

RESEARCH IN OPHTHALMIC SCIENCES

ARAVIND MEDICAL RESEARCH FOUNDATION



ANNUAL REPORT 2015 - 16

ARAVIND MEDICAL RESEARCH FOUNDATION

Aravind Medical Research Foundation is recognized as Scientific and Industrial Research Organization (SIRO) by the Department of Scientific and Industrial Research (DSIR)

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

Dr. G. Venkataswamy Eye Research Institute

Annual Report 2015 - 2016

ARAVIND MEDICAL RESEARCH FOUNDATION



Much has been done, but much remains to be done... we look to the future with renewed strength to continue the mission of providing quality eye care and hope that some of what we have learned will be useful to other eye care workers around the world.

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Dr. G. Venkataswamy Eye Research Institute

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FOREWORD



Aravind Medical Research Foundation, the research wing of Aravind Eye Care System (AECS) is committed to finding out the root cause of various sight threatening diseases prevalent in the country such as Glaucoma, Diabetic Retinopathy, Age-related Macular Degeneration and other eye problems. Research in any discipline ultimately aims to benefit the society at large and especially when this happens in the realm of healthcare, it results in a positive impact on the lives of many individuals. Studies at the institute have thrown light into the pathogenesis of many of ophthalmic diseases. The institute has intensified collaboration between clinicians and scientists, made possible in the unique situation of Aravind that its facilities are located close to each other. Translating the research findings into direct patient care will benefit a larger community, which can happen only through this collaboration. This collaboration has also resulted in the establishment a state-of-the-art Genetic Testing Centre for Retinoblastoma at AECS.

Of late, the institute has been focusing on the proteomics of eye diseases mainly Fungal Keratitis, Diabetic Retinopathy and Glaucoma. High throughput proteomics approaches are being carried out to study the specific proteomes that include information on protein abundances, their variations and modifications, their interacting partners and the networks they are involved in.

Continued growth of any research organization depends on the excellence of the core group of scientists and their commitment to research and education. Aravind Medical Research Foundation is fortunate to have a dedicated band of scientists who with their knowledge and expertise in relevant fields are a real asset to the organization. AMRF has added two more faculty scientists this year. Dr. Ramprasad will be concentrating on cell signalling in fungal keratitis, and micro RNAs in Diabetic Retinopathy. Dr. Rabbind Singh will be focusing on various cancers such as Uveal Melanoma. His expertise in fungal and microbial systems as well as in cancer biology will augment the institute's research capabilities. With the new faculty in place, AMRF is looking forward to intensifying research on significant eye problems and their management.

Being a research organization, educational activities such as Ph.D. programmes, symposia, workshops and courses are being conducted regularly to keep the staff, students and research scholars updated on the latest developments in the field. Going forward, the institute envisages to explore opportunities in the fields of nanotechnology and pharmacodynamics to further improve its translational research capacities.

Dr. P. Namperumalsamy
President, AMRF

INTRODUCTION



Research focus of AMRF continues to be understanding of ocular diseases. Elucidation of the mechanism as well as factors associated with disease phenotype are examined in detail. With the new faculties AMRF hopefully achieve some of the objectives in the future.

Whole genome analysis and functional genomics of eye diseases are the focus areas being pursued in AMRF by the genetics and bioinformatics groups. They work on diseases such as Glaucoma, Macular Corneal Dystrophies, Retinitis Pigmentosa and Retinoblastoma and actively pursue the leads they have obtained in the previous years. They have found some novel mutations in genes involved in some of the diseases and also examined additional mutations that have role to play in the diseases. A computational platform for mutation screening was developed in collaboration with the bioinformatics group. Analysis of single nucleotide variations allows the identification of mutations that are associated with a specific disease, which in the long run allow the elucidation of the mechanism underlying the disease. Bioinformatics group actively collaborate with the genetics group in analyzing the NGS data using the in-house developed pipelines.

Stem cell biology is having an expanding role in understanding the disease mechanisms and development of alternate strategies for treatment. Characterization and use of corneal epithelial stem cells are the focus of the group working on this aspect. This group is also analyzing the regulatory role of miRNA in stem cell differentiation.

Ocular microbiology and proteomics groups studies infection biology with special reference to bacterial and fungal keratitis. Role of autophagy in bacterial clearance in Pseudomonas keratitis has been the focus of microbiology group and recently they have started using an animal model of bacterial keratitis to better understand the persistence mechanism. Apart from analyzing fungal keratitis, proteomics group is also involved in the identification of biomarkers for diabetic retinopathy using serum and circulating micro vesicles.

Macular pigment density could be an indicator of Age Related Macular Degeneration that can be used as a predictor of AMD risk and this information has potential clinical use. Apart from this study, the ocular pharmacology group also studies the signaling in trabecular meshwork and its role in development of glaucoma with an objective to improve the management of Glaucoma.

Prof. K. Dharmalingam
Director - Research

MOLECULAR GENETICS

The most common eye disorders in Indian population are cataract, diabetic retinopathy, glaucoma, corneal and retinal dystrophies. The primary focus of the department is to identify putative genetic markers for diagnosis and genetic counselling in these eye diseases. The department of molecular genetics has given priority to study genetics of Primary Angle Closure Glaucoma (PACG) and identified five new loci and also determined the levels of cytokines in the aqueous humour of PACG patients. The lab is also interested in studying the role of *SIX6* gene in the pathogenesis of primary open angle glaucoma. Newer methods were developed for the genetic analysis of retinoblastoma patients. Mutational spectrum of *RB1* gene was further expanded with the analysis of promoter methylation. The next generation sequencing platform Illumina Miseq was established this year which enhances the understanding on the pathogenesis of retinoblastoma.



Molecular Genetics of Macular Corneal Dystrophy (MCD) in Indian population

Investigators : Dr. P. Sundaresan, Dr.N.V.Prajna, Dr.V.Lumbini, Dr.K.Rohan Agashe
Ph.D scholar : M.Durga
Funding agency : Cornea clinic research grant - Mutt study

Background

Macular corneal dystrophy (MCD) is an inherited autosomal recessive disorder of keratan sulfate (KS) metabolism. It is caused by mutations in the carbohydrate sulfotransferase-6 (*CHST6*) gene, encoding corneal N-acetyl glucosamine-6-O-sulfotransferase (C-GlcNAc-6-ST) enzyme. The abnormal accumulation of glycosaminoglycans (unsulfated keratan sulfates) in the stroma, Descemet's membrane, endothelial cells leads to severe visual impairment. The onset usually occurs

in the first decade of life, starting with a fine superficial stromal haze in the central stroma, followed by an accumulation of irregular, focal, grey white deposits.

MCD is most prevalent in Iceland followed by Japan, India and Saudi Arabia. In South Indian population, the high prevalence of MCD is probably a result of high frequency of consanguineous marriages. There is a limited study from the Indian population with regard to MCD genetics. Therefore, this study was undertaken to determine the spectrum of genetic variations in *CHST6* gene and understand its role in MCD pathogenesis. In this study, 55 unrelated families (90 study subjects with and without MCD) were recruited and screened for mutations in *CHST6* gene by Sanger sequencing. Out of 55 families, 30 families had consanguineous marriages, 20 families had the previous MCD case history. For this MCD study, 11 unaffected family members were also included as controls, along with 50 cataract controls.

Results & Conclusion

In this study, eight different novel mutations in 8 families and two hotspot mutations (frameshift, missense) in 20 families (Figure 1 shows the typical pedigree of MCD and Figure 2 shows one of the hotspot - frameshift (deletion) mutations in MCD family 11) were identified. No mutation was identified in 10 families. Further studies will be focused on the possible effects of these mutations using bioinformatics prediction tools.

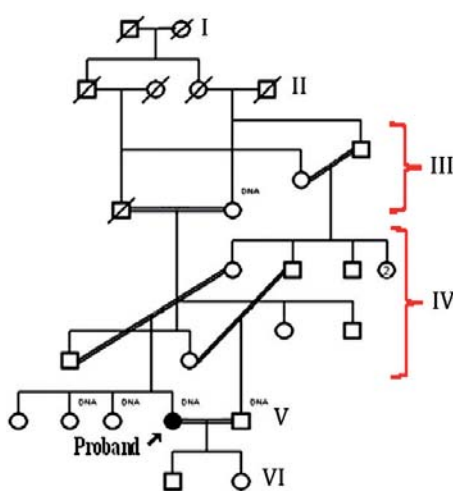


Figure 1- Shows the pedigree chart of MCD family 11

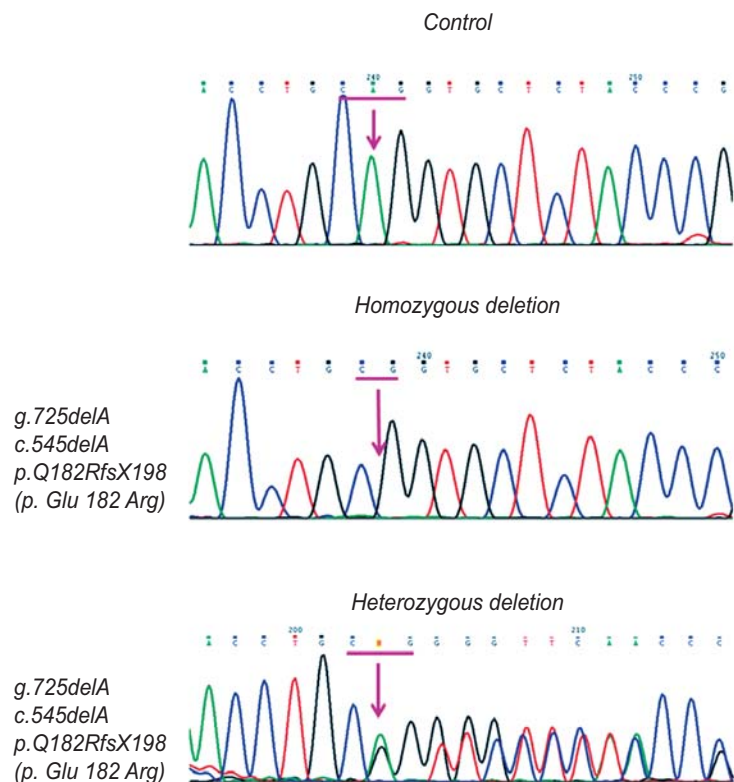


Figure 2- Shows the presence of hotspot - frameshift (deletion) mutation in MCD family 11

Genome wide association study identifies new susceptibility loci for Primary angle closure glaucoma

Investigators : Dr. P. Sundaresan, Dr. R. Krishnadas, Dr. R. Venkatesh,
Dr. Kavitha Srinivasan
Ph.D scholar : Roopam Duvesh
Funding agency : Aravind Medical Research Foundation

Introduction

Glaucoma is a complex neurodegenerative disorder characterized by progressive retinal ganglion cell damage, loss of optic nerve axons and distinctive optic neuropathy. It leads to optic disc cupping and concomitant loss of visual field and eventually blindness, if untreated. Glaucoma represents the second most leading cause of blindness in the world following cataract. Primary angle closure glaucoma (PACG) results from an appositional contact between peripheral iris & trabecular meshwork causing partial or complete anterior angle closure and thus hindering aqueous outflow. This leads to an increase in intra ocular pressure (IOP) and damage to the optic nerve. According to the recently published meta-analysis by *Tham et al*, the global prevalence of glaucoma for population aged 40-80 years is 3.54% and the prevalence of PACG is highest in Asians (1.09%). It is genetically heterogeneous disorder with complex genetic basis affecting many individuals within a family.

The significant association of SNP rs1015213 was previously reported in *PCMTD1-ST18* gene with PAC/PACG group ($p=0.002$) in South Indian population (Duvesh et al, 2013). Recently, a genome wide association study (GWAS) was conducted across 24 countries (Asia, Australia, Europe, North- and South America) including 10,503 PACG cases and 29,567 controls to identify the new susceptibility loci for PACG. This study showed significant evidence of disease association at five new genetic loci. These loci are at *EPDR1* rs3816415 ($p = 3.49 \times 10^{-15}$), *CHAT* rs1258267 ($p= 3.73 \times 10^{-16}$), *GLIS3* rs736893 ($p = 1.15 \times 10^{-14}$), *FERMT2* rs7494379 ($p = 6.32 \times 10^{-11}$), and rs3739821 mapping in between *DPM2* and *FAM102A* on chromosome 9 ($p = 6.77 \times 10^{-12}$). Apart from this, present study also confirmed significant association at 3 previously described loci for PACG ($p < 5 \times 10^{-8}$ for each of *PLEKHA7* rs11024102, *COL11A1* rs3753841, and *PCMTD1-ST18* rs1015213).

In collaboration with Singapore Eye Research Institute (SERI), 190 PACG cases and 288 controls were analyzed for these SNPs using Taqman allelic discrimination assay in South Indian population.

Results and conclusion

Highly significant association of SNP rs1015213 was observed in *PCMTD1-ST18* ($p=9.17E-05$) which was already reported from South Indian population, thus confirming prior reports. In addition, significant association of a new locus rs16856870 was also observed in *FNDC3B* gene ($p=0.0046$). *FNDC3B* is Fibronectin Type III Domain Containing 3B protein, which in earlier studies, has been associated with IOP and central corneal thickness (CCT) in primary open angle glaucoma (POAG) subjects.

Genetics and functional approaches of SIX6 gene to understand the pathogenicity of Primary open angle glaucoma

Investigators : Dr. P. Sundaresan, Dr. S.R. Krishnadas, Dr. Manju Pillai,
Ph.D scholar : Mohd Hussain Shah
Funding agency : Aravind Medical Research Foundation

Introduction

Primary open angle Glaucoma is a neurodegenerative disease characterized by the progressive loss of retinal ganglion cells, optic nerve degeneration and visual field loss, eventually blindness. It is a complex and genetically heterogeneous disease. Glaucoma is the second leading cause of irreversible blindness in the world. Many studies suggested POAG as an autosomal dominant inheritance with incomplete penetrance. However, the inheritance pattern of this disorder seems to be multifactorial resulting from the interaction of one or more genes and/or environmental stimuli. Although more than 33 genetic loci have been reported to be associated with the disease, only 3 genes - *MYOC* (myocilin), *OPTN* (optineurin), and *WDR36* were linked to POAG. In addition, genome wide-association (GWAS) has allowed the identification of candidate genes such as, *CAV1/CAV2*, *TMCO1*, *CDKN2B-AS1*, *TMCO1*, *TXNRD2*, *ATXN2* and *FOXC1* and *SIX1-SIX6*. In this study, *SIX6* gene was chosen, based on its functional analysis and association study with POAG in different ethnic population.

Results

In this study, 65 POAG cases and 65 controls were analysed. Two rare variants and two reported common variants (rs33912345 and rs10483727) in the *SIX6* gene were identified. To confirm the association of these two common variants with Indian cohort of POAG, 399 POAG cases and 383 controls were genotyped by Taqman based allelic discrimination Assay. No significant association of these variants was observed with POAG.

Conclusion

The variants-rs33912345 and rs10483727 are not associated with POAG in Indian population. The frequency of these alleles is almost the same in Indian primary open angle glaucoma cases and controls. Investigating association between genetic variants in genes associated with primary open angle glaucoma (POAG) will be research interest of the lab and increasing the number of samples may show significant differences. It may also suggest additional candidate or locus responsible for Indian POAG.

Genetic evaluation of genes involved in homocysteine metabolism and hyperhomocysteinemia with Pseudoexfoliation syndrome in South Indian population

Investigators : Dr.P.Sundaresan, Dr. Haripriya
Ph.D Scholar : G. Prakadeeswari
Funding agency : AEH-AMRF APEX project

Introduction

Pseudoexfoliation syndrome (PXF) is a common, multi-factorial, age-related systemic fibrillinopathy. It highly increases the risk of pseudoexfoliation glaucoma, a major cause of irreversible blindness worldwide. PXF is clinically diagnosed by the characteristic deposition of fibrillar material or abnormal production of extracellular matrix material. Histologically, these deposits are found to be in

the anterior segment of eye including lens capsules, lens zonules, iris, trabecular meshwork, cornea, ciliary body and the lamina cribrosa of the optic nerve posteriorly.

Homocysteine (Hcy) is a well-studied non-protein sulfur amino acid which is a known potential biomarker related to PXF. Hcy concentration is said to be consistently elevated in plasma, serum, aqueous humor and tears of PXF patients compared to the controls. Hcy is metabolized by one of the two pathways: Remethylation or Transsulfuration. The abnormal Hcy metabolism may be due to inherited defects in the controlling enzymes of Hcy metabolism including 5,10-methylenetetrahydrofolate reductase (*MTHFR*), methylenetetrahydrofolate dehydrogenase (*MTHFD1*) and cystathionine- β -synthase (*CBS*).

Hyperhomocysteinemia is known to have increased risk for pseudoexfoliation syndrome, retinal vein occlusion, vascular abnormalities, coronary artery disease (CAD) and hypertension. It is also a significant predictor of high mortality and patients generally have a shortened life span.

Rare Mendelian variants in *MTHFR*, *MTHFD1* and *CBS* may lead to the elevated plasma Hcy levels. The common functional polymorphisms rs1801133 (C677T) and rs1801131 (A1298C) in the *MTHFR* gene reduces the enzymatic activity which is essential for catalysing the conversion of the 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate. *MTHFR*, a co-substrate in homocysteine re-methylation pathway, produces mild hyperhomocysteinemia. But, there are many controversial reports exist on these genetic variants as the source of elevated Hcy levels in PXF.

Therefore, the aim of this study is to understand the correlation between the genes involved in the homocysteine metabolism and hyperhomocysteinemia in South Indian Pseudoexfoliation syndrome patients.

Results

Real-time Taqman allelic discrimination assay was performed for genetic variants rs1801133, rs1801131 (exon 4 & 7 of the *MTHFR* on chromosome 1) and intronic variant rs8006686 (*MTHFD1* on chromosome 14) using 860 PXF cases and 612 cataract controls (CC). Serum Hcy levels were also estimated by Enzyme-linked immunosorbent assay (IVD, DIALAB kit, Austria) in 804 PXF cases and 429 CC.

Serum Homocysteine levels were slightly higher in the PXF cases compared to cataract controls, but did not reach statistical significance ($p=0.710$). The minor alleles of *MTHFR* variants (rs1801133 & rs1801131) showed a protective effect for PXF in south Indian population, but the association did not reach statistical significance. In addition, the minor allele of *MTHFD1* rs8006686 showed an increased risk for PXF with marginally significant association ($p=0.069$).

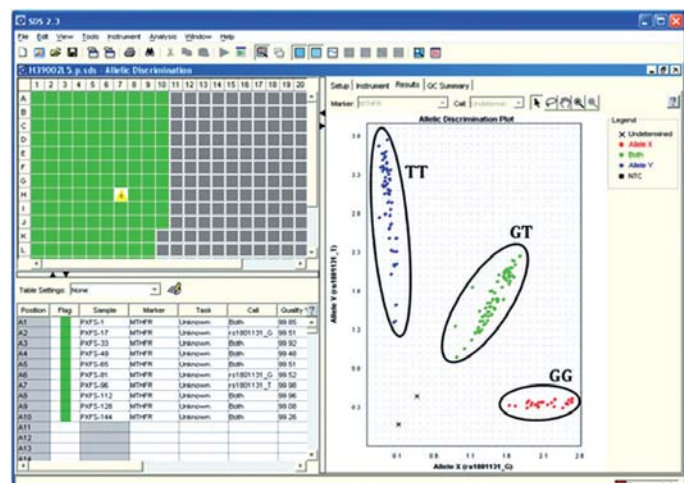


Figure: 1 Cluster plot of the real-time Taqman allelic discrimination assay

Conclusion

Overall, This study did not show any significant association with the SNPs of the *MTHFR* and the serum homocysteine levels but a marginal association of SNP rs8006686 in *MTHFD1* with PXF in South Indian population.

Whole-exome Sequencing Analysis Identifies Mutations in the *FAM161A* and *EYS* Gene in Retinitis Pigmentosa in Indian Population

Investigators : Dr. P.Sundaresan, Dr. R. Kim
Ph.D Scholar : Bibhuti Ballav Saikia
Collaborators : Dr. Xianjun Zhu and Dr. Zhenglin Yang
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Sichuan, China
Funding agency : Aravind Medical Research Foundation

Introduction

Retinitis pigmentosa is a highly heterogeneous genetic disease characterized by progressive visual loss caused by the impairment of retinal photoreceptors. The worldwide prevalence of RP is approximately one in 3,500–5,000 and it can be inherited as an autosomal recessive(ar) (50–60%), an autosomal dominant (30–40%) or an X-linked trait (5%–15%). So far, more than 70 genes and loci have been identified for RP. However, these genes account for only approximately 60% of RP cases. Therefore, unknown RP genes remain to be identified, and novel RP genes would provide valuable information for the diagnosis, prevention and treatment of RP. However, there has been limited success when using traditional approaches to screen potential genes for RP because many techniques for positional cloning and gene identification are relatively time consuming, expensive and inefficient. Recently, whole-exome sequencing (WES) by next-generation sequencing (NGS) has become an efficient method for identifying genetic variants at the whole-genome level.

Results

Whole exome sequencing was conducted on the probands of 16 RP families and 100 sporadic RP patients. A novel mutation was reported in *FAM161A* in RP-252 and RP-182 with two patients affected with RP in each family. In 100 sporadic Indian RP patients this homozygous mutation was present in one by whole exome analysis and validated by Sanger sequencing. Three compound heterozygous mutations and seven homozygous mutations in the *EYS* gene were also identified in two arRP families and eight sporadic RP patients. To confirm the accuracy of the mutations identified by exome sequencing, Sanger sequencing was performed to validate these mutations in the other family members and normal controls.

Conclusion

One frame shift novel homozygous mutation in *FAM161A*, three novel compound heterozygous mutations in *EYS* and seven novel homozygous mutations were identified in the Indian RP patients. This study not only expands the spectrum of *FAM161A* and *EYS* mutations for RP in the Indian population but also shows that WES can be an effective tool for identifying causative mutations in arRP patients and diagnosing genetic diseases.

Genetic testing of retinoblastoma

Investigators : Dr. A. Vanniarajan, Prof. VR.Muthukkaruppan, Dr. D.Bharanidharan
Dr. Usha Kim
Ph.D Scholar : A. Aloysius Abraham
Funding agency : Aravind Eye Foundation (AEF), USA

Background and Introduction

Retinoblastoma (RB) is primarily initiated by biallelic inactivation of *RB1* gene. Upon identification of mutations in the proband, the risk can be predicted for siblings and offspring, thereby enhancing the management of the disease. In order to understand whether the affected children carry a genetic change from their parents, the complete *RB1* gene was analyzed in 30 patients during this year and could detect the mutations in 21 patients. The increased risk of retinoblastoma was predicted in next child in two families through the analysis of the patient and their family samples. In twelve patients, de novo germline mutations which are not inherited from their parents but occurred during their development were detected. The analysis of tumor samples showed somatic mutations in seven patients, which will not be inherited to their sibling or next generation.

Newer methods were employed to increase the spectrum of mutations in *RB1* and other RB associated genes. Copy number changes are generally not identified by conventional Sanger sequencing and therefore additional techniques i.e. Multiplex ligation-dependant probe amplification (MLPA) and Real time PCR were employed. If there was no change detected at the genetic level, samples were further subjected for methylation analysis by MS-MLPA.



Results

Multiplex ligation-dependant Probe Amplification (MLPA) analysis:

Out of 30 patients recruited for the study, four patients had copy number alterations in *RB1* gene. Copy number alterations include deletion of a single exon, homozygous and heterozygous deletion of *RB1* gene and heterozygous duplication of *RB1* gene. The homozygous deletion was identified in the tumor sample but not in blood sample of the patient. (Fig 1).

Methylation Specific-MLPA (MS-MLPA) analysis:

Promoter methylation could be detected by the method of MS-MLPA, which shows the amplification of the probes when they were not digested by restriction enzyme. With the establishment of the MS-MLPA technique for methylation analysis in the RB patients, the mutation detection efficiency

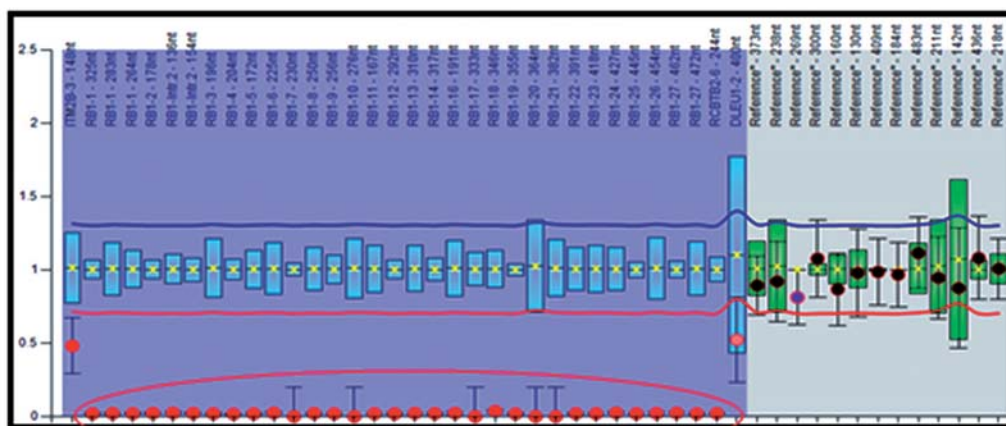


Fig 1: Tumor of Unilateral RB patient w/o any family history was found to have homozygous deletion of RB1 gene (circled in red).

