 age group. Here, the surgery rates for women were nearly double those of men (incidence rate ratio [IRR] 1.897; 95% CI, 1.885–1.908; p < 0.001), indicating a significant age-related disparity.

2. Sex Hormone-Dependent Chaperone Expression: Preliminary data suggest that chaperones, which help prevent protein disaggregation—a key factor in cataract formation—are hormone-dependent. This dependence could be a contributing factor to the earlier incidence of cataracts in women. The expression levels and activity of these chaperones may vary with hormonal changes, particularly those related to sex hormones, which are significantly different in women, especially during periods such as menopause.

Future Directions:

1. Expand Epidemiological Understanding through a Meta-Analyses of Population-Based Studies and Conduct a Comprehensive Multicentric Hospital based study.
2. Elucidate the Influence of Estrogen on Molecular Chaperone Dynamics in the Lens Epithelium
3. To Explore the Dynamics of Estrogen Receptor Splice Variants and Their Regulatory Effects on Molecular Chaperone Expression

Publication:

1. Narendran et al. Gender and Age Trends in Cataract Surgery Utilization in India: A Retrospective Multicenter Analysis of 3.3 Million Procedures. JAMA Network Open (under review)

Funding:

1. ICMR Small Grant – INR 35 Lakhs (2025-2027)

ADORE Objective II: Geographical Disparity in Diabetic Retinopathy

Investigators : Dr. Siddharth Narendran, Dr. R.Kim, Dr. Vignesh Elamurugan, Dr. Sujay, Dr. Anju Jose
Project students : Niraja, Karvannan.

Background:

Diabetic Retinopathy (DR) is a leading cause of visual impairment, particularly in India, where the escalating prevalence of Diabetes Mellitus (DM) poses a significant public health challenge. Notable geographical disparities exist in the prevalence of DR and Vision-Threatening Diabetic Retinopathy (VTDR), particularly between Tamil Nadu (TN) and Kerala (KL). Despite similar socioeconomic conditions and healthcare infrastructures, these disparities suggest that other factors such as diet, genetics, and environmental influences may significantly contribute. Addressing these disparities is crucial for optimizing healthcare resource allocation and improving strategies to reduce DR-related vision loss.

Results:

1. Phase 1: Retrospective Study (2016–2020, N=213,440): Higher DR prevalence in Kerala (21.22%) compared to Tamil Nadu (8.47%). Higher VTDR prevalence in Kerala (10.87%) compared to Tamil Nadu (3.26%). Adjusted analyses show patients from KL had 2.01 times higher odds of DR and 2.25 times higher odds of VTDR than those from TN.
2. Phase 2: Prospective Study (2023–2024, N=589): Coconut oil consumption linked to increased risk of DR (OR: 2.19) and VTDR (OR: 2.70).

Future Directions:

1. Gudalur Eye Study: This study will analyze the impact of diverse dietary habits on DR prevalence in a region where Tamil and Malayalam-speaking communities coexist. It will include detailed questionnaires on diet, socioeconomic status, demographics, and HbA1c.
2. Metabolomic Analysis: Comprehensive metabolomic analyses will be conducted to identify specific biomarkers associated with DR, including metabolic alterations linked to different dietary fats and their roles in DR progression.

Publications

1. Narendran S et al. Geographical disparities in the prevalence of diabetic retinopathy in two contiguous states of South India. AJO Int. 2025;2(1):100096.

ACER Objective I: Investigating Lens Regeneration by Analyzing Proteomic Shifts and Mechanistic Pathways Involved in Cataractogenesis

Investigators : Dr. Siddharth Narendran, Dr. Rekha, Dr. Jaishree
Project students : Kanmani

Background:

The progression of cataracts, a leading cause of visual impairment worldwide, is influenced by a complex interplay of environmental, behavioral,

and hormonal factors. In regions lacking primary healthcare, many suffer from advanced cataracts, leading to lens liquefaction. Our goal is to dissect the proteomic landscape of hypermature cataracts to understand the mechanisms driving lens liquefaction.

Results:

- Proteomic Analysis in Liquefied Cataracts: Leveraging mass spectrometry, we have identified specific proteins in the lens fluid of patients with hypermature cataracts that differ significantly in expression compared to controls.
- Protein Aggregation Assays: Our studies have shown that certain proteins in the intralenticular fluid (ILF) facilitate the disaggregation of crystallin proteins.