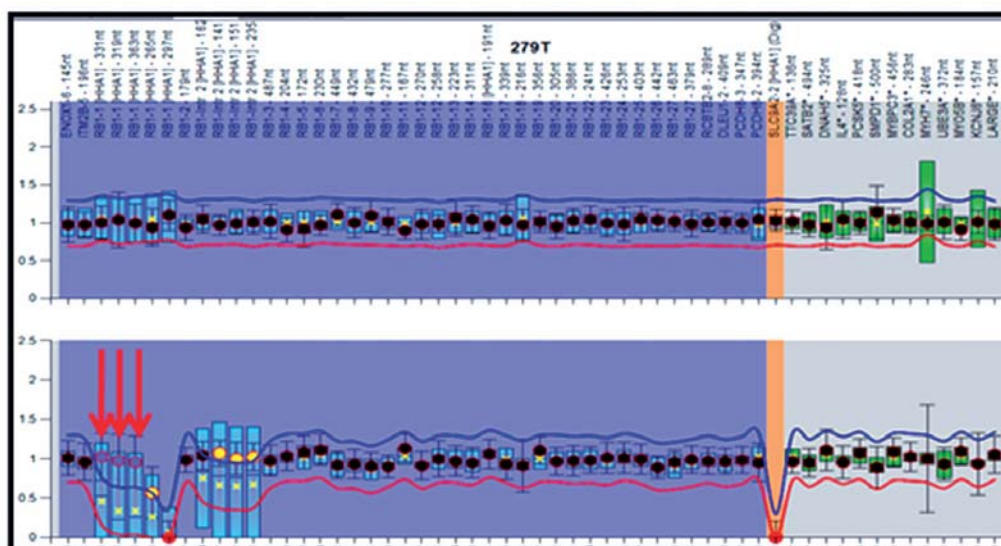


Fig 2: Tumor of Unilateral RB patient without any family history was found to have methylation of promoter in both alleles of RB1 gene (as indicated by red arrows). No other mutation was identified in this sample.

had improved. Promoter methylation was identified in four tumors. Methylation of both alleles was observed in one patient who showed the amplification of both alleles as shown below (Fig 2)

Analysis of *RB1* gene and RB related genes by NGS:

Next Generation Sequencing facility was established at AMRF this year. The first run on Miseq was carried out with the panel of genes (Complete gene analysis of 4 genes: *RB1*, *TP53*, *MYCN*, *BCOR* including introns and exons for small variant detections; Exonic analysis for copy number aberrations of 13 RB related genes and Hotspot analysis of 47 cancer related genes) in 16 samples. The Q30 scores which denote the quality of the run were above 91%. Variants were identified using the in-house pipelines developed by the bioinformatics team.

All known mutations in the positive controls were detected. In addition, two new *RB1* mutations were detected in two samples which were missed by Sanger sequencing possibly due to mosaicism and noise in the data of Sanger sequencing. In another sample with *RB1* duplication, two additional point mutations were identified. These variants need to be analysed further to know their role in pathogenesis.

Potential variants were found in *TP53* and *MYCN* but not in *BCOR*. In two samples, *TP53* mutations were detected, which did not have any *RB1* mutations. Till date there are no reports of *MYCN* variants in retinoblastoma patients. For the first time, a new variant was detected in exon1 of

MYCN gene in four retinoblastoma patients. Similarly several variants were observed in 47 cancer related genes. The variants will be further confirmed through resequencing and evaluation of their significance in retinoblastoma.

Conclusion:

Newer methods have improved the efficiency of detecting *RB1* mutations. Next Generation Sequencing has helped in identifying several cancer related genes that are involved in the process of tumorigenesis in retinoblastoma.

Genetic and transcript analysis of retinoblastoma

Investigators : Dr. A. Vanniarajan, Prof.VR.Muthukkaruppan, Dr. Usha Kim, Dr. Shanthi
Ph.D Scholar : Thirumalairaj Kannan
Funding agency : Indian Council of Medical Research

Introduction

Retinoblastoma is the intraocular tumor of children, primarily caused by the variations in the *RB1* gene. Variations can be either change of a single base or deletion of whole gene ultimately affecting the function of the retinoblastoma protein which controls the cell division. The alterations in the gene can be either inherited from parents or occur during the children's ocular development. When it is inherited, it is usually present in all the cells including the blood cells and there is high chance that the other siblings or their next generation may also be affected with the disease. If the mutation is present only in the tumor, it is less likely that it will be inherited. Thus it becomes important to understand the type of mutation and its inheritance pattern.

This study was aimed at analyzing mRNA from patient samples with splice site changes, frameshift mutations and deletions/duplications for understanding the nature of *RB1* transcript. RNA isolation from patient's blood and tissue samples were performed by Trizol (Invitrogen) and Swift Tissue Total RNA Kit (Shrimpex Biotech) respectively. RNA concentration was measured by Nanodrop spectrophotometer and integrity was checked by formaldehyde agarose gel electrophoresis. Further, 1µg of total RNA was converted to cDNA using Verso cDNA synthesis Kit (ABgene).

Results

In a patient's blood DNA, deletion of single base G was detected at the splice donor site (c.861+1delG) of exon 8 by Sanger sequencing (Fig 1). The cDNA of the patient showed the skipping of exon 8 because of this deletion of G due to altered splicing. The amplified product covering the region showed the normal and mutant transcript in the agarose gel electrophoresis. Sequencing of mutant transcript showed skipping of exon 8 in the patient blood sample because of the mutation in the canonical splice site (Fig. 1).

In another patient's tumor DNA, two heterozygous mutations, i.e. (1) deletion of two bases TC of exon 12 and (2) deletion of a single base T of exon 22 were identified. Sequencing of cDNA also showed the same two deletions causing frameshift and premature truncation (Fig 2).

Apart from point mutations and indels, *RB1* gene deletions and duplications are observed in retinoblastoma at a higher proportion. The *RB1* mRNA expression level was measured by quantitative Real Time PCR. Decreased expression of *RB1* mRNA was noted in patient with *RB1* gene deletion and increased expression in patient with *RB1* gene duplication (Fig 3).

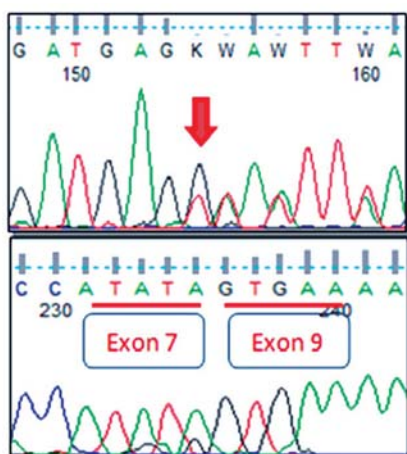


Fig 1. Sequence analysis showing the deletion mutation in the DNA marked by red arrow in the top panel and exon skipping in cDNA marked by boundaries of exons 7 and 9 in the bottom panel

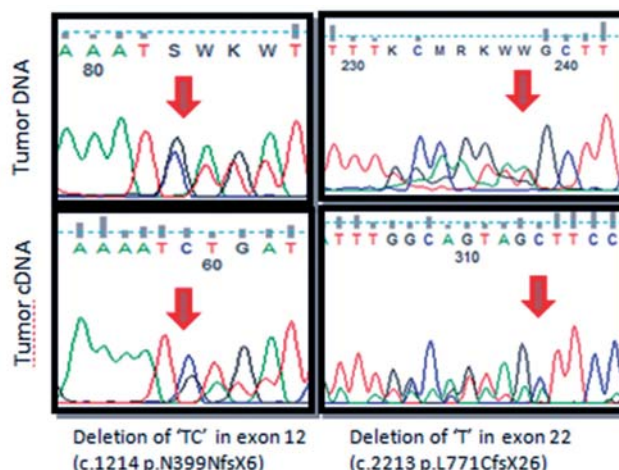


Fig 2. Electropherograms showing two frameshift mutations in patient's tumor DNA and cDNA leading to premature termination

Conclusion

Based on the data on the transcript analysis of the four RB patients, *RB1* gene mutations had been found to have effect on the mRNA in three different ways. If the mutation occurs in the splice donor site, exon skipping is observed in the transcript. Indels detected at DNA level were also mirrored at the mRNA level leading to the frameshift and premature truncation. The level of the *RB1* mRNA was directly proportional to the copy number changes noted in *RB1* gene.

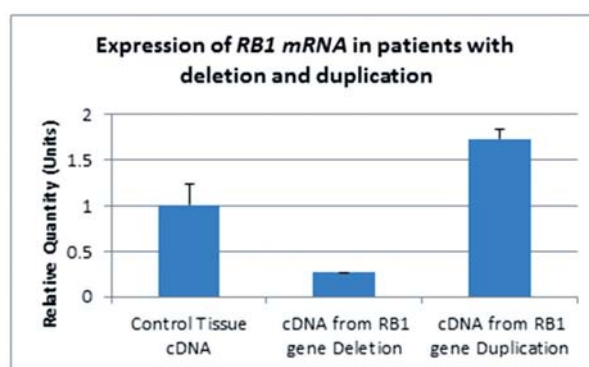


Fig.3. Relative expression of *RB1* mRNA in deletion and duplication of *RB* patients

Understanding the molecular basis of chemoresistance in retinoblastoma

Investigators : Dr. A. Vanniarajan, Prof. VR. Muthukkaruppan, Dr. Usha Kim,
 Project Fellow : T.S. Balaji
 Funding agency : Council of Scientific and Industrial Research (Fellowship)

Chemoresistance is clinically defined as either a lack of reduction of tumor size or recurrence of tumor following chemotherapy. Chemoresistance may be inherent or acquired during the course of treatment. ATP Binding Cassette (ABC) transporter mediated drug efflux has been suggested as the common mechanism for the chemoresistance. Other than ABC transporters, several studies suggested that loss of functional mutation in tumor suppressor genes such as *TP53* and *RB1* as other possible mechanisms for chemoresistance. This study will be focused on genetic analysis of oncogenes and tumor suppressor genes such as ABC transporters, *TP53*, *CHK2*, *ATM*, *RB1*, *E2F*, *BRCA1*, *BRCA2*, *CCNE1* and *BCL2* involved in chemoresistance in retinoblastoma. Retinoblastoma patients who did not show any chemo reduction with the current regimen along with disease progression will be included in the study.

STEM CELL BIOLOGY

The research focus of this department is to understand the basic biology of corneal epithelial stem cells. Till date, there is no exclusive marker for their identification and isolation. By combining two parameters – high expression of p63/ABCG2 with high nuclear to cytoplasmic (N/C) ratio, a specific method was established for their identification and quantification using confocal microscopy (Arpitha et al., IOVS, 2005; Micros. Res. & Tech., 2008; Cornea, 2008; Priya et al., 2013). Recently, a two-step protocol was established to enrich these stem cells to 80% by separating the limbal basal epithelial cells followed by laser capture microdissection of cells with high N/C ratio. Using such an enriched population, the current focus is to understand the molecular mechanisms - specific signaling pathways, molecular regulators like miRNA that are associated with the maintenance of stemness, and to identify stem cell specific markers.



Molecular signature of Corneal Epithelial Stem Cells (CESCs)

Investigator : Dr. Gowri Priya Chidambaranathan, Dr. VR. Muthukkaruppan,
Dr. N. Venkatesh Prajna
Research Scholar : M. K. Jhansi Rani
Funding Agency : Department of Biotechnology

Introduction including Background

Corneal epithelial stem cells reside in the basal layer of limbus at the corneoscleral junction and are responsible for maintaining the corneal epithelial homeostasis. CESCs account to 0.1-10% of total limbal epithelial cells and till date, there is no specific method for their isolation. Due to the low SC content in the population analyzed, no single specific positive marker has been identified for CESCs

and the molecular signaling mechanisms responsible for the maintenance of stemness is not clear. Loss of these stem cells results in limbal stem cell deficiency characterized by conjunctivalization, vascularization and loss of vision. Hence understanding the molecular signature of these stem cells is essential to identify the regulatory mechanisms associated with the maintenance of stemness, which will help in developing better treatment options for patients with limbal stem cell deficiency. Hence the objectives of the study are:

1. To enrich the SC content (>80%) from human donor limbal epithelium.
2. To analyze the transcriptional profile of enriched corneal epithelial stem cells.
3. To elucidate the relationship between high expression of nuclear transcription factor p63 isoforms and stemness.

This laboratory has earlier established the method to enrich the SC content up to 80% by isolating the limbal basal epithelial cells followed by Laser Capture Micro-dissection (LCM) of cells with N/C ratio > 0.7, which is proposed to be a better choice to understand the molecular signature of these CESC. Further it was demonstrated that the Δ Np63 α isoform was expressed in the enriched stem cell population and not in the differentiated cells. This indicates the purity of the enrichment and a probable role for this isoform in the maintenance of stemness. This year, the transcriptional profile of these enriched CESC was carried out.

Results

NGS was performed after preparing the cDNA using SMARTer universal low input RNA kit followed by cDNA amplification, low input library preparation and quantification using Qubit fluorometer. Raw reads were quality trimmed with phred >25 and adapter trimmed. More than 61 and 60 million reads from limbal basal cells with high N/C ratio and central corneal cells were processed with Partek software resulting in an average length of 71 and 68 respectively. The reads were further aligned with human genome hg19 using STAR aligner in Partek workflow. Only one fifth of the reads were aligned against exonic regions and it was further quantified with Hg19 transcripts. RPKM values were used to calculate the differentially expressed genes. Several (720) genes were found to be upregulated and 13 genes downregulated in the enriched stem cells compared to the differentiated central corneal cells. More than 320 differentially expressed genes were identified in the enriched stem cell population (fold change >100) compared to central corneal cells (differentiated cells). These include GPHA2, ANXA1, FTL, RPLP1, UBC, RAP2B, S100A6, RPS14, FTH1, RPS6, KRT14 and TPT1, which are reported to be stem cell specific. MALAT1, a long non-coding RNA, known to be associated with cancer stem cells was found to be highly upregulated more than 1000-fold.

Conclusions

Several stem cell specific genes were identified to be highly upregulated in the enriched stem cell population. Further analysis of these highly expressed genes and their signaling pathways are being carried out to confirm their role in the maintenance of stemness.

Title of the project: Limbal miRNAs and their potential targets associated with the maintenance of stemness

Investigators : Dr. Gowri Priya Chidambaranathan, Dr. Bharanidharan Devarajan,
Dr. VR. Muthukkaruppan, Dr. N. Venkatesh Prajna
Research Scholar : Lavanya Kalaimani
Funding : Department of Biotechnology

Introduction including Background

Reports are available on the role of miRNAs with reference to corneal wound healing and differentiation (Lee et al 2011; Peng et al., 2012). But no information exists concerning the miRNAs of the corneal epithelial SCs (CESCs) due to lack of a specific marker for their identification and hence, isolation. The lab has earlier established a method to enrich the SC content up to 80% by isolating the limbal basal epithelial cells followed by Laser Capture Micro dissection (LCM) of cells with high Nuclear to Cytoplasmic (N/C) ratio as described above. Using such an enriched CESC population, this study aims to understand the SC specific miRNA profile and to identify their functional target genes, specifically those associated with the maintenance of stemness.

Results

Whole globes from donors (age: 50-67 years) were obtained from Rotary Aravind International Eye Bank, Madurai within 24 hours of death. The limbal basal epithelial cells with high N/C ratio and the central corneal cells were isolated. Since the number of enriched stem cells from a single donor by laser capture microdissection is minimal and highly variable (26-1162 cells/donor), optimization of the small RNA library sequencing was carried out using 5ng and 500 pg of reference RNA and has been completed at Genotypic, Bangalore. Based on this, the small RNA sequencing of the CESCs enriched population and differentiated central corneal epithelial cells is now being carried out.

Conclusion

miRNA profile of a highly enriched population of CESCs in comparison to the differentiated corneal epithelial cells will enable us to understand the differential miRNA expression specifically associated with SCs.

miR-203 and its regulation of Δ Np63 α expression in human limbal epithelial stem cells (LESCs)

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Dr. N. Venkatesh Prajna
Research Scholar : M.K. Jhansi Rani
Funding : ICMR - SRF

Introduction with Background

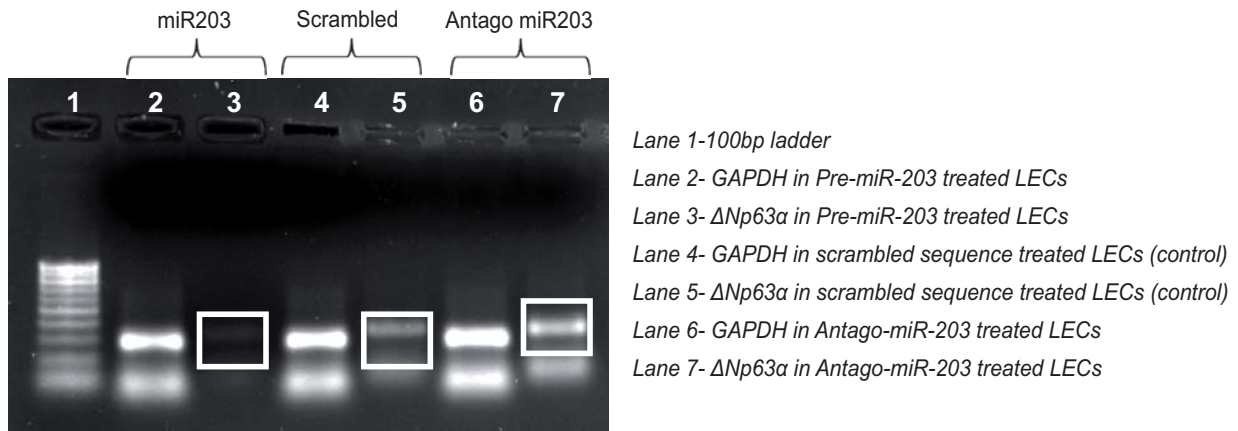
One of the isoforms of the nuclear transcription factor p63 - Δ Np63 α is expressed higher in CESCs in comparison to the differentiated cells. In the previous study from this lab, unique expression of this isoform was shown in enriched CESCs. In skin keratinocytes, miR-203 has been reported to repress stemness by inhibiting Δ Np63 α expression. This study aims to elucidate whether miR-203 has a similar influence in Δ Np63 α isoform expression and in the maintenance of stemness in the non-keratinized corneal epithelium.

Results

The regulation of Δ Np63 α expression by miR-203 and its role in CESCs maintenance was confirmed by transfecting cultured primary limbal epithelial cells (after 21 days) with 25nm pre-miR-203/antago miR-203, with scrambled sequence as control using Hiperfect transfection reagent. After 72 hours of transfection, RNA extraction was done from the transfected cells and analyzed for

- (i) miR-203 levels by real time quantification: The miR-203 level in miR-203 transfected cells was 106 fold higher compared to control and a 10 fold reduction was observed in antagomiR-203 transfected cells.

- (ii) Δ Np63 α mRNA levels by semi-quantitative RT-PCR: Δ Np63 α mRNA levels were reduced in miR-203 transfected cells whereas it was higher in antagomiR-203 transfected cells compared to control. This indicates that miR-203 regulates Δ Np63 α mRNA expression.



Δ Np63 α expression in miR-203 transfected samples. Primary LECs were transfected with pre miR-203, antago miR-203 and scrambled sequence for 72h. Expression of Δ Np63 α mRNA level was checked by semi-quantitative RT-PCR and normalized with GAPDH. Results of one experiment is represented from three biological triplicates.

- (iii) Colony forming assay to analyze the functional efficiency using the transfected cells: To further confirm the functional efficiency of transfected cells, after 72 hours of transfection, 2000 cells were seeded in mitomycin treated 3T3 feeder layer. After 7 days the epithelial colonies were stained with rhodamine. The colony forming efficiency (CFE) of antagomiR-203 transfected cells was 2 fold higher ($2.2 \pm 0.35\%$) than the control (scrambled sequence transfected cells) ($1.4 \pm 0.37\%$). Whereas the CFE of miR-203 transfected cells was ($0.1 \pm 0.35\%$) 10 times lesser than the control.

Conclusion

miR-203 specifically inhibits the proliferative potential in CECs by regulating Δ Np63 α expression. Further studies are essential to understand the signaling pathways associated with this molecular regulation of stemness.

Title of the project: Structural and functional integrity of corneal endothelium after storage in Cornisol, an indigenous intermediate stage corneal storage medium

Investigators : Dr. N. Venkatesh Prajna, Dr. Gowri Priya Chidambaranathan,
 Dr. Ganesh Gaikwad, Dr. Kishan A. Prajapati
 Research Scholar : S. Yogapriya

Introduction including Background

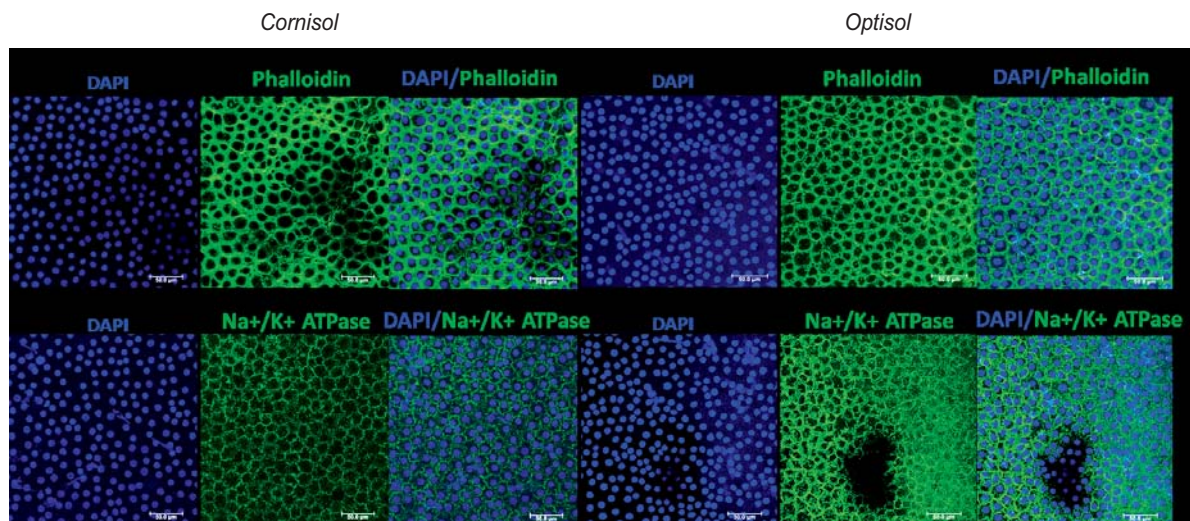
According to the Eye Bank Association of India, a total of 10 lakh people are affected with corneal blindness (2013). The only option for restoration of vision in these patients is corneal transplantation wherein the diseased or damaged cornea is removed and replaced by a healthy cornea from a deceased donor. Due to the acute shortage of donor eyes in our country most of the cases go untreated. On an average, the country requires 200,000 corneas each year but collects around only 44,800 (Eye Bank Association of India, 2013). For better utilization, the harvested healthy corneas from deceased donor are stored in a biochemically defined tissue culture medium. McCarey-

Kaufman (MK) medium is the widely used corneal storage medium in India. The limitation is that it is a short term storage medium which can preserve the corneal tissue only up to 3 days. One of the most widely used intermediate storage medium for optimal usage (14 days) of the donor tissues is Optisol GS. But due to its high cost, Optisol GS becomes unaffordable to the third world.

In order to overcome this issue, Aurolab, an integral part of Aravind Eye Care System manufactures Cornisol an intermediate storage medium which is available at an affordable cost for the storage of donor corneas. Preliminary studies by Aurolab established cornisol to be equivalent to optisol - as an intermediate storage medium based on the endothelial cell density and cellular viability. In continuation, this study evaluated the corneal endothelial cellular integrity as well as the Na⁺/K⁺ ATPase pump function by immunostaining the corneas stored in cornisol compared to optisol with specific markers.

Results

10 pairs of cadaver corneas, not suitable for transplantation (therapeutic -7/optical grade-3), received within 36 hours of death were used for this study. After treating the whole globe with antibiotics, the corneoscleral button were dissected and button of the right eye was stored in optisol and the left eye in cornisol for 7 or 10 days. The corneal endothelial density was 2550 ± 505 cells/mm² before storage, and the density after storage was 2250 ± 672 cells/mm² in cornisol and 2391 ± 714 cells/mm² in optisol. Immunostaining of the stored tissues with markers for cellular integrity like ZO-1 (tight junction protein), phalloidin (actin cytoskeletal protein), apoptosis– caspase 3 and functional status - Na⁺/K⁺ ATPase indicated no significant difference between the corneas stored in cornisol and optisol.



Confocal microscopic images of corneal endothelium stored in cornisol and optisol for 10 days, immunostained for the cytoskeletal protein phalloidin or Na⁺/K⁺ ATPase – FITC (green), counterstained with nuclear stain DAPI (blue). The staining pattern and the number of cells positive were similar between the tissues stored in cornisol and optisol.