Future Directions:

- Validating Novel Disaggregating and Chaperone Proteins: We plan to validate the function of newly identified disaggregating and chaperone proteins through Western Blotting and functional assays.

ACER Objective II: Deciphering Mechanisms Modulating Endogenous Retinal Pigment Epithelium Regeneration in Macular Degeneration

Investigators : Dr. Siddharth Narendran, Dr. Jaishree
Project Students : Karvannan

Background

Age-related Macular Degeneration (AMD) is a major cause of vision loss in the elderly, particularly in India where the aging population is rapidly increasing. Current treatments do not effectively halt RPE degeneration, highlighting the need for innovative strategies that harness the regenerative capabilities of RPE cells. This research aims to identify the cellular and molecular mechanisms underlying RPE regeneration, using advanced techniques like single-cell RNA sequencing to explore cellular dynamics post-injury.

Results:

- Regeneration Capacity of RPE: Following induced degeneration in mice, significant recovery in RPE structure and function was observed, particularly evident in improved phagocytic activity by day 28 post-injury, indicating potential for functional recovery.

Future Directions:

- Neogenesis and Cell Replication: Exploring the role of cell division and neogenesis in RPE repair and regeneration.
- Extracellular and Transcriptional Influences: Examining how extracellular factors and transcriptional co-factors like YAP and TAZ influence RPE regeneration.
- Inflammatory and Age-Related Factors: Investigating the impact of inflammation and aging on the regenerative processes, focusing on how these factors affect cellular responses and tissue recovery.

CONFERENCES / MEETINGS

Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO) 2024

Washington, 5-9 May 2024

Faculty members, scientists, and research scholars from AMRF, including Prof. K. Dharmalingam, Dr. P. Sundaresan, Dr. Gowri Priya Chidambaranathan, R. Sethu Nagarajan, and Shreya Dinesh, participated and presented papers at the ARVO annual meeting held in Seattle, Washington. To attend this meeting, Sethu Nagarajan received the ICMR Travel Grant, while Shreya Dinesh received the SERB Travel Grant. Notably, Shreya Dinesh's poster was selected as a Hot Topic.

- R. Sethu Nagarajan
  Inhibition of KIF14 supresses the retinoblastoma tumour progression with favourable outcome (Paper Presentation)
- Dr. P. Sundaresan
  Unravelling nuclear and mitochondrial contributions in Leber's Hereditary Optic Neuropathy (Poster Presentation)
- Dr. Gowri Priya Chidambaranathan
  MicroRNAs regulating human trabecular meshwork stem cells (Poster Presentation)
- Shreya Dinesh
  Dysregulated miRNAs and their role in Fusarium Keratitis (Poster Presentation)

National Symposium at Vivekananda – Pharmacy College for Women, Sankagiri, Salem

Salem, 10th August 2024

Dr. S. Senthilkumari was an invited speaker at the 2nd National symposium on Innovative Breakthrough in Pharmacare: Nurturing the future Pharmacists-2024, held at Vivekananda Pharmacy College of Pharmacy for Women, Sankagiri, Salem. She spoke on, "MicroRNA-based therapeutics for Glaucoma" which was well received by the participants.

30th Annual meeting of the ARVO-India 2024

New Delhi, 27-29 September 2024

Scientists, faculty members, and research scholars from AMRF participated in the 30th Annual Meeting of the Association for Research in Vision and Ophthalmology (ARVO)-India 2024, held at the All India Institute of Medical Sciences, New Delhi. They contributed through lectures, talks, and presentations. The programme focused on the theme, "Evolving Dimensions in Eye Research." During the event, Dr. P. Sundaresan delivered the Dr. D. Balasubramanian Oration Lecture on the topic, "Molecular genetics in ocular diseases: Laneways for personalised medicine," while Dr. S. Senthilkumari delivered the keynote lecture on, "MicroRNA-based therapeutics for steroid-induced ocular hypertension/glaucoma: Insights from Ex Vivo studies of human eyes."