Conclusions

This study on structural and functional status of corneal endothelium indicates that cornisol to be equivalent to optisol upto 10 days of storage.

PROTEOMICS

At the Department of Proteomics, various approaches are employed to understand the mechanisms underlying different eye diseases as well as to identify protein biomarkers with diagnostic and prognostic value. A well-equipped proteomics facility with two state-of-the-art mass spectrometers at AMRF helps to address the research questions in hand. The research focus of the department is currently on four ocular diseases, namely, Fungal Keratitis, Diabetic Retinopathy, Glaucoma and Keratoconus. In fungal keratitis, the key events that are triggered in the patients during fungal infection were identified using proteomics approach. This information throws light on why there is excessive corneal tissue damage in fungal keratitis patients. Many proteins that indicate the severity of the host response in these patients were also identified. Further, studies using human corneal epithelial cell lines are in progress that will allow us to understand the initial signalling events that take place immediately after fungal infection. The study on diabetic retinopathy aims to identify predictive biomarkers that can help in identifying the subgroup among diabetes who are at a higher risk of developing retinal complications. Towards this, 57 candidate protein biomarkers were identified in serum. Validation of these proteins using a larger sample size across different stages of the disease is currently underway. In addition to the proteome wide changes, the changes in the microRNA levels in different stages of DR were also analysed and this approach will provide the information on the functional role of microRNAs in the regulation of various genes during the progression of DR. Over the last year, collaborations were established with Institute Pasteur and University of Liverpool for expanding the research activities on fungal keratitis and keratoconus respectively. In the keratoconus study, the collaborative efforts are focussed on evaluating the effect of novel non-UV based chemical cross-linkers, which in turn will open up avenues for better treatment options for this disease.



A. Research on Human Mycotic Keratitis

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Funding	: Programme Support for Research on Human Mycotic Keratitis, Department of Biotechnology, Government of India

Background

Fungal keratitis is an important corneal disease, and is reported to constitute up to one-third of all cases of suppurative keratitis (infective corneal ulcer) in tropical parts of the world, resulting in severe visual impairment and blindness. *Aspergillus flavus* and *Fusarium* sp. are primary causative agents of keratitis in tropical countries. Fungal virulence factors, many of which are extracellular, facilitate hyphal invasion of the tissue. Combined with the induced inflammatory response of the host, fungal infection leads to the destruction of corneal tissue even in otherwise healthy and immunocompetent individuals. Treatment failure occurs in up to 60% of patients, who may require at least one and sometimes repeated corneal transplantation; and in severe cases enucleation of the infected eye. The differential response among keratitis patients to antifungal treatment, indicates variation in the individual's response. Hence, it is important to identify both the host and fungal factors responsible for disease outcome, markers to predict the treatment outcome and to modify treatment strategy. Towards this, the objectives include

1. Understanding of host immune response to fungal infection
2. Identification of factors contributing to the virulence of fungal pathogens

1. PROTEOMICS OF HOST IMMUNE RESPONSE TO FUNGAL INFECTION

1.1 Proteomics of tear of *A.flavus* keratitis patients

To understand the changes in the host in response to *A.flavus* infection, an in-depth proteome profiling of tear from both healthy controls and keratitis patients was performed. In this analysis, 2897 proteins were identified in control tear and 1966 proteins in tear from *A.flavus* keratitis patients. Combining the proteins identified from control and patient tear, a total of 4260 tear proteins were identified reliably. Comparative proteome analysis combined with label-free quantitation revealed a set of 177 proteins to differ significantly in abundance in the keratitis patient tear. In addition, 1235 proteins were identified exclusively in tear from keratitis patients, indicating the synthesis and secretion of new proteins in response to fungal infection.

Activation of Complement pathway in response to fungal infection

Examination of the tear protein identification data shows that majority of the complement proteins were not found in control tear. However, in the patient tear, proteins representing all the three complement pathways were found at high levels indicating that the complement pathways are activated upon *A. flavus* infection and reflects an antifungal mechanism elaborated by the host to deal with the infection. Interestingly, regulators of complement pathways (factor H, factor I, vitronectin, clusterin) were also found to be upregulated in the patient tear. Identification of these inhibitors shows that a fine balance exists between activation and down modulation of complement pathways.

Neutrophil-mediated defense functions in tear proteins

Many previous reports have shown that neutrophils and their components provide active defense against fungal infection. Several components of neutrophil-mediated defense proteins were identified in tear from patients but not in control tear. Some proteins that have been shown to be the components associated with neutrophil extracellular traps were found only in patient tear. Identification of proteins of the primary granules along with nuclear-localized histone proteins in the patient tear indicated that the neutrophils might employ NETosis for killing *A. flavus* hyphae. These

results suggest that NETs may be a defense mechanism against *A. flavus* as has been shown for other fungal pathogens such as *C. albicans*, *A. fumigatus* and *A. nidulans*.

Coagulation cascade and wound healing are also upregulated in patient tear

The above mentioned processes can act as anti-fungal defense but also can induce tissue damage. The host activates wound healing responses under such instances. Similar presence of coagulation cascade proteins and proteins involved in wound healing were also demonstrated in corneal infection. Early induction of these proteins indicates the interplay of various regulators of antifungal defense and activators of host recovery.

Human tear from mycotic patients reflects the ongoing defense mechanisms activated in response to the fungal infection (fig 1.1.1).

The presence of proteins that down modulate these responses indicate an intricate equilibrium between the clearance of infection and overt host response. The differentially expressed proteins across the different stages of infection are currently being validated.

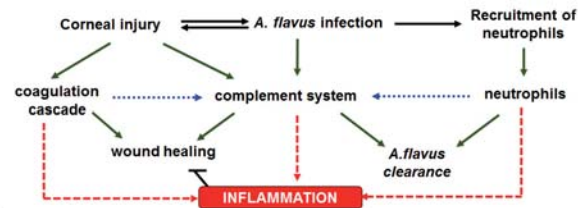


Fig 1.1.1. Summary of the host events activated during *A. flavus* infection of the cornea and their probable role in inflammation

Analysis of expression of complement factor H in tear from keratitis patients

Complement Factor H (CFH) is an important regulator of the complement pathways, which showed seven-fold upregulation in the tear from keratitis patients. This observation was validated by performing antibody based assay (western blot) for individual tear samples from both control and keratitis patients at three different stages of infection (figure 1.1.2).

Tear samples (15 µg) were resolved by 1D-SDS PAGE, transferred to NC membrane and probed with anti-CFH antibody. C, control; E, Early stage keratitis patients; I, Intermediate stage keratitis patients; L, Late stage keratitis patients.

CFH is a 139 kDa protein with multiple glycosylation sites. Hence, it is detected at a molecular weight of close to 180 kDa in the SDS-PAGE. Although there is a difference in the level of expression, it is clear that complement factor H is expressed at all stages of infection, whereas it is not detected in healthy controls. Interestingly, two other low molecular weight proteins (~50 kDa and ~66 kDa) were also consistently detected in all the keratitis tear samples. Experiments are in progress to identify these proteins which are also regulated similar to CFH.

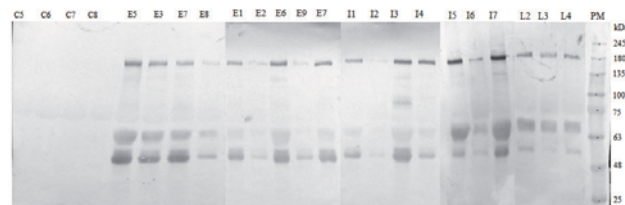


Fig. 1.1.2. Comparison of the expression levels of CFH in keratitis patients.

1.2. Enrichment and profiling of tear glycoproteins

To examine the glycosylation profile of tear proteins from control and patients, glycoproteins in tear were enriched using ConA columns. Comparative analysis of the proteins identified in N-glycosylated and non-N-glycosylated fractions of tear from control and keratitis patients was carried out. Amongst the 6962 proteins identified in tear, 2112 proteins were bound by Con-A column and represent the

N-glycoproteins in tear. Further, of the 1003 proteins found to be exclusively glycosylated in control tear, only 154 proteins were found to be exclusively glycosylated in tear from patients. Similarly, out of the 924 proteins that were not glycosylated in healthy tear, only 121 were found to be non-glycosylated during infection. Interestingly, 111 proteins identified in neat control tear were identified in the glycofraction of the infected tear but not in control tear.

Interestingly, glycoproteome analysis indicated that glycosylation of tear proteins are altered during fungal infection. These observations state that the disease condition alters the post-translational modification of proteins in tears which resulted in glycosylation of some proteins during fungal infection. Even though changes in glycosylation of proteins have been reported in cancer, limited reports are available on such changes in infectious diseases. “Glyco-Evasion” mechanism was suggested as a method used by pathogens to evade host immune system. Although it is not clear whether host or pathogen induce the changes in glycosylation of tear proteins, protein glycosylation can have a profound effect on the functions of the proteins and thereby, the outcome of the disease.

1.3. Differential expression of Zinc-alpha-2 glycoprotein in fungal keratitis

ZAG is one of the most abundant proteins in human tear, contributing to 12% of the total tear proteins. Through quantitative DIGE analysis of tear samples from keratitis patients in different stages of infection, it was evident that ZAG levels decrease as the infection progresses. This protein was also found to exist as multiple proteoforms and hence it is important to understand the role of ZAG and the mechanism of its decrease in fungal infection.

In Human tear samples, three proteoforms of ZAG were observed in the pH range of 4-7. However, when the tear proteins were resolved on a zoom-in pH range of 3-5.6, seven proteoforms of ZAG were observed.



Fig 1.3.1 ZAG proteoforms. Proteoforms of ZAG in the pH range of 4-7 (A) and in 3-5.6 (B).

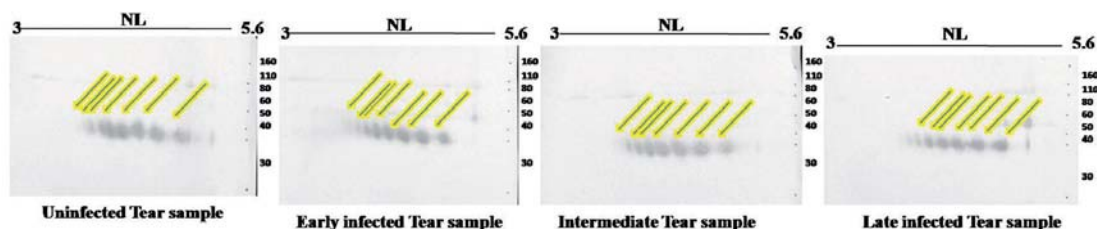


Fig 1.3.2. 2D Proteome profile of normal control tears and *A.flavus* keratitis tears.

To determine the regulation of the seven proteoforms of ZAG during the *A.flavus* infection of the cornea, two dimensional differential gel electrophoresis was performed, followed by western blot of infected tear proteins (fig 1.3.2).

Quantitative DIGE analysis was also performed to see the quantitative difference in the levels of the seven proteoforms as the infection progresses. Four out of the seven proteoforms showed a decrease in level as the infection progresses, while three proteoforms showed an increase in level of ZAG during the onset of disease (fig 1.3.3).

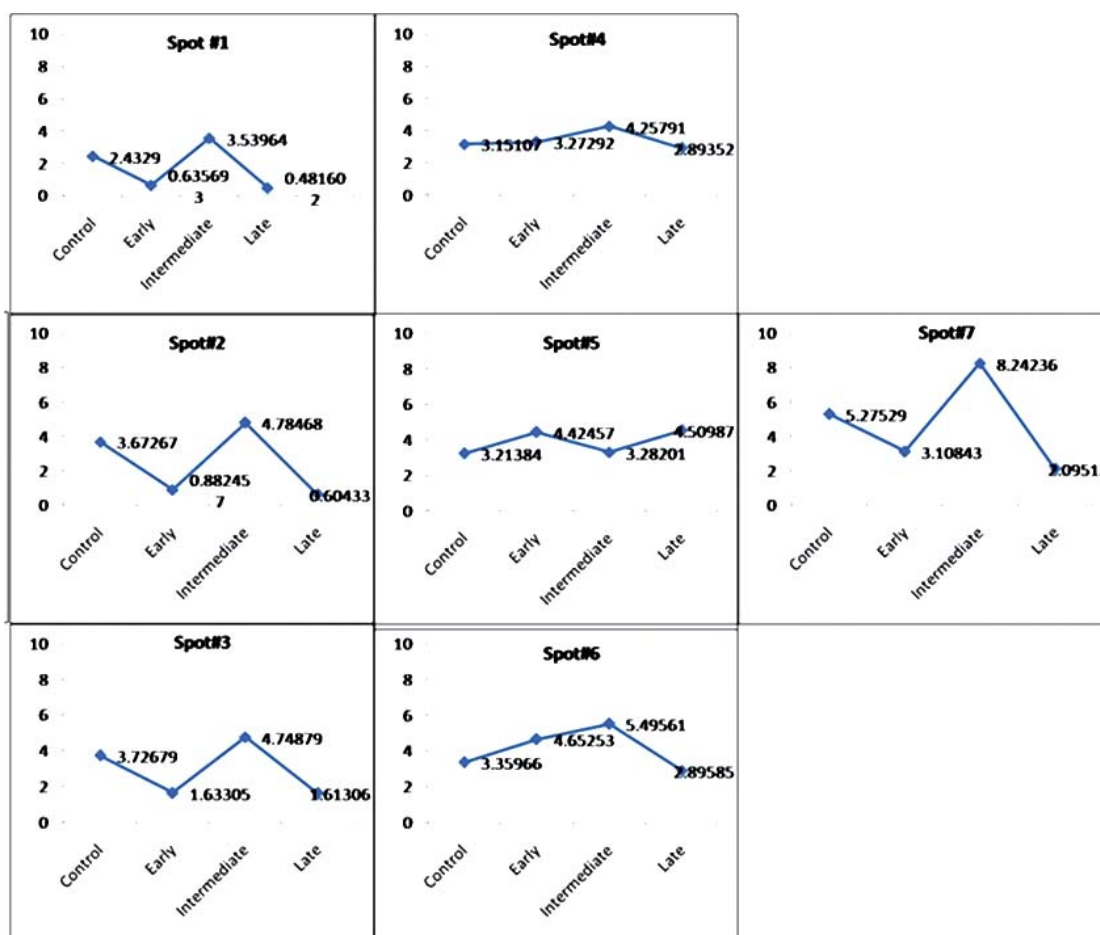


Fig 1.3.3. Quantitative analysis of the seven proteoforms of ZAG. Graphical views showing the standardized log abundance of spot volume (Y-axis) is plotted as a function of the stage of infection (X-axis).

Experiments are in progress to identify the PTMs contributing to the seven proteoforms of ZAG and to understand why some isoforms are upregulated while some downregulated.

1.4. Human Corneal Epithelial cell line as a model system to study fungal infection

1.4.1. Zymosan induced changes in Human Corneal Epithelial (HCE) Cell line

Zymosan is carbohydrate fraction of yeast and has been used by several investigators to examine the induction of fungal carbohydrate induced changes. The previous experiments from the lab showed marginal changes in zymosan treated HCE cells. In this period the 2D DIGE profile of HCE cell treated with zymosan was examined and there were no significant changes in the protein profile of treated cells compared to control untreated cells.

1.4.2. 2D reference map of Human corneal epithelial cell line:

A reference map of HCE cell line is valuable for several studies including infection-induced changes in the proteome. Towards this, construction of a 2D reference map was initiated (fig 1.4.2.1).

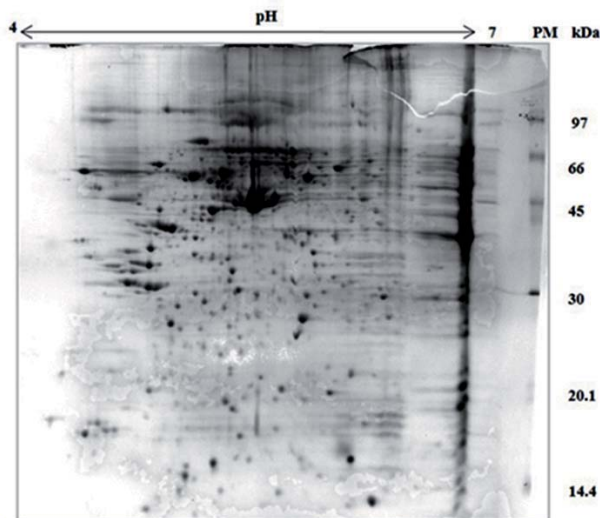


Fig 1.4.2.1. Preparative gel for HCE total cell lysate. Control and zymosan treated HCE cell lysate were pooled. Protein sample (450 µg) was separated in the first dimension based on pI using 18cm linear IPG strip in the pH range 4-7 and based on molecular weight in the second dimension in 12.5% polyacrylamide gel.

The gel was analyzed using Image Master Platinum 2D-7 software to determine the total number of spots, spot volume, molecular weight and pI. In total 350 spots were detected by the software and all the separated protein spots are now being identified. Identification of these protein spots are in progress.

1.4.3. Proteome changes of Human Corneal Epithelial cell line upon interaction with live and heat inactivated *A.flavus* spores

The morphology between uninfected control and cells infected with ATCC 200026 heat killed *A.flavus* spores were examined under inverted phase contrast microscope (fig 1.4.3.1). The cells infected with live germinated spores formed a mat over HCE cells after 16hrs. Hence the morphology of HCE cells couldn't be examined.

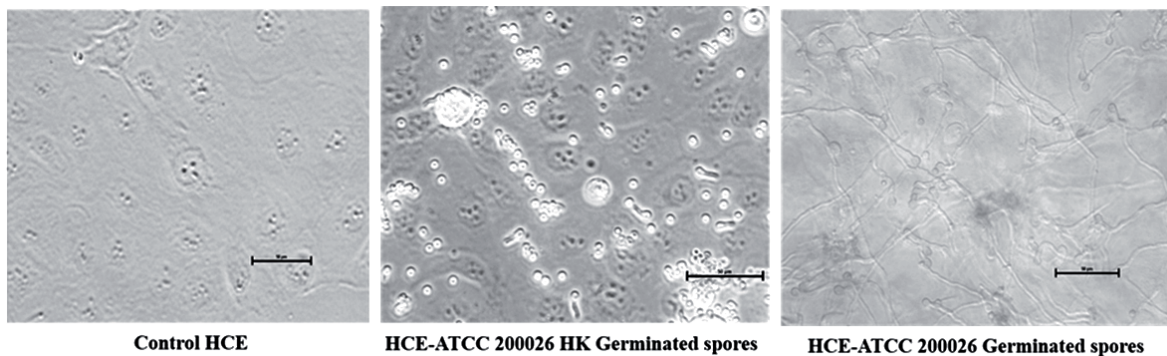


Figure 1.4.3.1 Morphological examination of HCE cells infected with ATCC 200026 heat killed germinated spores and germinated spores for 16hrs at 37°C in 5% CO₂ incubator observed under 400x magnification. HK germinated spores were observed as a clumps. The germinated spores after 16hrs formed a mat and it completely covered the HCE cells.

Experiments are in progress to identify the differentially expressed proteins upon infection with *A.flavus* (ATCC 200026) by mass spectrometry.

1.5 Modulation of signaling pathways in corneal epithelium during *Aspergillus flavus* keratitis

Introduction:

The human cornea is made up of the outermost corneal epithelium, followed by the stroma and the endothelial cells. Being the outermost layer of the cornea, the corneal epithelium presents the first line of defence against the microbes. The epithelium is made up of 5-7 stratified layers of self-renewing cells, about 50 µm thick and the cells are connected with each other using Junctional adhesion molecules and occludins.

Rationale of the study

Fungal cells secrete several compounds known as Pathogen associated molecular patterns (PAMPS), which recognize Pathogen responsive receptors (PRRs) in the corneal epithelium and stroma. The expression of PRRs upon recognition of the fungal PAMPs has been studied well in a myeloid lineage cells such as macrophages, dendritic cells and even in corneal stroma. However, the role of signalling molecules in the corneal epithelium in the pathogenesis of the disease remains largely unexplored. Hence, understanding global changes in signalling patterns of the proteins and its phosphorylation status in non-phagocytic cells such as epithelial cells after fungal infection would give an insight to the initial signalling events in the corneal epithelium leading to the activation of innate immune system molecules. The role of *Aspergillus flavus* components in the induction of signalling molecules in corneal epithelial cells is least understood and this will be examined in this study.

Major objectives

The major objective of this study is to understand the global changes in the signalling molecules and their functional role in the human corneal epithelial cell line (non-phagocytic cell line) either after treatment with fungal ligand Zymosan or after infection with *Aspergillus flavus* germinating conidia. The *Aspergillus flavus* infected corneal epithelial scrapings from patients can be used to gain an insight into the initial signalling events in the corneal epithelium leading to the activation of innate immune system molecules.

Results and Conclusion

Initial experiments were performed to analyse the differential regulation of transcripts in human corneal epithelial cell line after treatment with the fungal ligand zymosan. Zymosan is known to bind to the TLR-2 and Dectin-1 receptors in many cell types. TLR-2, MyD88, Dectin-1, E-cadherin, caspase-3, caspase-8, MMP-7, MMP-8, and pro-inflammatory cytokines IL-6 and IL-1 β transcripts were analyzed by end-point PCR and quantitative Real-time PCR. Out of all the transcripts, only IL-6 showed a 3.34 fold upregulation in the Zymosan treated cells in comparison to the untreated cells. This was confirmed by real-time PCR analysis of the IL-6 transcript where GAPDH was used as an internal control for normalization (fig 1.5.1). Other transcripts didn't show any significant changes after Zymosan treatment. Zymosan treatment is able to elicit an upregulation of the IL-6 cytokine in human corneal epithelial cells.

Further studies: Studies are in progress to see the changes at the transcript level in human corneal epithelial cells after treating them with germinating conidia of the *Aspergillus flavus*. In the future, mass spectrometry based proteomics approach will be done to analyze changes in the phosphorylation levels of the signalling molecules and the total proteins in the Zymosan treated as well as germinating conidia infected human corneal epithelial cells.

2. PROTEOMICS OF FUNGAL PATHOGENS

The outcome of a fungal infection in an immunocompetent human eye depends on the ability of the fungus to overcome the host defense and propagate itself. In this process, the earliest events with respect to the fungal proteins involved include the secretory proteins of the invading organism. As a first step towards understanding the role of the extracellular proteins, exoproteome profile of the fungal isolates was generated. When examining the exoproteome, one protein was found to be expressed abundantly and more interestingly, in multiple proteoforms. The role of this protein is being studied in great depth to understand its role in the virulence of *A. flavus* corneal isolates.

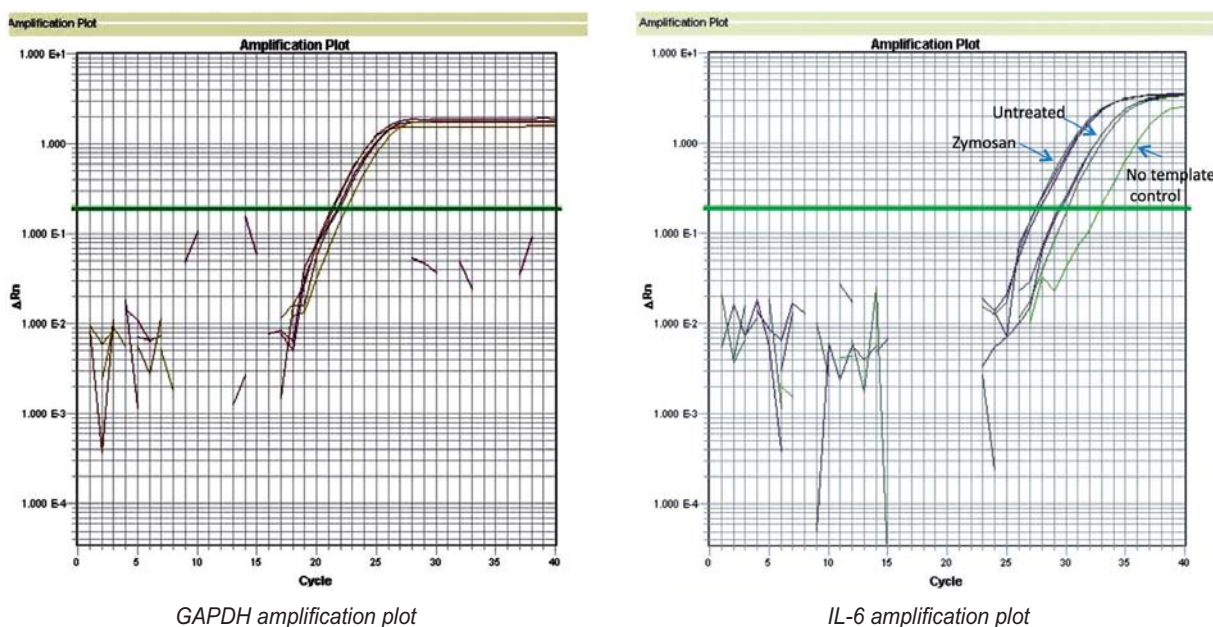


Fig. 1.5.1: Amplification plots of GAPDH and IL-6 of the untreated and Zymosan treated human corneal epithelial cells (in triplicates).

2.1. Characterization of alkaline protease protein, Alkaline protease activity and Alk protein level in clinical isolates of *A.flavus*

Alkaline protease is one of the most abundant exoproteins of *A.flavus*. Selvam et al., (2015) has shown alkaline protease (Alk) to exist in 24 different proteoforms. This study aims at characterizing the modifications of *alk* at both the transcript and protein level that results in multiple proteoforms. Identification of the modifications that contribute to multiple isoforms will allow us to understand the functional significance of this protein, in particular reference to the virulence of *A. flavus*.

Earlier the Alk protein was purified from the exoproteins and two forms of this protein were seen in the purified fraction (37 kDa and 28 kDa). Both these forms were confirmed as Alk proteins by western blot as well as MS identification. The gene architecture and the processing of Alk is shown in fig 2.1.1.

To determine the levels of Alk secreted protein in different clinical isolates of *A. flavus*, 13 strains were selected and the exoproteins were prepared using solid state fermentation method. The exoproteins were resolved by 1D SDS-PAGE and transferred to NC membrane, which was then probed with anti-alk antibody.

This western blot data shows that all the clinical isolates express both the 37 kDa and 28 kDa Alk protein. In all the strains, level of the 37 kDa protein is more than that of 28 kDa (fig.2.1.2). Few strains also seem to express additional forms of Alk. The expression levels of Alk were quantitated based on densitometry analysis. The expression level of Alk in ATCC26 was used as the reference to calculate the fold change in expression of the 37 kDa and 28 kDa forms of Alk in all the other strains (figure 2.1.3).

This analysis reveals that the expression level of the 37 kDa protein does not vary significantly across the clinical strains with the fold change not exceeding 3. However, the fold change for the 28 kDa proteins seems to vary significantly for many of the clinical strains with a maximum of 23-fold increase for CI22537. Overall, it appears that all the strains secrete Alk as the preprotein, 37 kDa form. However, there seems to be a difference in the processing of the 37 kDa form to the protease form, 28 kDa between the strains.

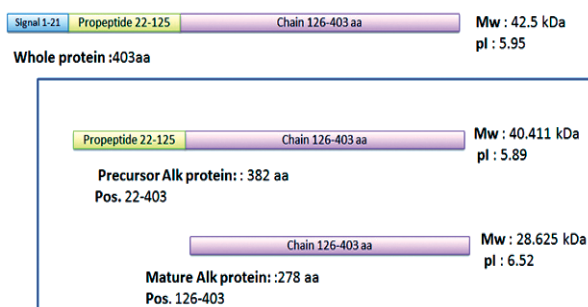
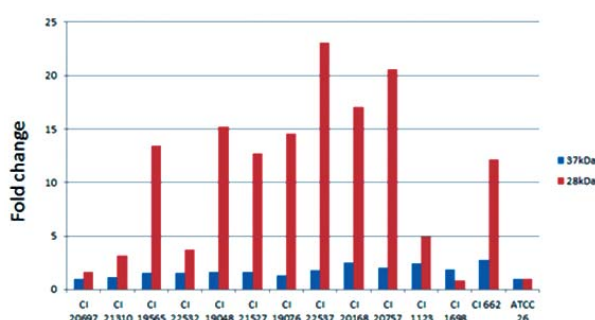


Fig. 2.1.1.1. Alk protein architecture along with its processed forms



1. predict the subgroup of Diabetics who are at high risk of developing DR
2. monitor the disease progression of DR patients

1. Serum as a source of biomarkers

The initial discovery phase study focusses on the identification of protein level changes in the stages at the two extremes of the disease spectrum – type II diabetes mellitus (DM) and Proliferative DR (PDR).

1.1. Quantitative comparative proteomics

A comparative proteomics approach was employed to analyse the differences in the expression of proteins in the serum of PDR patients using DM as control. The different approaches adopted for this comparative analysis is shown below.

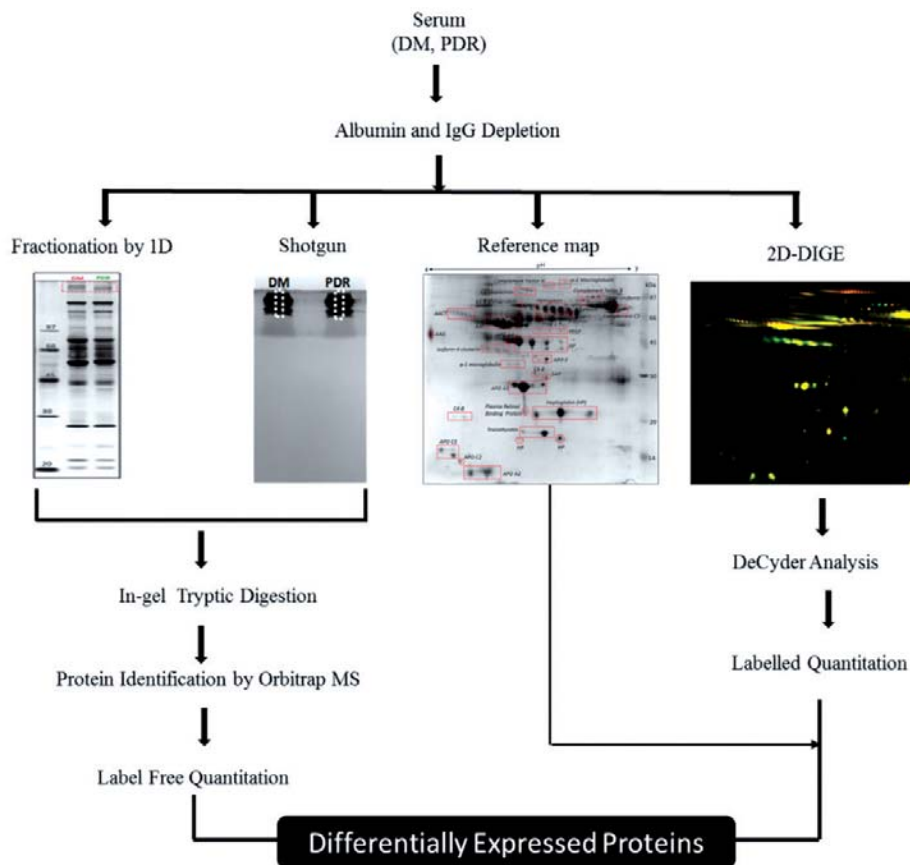


Fig. 1.1.1. Workflow adopted for comparative proteome analysis of serum from DM and PDR patients.

Combining the results from the three different approaches, 57 proteins were identified, that were differentially expressed in the serum of PDR patients (figure 1.1.2). Global search for previous literature indicated association of three functional protein families with type 2 diabetes namely Complement, lipoproteins and Serine Protease Inhibitors (SERPINs). This was in consensus with data from this lab. Thirty proteins out of fifty seven differentially regulated proteins could be categorized under these three protein families.

Interestingly, some proteins were present in both the up and down regulated list. These are the proteins which undergo processing and there is a differential regulation of the processed forms. One example of such a protein is Complement component C3. As reported by other groups, down

regulation of C3 in PDR condition was found in this study. However, through this 1D-LFQ approach, the C3b form (110 kDa) is 7-fold downregulated while the C3dg processed form shows a marginal upregulation in PDR. This could be due to the increased rate of processing of C3b and experiments are under progress to validate this hypothesis.

Currently, the changes in the levels of these proteins are being validated in different stages of DR – Control (no DM, no DR), DM (no DR), NPDR (mild, moderate, severe), PDR.

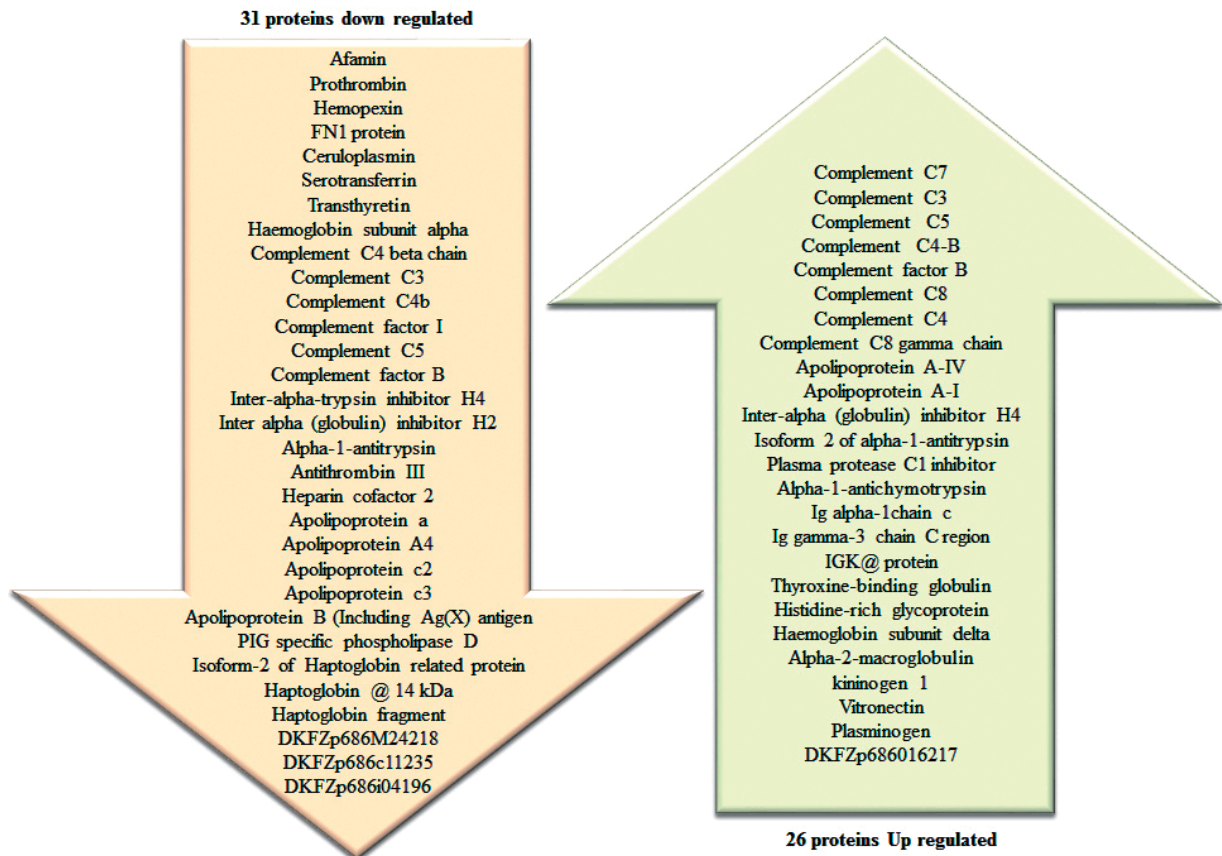


Fig 1.1.2. List of putative serum biomarkers for PDR. From the three different approaches based on the fold change and statistical significance, 57 proteins were shortlisted as putative serum biomarkers for PDR.

2. Microparticles as a source of biomarkers

Serum has an inherent problem of proteins spanning 12 orders of magnitude. Due to this dynamic range in serum proteins, it is difficult to identify the changes in low abundant proteins. Further, the changes related to DR might be masked if the patient has other systemic problems. To overcome this problem to a certain extent, microparticles can be used as a source of biomarkers. The isolation protocol for microparticles is optimized and the proteome of microparticles isolated from serum as well as plasma were compared. Based on this analysis, serum was found to be suitable for isolating microparticles. A shotgun analysis of microparticles was also performed from healthy controls, DM, NPDR and PDR followed by label-free quantitation. The preliminary analysis shows that many proteins were found to be differentially expressed in PDR that were not identified in serum. Further, among the 57 candidate biomarkers identified in serum, 49 proteins were identified in serum microparticles also and ten of these show similar expression pattern in both serum and serum microparticles. Quantitative differences in the proteins of the microparticle using iTRAQ labelling is currently in progress.

3. Proteoforms as putative biomarkers

Results from 2D-DIGE analysis also revealed the differential expression of proteins at the proteoform level between the DM and PDR condition. As seen from the 2D protein profile of serum, multiple proteoforms of the proteins were identified which could be attributed to the post translational modification occurring within the biological system. Two proteins show obvious difference at the proteoform levels in PDR and is discussed here

- Complement factor B resolves as five proteoforms at a pI of 6-7 when separated on a pH range strip of 4-7. The two highly basic forms of CFB were found to be upregulated in PDR (fig 1.3.1). Based on computational analysis, it is hypothesized that these isoforms differ in the number of phosphates that they harbour – the basic form representing the phosphorylated form with one phosphate while the most acidic form with five phosphate groups.
- Alpha-1-antitrypsin (AAT) does not show significant difference in their expression between PDR and DM serum. However, the 2D DIGE clearly showed there is a difference in the mobility of AAT in PDR serum in comparison to that in DM serum (fig 1.3.2).

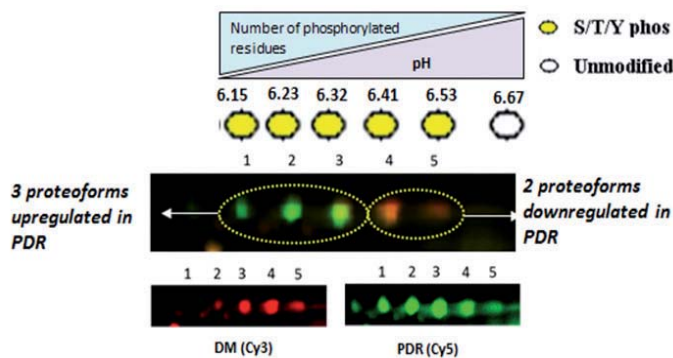


Fig 1.3.1. Differential expression of Complement factor B proteoforms in PDR.

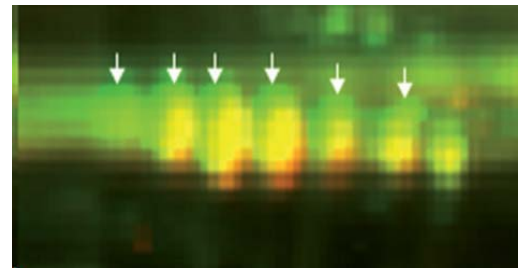


Fig 1.3.2. Differential Glyco-modification of Alpha-1 antitrypsin proteoforms in PDR.

Functional analysis of circulating microRNAs and their regulatory role in Diabetic retinopathy

Investigators : Dr. O.G. Ramprasad, Prof. K. Dharmalingam, Dr. D. Bharanidharan, Dr. Kim Ramasamy

Funding : SERB-Early career research grant (2016-2019) and the Mindtree grant.

Background

MicroRNAs (miRNAs) are a novel group of non-coding small RNA molecules with marked tissue specificity that post-transcriptionally control gene expression and are implicated in a large variety of physiological and pathophysiological processes (Ha and Kim, 2014). MicroRNAs have been detected in various body fluids including serum. Levels of miRNAs in the serum of humans have been shown to be stable, reproducible, and consistent amongst healthy individuals but show changes during pathophysiology, allowing them to be of potential value as clinical biomarkers of diseases including cancers and metabolic disorders (Gilad et al., 2008).

Rationale

The role of serum microRNAs in the progression of DM to NPDR and NPDR to PDR in humans is largely unexplored. Therefore, the rationale of this study is to understand the regulatory function of microRNAs in the progression of microvascular complications among diabetic patients and also at the exploration of validating miRNAs as biomarkers.

Objectives

The objective of this study is to reveal disease specific miRNAs from serum of PDR, NPDR and DM patients and compare it with serum samples of control healthy patients. Further, identification of potential molecular targets of differentially expressed miRNAs and their regulatory networks towards the understanding of disease pathogenesis will be analyzed using computational tools. The differentially expressed miRNAs will be validated for their functional implication using human retinal endothelial cell line grown under different glucose conditions and tissue biopsies from patients.

Results and Conclusion

The main methods utilized for the preliminary studies of the project involve miRNA isolation from serum samples and analysis of select miRNAs in purified RNA samples using quantitative real-time PCR. The results from the preliminary studies are described here. Isolation of total RNA including microRNAs from 150 μ l of the serum samples of DM, PDR patients and control healthy patients yielded 313.2 ng, 319.2 ng and 164.4 ng of total RNA respectively. The DM and PDR serum samples were having more RNA than the normal control samples.

Quantitative real-time PCR amplification of specific miRNAs were done in all the serum samples. C.elegans miR-39 spike-in control was used for the normalization process during the relative quantification of the fold changes in the miRNA expression levels. Preliminary results indicate that miR-21 is upregulated by 2.63 folds in PDR patients and 3.82 folds in DM patients compared to control patients. miR-21 targets the angiogenesis process by activating AKT and ERK1/2 signalling pathways, thereby enhancing HIF-1 α and VEGF expression which promotes neovascularization.

Studies in progress: Studies to analyze the changes in levels of miR-181c, miR-1179, miR-184, miR-200b, miR-31 and miR-125b are in progress. Subsequent studies will include the next generation sequencing of the miRNAs, their target prediction and regulatory networks using computational tools, their validation and functional analysis.

OCULAR PHARMACOLOGY

The main research focus of the ocular pharmacology department is to understand the pathogenic mechanisms and to identify a better therapeutic strategy for age-related eye diseases such as age-related macular degeneration (AMD) and glaucoma. The department also focuses on understanding the reservoir function of human amniotic membrane (HAM) with different classes of drugs in ocular use.



Indian Macular Carotenoids Research (IND-MACARE) - A Feasibility Study

Investigators : Dr. Senthil kumari, Dr. Anand Rajendran
Senior Research Fellow : Madhavi Latha Yelchuri
Funding Source : Indian Council of Medical Research (ICMR) (2015-2017) and Aravind Eye Foundation (AEF), New York (MPS II).

Introduction:

Age-related macular degeneration (AMD) is the leading cause of irreversible blindness in the elderly population (age above 60 years). Macula is the central part of the sensory retina which appears yellow due to the accumulation of carotenoids especially lutein (L), zeaxanthin (Z) and meso-zeaxanthin (MZ). It functions as a blue-light filter and shields retina from photo-oxidative damage.

Substantial evidences suggest that the deficiency of these pigments is one of the risk factors for the development of AMD. Carotenoids cannot be synthesized by the human body and hence it has to be supplemented through diet rich in lutein and zeaxanthin. Previous studies from this laboratory (using HPLC) demonstrated that south Indian donor eyes had significantly very low levels of macular carotenoids. Hence, screening of macular pigment density is crucial in Indian population to identify the susceptibility of patients for the development of AMD.

Macular pigment can be monitored clinically (as macular pigment optical density (MPOD) to predict the risk for the development of AMD due to carotenoids deficiency. Such information related to Indian population is very limited. Therefore, the Indian macular carotenoids study aims to assess the macular pigment density in Indian eyes of different age-groups and also in patients with AMD. This will help in identifying individuals at reduced, medium and elevated risk for age-related eye disease based on high, medium and low central MPOD levels respectively and their genetic predictors in Indian population.

Results

The MPOD of the study participants was assessed using MPS II (Electron Technology, UK) after completing their ocular examination (fundus photography, OCT, visual acuity and lens status).

MPS II is based on heteroflicker photometry which involves a frequency of blue green light. MPOD was measured at 1° eccentricity (pigment level is high) for central whereas the measurement at 8° eccentricity (where there is no pigment) for periphery. MPOD was calculated based on ratio of central minima 1° to peripheral minima 8°.



In Vivo Measurement of Macular Carotenoids using MPSII (Electron Technology, UK)

MPOD normal values	: 0.3 and above
Poor MPOD	: below 0.25

Repeatability and Re-reducibility of MPOD by MPS II

The overall mean (\pm SD) MPOD was found to be 0.51 ± 0.11 ($0.23 - 0.74$; $N=40$ eyes of 20 volunteers). There was no variation between the observers and good reproducibility was observed between test and retest using MPS II.

In this report, normative data of 110 eyes of 55 volunteers was given. The measured parameters for the volunteers are listed in the table below.

PARAMETERS	FEMALE	MALE	Total
AGE \pm SD	30.27 ± 8.29	34.65 ± 8.29	31.88 ± 10.91
MPOD Right eye \pm SD	0.49 ± 0.12	0.44 ± 0.11	0.49 ± 0.15
MPOD Left eye \pm SD	0.50 ± 0.17	0.52 ± 0.16	0.47 ± 0.15
Haemoglobin% \pm SD	12.72 ± 1.0	15 ± 1.03	13.62 ± 2.30
Fasting glucose% \pm SD	86.62 ± 10.4	91.03 ± 8.1	87.29 ± 13.99
Total cholestrolmgs% \pm SD	149.4 ± 20.40	162 ± 28.35	153.31 ± 30.53
Triglyceridesmgs%L \pm SD	93.82 ± 29.71	115.15 ± 56.70	102.43 ± 45.67
HDL Cholestrolmgs%L \pm SD	40.34 ± 4.9	40.96 ± 5.12	40.00 ± 6.81
LDL cholestrolmgs%L \pm SD	94.68 ± 17.9	97.96 ± 23.1	94.83 ± 22.59
VLDL cholestrolmgs%L \pm SD	18.73 ± 6.07	22.30 ± 10.08	20.13 ± 8.40
Total cholestrol/HDL% \pm SD	3.73 ± 0.55	4.01 ± 0.90	3.80 ± 0.85
LDL cholestrol/HDL%L \pm SD	2.28 ± 0.48	2.46 ± 0.74	2.33 ± 0.66
Systolic Blood pressure (mmHg) \pm SD	115.17 ± 9.1	113.84 ± 6.9	112 ± 16.08
Diastolic Blood Pressure (mmHg) \pm SD	73.10 ± 7.12	71.92 ± 6.93	71.40 ± 11.06

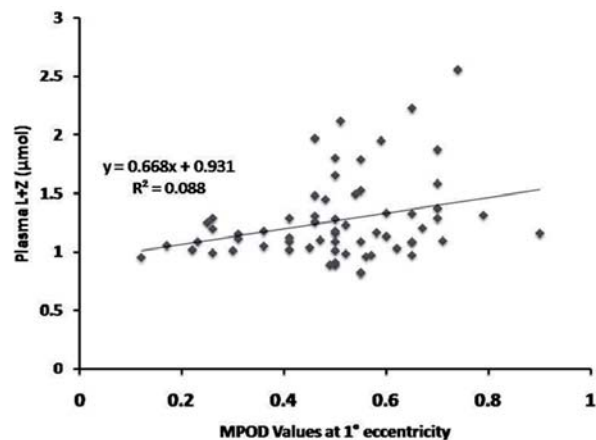
The overall mean MPOD was found to be 0.49 ± 0.14 at 1° eccentricity. There was no significant association between MPOD and age. Male participants showed marginal increase in MPOD values (0.52 ± 0.17) as compared to female participants (0.47 ± 0.12) but found to be statistically insignificant ($p=0.298$).

MPOD Vs Age

As AMD is a progressive degeneration of the central macula with the increase in the age, correlation of age with that of MPOD was assessed. There was no significant correlation of MPOD with Age.

MPOD and plasma carotenoids

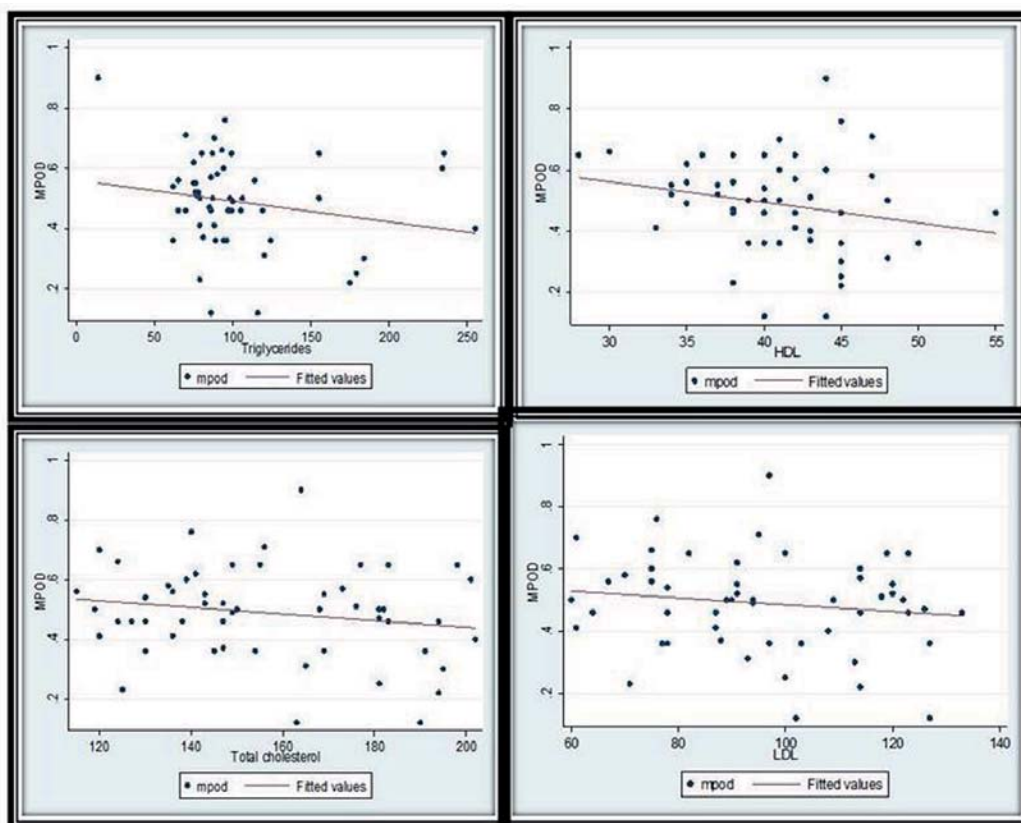
Plasma L and Z of the study volunteers were estimated by HPLC using PDA detector. The relationship between plasma L+Z and MPOD was investigated and found positive but weak association in these study participants.