Dr. Anwar Azad gave an invited talk at the symposium cum workshop on Advanced Techniques to Augment Innovations in Vision Sciences on the topic, "Challenges in retinal organoids and potential

AMRF team at the Annual Meeting of ARVO held in Seattle, Washington

Dr. S. Senthilkumari being honoured with a memento at the National Symposium, Salem

AMRF team at the ARVO-India Annual Meeting

advancements." Prof. K. Dharmalingam delivered an invited talk at the symposium on HRMS using Orbitrap Technology on the topic, "Proteomics of ocular extracellular vesicles." Additionally, Iswarya and R. Sethu Nagarajan received travel fellowships, and Sethu Nagarajan was honoured with the Young Investigator Award for Best Poster. Several other participants from AMRF also presented posters and papers as follows:

- K. Saraswathi

  Genomic and transcriptomic profiling identifies distinct molecular signatures in Recurrent Ocular Adnexal B-Cell Lymphoma (Oral Presentation)
- Dr. Swagata Ghosh

  Corneal transcriptomic signatures in fusarium keratitis patients with distinct disease trajectories: Deciphering protective and pathogenic host responses (Oral Presentation)
- Dr. Daipayan Banerjee

  Epigenetic alterations in pterygium: Genome-wide methylation profiling reveals potential oncogenic pathways (Oral Presentation)

Dr. P. Sundaresan being honoured for delivering the Dr. D. Balasubramanian Oration Lecture, New Delhi

- R. Sethu Nagarajan

  Patient-derived three-dimensional spheroid culture: A promising model for drug screening in retinoblastoma (Poster Presentation)
- R. Kanthimathi

  Integrative metadata analysis and validation of putative markers for adult retinal pigment epithelial stem cells (Poster Presentation)
- P. Thushmitha

  Decline in YAP nuclear expression in lens epithelium of cataractous donors compared to healthy donor lenses (Poster Presentation)
- R. Iswarya

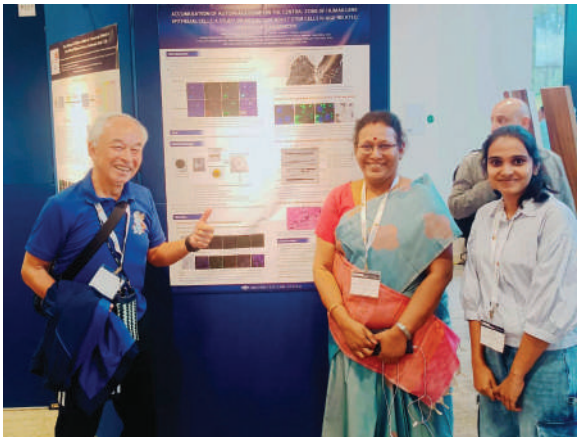
  Trabecular meshwork stem cell exosomes: Identifying the cargo and functional efficacy for a cell-free therapy for glaucoma (Poster Presentation)
- T. Keerthana

  Characterisation of pearl/regeneratory posterior capsular opacification (Poster Presentation)
- R. Praveen Kumar

  Identification of human corneal miRNAs in the disease severity of pseudomonas aeruginosa keratitis (Poster Presentation)
- Shiva Shankari

  Clinical profile of neuro-ophthalmological conditions at a tertiary eye care centre: A six-year retrospective study (Poster Presentation)
- G. Hariharan

  Proteomic analysis of extracellular vesicles from saprophytic and clinical isolates of Aspergillus Flavus: A comparative study (Poster Presentation)
- Aadhithiya T. G. (Participant)



Dr. Gowri Priya Chidambaranathan (centre) presenting her poster

Dr. S. Senthilkumari at APPICON 2024, Chennai

Singapore International Symposium on "Stem Cells in Tissue Maintenance, Repair, and Aging"

Singapore, 11-13 December 2024

The International Society for Stem Cell Research, in partnership with the Stem Cell Society Singapore, conducted a unique symposium at Nanyang Technological University, Lee Kong Chian School of Medicine, Singapore. The event brought together scientists from across the globe to share their latest research on tissue stem cells and their roles in maintenance, repair, and aging. Faculty members and research scholars from the Department of Immunology and Stem cell Biology, AMRF participated in the symposium and presented posters. Dr. Gowri Priya Chidambaranathan presented a poster on "Accumulation of autophagosomes in the central zone of human lens epithelial cells: A study on biology of adult stem cells in age-related cataract pathogenesis," and Sneha Nair presented a poster on "MiRNAs regulating human trabecular meshwork stem cell maintenance and their decline with ageing." Before the symposium, the team visited Singapore Eye Research Institute (SERI) and met Dr. Gary SL Peh, Junior Principal Investigator, Tissue Engineering and Stem Cell Group, and Dr. A.R. Muralidharan, Assistant Professor, Visual Neuroscience Department. They also visited the 10X Genomics Office at Singapore to discuss spatial transcriptomics technology.

Annual Conference of Physiologists and Pharmacologists of India - APPICON 2024

Chennai, 12th December 2024

Dr. S. Senthilkumari has been invited to deliver a talk at the 70th Annual conference of Physiologists and Pharmacologists of India - APPICON 2024, organised by the ESIC Medical College & Hospital, K.K. Nagar, Chennai. She spoke at the scientific symposium on the topic "Drug Penetration across Blood-ocular Barriers - P-glycoprotein & Efflux Transporters of Blood Ocular Barriers".

Participants of the workshop on Extracellular Vesicles: Isolation, Characterisation, and Applications

CONFERENCES CONDUCTED

Workshop on Extracellular Vesicles: Isolation, Characterisation, and Applications

AMRF, 24-25 October 2024

Organised by AMRF as part of the October Summit and the Scientific Social Responsibilities (SSR) initiative of the ongoing SERB-SRG project, this two-day hands-on workshop featured lectures by Dr. Arup Banerjee, Professor, Regional Centre of Biotechnology, Faridabad, Dr. SM Salahudeen, Director, Tricell Biologics, Chennai, Prof. K. Dharmalingam, and Dr. Gowri Priya. Participants conducted hands-on experiments to isolate extracellular vesicles (EVs) from plasma using two distinct techniques and observed a demonstration of EV isolation from Platelet-Rich Plasma (PRP). They also performed EV characterisation using Nanoparticle Tracking Analysis (NTA), protein isolation, Western blot, and proteome analysis.

During the event, Dr. Daipayan Banerjee, the Principal Investigator and Convenor, emphasised the growing significance of EV research in diagnostic and therapeutic applications and conducted practicals with student resource persons. Researchers from AMRF, including Hariharan Gnanam, Kanmani, Aruna Devi, Karthik, Mathan, Dr. Viswanathan, Aadhithiya, and Iswarya, served as resource persons. A total of 17 participants, including faculty members, Ph.D. research scholars, and M.Sc. students from 11 institutions across Tamil Nadu, attended the workshop.

AMRF – Dartmouth Education and Research Conference

AMRF, 3-4 December 2024

AMRF hosted a two-day Education and Research Conference in collaboration with Dartmouth College, Hanover, USA. This partnership began in 2016 through discussions between Dr. N. Venkatesh Prajna, Chief, Cornea Services, Aravind-Madurai, and Dr. Michael E. Zegans, Chief, Ophthalmology Department, Dartmouth-Hitchcock Medical Centre. The conference was organised by Prof. K. Dharmalingam, and coordinated by Dr. D. Bharanidharan, Scientist, Microbiology and Bioinformatics, AMRF, alongside Dawn E. Carey, Senior Associate Director, Global Health and Development, Dartmouth College.

During the morning sessions, ten students from Dartmouth College presented their research proposals on various eye diseases to an audience group of clinicians, scientists, and research scholars. The presentations were highly praised by the invited experts, including Dr. R. Kim, CMO, Aravind-Madurai, Dr. N. Venkatesh Prajna, Dr. S. R. Rathinam, Dr. Usha Kim, Dr. S. Lalitha Prajna, and Dr. Mahesh Kumar. In the afternoon, AMRF research scholars displayed their research activities through poster presentations. During lab visits, AMRF scientists showcased the core research facilities and explained the ongoing projects to the Dartmouth delegates. Additionally, there were cultural events, performed by students from both institutions. The conference served as a unique opportunity for both institutions to share educational and research activities, paving the way for future collaborative research projects and long-term partnerships.

Participants of the AMRF – Dartmouth Education and Research Conference

AWARDS

ICMR Emeritus Scientist

Dr.P. Sundaresan, Senior Scientist, Department of Genetics has been appointed by Indian Council of Medical Research as ICMR Emeritus Scientist for the year 2024 and ICMR supporting to continue his biomedical research at Aravind Medical Research Foundation.