Graph showing the relationship between MPOD and plasma L+Z

MPOD and Lipid Profile

Serum triglycerides, total cholesterol, LDL and VLDL showed an inverse association with MPOD in Indian Cohort but not statistically significant ($p=0.113$).



Correlation between MPOD and Total cholesterol, triglycerides, HDL and LDL

Conclusion

The findings of the present study indicate that, MPOD is positively associated with plasma carotenoids, adding further evidence that additional intake of LZ may be beneficial in delaying the risk of AMD in Indian population. Future studies related to dietary intake, lifestyle and genetic analysis of study participants will help us to understand the role of carotenoids in preserving vision with age.

Studying the role of Rho A / Rock signaling in conventional outflow pathway using Human Organ culture anterior segment (HOCAS), an implication in Glaucoma therapy

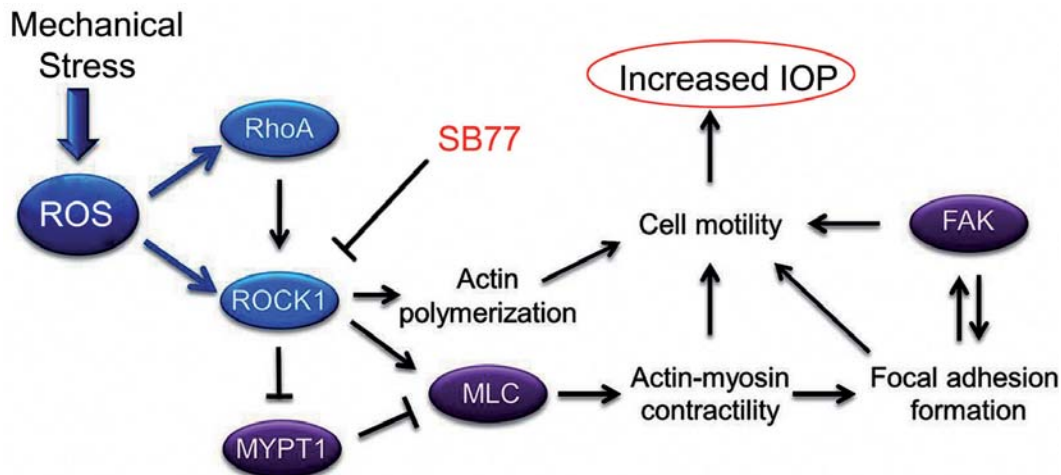
Investigators : Dr. S. SenthilKumari, Dr. C. Gowri Priya, Dr. Krishnadas
Junior Research Fellow : S. Ashwin Balaji
Funding Source : Science and Engineering Research Board (SERB) (2015-18)

Introduction

Glaucoma is a multifactorial optic neuropathy leading to irreversible blindness. Primary open angle glaucoma (POAG) is the predominant form of glaucoma and elevated intraocular pressure (IOP) is the only modifiable risk factor. The elevated IOP is due to impaired outflow through the trabecular

meshwork as a result of changes in cytoskeletal organization, increased tissue stiffness, increased expression and deposition of ECM proteins, as well as elevated levels of specific cytokines and growth factors.

RhoA/ROCK signaling plays an important role in the modulation of the cytoskeletal integrity of cells, the synthesis of extracellular matrix components in the aqueous humor outflow tissue and the permeability of Schlemm's canal endothelial cells. Activation of the RhoA/Rho associated kinase (ROCK) pathway via secreted bioactive molecules or via integrin activation after extracellular matrix



A schematic diagram showing Rho-ROCK signaling

binding lead to polymerization of actin stress fibers and formation of focal adhesions. This result in increasing the resistance to aqueous humor outflow by inducing alterations in TM cell contraction, actomyosin assembly, cell adhesion and ECM synthesis. Inhibition of ROCK pathway leads to decrease in flow resistance, increase the aqueous humor outflow and thus have a potential role in glaucoma therapy. The purpose of the present study was to investigate the effect of SB77 (ROCK inhibitor) in enhancing the human aqueous outflow using human organ culture anterior segment (HOCAS) *ex vivo* model system.

For establishing this *ex vivo* model, whole globes of human donor eyes not suitable for corneal transplantation were obtained from Rotary Aravind International Eye Bank, Madurai and mounted into a Petri dish specially designed for human eye. Intraocular pressure (IOP) and outflow facility (OF) were monitored continuously using Power Lab system with Lab pro chart software. After baseline stabilization for 2-3 days, one eye of each pair was exchanged with SB77 at 200µl/min flow rate and the contralateral eye received the respective vehicle. IOP was monitored for 24 hours post treatment; the eyes were then fixed by perfusion using 4% paraformaldehyde and the aqueous outflow tissues were assessed using light microscopy. Immunofluorescence analysis was carried out to study the distribution and localization of ROCK1 expression in aqueous outflow pathway and investigated its expression level after treatment with SB77.

Results

A representative picture showing the effect of SB77 on IOP is given below. SB77 showed dose-dependent increase in outflow facility in HOCAS model. SB77 at 10µM increased OF by 21% (N=8) in 24 hours ($p < 0.005$) where as 50µM showed 40 % increase (N=8; $p < 0.005$) as compared to its control. The nano molar concentration (100 nM) didn't show any significant change in outflow facility.

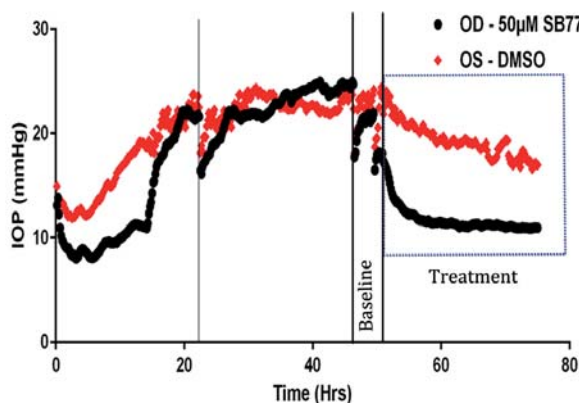
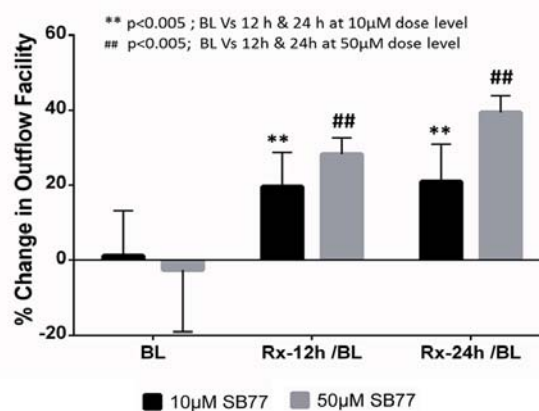
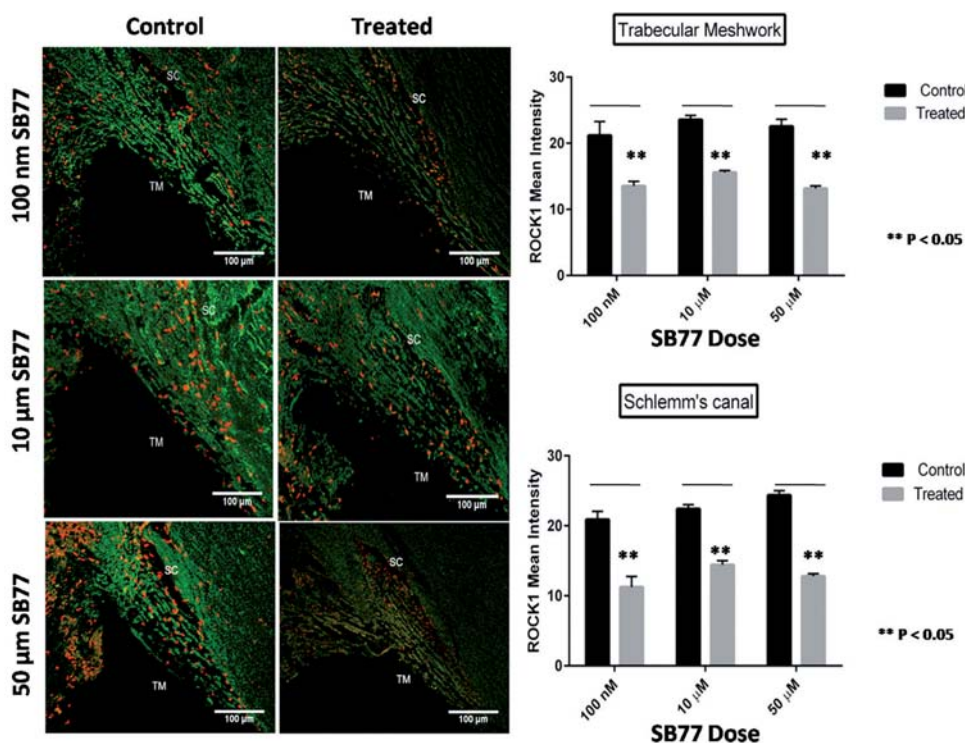


Figure showing intraocular pressure (IOP) graph of the anterior segment receiving 50 μ M SB77 versus vehicle in fellow control anterior segment. The presence of SB77 decreases IOP and increase outflow facility as compared to its vehicle control.



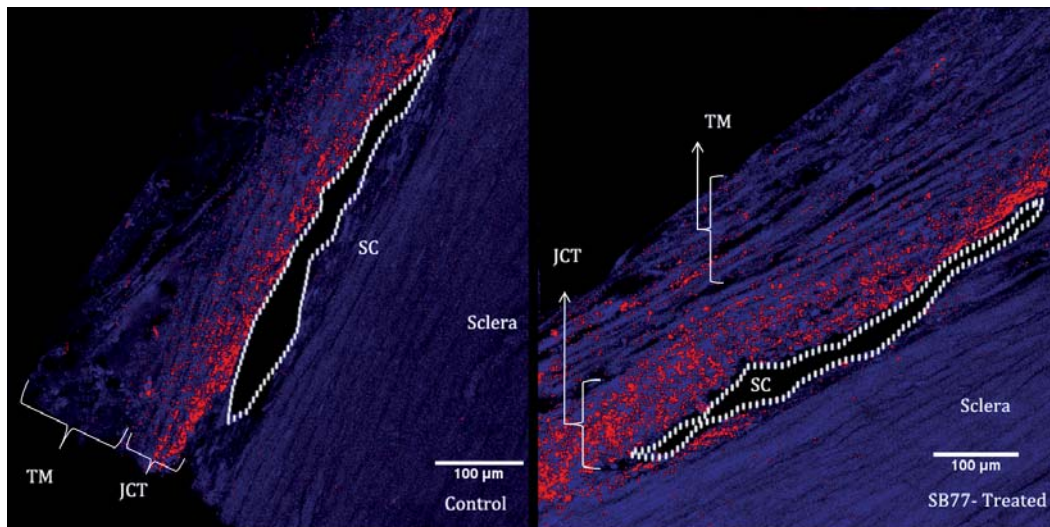
Graph showing a percentage change in outflow facility in SB77-treated and vehicle control eyes. Statistical significance was calculated using paired t-test.

Immunofluorescence analysis showed a significant reduction of ROCK1 expression in TM and SC of SB77 treated eyes as compared to its vehicle treated eyes.



Immunofluorescence Analysis of ROCK1 expression in human aqueous outflow tissues (A) Representative image of ROCK1 (green) merged with PI (Red) in Vehicle Control and Treated TM tissues. Significant reduction in ROCK1 expression was observed in the SB77 treated eyes as compared to Vehicle Control eyes. (B) Densitometry analysis of the HTM area and SC on each tissue section demonstrated a significant decrease in relative intensity of ROCK1 in SB77 treated eyes as compared to its vehicle treated control. Relative intensity data of the ROCK1 isoform are represented as the Mean \pm SD. Statistical significance was calculated using unpaired Student's t-test; * p < 0.05). TM – Trabecular meshwork and SC-Schlemm's canal.

To label the hydrodynamic pattern of outflow after treatment with SB77, the anterior chamber contents of all eyes were then exchanged (7 mL) with media containing red fluorescent microspheres (0.2 μm , 0.002% v/v) (at 200 $\mu\text{L}/\text{min}$ flow rate) followed by 0.5 mL perfusion of this same solution at the flow rate of 2.5 $\mu\text{L}/\text{min}$ to deliver an equivalent volume of tracer to the trabecular meshwork of each eye. Finally anterior segment was fixed by perfusion and analyzed for changes in the hydrodynamic pattern using confocal microscopy.



Tracer-decorated aqueous outflow pattern is shown in the figure. Segmental distribution of fluorescent tracer was observed along the SC in both control and SB77-treated eyes. More tracer distribution is seen in SB77 treated eyes as compared to its control. TM – Trabecular meshwork; JCT- Juxta canalicular trabecular meshwork and SC-Schlemm's canal.

Conclusion

The present study reveals that, SB77 at the studied concentration was effective in enhancing OF in HOCAS. Immunostaining showed significant reduction in the expression of ROCK1 in both Trabecular Meshwork and Schlemm's canal regions. Hydrodynamic pattern study with fluorescent microspheres showed more uniform distribution along the inner wall of Juxtacanalicular and inner wall expansion. Thus ROCK inhibitor (SB77) increases the outflow facility and may be a potential clinical candidate for the management of glaucoma therapy. In addition, the effect of cyclic mechanical stress on the Rho A –ROCK signaling in altering the contractility of intact trabecular meshwork in mediating aqueous outflow resistance and also the role of Rho Kinase inhibitors in reducing such resistance generated by cyclic IOP are being investigated.

Is Human Amniotic Membrane (HAM) a Suitable Reservoir System for the Release of Drugs in Ocular use?

Investigators : Dr.S.Senthil kumari, Dr. Venkatesh Prajna
 Research Scholar : Madhavi Latha Yelchuri
 Clinical Fellows : Dr. Bhagyashree Madhvi, Dr. Nilam Gohil, Dr. Hithasara Sajeev
 Funding : MUTT – Study (AEH)

Introduction

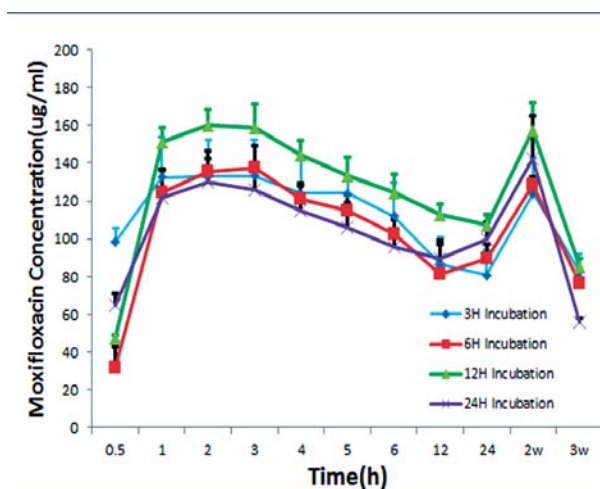
Human amniotic membrane (HAM) is the innermost layer of placenta. It has been shown to have anti-inflammatory, anti-fibrotic, anti-angiogenic as well as anti-microbial properties. Also because

of its transparent structure, lack of immunogenicity and the ability to provide an excellent substrate for growth, migration and adhesion of corneal and conjunctival epithelial cells, it is being used increasingly for ocular surface reconstruction in a variety of ocular pathologies including corneal disorders associated with limbal stem cell deficiency, surgeries for conjunctival reconstruction, as a carrier for ex vivo expansion of limbal epithelial cells, glaucoma surgeries, scleral melts and perforations. In all such cases, topical treatment with antibiotics or anti-inflammatory drugs is essential after HAM transplantation.

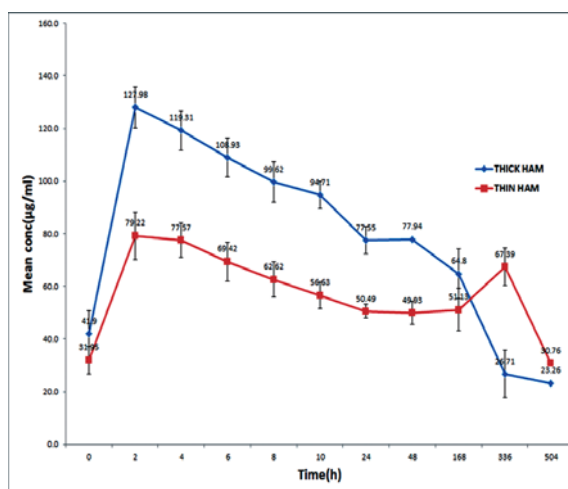
Apart from these advantages, HAM has been shown to act as a reservoir for the release of anti-infective drugs. The permeability behaviour of HAM has been demonstrated in an *in vitro* model system with netilmycin; ofloxacin and bevacizumab. However, the information related to the permeability characteristics of HAM for drugs that are used routinely in ocular practise is very limited. So the current interest is to investigate the reservoir function of HAM to different classes of drugs (moxifloxacin, cefazolin, prednisolone, anti-VEGF agents and voriconazole) in ocular use.

Results

Moxifloxacin, a fluoroquinolone antibacterial that is widely used in bacterial keratitis was selected for this study. The release kinetics of topical moxifloxacin (0.5%) after incubating HAM (12 mm punch) with moxifloxacin at different time intervals were evaluated.



Graph showing the release of 0.5% moxifloxacin after incubating HAM with moxifloxacin at different time intervals



Comparative release profile of 0.5% moxifloxacin from thin and thick HAM

In another study, the release kinetics of moxifloxacin were evaluated from two different types of HAMS (a) Thin HAM (N=3; mean wt: 46.3 ± 3.7 mg) and b) Thick HAMs (N=8; Mean wt: 73 ± 5.6 mg). The cumulative release of thick HAM was found to be $861.71 \mu\text{g/ml}$ whereas thin HAM showed $627.11 \mu\text{g/ml}$ of moxifloxacin upto 5 weeks' time period.

The results of the present study reveal that around 10-12% of the drug was loaded into the HAM membrane and showed detectable amount of moxifloxacin until 6 weeks of analysis. However, this findings further warrants the necessity to evaluate its suitability in a dynamic *in vitro* / *in vivo* system

In order to visualize the uptake of moxifloxacin by HAM, the inherent fluorescent property of moxifloxacin (Ex : 296 nm; Em: 495 nm) was utilized. HAM without moxifloxacin served as a control.

HAM impregnated with moxifloxacin showed blue fluorescence under fluorescence microscope.



a) HAM

b) HAM + Moxifloxacin

Picture showing blue fluorescence in moxifloxacin impregnated HAM as compared to HAM with tear buffer under fluorescence microscope. (Ex : 296 nm; Em: 495nm)

Conclusion

The data from this study indicates that prolonged and sustained anti-bacterial effect may be obtained by using drug impregnated HAM in the case of infective keratitis. Currently, other class of drug molecules like anti-VEGFs, anti-fungal, steroids are being screened to explore the suitability of HAM as a drug delivering tool to cornea. This product upon further validation may be commercialised with the concerted effort of AMRF and Aurolab.

BIOINFORMATICS

The department primarily focuses on developing bioinformatics methods of omics analysis and noncoding RNAs for eye diseases. The availability of huge omics data from genome projects and high-throughput technology (next generation sequencing and microarray) has brought a great challenge to understand the complexity of biological process and disease mechanism in eye research. Bioinformatics facility has reliable infrastructure and framework comprised of LINUX, Windows based servers and desktop workstations, which allow us to integrate those data and study them at systems level. It further provides to customize data analysis tailored to the needs of individual research projects across all the research groups. Current research foci are development of machine-learning approaches for variant prioritization, network approaches and multi-omics data integration for microRNA and their potential targets in eye diseases. In addition, the comparative genome analysis of ocular multidrug resistant pathogens is used to understand and identify resistance mechanisms.



Clinical exome analysis pipeline for eye disease next-generation sequencing panel

Investigator : Dr. D. Bharanidharan
Research Scholar : K. Manojkumar
Funding : DST-SERB

Introduction

The exome sequencing studies primarily aim at the discovery of single nucleotide variants (SNVs), and small insertions and small deletions (INDELs) of coding region that is about 85% of mutations among all the genetic variations. Exome sequencing method has been widely used to elucidate the genetic causes of many eye diseases, starting from single gene disorders to more complex genetic eye disorders, including complex traits and cancer. Although the exome sequencing has demonstrated identifying clinical variants, bioinformatics challenges are being faced as the current

bottleneck in exome/genome methods - shifted from sequence generation to data management and analysis. In order to identify potential clinical variants, each step of the analysis workflow needs to be carefully considered, and specific tools need to be used for complete analysis in this clinical setup. Moreover, based on the diversity and the lack of standards for NGS analysis, many different tools and data formats were introduced, posing a problem when combining different methods to conclude the analysis and obtain meaningful clinical variants. To overcome these challenges, a comprehensive analysis pipeline is the possible solution with the selection of adequate tools, applying appropriate parameters, and combining them. For the pipeline development and reliable results, the accuracy of sequence alignment, consensus calling and variant detection is of paramount importance. Thus, this study is aimed to assess the performance of widely used tools for the standard and eye-disease related human exome data.

TABLE 1.1

Type	Software	Version
Aligner	BWA-MEM	0.7.12
Aligner	Bowtie	2.2.6
Aligner	Novoalign	3.04.0
Aligner	Mosaik	2.2
Aligner	SOAP	2.21
Variant Caller	GATK	3.5
	Haplotype Caller	
Variant Caller	SAMtools	1.5
	mpileup	
Variant Caller	Freebayes	0.9.10

Results and Conclusions

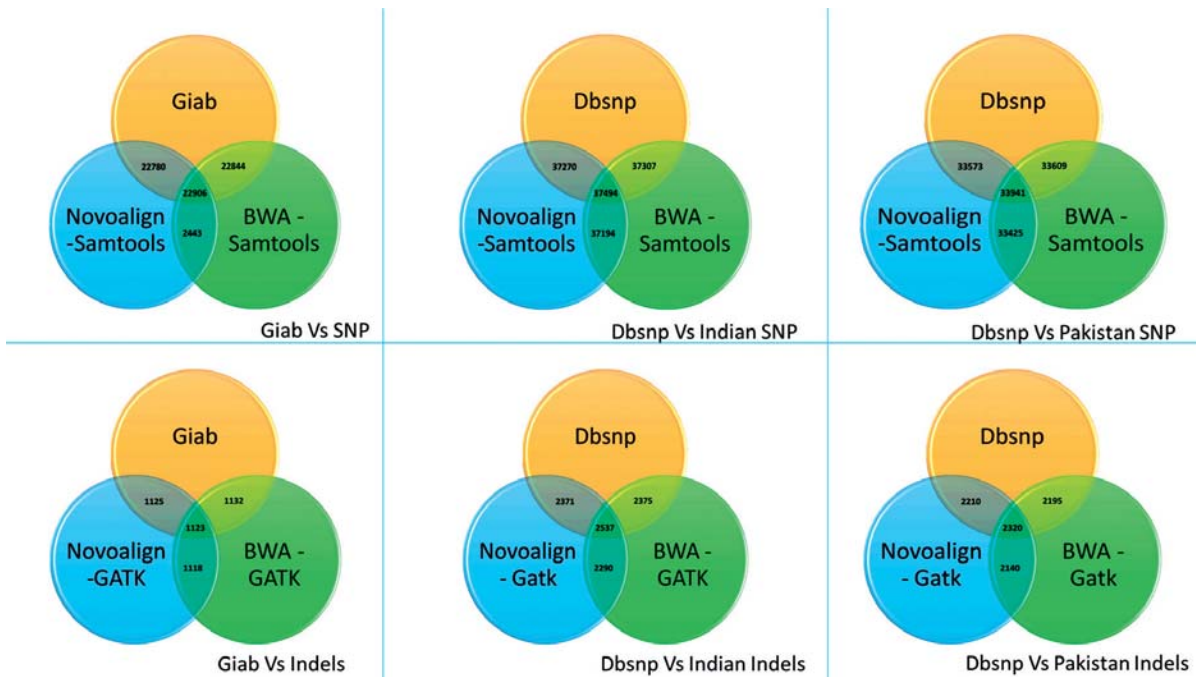
In order to develop streamlined pipeline for SNVs and INDELs separately for eye diseases, benchmarking was done with 5 alignment tools and 3 variant callers (Table 1.1) on three whole exome data. First HapMap/1000 CEU female, NA12878 whole human exome data was used as a reference data since it has highly accurate and well characterized set of genome-wide reference material of NA 12878, including BED and VCF file of high-quality sequence regions and variant calls respectively, developed through the Genome in a Bottle Consortium (GIAB), the National Institute of Standards and Technology (NIST). Moreover, the GIAB call sets were built from the integration of eleven NA12878 whole human genome data sets and three exome data sets, generated across five sequencing platforms to eliminate bias from any single platform. Therefore, the NA12878 can be used for producing and comparing the analytical performance aligners and callers compare to simulated data. Next, one normal Indian human exome and one eye-disease associated clinical exome data was used, where dbSNP build 146 was used for comparison. All the evaluation work was performed for SNVs and InDels separately.