Prize at the 6th International Conference on Genome Biology (ICGB)

Madurai, 28th February - 1st March 2025

Sneha, Junior Research Scholar, Department of Immunology and Stem Cell Biology winning First Prize in Oral Presentation at the 6th International Conference on Genome Biology (ICGB): Cell - Organism - Environment & the 56th Aqua-Terr Annual Day, organised by the School of Biological Sciences, Madurai Kamaraj University, Madurai.
Title: MiRNAs associated with the maintenance of human trabecular meshwork stem cells: Changes with ageing and in glaucoma

Ph.D awarded by Alagappa University

R. Kadarkarai Raj
Department of Genetics
Thesis: Molecular Genetics of ABCA4 Gene in Patients with Retinal Dystrophies
Guide: Dr.P.Sundaresan

C. Prakash
Department of Genetics
Thesis: Investigating the Cross Talk between Nuclear and Mitochondrial Genome in Patients with Leber's Hereditary Optic Neuropathy
Guide: Dr.P. Sundaresan

A. S. Sriee Viswarubhiny
Department of Genetics
Thesis: Molecular Characterization of Leber's Congenital Amaurosis in South Indian Cohort
Guide: Dr. P. Sundaresan

Ph.D awarded by Madurai Kamaraj University

T. Shanthini
Department of Molecular Genetics
Thesis: Molecular characterization of tumor progression in Retinoblastoma
Guide: Dr. A.Vanniarajan

Prof. VR.Muthukkaruppan Endowment Award 2024

In 2014, students and colleagues of Prof. VR.Muthukkaruppan established an endowment in his name. From this endowment fund, an annual award is bestowed upon the best researcher of the institute, selected based on the scientific merit of their research abstracts and presentations. This award includes a certificate and cash prize.

Saranya P, Department of Immunology and Stem Cell Biology won the award for her outstanding research work on "Functional decline and morphological changes in the human lens epithelial stem cells in cataractous lens"

P. Saranya receiving Prof. VR. Muthukkaruppan Endowment award

GUEST LECTURE

Dr. E.Nalini, Assistant Professor, Department of Integrative Biology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore

Topic : “Bacterial Membrane Vesicles in Health and Disease”

9th April 2024

Dr. Gordon W. Laurie, Professor, Departments of Cell Biology, Biomedical Engineering and Ophthalmology, UVa Health System, University of Virginia, Charlottesville, VA 22908-0732

Topic: “Returning the Eye to Normal with Lacritin: Fruit of an Unbiased Screen”.

22nd November 2024

Prof. Michael E. Zegans, Chief of Ophthalmology - Dartmouth-Hitchcock Medical Center, Professor of Surgery (Ophthalmology), Geisel School of Medicine at Dartmouth, Francis L’Esperance Vision Scholar, Lebanon, USA

Topic : “Antifungal activity of beta-adrenergic receptor antagonists”.

5th December 2024

Dr. D. Vasudharani, Associate Professor and Associate Dean, IISER Tirupati

Topic : “Metabolism Induced Structure and Functional Changes in Neurons: Implications in Diabetic Retinopathy”.

1st February 2025

PUBLICATIONS 2024 - 2025

Susmita Chowdhury, Prakash Chermakani, Girish Baliga, Rupa Anjanamurthy, Sundaresan P

- *"Genotypic and Phenotypic diversity in X linked retinoschisis: Findings from a South Indian patient cohort"*

Indian J Ophthalmol 72(6): p 902-911, June 2024.

Ayyasamy Vanniarajan, Puja Maitra, Karuvel Kannan Saraswathi, Parag K.Shah

- *"Impact of RB1 gene screening from blood collected on a single day from 411 family members of 113 Retinoblastoma survivors in India"*

Eye 2024 Jun;38(8):1575-1580

G. Neethirajan, Krishnadas S. R., Vijayalakshmi P, Shashikanth Shetty and Sundaresan P

- *"Semi-Quantitative Analysis of PAX6 Gene in Ocular and Non-Ocular Tissues"*

New Visions in Medicine and Medical Science Vol. 3, 30 March 2024 , Page 47-59 (book chapter)

Ondippili Rudhra, Hariharan Gnanam, Sivaramakrishnan Sivaperumal, Venkatesh Prajna Namperumalsamy, Lalitha Prajna, Dharmalingam Kuppamuthu

- *"Melanin depletion affects Aspergillus flavus conidial surface proteins, architecture, and virulence"*

Applied Microbiology and Biotechnology 2024 108(1):291

Hanith Raj Deivarajan, Vignesh Elamurugan, Padmapriya Sivashanmugam, Jaishree Pandian, Karvannan Sevugamurthi, Gunasekaran Rameshkumar, Swagata Ghosh, Daipayan Banerjee, Anitha V, Anju Jose, Ram Rammohan, Anita Raghavan, Revathi Rajaraman, Dharmalingam Kuppamuthu, Lalitha Prajna, Venkatesh N. Prajna, Siddharth Narendran

- *"Development and Clinical Evaluation of a CRISPR/Cas12a-based Nucleic Acid Detection Platform for the Diagnosis of Keratomycoses"*

Ophthalmology Science 2024 Mar 28;4(5):100522.

Rathinam SR, Jeya Kohila G, Balagiri K, Gowri Priya C, Vedhanayagi R, Radhika M, Venu Nadella

- *"Utility of demographic and clinical signs as diagnostic predictors for leptospiral uveitis: A retrospective study"*

Indian J Ophthalmol. 2024 Mar 8;72(6): 869-877

Arif Waseema, Narendran Siddharth, Kannan Naresh Babu, Ramasamy, Kim, Veerappan, Muthukkaruppan, Chidambaranathan, Gowri Priya

- *"Age-related reduction in the functional properties of adult stem cells located in the peripheral region of human retinal pigment epithelium"*

Indian Journal of Ophthalmology 2024 Apr 16;72 (Suppl 4):S688–S695

Sekaran Balaji, Anindita Rao, Karuvel Kannan Saraswathi, Rathinavel Sethu Nagarajan, Radhakrishnan Santhi, Usha Kim, Veerappan Muthukkaruppan, Ayyasamy Vanniarajan

- *"Focused cancer pathway analysis revealed unique therapeutic targets in retinoblastoma"*

Medical Oncology (2024) 41:168

Kumar Jeyaprakash, Manojkumar Kumaran, Usha Kim, Radhakrishnan Santhi, Veerappan Muthukkaruppan, Bharanidharan Devarajan, Ayyasamy Vanniarajan

- *"Investigating druggable kinases for targeted therapy in retinoblastoma"*

Journal of Human Genetics 69, 467-474 (2024)

Senthilkumari S, Anand Rajendran, Bala P, Mohammed Sithiq U

- *"Association of macular pigment optical density with plasma macular carotenoids levels and serum lipids in south Indian healthy volunteers and patients with early age-related macular degeneration"*

Eur J.Ophthalmol August 2024

Karuvel Kannan Saraswathi, Radhakrishnan Santhi, Usha Kim, Ayyasamy Vanniarajan

- *"Investigating the frequency of somatic MYD88 L265P mutation in primary ocular adnexal B cell lymphoma"*

Molecular Biology Reports (2024) 51:973

Deivarajan HR, Sehupathy RP, Elamurugan V, Akshayaa VS, Reega P, Dharani C, Harivignesh S, Elakkiya Nandhini G, Kanmani M, Dharsini N, Sevugamurthy K, Saravanan VR, Anuradha K, Shah PK, Rammohan R, Nair A, Shah K, Jose A, Jaishree P, Lalitha P, Prajna NV, Narendran S

- *"Clinical Evaluation of a Novel CRISPR-cas 12a based RID-MYC assay for the diagnosis of fungal endophthalmitis"*

Ophthalmology Retina Nov 2024

Durga M, Roopam D, Sindura Devi A, Prajna NV, Prakash C, Sundaresan P

- *" Genetic implications of CHST6 gene mutations and their corneal microstructural changes in macular corneal dystrophy patients"*

Molecular vision 2024; 30:305-318.

Adhithya Subramanian Gopalakrishnan, Sumaiya Sirajudeen, Nasrin Banu, Jessica Nunes, Divya T. Rajendran, Seema Yadav, Namperumalsamy Venkatesh Prajna, Rachel Williams, Dharmalingam Kuppamuthu, Ramprasad Obula Giridhara Gopalan

- *"Inhibition of matrix metalloproteases by a chemical cross-linker to halt the corneal degradation in keratoconus"*

Experimental Eye Research 251 (2025) 110208

Karuvel Kannan Saraswathi, Pragya Saini, Radhakrishnan Santhi, Veerappan Muthukkaruppan, Ayyasamy Vanniarajan, Usha Kim

- *"Ocular adnexal lymphoma – A single-center observational study of survival outcomes"*