The comparison of SNVs and InDels of NA12878 whole exome data against NIST reference call set showed different performance between aligners and callers as shown the precision and sensitivity of pipelines in Table 2. Moreover, the algorithms perform differently for SNVs and InDels. Of 15 pipelines, SOAP_GATK and Novoalign_Samtools showed high precision while BWA_Samtools and Bowtie_freebayes showed high sensitivity for SNVs. By combining both Precision and sensitivity (F-Score), it was found that BWA_Samtools and Novoalign_Samtools performed well for SNVs. On the other hand, Novoalign_GATK and BWA_GATK performed well for InDels. These results surmise that BWA and Novoalign are best aligners compare to others for both SNVs and InDels, wherein both would be used for better results. In contrast, Samtools and GATK could be used separately for SNVs and Indels for both the alingers and could be merged. Therefore, ensemble of variants from Samtools and GATK for SNVs and InDels respectively would help us to identify most true positives.

Pipeline	SNV						InDel					
	TP	FP	FN	Precision	Sensitivity	F-Score	TP	FP	FN	Precision	Sensitivity	F-Score
Bowtie-Freebayes	22831	2771	855	0.892	0.964	0.926	1036	154	222	0.871	0.824	0.846
Bowtie-GATK	22572	1580	1114	0.935	0.953	0.944	1090	49	168	0.957	0.867	0.909
Bowtie-Samtools	22781	942	905	0.960	0.962	0.961	926	290	332	0.762	0.736	0.749
BWA-Freebayes	22812	1467	874	0.940	0.963	0.951	1026	90	232	0.919	0.816	0.864
BWA-GATK	22661	1622	1025	0.933	0.957	0.945	1123	61	135	0.949	0.893	0.920
BWA-Samtools	22844	762	842	0.968	0.964	0.966	939	246	319	0.792	0.746	0.769
Mosaik-Freebayes	22715	3368	971	0.871	0.959	0.913	1032	355	226	0.744	0.820	0.780
Mosaik-GATK	1655	392	22031	0.809	0.070	0.129	61	20	1197	0.753	0.049	0.091
Mosaik-Samtools	-	-	-	-	-	-	-	-	-	-	-	-
Novoalign-Freebayes	22688	3333	998	0.872	0.958	0.913	1029	84	229	0.925	0.818	0.868
Novoalign-GATK	22651	1848	1035	0.925	0.956	0.940	1125	62	133	0.948	0.894	0.920
Novoalign-Samtools	22780	549	906	0.976	0.962	0.969	950	216	308	0.815	0.755	0.784
SOAP-Freebayes	22242	7862	1444	0.739	0.939	0.827	152	14	1106	0.916	0.121	0.213
SOAP-GATK	21711	239	1975	0.989	0.917	0.951	39	4	1219	0.907	0.031	0.060
SOAP-Samtools	-	-	-	-	-	-	-	-	-	-	-	-

Table 1.2: The pipeline results for SNVs and Indels using NA12878 whole human exome data.

Similarly, the benchmarking was performed on whole exome data of Normal Indian and human exome data from patient with Leber congenital amaurosis (LCA), which was published by Marni et al. in Nat Genet. 2012 44(9): 1040–1045. The data, sequenced by illumina platform, was download from NCBI Sequence Read Archive, accession SRP013517. The comparison of SNVs and InDels was carried out against dbSNP146. Here, the false positives and false negatives could not to be considered as it was compared with polymorphism data. Moreover, there is no separate call set for SNVs and InDels from dbSNP146. Nevertheless, the pipelines were assessed based on F-Score, which would give maximum score based on high concordant variants (TP). In agreement with previous comparison, BWA and Novoalign are best aligners compared to others for both SNVs and InDels, wherein both would be used for better results. In contrast, Samtools and GATK could be used separately for SNVs and Indels for both the aligners and could be merged.



Conclusion

In conclusion, different aligners and variant callers should be used for SNVs and InDels separately for any exome data, even for the patient with eye diseases. In progress, top two pipelines (as presented in the Venn diagram) are merged to produce ensemble of SNVs and InDels separately. The same data and one simulated data are now being analyzed with current human genome reference sequence build, GRCh38, which would reconfirm these results to proceed further for complete automation.

Comparative genome analysis to identify genomic variants and genes associated with drug resistance in Methicillin-Resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa* ocular strains

Investigators : Dr. D. Bharanidharan, Dr. M. Vidyarani, Dr. Lalitha Prajna
Research Scholar : K. Kathirvel, Project Fellow
Funding : AMRF

Background

The evolution of drug resistance in ocular pathogens remains largely unexplored. Majority of bacterial ocular infection cases reported from Aravind Eye hospital is caused by MRSA and *P. aeruginosa*. An array of virulence factors contribute to the pathogenicity of *P. aeruginosa*. Cell-associated structures, including flagella, pili, fimbriae, and endotoxin (lipopolysaccharide), as well as extracellular products, such as proteases and exotoxins, are associated with virulence, invasiveness, and colonization. Also, clinical isolates of *Pseudomonas* often exhibit multiple resistances to antibiotics. On the other hand, in recent times, MRSA clones have been increasingly reported in Indian communities. Studies have shown an increase in the pervasiveness of ocular MRSA infections. Hence, to efficiently control bacterial infections, it is mandatory to understand the intrinsic and extrinsic virulence mechanisms of this bacterium. The increasing divergence between same bacterial strains is determined by their additional chromosomal substances such as plasmids and blocks of DNA inserted at various loci in the chromosome. Since bacterial genomes are less conserved than human genomes, comparative whole-genome analysis can provide a chance to study about the complex biology such as niche specification and pathogenicity. This study proposes to identify drug resistance mechanisms in ocular isolates at the genome level and correlate it with the clinical outcome in patients.

Table 2.1	<i>P.aeruginosa</i>					MRSA				
Strain ID	2808	25832	28923	36155	38529	13220	16825	19084	21352	3091
Number of Contigs	48	153	81	76	127	58	39	67	76	54
N50	344632	202606	222889	350853	191866	146689	152728	145935	122754	159068
Total Size (MB)	6.3	7.1	6.3	6.5	7.1	2.8	3.2	2.8	3	2.8
Number of Coding Sequences	5914	6494	5895	5982	6733	2649	2770	2755	2813	2739
Number of RNAs	58	55	60	58	63	58	61	63	60	62

Here, five MRSA and *P. aeruginosa* ocular isolates were selected for genome sequencing analysis based on their virulence, resistance to antibiotics and clinical history of the patients. The whole genome sequencing was performed by Illumina Nextseq platform with paired-end method at Genotypic, Bangalore. Raw data were adapter trimmed and quality filtered for better assembly. The processed reads were assembled using CLC Genomics workbench version 8. Genes were annotated by RASTtk and PROKKA. Table 2.1 shows the assembly and annotation results of ten ocular isolates. Pan-genome analysis pipeline (PGAP) toolkit was used for comparative gene analysis using annotated genomes. Here, gene distribution among the five *P. aeruginosa* of ocular isolates with reference strain PA01 based on the homology cluster generation method was given. This (Figure 2.1) showed that the core genome of 5375 genes was shared among all strains.

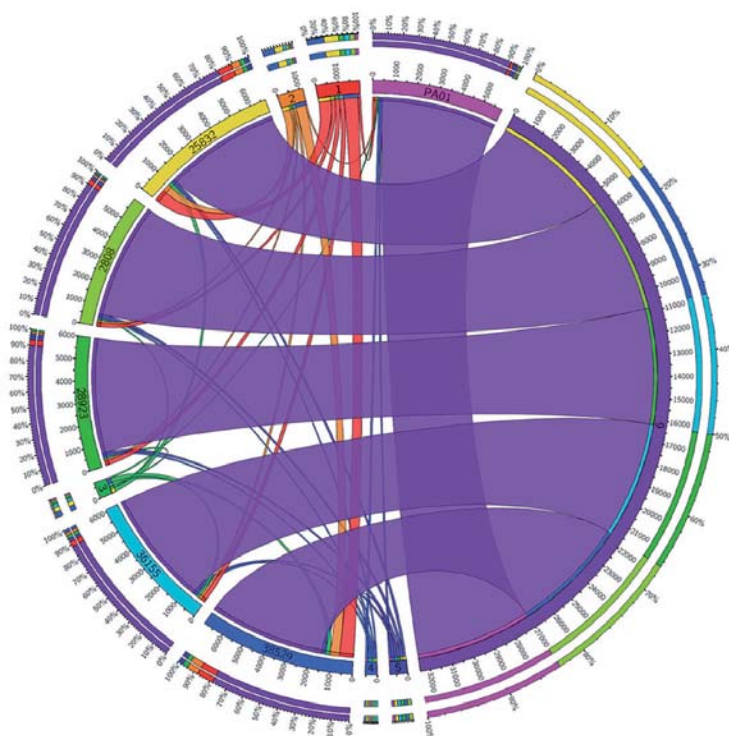


Figure 2.1. Gene distribution by cluster conservation in five ocular isolates with reference strain PA01.

The numbers 1-6 represents a set of genes that shared by each strains and the number represents the number of strains that share the genes. For example, the Number 6 consists of six colors (outer circle) for each strain and all the genes are shared by each strain (the purple color of inner circle. Magenta, Yellow, Yellowish green, Green, Aquamarine and Cyan colors represents the strains PA01, 25832, 2808, 28923, 36155 and 38529 respectively.

A subset of genes was shared by one or more strains. Interestingly, More than 529 genes were only acquired in ocular isolate (In Fig. 2.1, Number 1 with cyan color) ID: 38529. While the 429 genes in ocular isolate (In fig.1, Number 1 with yellow color) ID: 25832 consists of Phage regions, resistance genes including Beta-lactamase, TerD, TerB, ChrB, CzcD and CusA, CRIPER-associated proteins, pili proteins and proteins associated with small-colony variant.

In summary, the ocular strains of *P. aeruginosa* acquired subset of genes, shared among them and expressed uniquely, which requires in-depth analysis to identify genetic variations and genes associated with resistance mechanisms in all strains. Comparative genome analysis of ocular MRSA is being carried out.

OCULAR MICROBIOLOGY

The department conducts basic research to understand the molecular basis of ocular infectious and inflammatory diseases. Studies are focused on the bacterial virulence and drug tolerance mechanisms, host-pathogen interactions, and the genetic dissection of pathogenicity and drug resistance in ocular pathogens. The host pathogen interactions are studied using *in vitro* cell culture models, *ex vivo* analysis of ocular tissue samples and an *in vivo* mouse model of corneal infection. Ocular fluids from uveitis and endophthalmitis patients are used to establish a representative cytokine profile for each condition to provide a closer understanding of the pathophysiology. Overall, the knowledge obtained from these studies will help to understand the reasons behind treatment failure and suggest appropriate ways for disease management.



Analysis of bacterial persistence mechanisms in recalcitrant ocular *Pseudomonas aeruginosa* infections

Principal Investigator : Dr. Vidyarani Mohankumar
Funding Agency : SERB

Background

Keratitis caused by *Pseudomonas aeruginosa* could lead to corneal scarring and severe visual disability in treatment non-responders. In some patients, even the drug susceptible bacteria are not cleared completely by antibiotics, leading to persistent infection. Such bacterial persistence could be due to inefficient clearance of bacteria from the host or due to the recalcitrant nature of the bacterium which makes it tolerant to the antibiotics. This study proposes to identify the bacterial persistence

mechanisms by comparing the drug tolerance and intracellular survival mechanisms of *P. aeruginosa* isolates obtained from keratitis patients who did not respond to standard antibiotic treatment.

Results

Intracellular clearance of *P. aeruginosa* by autophagy

Ocular isolates of *P. aeruginosa* invade and replicate inside human corneal epithelial cells (HCET) irrespective of their type three secretion system (T3SS) genotype. Autophagy, a cellular catabolic process, was recently shown to play a role in the detection and elimination of intracellular pathogens. This work is aimed to study the role of autophagy in limiting intracellular bacterial infection in *P. aeruginosa* keratitis. Earlier it was found that in HCET cells infected with T3SS negative strains, the bacterial load was relatively higher when the cells were pretreated with autophagy inhibitors, whereas no such effect was seen in cells infected with invasive ST strains. In the current work, *P. aeruginosa* isolates with cytotoxic T3SS genotypes (UT & UST) were used to study the influence of T3SS toxins on host cell autophagy.

LC3-GFP (autophagy marker) transfected HCET cells when infected with cytotoxic ocular *P. aeruginosa* isolates showed increased LC3 punctation as observed under Leica TCS SP8 confocal microscope. The transcript levels of autophagy associated protein, Beclin-1 was also upregulated in infected cells. To elucidate the role of autophagy in the clearance of intracellular bacteria, gentamicin survival assay was done in the presence of inhibitors /inducers of autophagy. The cells were cultured with EBSS to mimic starvation induced autophagy, whereas 3-Methyl adenine and chloroquine were used to inhibit autophagosome formation and lysosomal degradation respectively. Cells infected with UT strain, compared to UST strain, had significantly higher bacterial load upon inhibition of autophagy. Overall, the data suggest that the presence of T3SS toxin, exoenzyme S may subvert or prevent the intracellular clearance of *P. aeruginosa* by the autophagic machinery. Further these results are being validated with western blot analysis of LC3-II protein levels in infected HCET cells.

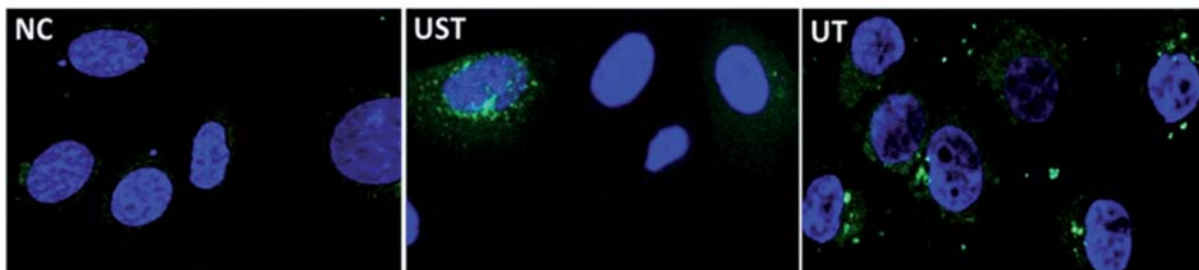


Figure 1. A

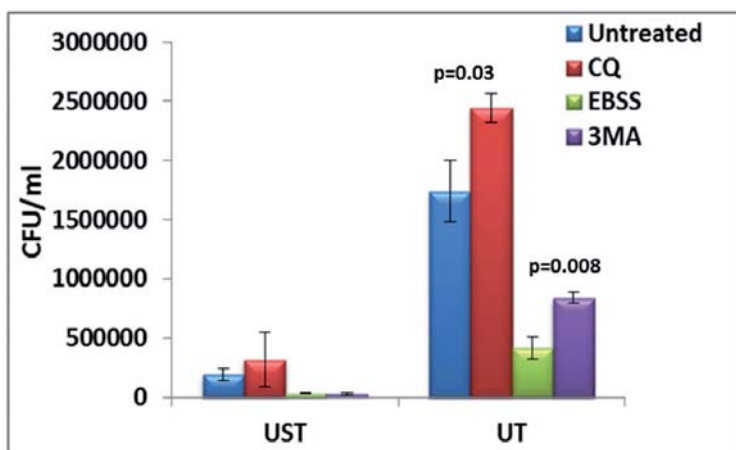


Figure 1. B

Fig 1. A. LC3-GFP puncta in HCET cells infected with cytotoxic *P. aeruginosa* isolates; B. Intracellular bacterial load of cytotoxic isolates upon modulation of autophagy

Determination of bacterial persisters following exposure to antibiotics

In ocular infections that were refractory to antibiotic treatment, the isolates were observed to be sensitive to most of the antibiotics tested *in vitro*. In these cases, treatment failure could possibly be due to the selection of high persister cells, that survive the exposure to a given cidal antibiotic and revive under highly specific conditions. To determine the number of persisters following an exposure to antibiotics, *P. aeruginosa* isolates obtained during the initial and later visits of two keratitis patients (isolates cultured from corneal scrapings and corneal buttons) were selected. The stationary phase cultures were treated with three different concentrations of aminoglycoside drugs, gentamicin, amikacin and tobramycin (25, 50, 100 µg/ml) and fluoroquinolones, moxifloxacin and gatifloxacin (10, 50, 100 µg/ml) for 6h in a 96 well plate. After incubation, an aliquot of the antibiotic treated cultures was plated for colony counting and the cultures were diluted 1:100 in LB medium, and incubated in an automated OD plate reader for 40h. After incubation, the culture supernatants were plated in LB agar and CFU/ml was calculated. From the growth curve, difference in lag phase (Δt) between untreated and antibiotic treated cultures was calculated and used as a parameter for comparison between isolates. The minimum inhibitory concentration (MIC) of these drugs was also determined against all the isolates.

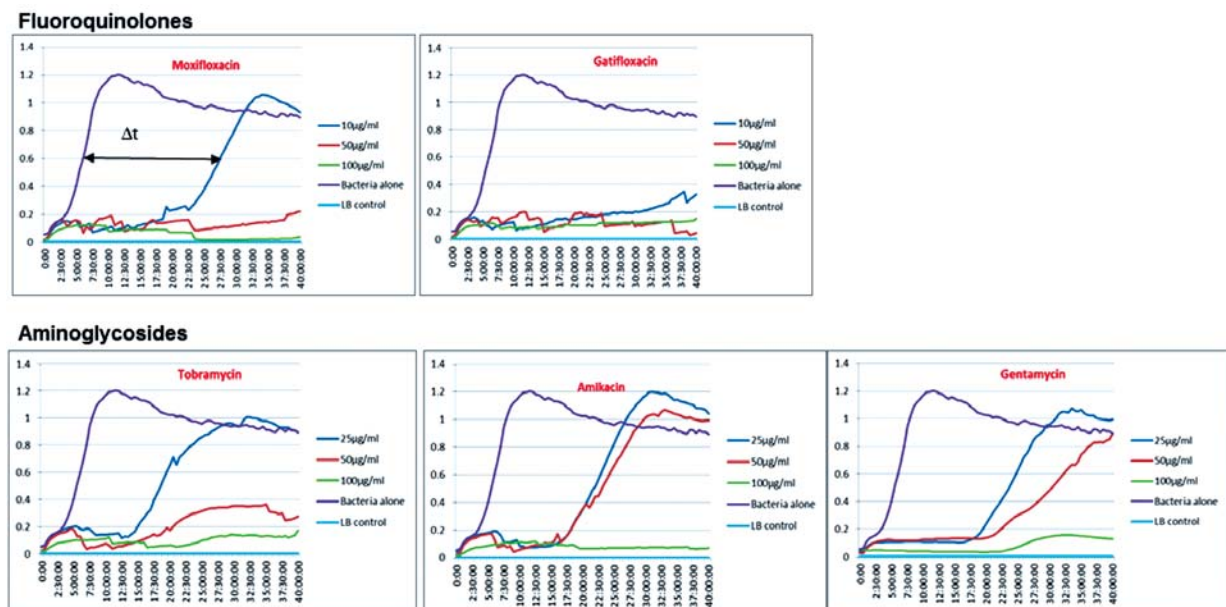


Fig 2. Representative growth curves showing the revival of persisters following an exposure to fluoroquinolone and aminoglycoside antibiotics.

Table1: Δt (in hours)

Isolate No.	Moxifloxacin (µg/ml)			Gatifloxacin (µg/ml)			Gentamycin (µg/ml)			Amikacin (µg/ml)			Tobramycin (µg/ml)		
	10	50	100	10	50	100	25	50	100	25	50	100	25	50	100
P1	10	14	22	12	13.5	15.5	11.5	15.5	23	12	15	22	12.5	20.5	-
P2	10	14	22	12	13.5	15.5	11.5	15.5	23	12	15	22	12.5	20.5	-
P3	21.5	-	-	-	-	-	19	26.5	-	17	18.5	-	13.5	-	-
P4	21.5	-	-	-	-	-	13	19.5	-	12.5	23.5	-	19	-	-

Table1. Difference in lag phase (Δt) between untreated and antibiotic treated cultures of four isolates is shown. Lag phase is the time taken to reach an OD of 0.6 at 600nm. Isolates P1, P2 - Early and late isolates from patient 1; P3, P4 - Early and late isolates from patient 2.

In vivo animal model of bacterial keratitis

To validate the *in vitro* results, a mouse model of keratitis was developed in collaboration with Sastra University, Thanjavur. Corneas of C57BL/6 mice were scarified with three parallel 1-mm-long abrasions using a sterile needle and 1×10^6 *P. aeruginosa* was applied to the scarified cornea in both the eyes. In control animals, sterile PBS was applied to the abraded cornea as a trauma control. Two different strains, one reference (PA01) and one clinical strain were used for infection. In one of the eyes, moxifloxacin (5mg/ml) was added topically every four hours. Mice were examined under a stereomicroscope for corneal opacification, ulceration, and perforation. At specific time points after infection, images of the cornea were captured to grade the disease progression. The first signs of infection were visible after 12h p.i in the infected eyes, whereas no infection developed in the moxifloxacin treated eye. Severe corneal ulceration was seen at the end of 24h, and the pathology was relatively worse in mice infected with the clinical isolate. Animals were sacrificed at the end of 24h and 48h and the eyes were enucleated. Corneas were excised and stored for RNA and protein analysis.

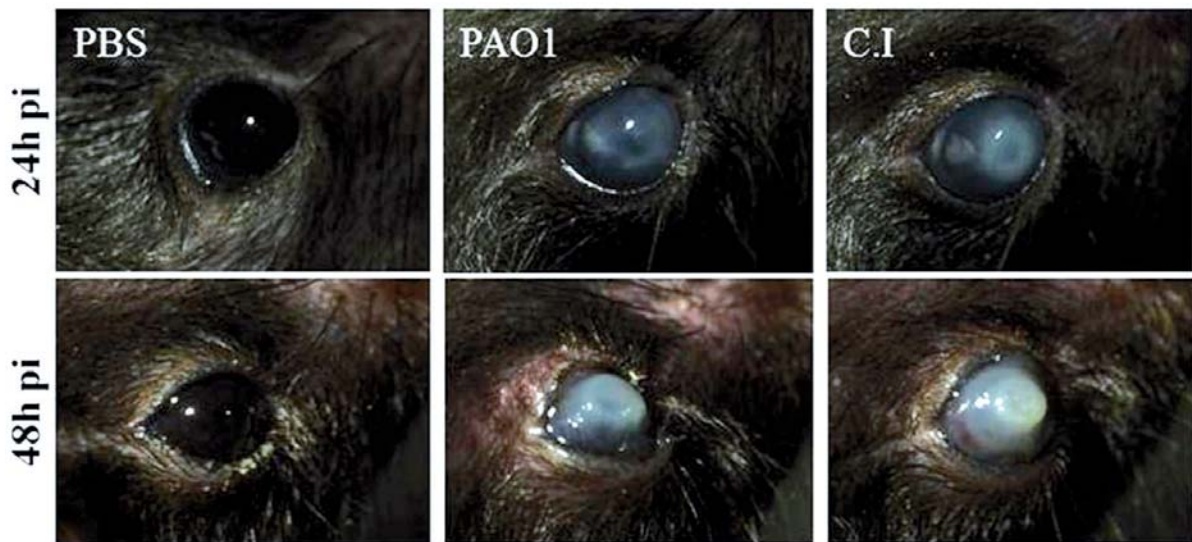


Fig 3. Corneal ulcer induced in C57BL/6 mice upon infection with PA01 and clinical isolate at 24h and 48h p.i.

Conclusion

Ocular *P. aeruginosa* isolates induce autophagy in human corneal epithelial cells irrespective of their T3SS genotype. Induction or inhibition of the autophagic process results in variable intracellular bacterial load, particularly with cytotoxic strains and strains lacking T3SS toxins. These results suggest that modulation of host cell autophagy may impact final disease outcome in *P. aeruginosa* keratitis. Further validation with animal models will provide an insight into the role of autophagy in disease pathology. Persister cells form a fraction of drug sensitive isolates and survive antibiotic concentrations much higher than their MIC levels. Since the presence of such persisters may lead to treatment noncompliance, the need to understand the molecular mechanisms of persistence is acute.