Indian J Ophthalmol 2025 Feb 1;73(2):261-266

ONGOING RESEARCH PROJECTS

No	Projects	Funded by	Investigators	Research Scholar
PROTEOMICS				
1.	Isolation and Characterisation of Extracellular Vesicles from Human tear film & Multiple isoforms Human Tear lysozyme are localised in the Extracellular Vesicle	AMRF 2024-2025	Prof. K.Dharmalingam Dr. N. Venkatesh Prajna Dr. Lalitha Prajna	Karthik A
2.	Stress induced alterations in the Extracellular Vesicles of Aspergillus flavus	AMRF 2024-2025	Dr. N. Venkatesh Prajna Prof. K. Dharmalingam Dr. Lalitha Prajna	G.Hariharan
3.	Comparative Proteomic Profiling of Plasma-Derived Small Extracellular Vesicles Across Progressive Stages of Diabetic Retinopathy	Sun Pharma March 2023-May 2024	Dr. Daipayan Banerjee Dr. Bhavani S Dr. K. Naresh Babu Dr. R. Kim Prof. K. Dharmalingam	Aadithiya T Gr
4.	Proteomics analysis of Cell-derived Nanovesicles from myofibroblasts and pterygium fibroblasts.	SERB 1st November 2022-31st October 2024	Dr. Daipayan Banerjee	L.Mathan
5.	Pharmaceutical inhibitors to attenuate myofibroblasts differentiation in pterygium	SERB 1st November 2022-31st October 2024	Dr. Daipayan Banerjee	L.Mathan
6.	Understanding the mechanism of action of a novel chemical cross-linker designed to treat keratoconus	ICMR	Dr.O.G.Ramprasad Prof. K. Dharmalingam Dr. N.Venkatesh Prajna Dr. Naveen Radhakrishnan	
7.	Analysis of tear MMPs from Down's syndrome patients to assess the predisposition of Keratoconus among Down's syndrome patients	AEH	Dr. O. G. Ramprasad Prof. K. Dharmalingam Dr. A. Vanniarajan Dr. Meenakshi Dr. Anitha	Agnes Angel
MICROBIOLOGY				
8.	Development and validation of a non-invasive point-of-care diagnostic tool for fungal keratitis	VELUX STIFTUNG 01.08.2021 – 31.07.2025	Dr.N. Venkatesh Prajna Dr. Lalitha Prajna Dr.K. Dharmalingam Dr.Thulasiraj Ravilla Dr.N. Siddharth	Hanithraj D. Padmapriya S Hari Vignesh Kanmani
9.	Role of Human Corneal MiRNAs in the onset and severity of Fungal Keratitis	ICMR Nov.2021- Nov 2024	Dr. D. Bharanidharan Dr. K. Dharmalingam Dr. N. Venkatesh Prajna Dr. Lalitha Prajna	Gayathri M
10.	Dysregulated human Corneal miRNAs and their role in disease progression in *Fusarium* keratitis	ICMR-SRF 2022-2025	Dr. D. Bharanidharan	Shreya Dinesh

11.	Investigation of ocular immuno-inflammatory signatures in uveitis patients for prognostication and risk stratification	AEH	Dr.R.Rathinam	
MOLECULAR GENETICS				
12.	Investigation of nuclear genes involvement in a Mitochondrial Disorder: Leber's Hereditary Optic Neuropathy	SERB 18.08.2023 – 17.08.2026	Dr.P.Sundaresan Dr. S. Mahesh Kumar	
13.	Decoding the Unknown Genetic Etiology to Ameliorate the Molecular Diagnosis of Leber's Congenital Amaurosis	DHR-GIA, ICMR 06.11.2023 – 05.11.2026	Dr. P. Sundaresan Dr. S. Senthilkumari Dr. Rupa Anjanamurthy	S. Shiva Shankari
14.	Mitochondrial Association with Transcriptomic Signatures for Trabecular Meshwork Damage in Glaucoma: A tissue-specific omics approach	ICMR 2025-2027	Dr.P.Sundaresan Dr.S. R. Krishnadas Dr. Ann Mary Mathews	S. Hari Vignesh
15.	Early Detection of Glaucoma by Genome Analysis	Aravind Eye Foundation 2024-2025	Dr. A.Vanniarajan Dr. K. Dharmalingam Dr. D. Bharanidharan Dr. Indira Durai Dr. SR Krishnadas	Dr.Viswanathan
16.	Molecular characterization of ocular lymphoma for improved disease prognosis	Lady Tata Memorial Trust 01.08.2020 31.07.2025	Dr.A. Vanniarajan	K. Saraswathi
17.	Elucidating the role of cancer stem cells in chemoresistant retinoblastoma and their therapeutic implications	ICMR-SRF 2022-2025	Dr. A. Vanniarajan	R. Sethu Nagarajan
IMMUNOLOGY AND STEM CELL BIOLOGY				
18.	Characterization of adult human lens epithelial stem cells in the maintenance of tissue homeostasis throughout life and their functional status in cataractous lens	Lady Tata Memorial Trust – SRF 01.08.2021 – 31.07.2024	Dr. Madhu Shekhar Dr. Gowri Priya Chidambaranathan Dr.Haripriya Aravind Prof. VR.Muthukkaruppan	P. Saranya
19.	Identification and Characterization of adult human retinal pigment epithelial stem cells	Part time PhD from 17.03.2022	Dr. Gowri Priya Chidambaranathan	A.Waseema
20.	Adult stem cell derived extracellular vesicular miRNAs for trabecular meshwork regeneration in glaucoma	UGC-SRF 03.06.2022– 02.06.2025	Dr. Gowri Priya Chidambaranathan Dr. SR. Krishnadas Prof. VR. Muthukkaruppan	R. Iswarya
21.	Molecular regulators associated with the maintenance of human trabecular meshwork stem cells in relation to their reduction in ageing and glaucoma	SERB Oct.2021 – Apr. 2025 LTMT –JRF Dec 2024 – Dec 2026	Dr. Gowri Priya Chidambaranathan Dr. D. Bharanidharan Dr. S. R. Krishnadas Prof. VR. Muthukkaruppan	Sneha Nair

22.	Molecular characterization of human retinal pigment epithelial stem cells and their role in age related macular degeneration	ICMR Dec 2022- Dec 2025	Dr. Gowri Priya Chidambaranathan Dr. D.Bharanidharan Dr. R.Kim Prof. VR.Muthukkaruppan Dr.Siddharth Narendran	R.Kanthimathi
23.	Molecular regulation of adult human lens epithelial stem cells: changes with aging and cataract	ICMR-JRF Oct 2023- Oct 2025	Dr. Gowri Priya Chidambaranathan	P. Thushmitha
24.	Characterization of Pearl/ Regeneratory Posterior Capsular Opacification	Institutional Research Fellow Feb 2023 – Jan 2025	Dr. Gowri Priya Chidambaranathan Prof. K. Dharmalingam	T. Keerthana
25.	A scalable system for the production of human induced pluripotent stem cell derived functional retinal pigmented epithelial cells	04.10.2023 – 03.10.2026	Dr.P. Anwar Azad	S.Gopika
OCULAR PHARMACOLOGY				
26.	Evaluation of IOP lowering property and Anti-fibrotic property of Relaxin on TGFβ2-induced Elevated IOP Ex vivo model of glaucoma using Human Organ Cultured Anterior Segment (HOCAS)	SERB-CRG grant (2023-2026)	Dr. S. Senthilkumari Dr. R. Sharmila	Lakshmi Devi Priya
27.	'Relaxin' the pressure in glaucoma	Fight for Sight PhD Studentship 2022 programme (UK) (Oct 2023-Oct 2026)	Prof. Colin Willoughby (UK) Dr. S. Senthilkumari Dr Kazuhiro Yamamoto (UK)	Ms. Eva Mihalovova
28.	Role of vitamin D3, dopamine and serotonin levels in myopia development and progression	AEH, Madurai 02-Aug-2023 - 31-Jul-2024	Dr. Sahithya Dr. Elackiya Dr. P. Vijayalakshmi Dr. S. Senthilkumari	
29.	Prospective Study Analyzing the Role of Cytokine- Mediated IOP reduction in POAG /OHTN Patients Receiving Therapeutic Ultrasound for Glaucoma (TUG) Treatment	EyeSonix, USA	Dr. Sharmila Rajendrababu Dr. S.Senthilkumari Prof. K. Dharmalingam Dr. Donald Schwartz Mr. Eric Schultz	M. Fazil
BIOINFORMATICS				
30.	Expression profiling of human corneal miRNAs and their role in Pseudomonas aeruginosa induced keratitis	DBT-BET 2022-2027	Dr. D. Bharanidharan	R.Praveen kumar
31.	Identification and Analysis of Alternative Transcripts in Retinoblastoma Progression	ICMR-SRF 2022-2025	Dr. D. Bharanidharan	Mohd Hameed Aslam

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