Characterization of the virulence determinants of *Pseudomonas aeruginosa* causing keratitis

Investigators : Dr.Vidyarani Mohankumar, Dr.Lalitha Prajna
Funding Agency : AMRF
Research Scholar : J. Lakshmi Priya

Background

The different secretion systems of *P. aeruginosa* secrete exo proteins or proteases that mediate host cell death, degradation of stromal collagen, cleavage of host cell surface molecules or induction of inflammatory response. The purpose of this study is to elucidate the role of important virulence mechanisms of *P. aeruginosa*.

Results

To check the relative expression levels of exotoxin A and alginate D, twelve ocular *P. aeruginosa* isolates were selected based on their T3SS genotypes. All the isolates were analyzed for their ability to form biofilms, and the transcript levels of *toxA* and *algD* were determined by real time qPCR. The relative expression fold was calculated with reference strains, PA01 and PA14 as controls. Almost all the isolates had the ability to form moderate to strong biofilms, with correspondingly increased transcript levels of *algD* gene in most of the isolates. Exotoxin A gene was also highly expressed in nearly half of the isolates. The expression levels of neither gene correlated with the final disease outcome.

S. no	T3SS genotype	<i>toxA</i>		<i>algD</i>		Biofilm density	Clinical outcome
		PA14 as control	PA01 as control	PA14 as control	PA01 as control		
1	ST	19.56	0.85	38.05	2.08	Strong	TPK
2	ST	107.63	4.69	62.68	3.43	Strong	Enucleation
3	ST	165.42	4.38	116.16	3.46	Moderate	TPK
4	ST	310.83	8.22	867.00	16.90	Strong	TPK
5	S	250.73	10.93	144.01	7.89	Moderate	Healed ulcer
6	UT	9.00	0.39	5.70	0.31	Strong	TPK
7	UST	666.29	17.63	230.72	6.87	Strong	Healed with scar
8	UST	0.44	0.02	0.95	0.05	Strong	Healed with scar
9	N	261.38	6.92	82.71	2.46	Strong	Healed ulcer
10	N	1.18	0.03	4.79	0.14	Strong	No follow up
11	T	2.11	0.06	0.95	0.03	Strong	Healed with scar
12	T	0.49	0.02	17.63	0.97	Strong	TPK

Table1. Relative expression levels of exotoxin A and alginate D genes in *P. aeruginosa* isolates. TPK- Therapeutic penetrating keratoplasty

Small colony variants of *P. aeruginosa*

Small colony variant (SCV) phenotype confers a fitness advantage under chronic infection conditions, and thus plays an important role in the pathogenesis of *P. aeruginosa* infections. The analysis of SCVs has contributed significantly to the understanding of bacterial persistence in cystic fibrosis patients.

HCET cells infected with *P. aeruginosa* were treated with gentamycin for 3hrs and the supernatants were plated for colony counting. Notably, the colony morphology of two cytotoxic strains had transformed completely from wild type to SCV phenotype. The SCVs did not revert back to the original phenotype even after twenty passages. To study if the SCVs had an altered expression of virulence genes, the relative expression levels of *toxA* and *algD* genes was analyzed (real time qPCR) in the wild type (P0) and SCV morphotypes (passage-1(P1) & passage-12 (P12)) of one cytotoxic isolate. Expression of both *toxA* & *algD* was increased in the SCVs than the parent strain, and the highest expression of *toxA* was noted in the later passage, p12.

Figure 1 A

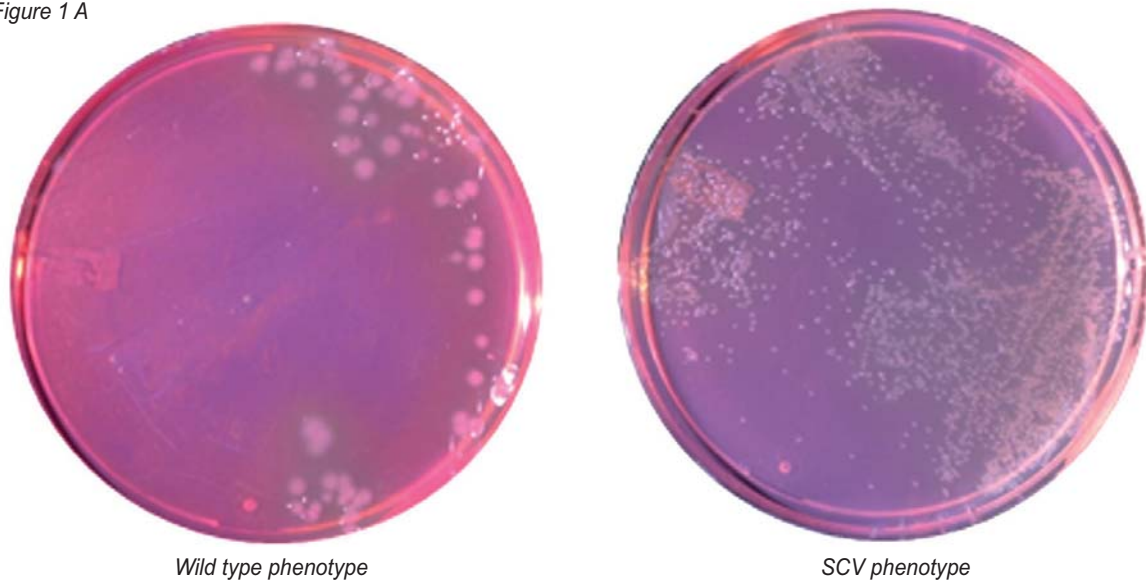


Figure 1 B

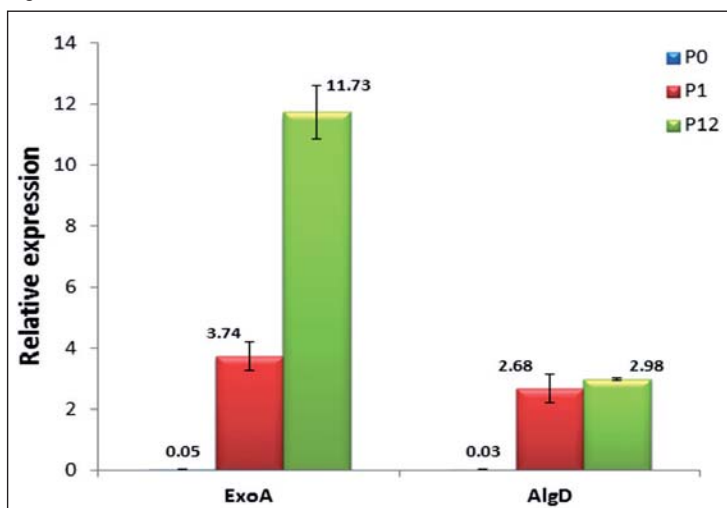


Fig 1. A.Colony morphology of wild type and small colony variant B. Relative expression levels (values denoted as data labels) of exotoxin A and alginate D genes in the wild type and SCV morphotypes, with PA01 as control.

Conclusion

The ocular isolates had an increased expression of alginate and exotoxin A genes that may have a role in corneal colonization and pathogenesis. The formation of small colony variants following exposure to antibiotics, and an increased expression of virulence genes in the SCV may have clinical significance in keratitis.

Regulatory role of human MicroRNAs in Microbial Keratitis

Investigators : Dr. Vidyarani Mohankumar, Dr. D. Bharanidharan, Dr. Lalitha Prajna,
Dr. N.V. Prajna
Research Associate : Dr. B. Hema Devi
Funding Agency : AMRF

Background

Microbial keratitis due to either fungus or bacteria is a major cause of blindness in India. Majority of times in spite of adequate medical management the ulcer does not heal and may require a corneal transplant. One of the major reasons for a poor outcome in these diseases is the excessive inflammation that is mounted by the host to overcome these infections. Dysregulations in miRNA expression could be associated with excessive corneal inflammation and impaired wound healing in fungal keratitis. Of late, therapeutic modulation of miRNA expression has evolved as a promising new approach to treat human inflammatory disorders. The previous studies from this lab on the human miRNA expression profile in fungal keratitis corneas showed significant miRNA expression profile and highlighted the regulatory role of miRNAs in corneal wound inflammation (Hemadevi *et al.*, IOVS, 2015). Hence this work is aimed to study select dysregulated miRNAs in microbial keratitis compared to non-infectious corneal inflammatory disease.

Results

The earlier study from this lab was based on the miRNA expression profile in human cornea from fungal keratitis patients infected with *A. flavus*. By Illumina deep-sequencing, a total of 75 miRNAs were significantly differentially expressed in pooled keratitis corneas compared to normal: 43 miRNAs were up regulated and 32 were down regulated. More than 590 target genes were predicted for 75 miRNAs. Of these, highly upregulated hsa-miR-21-5p, hsa-miR-223-3p, hsa-miR-142-5p, hsa-miR-155-5p, hsa-miR-511-5p were found to highly regulate inflammatory and immune responses, involving Toll like receptor signaling pathway. Hsa-miR-451a with an increased expression (>8 fold) in keratitis may have a role in wound healing by targeting Macrophage Migration Inhibitory Factor (MIF), which is reported as a direct target of hsa-miR-451a. In continuation, to identify their role in other infectious (Fusarium) keratitis as well as their specific expression compared to non-infectious corneal inflammatory disease, hsa-miR-223-3p, hsa-miR-142-3p, hsa-miR-204-5p, hsa-miR-511-5p, hsa-miR-451a and novel mir-cornea-3p were selected. Corneas from bullous keratopathy patients were used to represent noninfectious inflammatory disease. Relative expression of miRNA was analyzed by real time qPCR using cadaver corneas as controls.

A significant variation was found in the magnitude of miRNA expression between the two fungal infections, especially with miR-204-5p and miR-cornea-3p (Table 1). Notably, expression of miR-223-3p was several-fold higher in fungal keratitis corneas compared to bullous keratopathy, which further indicates the infiltrating neutrophils as a major source of this miRNA during corneal infections.

MiRNA	Fusarium	<i>A.flavus</i>	Non-Infectious
miR-223-3P	847	1531	2.6
miR-451-a	1159	240	189
miR-204-5p	17	-10.6	14
miR-511-5p	493	32	14
miR-142-3p	1582	371	37
miR-Cornea-3p	4786	14	55

Table 1. Select miRNAs mean fold change calculated by $2^{-\Delta\Delta Ct}$ method, normalized with RNU6B (U6) snRNA, in human cornea infected with *Fusarium*, *A. flavus* and Non-infected bullous keratopathy cornea.

Conclusion

This work indicates that miRNAs play important regulatory roles in corneal inflammation and further investigation is required for selected miRNAs as candidates for clinical use.

Genotypic characterization and analysis of virulence factors in Methicillin resistant *Staphylococcus aureus* causing ocular infections

Principal Investigator : Dr. Lalitha Prajna
 Research Scholar : V. Nithya
 Funding : AMRF

Background

The community associated methicillin- resistant *Staphylococcus aureus* (CA-MRSA) is predominant in causing various ocular infections. ST772 clone is prevalent in eye infections and known to harbor panton-valentine leukocidine (PVL). Apart from ST772, other clones like ST22, ST30, ST672 are also associated with ocular infections. *S. aureus* expresses a wide range of exotoxins like α - hemolysin (Hla), Panton valentine leukocidine (PVL) and α -type phenol soluble modulins (PSM) that have been found to be important virulence factors in CA-MRSA strains.

Results

Eight different MRSA strains belonging to ST772, ST22, ST2066 and ST672 which were predominant in causing ocular infections were analyzed for the expression of toxin genes upon infection. HCET cells infected with a MOI of 50 exhibited 10% cell death at 1h p.i and upto 20% cell death after 3h of infection. Expression of PVL, PSM α and Hla toxins was analyzed by real time PCR. PVL expression was comparatively higher in ST772, ST1037 whereas PSM expression was significantly higher in strain 3091 (ST22), which is a PVL negative strain. Expression of toxins in methicillin-susceptible strains (ST2066) was comparatively lower than MRSA strains. Expression of Hla was not detectable even when the cells were infected for three hours with MRSA and MSSA strains.

Whole genome sequencing of seven MRSA isolates belonging to ST772, ST22, ST2066 and ST672 clones was done in an Illumina platform. Genome assembly was done with CLC workbench and prediction of virulence genes was done using VirulenceFinder - 1.5 server. 90% of threshold identification was set to predict the virulence genes. An exoenzyme *aeu* coding for aureolysin

and a cluster of enterotoxin genes were observed in all the seven MRSA isolates. Among these enterotoxins, seg, seu, sem, sei, sen, and seo were present only in two strains of ST772 clone. In the sequence type ST2066, only enterotoxin B (seb) was present with other virulence genes, whereas in case of ST22, enterotoxins like seq, sek along with seb were present. Genes coding for Panton-Valentine leukocidins (PVL), LukS-PV and LukF-PV were present in strains of ST772, ST2066, ST672 and one strain of ST22 (19983) but these two genes were absent in strain 3091. In addition to PVL coding pore forming subunits, other leukocidin subunits like LukE and LukD were present in ST772 and ST22. Four different genes coding for hemolysins including, beta hemolysin (hly), gamma hemolysins (hlyA, hlyB, hlyC) were present in all the genomes. Other virulence genes coding for serine proteases like splA and splB were present in two strains of ST2066 and in ST22 but absent in two strains of ST772. The host immune evasion factors of *S. aureus* such as Staphylococcal complement inhibitor coded by scn was found to be present in all the seven MRSA isolates. In addition, one more immune evasion factor staphylokinase coded by sak was present in ST22 and ST2066 but absent in ST772.

Conclusion

An increased expression of PSM toxin may have a role in corneal pathogenesis. Characterization of PVL carrying strains is very important since the most prevalent CA-MRSA strains are found to harbor PVL gene. Understanding the virulence mechanisms of the predominant clonal types ST772 and ST22 in ocular infections would aid in disease management.

Aqueous and vitreous cytokine and chemokine profile in infectious and non-infectious ocular inflammation.

Investigators : Dr. S.R Rathinam, Dr. Lalitha Prajna
Research Scholar : Lalan Kumar Arya
Funding Agency : ICMR

Background

Uveitis is a leading cause of blindness in both developed and developing countries, including India. The inflammation in eye can be due to a variety of causes – infections and autoimmune processes. Components of the aqueous and vitreous humor reflect the state of intraocular tissues, and change rapidly with the development or progression of inflammation. Abnormal concentrations of various cytokines have been reported in aqueous associated with different causes of uveitis, and distinct cytokine profiles may be used to identify specific diseases.

Results

In this study, the aqueous humour cytokine profile of patients was analyzed with trematode induced uveitis (n=14), tuberculous uveitis (n=8), lens induced uveitis (n=5) and endogenous uveitis (n=8) in comparison with non-inflammatory cataract controls with no other ocular pathology (n=17). Similarly, cytokine profile was studied in vitreous humour of patients with endophthalmitis (n=10) in comparison with non-infectious retinal detachment (n=5) patients. Bio-Plex human Cytokine Group I -17 and 27 plex Assay was done for the cytokine and chemokine analysis. Mann-Whitney U tests with bonferroni correction was used to assess the difference between two groups and a p-value less than 0.05 considered as statistically significant.

The Trematode induced uveitis group showed significantly higher levels of interleukin-6 (IL-6; $p<0.001$), interleukin-8 (IL-8; $p<0.001$), interferon-gamma induced protein 10 (IP-10; $p<0.001$), RANTES ($p<0.001$), IL-17 ($p<0.001$) and IFN- γ ($p<0.001$) compared to the controls. The Tuberculous uveitis group showed significantly higher levels of IL-1 β ($p=0.006$), IL-2 ($p=0.001$), IL-4 ($p=0.001$), IL-5 ($p<0.001$), IL-6 ($p=0.004$), IL-8 ($p=0.001$), IP-10 ($p<0.001$), TNF- α ($p<0.001$) and IL-1ra ($p=0.003$). Whereas Lens induced uveitis group showed significantly higher levels of IL-1 β ($p=0.006$), IL-4 ($p=0.006$), IL-8 ($p=0.002$), TNF- α ($p<0.001$), IL-1ra ($p=0.003$) and G-CSF ($p=0.005$). The Endogenous uveitis group showed significantly higher levels of IL-5 ($p<0.001$), IL-6 ($p=0.006$), MCP-1 ($p<0.001$) and TNF- α ($p=0.003$) whereas the endophthalmitis group showed significantly higher levels of IL-5 ($p=0.002$), IL-10 ($p=0.003$), IL-12 ($p=0.007$) and IL-13 ($p=0.003$) compared to controls.

Conclusion

In this study, predominant up regulation of Th1, Th2 and other proinflammatory cytokines and chemokines in aqueous samples of patients with trematode induced uveitis was observed, which was markedly different from other forms of uveitis. IP-10 levels were highly upregulated in both trematode and tuberculous uveitis which suggests a dysregulated immune homeostasis. Comparison of vitreous cytokine profile in endophthalmitis cases and controls did not show much difference. Ocular cytokine mapping may contribute to a closer understanding of the pathophysiological characteristics and also provide guidance for new therapeutic targets.

CORE RESEARCH FACILITIES

Leica TCS SP8 confocal laser scanning microscope is an inverted microscope designed for optical imaging with optimal photon efficiency and high speed; facilitating optical sectioning. All optical components are matched towards increasing optical resolution using point illumination and a spatial pinhole to eliminate out of focus light in specimens by preserving fluorescence photons for image contrast and to improve cell viability in live cell imaging. Backing up this sensitive detection are a high speed scanning system with up to 428 frames per second, large field of view of field number 22 and accelerated Z-stacking by a novel mode for the SuperZ galvanometer called Galvoflow. This microscope is equipped with 4 laser ports namely UV/405, laser blue 488 nm, laser green 552 nm and laser red 638 nm. In addition to PMT, the Leica HyD has been integrated into Leica TCS SP8 system. With its high quantum efficiency, low noise and large dynamic range, the Leica HyD is the most versatile detector in the Leica TCS SP8 confocal platform. It synergizes perfectly with the filter-free spectral detection system and the acousto-optical beam splitter (AOBS) in the gapless light detection with maximum photon efficiency. This makes the Leica TCS SP8 ideally suited for quantitative measurements and all-purpose imaging.



LEICA TCS SP8 Confocal Laser Scanning Microscope

Applications

Stem Cell Biology

- Two parameter analysis (high ABCG2 expression in cells with high nuclear-cytoplasmic ratio) for limbal epithelial stem cell identification
- Identification and characterization of trabecular meshwork stem cells
- Assessment of the structural and functional integrity of corneal endothelium after storage of cadaver corneas in cornisol, an indigenous intermediate corneal storage media

Microbiology

- Host cell (human corneal epithelial cell) autophagy in response to bacterial infection.

Ocular Pharmacology

- Characterization of Human Trabecular Meshwork Cells by expression analysis.
- Evaluating the role of lutein & zeaxanthin in inhibiting the accumulation of A2E in ARPE-19 cell line using autophagy markers.
- Expression analysis of ALR and VEGF in ARPE-19 cells challenged with different glucose concentration under normoxia and hypoxia.

MASS SPECTROMETRY FACILITY

A Core Mass Spectrometry facility is available at Aravind Medical Research Foundation with the state-of-the-art high throughput Mass Spectrometers. This facility was established primarily to

support proteomics research on different eye disease in the Institute. However, this facility also provides mass spectrometry services for proteomics research from other Institutes and Universities.

Service Areas

1. Identification of proteins and peptides in complex samples
2. Detection of low-abundance peptides and proteins
3. Label-free quantification of proteins
4. Characterization of the post-translational modifications of proteins
5. Accurate quantitation of target proteins in complex mixtures
6. High throughput screening of large number of samples

FACILITY

The Facility is equipped with two high performance mass spectrometers, both connected to Ultra high pressure nanoLC system.

1. Easy-nLC 1000

- Fully integrated nano-LC system that works upto 1000 bar (15000 psi.)
- Narrow column ID to increase analyte and improve detection sensitivity
- Seamless integration with state-of-the-art mass spectrometers

2. Orbitrap Velos Pro™ Hybrid Ion Trap-Orbitrap Mass Spectrometer

Orbitrap Velos Pro™ combines a Thermo Scientific™ Orbitrap™ mass analyzer and Velos Pro ion trap technology to deliver high resolution, speed, sensitivity, and flexibility.

- Has a resolving power up to 100,000 FWHM and mass accuracy better than 1ppm
- Fast scanning and parallel MS and MSn analysis
- Complementary fragmentation techniques—CID, HCD, and ETD—with the high mass resolution and mass accuracy
- Multiple activation types and Data Dependent Decision Tree capabilities for high-confidence peptide identifications
- Parallel acquisition capabilities to enable high-throughput sequencing
- High-resolution accurate-mass (HR/AM) capabilities for identification of unexpected PTMs

3. TSQ Quantum Ultra™ triple quadrupole mass spectrometer

This MS allows Multiple Reaction Monitoring, the principal and powerful method for quantitative measurement of target proteins.



- Ion Source with HESI-II probe
- HyperQuad™ Mass Filter to reduce noise and increase sensitivity
- Quantitation-enhanced data-dependent MS/MS (QED-MS/MS) for simultaneous compound confirmation and quantification
- High-resolution selected reaction monitoring (H-SRM) for quantification of many compounds in a single run
- Extended Mass Range for Large-Molecule Analysis



4. Data Analysis

A dedicated computational facility for the analysis of Orbitrap MS generated high throughput data is also available along with additional support from AMRF Biocomputing Center.

Service Description

Proteomics Service offered	What is performed in the facility
In-solution digestion	<ul style="list-style-type: none"> • In-solution protein digest • Zip-tip purification • Nano-LC-MS/MS analysis • Database search and report of results
In-gel digestion	<ul style="list-style-type: none"> • In-gel protein digest • Extraction of peptides • Zip-tip purification • Nano-LC-MS/MS analysis • Database search and report of results
Peptide sequencing and Protein identification	<ul style="list-style-type: none"> • Zip-tip purification • Nano-LC-MS/MS analysis • Database search and report of results
Custom database searches with MASCOT and SEQUEST	<ul style="list-style-type: none"> • Create a custom database with your specific protein sequences to be used for protein ID or modification searches
De novo peptide sequencing using PEAKS studio	<ul style="list-style-type: none"> • De novo peptide identification for proteins from species whose genomes are not available
Label-free quantitation	<ul style="list-style-type: none"> • LFQ based on spectral counting and intensity
Search for additional, custom or non-standard PTMs modifications	<ul style="list-style-type: none"> • Searches performed using user specified

This facility includes

- One server and two workstations to handle computationally intensive workloads.
- Two dedicated network storage devices are available for storing raw as well as analyzed MS data.

5. Software

- Proteome Discoverer 1.4
- PEAKS studio 7
- PINPOINT 1.4
- MASCOT 2.4

Data Processing, Results and Storage

At the end of the analysis, the user will be provided with the instrument generated RAW data (.raw format), analyzed data (.msf and .xlsx format) along with the sample analysis report (.pdf). Depending on the size, data will be sent to the user by email or copied onto a CD or DVD a minimal fee. All the data will be archived into the facilities Network attached storage and stored for three months.

ILLUMINA MISEQ

The next generation sequencing facility was established in AMRF during this year. For library preparation which involves fragmentation, Covaris was also setup for mechanical shearing of the starting material. Along with the system, Agilent bioanalyser, Qubit and ABI real time system are in place for the checking the quality and quantity of the libraries prepared for the run.

Typical NGS workflow involves library preparation, cluster generation, sequencing and data analysis. Illumina Miseq is the bench top sequencer which includes cluster generation, paired-end fluidics and computing facility for primary analysis in a single machine. It is automated completely with the single use reagent cartridge and easy positioning of flow cell.

Miseq enables up to 15 GB of output with 25 M sequencing reads and 2x300 bp read lengths. Miseq system enables a broad range of applications, from targeted gene sequencing to metagenomics, small genome sequencing, targeted gene expression analysis, ChIP Sequencing and more.

For the initial test run, the control DNA Phix was used and the calibration of the machine was done with this known control DNA. The results were good and it is clear that the instrument Illumina-Miseq is functioning perfectly. Further runs with the retinoblastoma patients with the custom panel also yielded good results with RB1 and other related genes.



Next Generation Sequencing: Illumina Miseq



7900 Real Time PCR



3130 - Genetic Analyser

7900 Real Time PCR

Experiments done in the projects using the facility

Real Time PCR is essential for multiple experiments. Department of Genetics utilized 100% of this instrument and analyzed huge number samples of patients and controls. In addition, other departments (Microbiology, Ocular Pharmacology Stem Cell Biology and Proteomics) also using this instrument routinely.

3130 -Genetic Analyser

Experiments done in the projects using the facility

Genetic analyzer is essential for multiple experiments. Department of Genetics utilized 100% of this instrument and analyzed huge number of samples of patients and controls. In addition, other departments (Microbiology, Ocular Pharmacology Stem Cell Biology and Proteomics) also using this instrument routinely

Human Organ Culture Anterior Segment (HOCAS) Facility for Trabecular Meshwork Outflow Studies

HOCAS facility has been established at AMRF in 2013 in collaboration with Prof. Paul L.Kaufman and B'Ann Gabelt, Department of Ophthalmology & visual sciences, University Wisconsin, Madison, USA. This facility was funded by Aravind Eye Foundation (AEF), New York, USA.

The human organ cultured anterior segment system (HOCAS) has been developed as an intermediate step between cell culture and whole animal or human studies for determining trabecular meshwork outflow facility responses to investigational drugs. This system allows direct experimentation of candidate drugs on human/bovine/ pig and monkey eyes and thus provides direct and meaningful information about the candidate drugs for glaucoma. They are also useful for investigating the potential of gene therapy to target the trabecular outflow pathway.



HOCAS: Instrument Set-up

The human anterior segment mounted onto a specially designed Petri dish is maintained at 37°C with 5% CO₂ incubator (Thermo Fisher, USA) and continuously perfused with nutrient medium using PH D infusion only pump (Harvard Apparatus, USA). The other end of the eye is connected to APT 300 pressure transducers (Harvard Apparatus, USA) and the pressure is monitored on a computer connected to the Powerlab system (8/35) (Quad Bridge Amplifier) with Lab Chart Pro software (Harvard Apparatus, USA).

HOCAS: Services

Training: Hands-on training on Dissection, HOCAS set up, Drug treatment protocol and data analysis is being provided to International students as a part of research collaboration.

HOCAS: Research Activities

Currently in use

- To investigate the effect of cyclic stress on human aqueous outflow facility and the effect of Rho kinase inhibitors in increasing outflow facility.
- To establish cell loss model using saponin to investigate the role of TM stem cells in maintain IOP homeostasis

AMRF BIOCOMPUTING CENTER (ABC)

The AMRF Biocomputing Center (ABC) provides a core computational facility with a reliable infrastructure and framework comprised of LINUX and Windows based servers and desktop workstations to support interdisciplinary and computational research by developing and maintain computing facilities including data storage, database development and maintenance, algorithm development, analysis software tools, hardware support. It is a multidisciplinary research environment that provides to customize data analysis tailored to the needs of individual research projects across all the research groups and extend this service to others on mutually acceptable terms. In addition, it helps to train manpower by way of workshops and short training courses.



1. Resources

- Equipped with Dell T630 Server (With Ubuntu 14.04) and HP DL580R07 (E7) CTO Server,
- Two Dell workstations and five Intel i7-3370 3.5GHz workstations
- Two Iomega 12TB storage devices and LINUX and Windows based servers with disk storage and backup systems are available.

2. Projects

- Developing an exome analysis software pipeline to detect and filter clinical variants for genetic eye disorders using Next-generation sequencing clinical data
- Comparative bacterial genome analysis to find genome wide differences between ocular isolates and to find mutations and genes associated with drug resistance mechanism using whole genome short-gun sequencing projects

- Transcriptome analysis of predicting target genes associated with the maintenance of stemness using next-generation RNA sequencing (RNA-seq) data.
- Developed In-house Bioinformatics Pipeline to Identify Pathogenic Variants of Retinoblastoma (RB) and is being extended to other ocular tumors.
- Target Gene Prediction and Functional Analysis of Differentially Expressed MicroRNAs in microbial infections
- RNA-seq analysis of saprophyte and corneal isolates of *A.flavus* at two different growth temperatures

3. Data in public repository for open access

- *Pseudomonas aeruginosa* BK1, whole genome shotgun sequencing project, (2014) Lakshmi Priya, J. Logambiga P. Sivakumar, N. Lalitha, P. Vidyarani, M and Bharanidharan, D. GenBank Accession Number: JBTQ00000000.
- *Staphylococcus aureus* subsp. *aureus* AMRF2, whole genome shotgun sequencing project, (2014) Nithya, V. Logambiga P. Sivakumar, N. Lalitha, P. Vidyarani, M and Bharanidharan, D. GenBank Accession Number: JASM00000000
- *Staphylococcus aureus* subsp. *aureus* AMRF2, whole genome shotgun sequencing project, (2014) Nithya, V. Logambiga P. Sivakumar, N. Lalitha, P. Vidyarani, M and Bharanidharan, D. GenBank Accession Number: AZTC00000000.
- Human Corneal microRNA Expression Profile in Fungal Keratitis, Small Non-coding RNA deep-sequencing. (2014) Lalitha, P and Bharanidharan, D, GEO Accession Number: GSE64843.
- Exoproteome of *Aspergillus flavus* corneal isolates and saprophytes, (2015) Selvam RM, Nithya R, Devi PN, Shree RS, Nila MV, Demonte NL, Thangavel C, Maheshwari JJ, Lalitha P, Prajna NV, Dharmalingam K, ProteomeXchange ID: PXD001296

4. Services

- Next-generation sequence data processing and analysis: The resource has developed processing and analysis pipelines for illumine and ion data such as
- Exome/targeted data analysis
- De novo assembly and genome comparison
- RNA-seq data analysis
- Small-RNA seq data analysis

The input to the NGS pipeline is either raw reads from the sequencing machines or mapped reads from alignment software.

- Microarray data processing and analysis: This includes background correction, normalization, summarization, quality control, detecting differentially expressed genes, and correlation of gene expression with phenotypes or clinical variables.
- Bioinformatics: The resource is available to help investigators with bioinformatics analysis such as pathway and gene function enrichment analysis and gene network analysis.
- Database: Services include design and implementation of interactive web applications as well as the underlying database back-ends. Support is available for investigators with problems concerning data acquisition, management, and analysis.
- Training: NGS and proteomics data analysis training will be provided

CONFERENCES ATTENDED

American Association for Cancer Research (AACR) Meeting 2015

Pennsylvania, USA, April 18-22, 2015.

DR. A. VANNIARAJAN, SCIENTIST

- He presented a poster on his work entitled "Advanced Paternal age as a possible risk factor for retinoblastoma".

ARVO Meeting 2015

Denver, Colorado, USA, May 03-07, 2015

Posters

DR. P. SUNDARESAN

- Establishment of Retinal Mitoscriptome Gene Expression Signature for Diabetic Retinopathy Using Human Cadaver Eye

DR. M. VIDYARANI

- *Pseudomonas aeruginosa* induces autophagy in human corneal epithelial cells

DR. D. BHARANIDHARAN

- Human Corneal microRNA Expression Profile in Fungal Keratitis

DR. A. VANNIARAJAN

- A New Sequential Screening Strategy for Rapid Diagnosis of Retinoblastoma

MS. K. JHANSIRANI

- *miR-203* inhibits Δ Np63 α dependent clonogenicity in corneal epithelial stem cells (CESCs)

MS. ROOPAM DUVESH

- Cytokines and chemokines profile in aqueous humor of PACG EYES

The presentations were very much appreciated and positive feedback was provided by the peer group.

22nd Annual meeting of Indian Eye Research Group (IERG) - ARVO - India Chapter

LV Prasad Eye Institute, Hyderabad - July 25-26, 2015

Research Scholars from Aravind Medical Research Foundation participated and presented their work in the 22nd IERG-ARVO-IC Meeting at LV Prasad Eye Institute, Hyderabad. Dr. B. Hemadevi and K. Jhansi Rani received the travel grants for attending the meeting

Oral presentations

K. THIRUMALAI RAJ

- Identification of novel indels and splice variants in retinoblastoma patients

K. JHANSI RANI

- Role of *miR-203* in suppressing stemness in human corneal epithelial stem cells (CESCs) through Δ Np63 α inhibition

Poster presentation

B. HEMADEV

- Differentially expressed micro RNAs in human cornea with fungal keratitis

V. NITHYA

- Comparative genome analysis of ocular methicillin resistant *Staphylococcus aureus* isolates

ROOPESH R. PAI

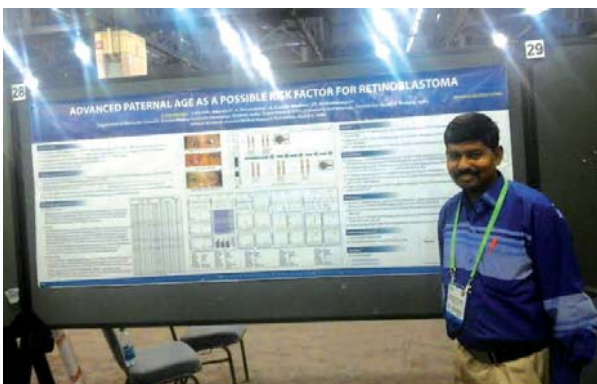
- Quantitative proteomics of serum biomarkers for proliferative diabetic retinopathy

SANDHYA KRISHNAN

- Glycoproteome analysis of tear from *aspergillus flavus* keratitis patients

Ms. Dhivya Participated in the meeting as an observer.

Dr. A. Vanniarajan at the AACR Meeting 2015



Dr. D. Bharanidharan, Dr. P. Sundaresan, M.K.Jhansi Rani, D. Roopam, Dr. M. Vidyarani and Dr.A. Vanniarajan at ARVO Meet 2015





Research Scholars attended 22nd IERG-ARVO-IC Meeting at LV Prasad Eye Institute, Hyderabad.



Dr. J. Jeya Maheshwari at HUPO meeting, Vancouver, Canada

Workshop on Formulation of National Action plan for Low vision and rehabilitation services

All India Institute of Medical Sciences, New Delhi, September 26-27th, 2015

Dr. P.Sundaresan attended Genetics group meeting and workshop on Formulation of National Action plan for Low vision and rehabilitation services on 26-27th September 2015 at Dr. R.P. Centre for Ophthalmic Sciences, All India Institute of Medical Sciences, New Delhi.

14th FAOBMB Congress & 84th Annual meeting of SBC (I) Current Excitements in Biochemistry and Molecular Biology for Agriculture and Medicine

CCMB, Hyderabad 27-30 November 2015

DR.P.SUNDARESAN

- Delivered an Invited talk on "Ocular diseases gene discovery"

14th Human Proteome Organization World Congress (HUPO 2015),

Vancouver, British Columbia, Canada (September 27– 30, 2015)

The Human Proteome Organization (HUPO) is an international scientific organization representing and promoting proteomics through international cooperation

and collaborations. The HUPO congress is an yearly event and this year, the 14th Human Proteome Organization World Congress (HUPO 2015), was held during September 27 – 30, 2015 at Vancouver, British Columbia, Canada. The theme of this meeting was 'Translating Proteomics and Allied -Omics to the Clinic'. Dr. J. Jeya Maheshwari from the Proteomics Department at Aravind Medical Research Foundation attended this HUPO 2015 congress, which served as a wonderful forum for refreshing the knowledge base in the field of proteomics as well as to explore the innovations in proteomics and genomics.

She also presented the research work carried out in AMRF as a poster entitled "Proteomics of Ocular Diseases". This meeting brought together the pioneers in the area of proteomics who discussed their latest innovations and research to a gathering of more than 1000 delegates from across the world. Dr. Jeya Maheshwari received the International Travel Support from Science and Engineering Research Board, Department of Science and Technology, Government of India to attend this conference. She also visited the research facility at the University of British Columbia's Eye Care Centre and discussed the research work carried out here at AMRF with the UBC faculties.

Dr. P. Sundaresan in Genetics group meeting at AIIMS, New Delhi



Dr. P. Sundaresan attended 14th FAOBMB Congress meeting at CCMB, Hyderabad.





Dr. P. Sundaresan with Dr. Sai Nair and Dr. Sugantha Lakshmi at University of California, San Francisco



Sushil Kumar Dubey defending his Ph.D thesis

Professional Visits abroad

Visit to University of California, San Francisco, May 02-11, 2015

Dr.P.Sundaresan visited University of California, San Francisco after ARVO 2015 meeting. He met one of his collaborators Dr. Sai Nair at the Department of Ophthalmology, UCSF, School of Medicine, San Francisco CA and discussed to develop animal models for some of the eye diseases.

Ph.D Awarded

Mr.Sushil Kumar Dubey, Department of Molecular Genetics, defended his Ph.D thesis entitled "Identification and Characterization of Mutations in Candidate Genes involved in major Congenital Globe Anomalies" on 31st March 2015. He has carried out his studies under the guidance of Dr.P.Sundaresan.

Mr.Lalan Kumar Arya, Department of Microbiology defended his Ph.D thesis entitled "Etiology and Immunopathogenesis of Subconjunctival and Anterior Chamber Granulomatous Uveitis in Children of South

India" on 3rd February 2016. He has carried out his studies under the guidance of Dr.SR.Rathinam

Mr.Anshuman Verma, Department of Molecular Genetics, defended his Ph.D thesis entitled "Molecular Studies of Leber Congenital Amaurosis (LCA) in Indian Population" on 5th February 2016. He has carried out his studies under the guidance of Dr.P.Sundaresan.



Anshuman Verma defending his Ph.D thesis

Lalan Kumar Arya defending his Ph.D thesis



Prasanthi Namburi, defending her Ph.D thesis



Ms.Prasanthi Namburi, Department of Molecular Genetics, defended her Ph.D thesis entitled “Identification of Genetic Variants in Genes Associated with Primary Open Angle Glaucoma in Indian Population” on 23rd March 2016. She has carried out her studies under the guidance of Dr.P.Sundaresan

Training of Project Students

- Ms. A. Priya, M.Sc, Biotechnology, Bharathidasan University, Tiruchirappalli.
- M. Iswarya, M.Sc. Biotechnology, Bharathiyar University, Coimbatore
- N.Nafia, M.Sc Biotechnology, Bharathiyar University, Coimbatore
- S. Mohamed Imran, M.Tech. Biotechnology, Bharathidasan University, Trichy
- S.Vaishnavi, M.Sc. Biomedical Sciences, VIT University, Vellore
- Ms.R.Sangeetha, MSc Microbiology, Bharathidasan University, Tiruchirappalli
- Ms.M.Kanmani, M.Sc Biotechnology, Alagappa University, Karaikudi
- P.Gowri, B.Tech, Biotechnology, Bharathidasan University, Tiruchirappalli
- M. Priyatharshini, B.Tech, Biotechnology, Bharathidasan University, Tiruchirappalli

CONFERENCES / WORKSHOPS CONDUCTED

World Immunology Day

29th April 2015

World Immunology Day is celebrated on 29th April, since 2007, with the purpose to establish a community of immunologists dedicated to improve the health of people around the world. Under the initiative of Indian Immunology Society, New Delhi, World Immunology day was celebrated in 20 institutes in India this year including AMRF. A seminar on "Immunology Today" was organized and a total of 76 participants attended the meeting including 45 postgraduate students/research scholars from five nearby colleges (Lady Doak College, Madurai (7); The American College, Madurai (8); Thiagarajar College of Arts and Science, Madurai (6); Kamaraj College of Engineering, Virudhunagar (11); MEPCO Schlenk Engineering College, Sivakasi (13) and 31 participants from AMRF

Five speakers were invited for the occasion:

1. Dr. S. Vijaya, IISc, Bangalore - Human immune responses to Japanese encephalitis virus highlight the importance of interferon gamma in protection
2. Dr. P. Rajaguru, Anna University, Trichy - Multiple targeting for effective cancer treatment
3. Prof. VR. Muthukkaruppan, Advisor – Research, AMRF - Nobel Laureates in Immunology
4. Prof. K. Dharmalingam, Director – Research, AMRF - Adaptive Immunity in Bacteria
5. Dr. S. R. Rathinam, Professor and Head, Uvea Clinic, Aravind Eye Care System - Doctors corner: How do we treat the patients with auto immune disease?

The students and faculty from the colleges had a good interaction with the invited speakers and staff at AMRF. All the participants were taken on a tour to the departments to highlight the kind of basic research carried out at Aravind Medical Research Foundation and the facilities available.

5th Annual conference of the Society for Mitochondrial Research and Medicine (SMRM)

November 6-7, 2015

5th Annual conference of the Society for Mitochondrial Research and Medicine – India (SMRM) on Mitochondria in Cancer and Neurodegenerative Disorders was organized by AMRF, Madurai during November 6-7, 2015.

The conference was inaugurated on 6th Nov 2015. The dignitaries of SMRM and AMRF lighted the lamp. The Organizing Secretary Dr. Sundaresan welcomed all the delegates. Prof. VR. Muthukkaruppan, Advisor-Research, AMRF detailed the activities of Aravind Medical Research Foundation. Prof. K. Satyamoorthy, President, SMRM provided the overview of the SMRM. Prof. K. Dharmalingam, Director-Research, AMRF highlighted the recent developments in the mitochondrial biology in his presidential address. The need for the translational research on mitochondrial medicine was emphasized in his inaugural address by Prof. P. Namperumalsamy, President, AMRF. The conference proceedings and Annual report of AMRF were released by Mr. G. Srinivasan, Director-Finance, AEH, Madurai. Vote of thanks was proposed by Dr. Vanniarajan, AMRF.

Participants of the seminar on Immunology Today





Participants of SMRM 2015

The conference had 8 sessions and 2 plenary talks. This meeting served as a common podium for basic scientists, clinicians and young researchers to discuss recent advances in mitochondrial biology, diagnosis and treatment of mitochondrial diseases. About 170 participants including 9 national and 9 international invited faculties attended the meeting.

The major points of discussion in this meeting include (1) Utilizing the deep sequencing methods for the diagnosis of mitochondrial diseases (2) Employing reprogramming strategies to provide patient specific cell types for developing therapies (3) Providing an update of the ongoing clinical trials for mitochondrial diseases. Involvement of the mitochondrial dysfunction in ocular diseases such as Leber Hereditary Optic Neuropathy, glaucoma and diabetic retinopathy.

The theme of this meeting being the cancer and neurodegenerative diseases, role of genetic and epigenetic factors in breast, cervical cancers and multiple neurological diseases were discussed in detail. Importance of clinical diagnosis and histopathology for mitochondrial diseases has been reemphasized. The meeting also covered the possible role of mitochondria in Asthma, infertility and cardiac problems. Role of mitochondria in regulating nucleotide metabolism and calcium signaling were also demonstrated in the talks. Necessity of suitable bioinformatic tools in the analysis of the mitochondrial diseases was explained.

Participants of the workshop on Experimental approaches to Proteomics



Overall the meeting was a perfect scientific feast for the clinicians, basic researchers, industrial participants and young students.

[Workshop on “Experimental approaches to Proteomics”](#)

2-5 March 2016

The workshop on “Experimental Approaches to Proteomics” was conducted during 2-5 March 2016 at Aravind Medical Research Foundation. The main objective of this workshop was to provide the participants a comprehensive hands-on training on the bottom-up proteomics approaches. There were twenty-two participants, both research scholars and faculties who were from different Universities and Institutes. This four-day workshop was designed to cover four modules on the basics as well as experiments done routinely in the mass spectrometry based proteomics studies. As the workshop participants were a heterogeneous group in terms of their familiarity to Proteomics, the workshop started with an Introductory lecture on Proteomics by Prof. K. Dharmalingam. Subsequently, the participants were given hands-on training in the experiments such as protein quantitation, different methods of sample preparation and clean-up of samples for mass spectrometry analysis. Every participant was given the opportunity to do the experiments individually. Two days of the workshop was dedicated for providing training on analysis of MS data, both identification as well as quantitation of proteins using Proteome Discoverer and Pinpoint softwares. The workshop also included invited lectures by Prof. Balamurugan from Alagappa University, Karaikudi, Dr. Krishna Tej from Narayana Nethralaya, Bengaluru and Dr. Mahesh Kulkarni from National Chemical Laboratory, Pune. The workshop concluded with a discussion with the participants on their feedback as well as their expectations on future events.

[Workshop on Mycology and Ocular Microbiology](#)

30th November to 4th December, 2015

The Ocular Microbiology Department of Aravind Eye Hospital, Madurai and Aravind Medical Research Foundation conducted a five day workshop on Mycology



Dr. Lalitha Prajna addressing the participants of Mycology workshop

and Ocular Microbiology. The programme included informative lectures by invited speakers and the faculty of AMRF working in the field of mycology, ocular microbiology and molecular biology. The workshop had been designed to give a basic working knowledge about handling and processing of ocular specimens and interpretation of cultures. It also included plenty of hands-on training experience in basic mycology and molecular biological techniques. A total of 19 participants attended the workshop, including MD Microbiology students and people working in the field on clinical microbiology from TamilNadu and New Delhi.

Invited speakers:

DR. M. R. SHIVA PRAKASH, PGIMER, CHANDIGARH

- Introduction to Medical Mycology
- Conidiogenesis
- Approaches for laboratory diagnosis of fungal infections
- Superficial and subcutaneous fungal infections

DR. ANUPMA JYOTI KINDO, SRI RAMACHANDRA UNIVERSITY, CHENNAI

- Collection and processing of specimens
- Antifungal Susceptibility test for molds & yeasts
- Opportunistic fungal infections

DR. JAYANTHI SAVIO, ST. JOHN'S MEDICAL COLLEGE, BANGALORE

- Yeast identification including *Malassezia*

Prof.VR.Muthukkaruppan Endowment Award

Students and colleagues of Prof. VR. Muthukkaruppan created an Endowment in his name in 2014 out of which an award will be given to the best researcher at Aravind Medical Research Foundation every year.

The award is given based on the scientific merit of abstracts and poster presentation by the research scholars of Aravind Medical Research Foundation. The award carries a certificate and cash prize of Rs.25,000/-.

This year Prof.VR.Muthukkaruppan Endowment award was given to Ms. Kasinathan Jhansi Rani, Senior Research Fellow, Department of Stem Cell Biology, for her outstanding research work on "miR-203 inhibits $\Delta Np63\alpha$ dependent clonogenicity in corneal epithelial stem cells (CESCs)". The selection committee included Prof.Anuranjan Anand, JNCASR, Bangalore, Dr.D.Bharanidharan, AMRF, Dr.D.Karunakaran, IIT, Madras, Dr.Kumaravel Somasundaram, IISc, Bangalore and Dr.C.Mohan Rao, CCMB, Hyderabad.



Jhansi Rani receiving the Prof.VR. Muthukkaruppan Endowment Award

GUEST LECTURES DELIVERED BY VISITING SCIENTISTS



DR. T. RAMASAMY, Former Secretary, Department of Science and Technology & Chairman, Oversight Committee, Science and Engineering Research Board visited AMRF on 25th August 2015 and gave a lecture on "Approaches for affordable biomedical devices and critical care"



DR. MICHAEL E. ZEGANS, Section Chief, Ophthalmology Cornea and Uveitis Geisel School of Medicine at Dartmouth, Professor of Surgery (Ophthalmology), Dartmouth Hitchcock Medical Center Lebanon NH 03756 visited AMRF on 17th March 2016 and gave a lecture on "Biofilm, PSL and LasR among *P.aeruginosa* isolates in the SCUT study"



PROF. P. VENKATACHALAM, Department of Human Genetics, Sri Ramachandra University, Porur, Chennai visited AMRF on 23rd March 2016 and gave a lecture on "Approaches to Fluorescence In Situ Hybridization (FISH) and its diagnostic potential"

SUSHIL KUMAR DUBEY, MAHALAKSHMI, PERUMALSAMY VIJAYALAKSHMI, PERIASAMY SUNDARESAN

- *Mutational analysis and genotype-phenotype correlations in sporadic and familial aniridia patients from southern India.*

Molecular Vision 2015;21:88-97

SAUMI MATHEWS, JAYA DEVI CHIDAMBARAM, SHRUTI LANJEWAR, JEENA MASCARENHAS, NAMPERUMALSAMY VENKATESH PRAJNA, VEERAPPAN MUTHUKARUPPAN, GOWRI PRIYA CHIDAMBARANATHAN,

- *In vivo confocal microscopic analysis of normal human anterior limbal stroma*

Cornea 2015;34:464-470

TIN AUNG ET AL.,

- *A common variant mapping to CACNA1A is associated with susceptibility to Exfoliation syndrome.*

Nature Genetics 2015 Apr; 47(4):387-92.

SUSHIL K. DUBEY, JAMES F. HEJTMANCIK, SUBBIAH R. KRISHNADAS, RAJENDRABABU SHARMILA, ARAVIND HARIPRIYA, PERIASAMY SUNDARESAN

- *Evaluation of Genetic Polymorphisms in Clusterin and Tumor Necrosis Factor-Alpha Genes in South Indian Individuals with Pseudoexfoliation Syndrome*

Current Eye Research, Early Online, 1-7, 2015

LI Z, ALLINGHAM RR, NAKANO M, JIA L, CHEN Y, IKEDA Y, MANI B, CHEN LJ, KEE C, GARWAY-HEATH DF, SRIPRIYA S, FUSE N, ABU-AMERO KK, HUANG C, NAMBURI P, BURDON K, PERERA SA, GHARAHKHANI P, LIN Y, UENO M, OZAKI M, MIZOGUCHI T, KRISHNADAS SR, OSMAN EA, LEE MC, CHAN AS, TAJUDIN LS, DO T, GONCALVES A, REYNIER P, ZHANG H, BOURNE R, GOH D, BROADWAY D, HUSAIN R, NEGI AK, SU DH, HO CL, BLANCO AA, LEUNG CK, WONG TT, YAKUB A, LIU Y, NONGPIUR ME, HAN JC, HON DN, SHANTHA B, ZHAO B, SANG J, ZHANG N, SATO R, YOSHII K, PANDA-JONAS S, ASHLEY KOCH AE, HERNDON LW, MOROI SE, CHALLA P, FOO JN, BEI JX, ZENG YX, SIMMONS CP, BICH CHAU TN, SHARMILA PF, CHEW M, LIM B, TAM PO, CHUA E, NG XY, YONG VH, CHONG YF, MEAH WY, VIJAYAN S, SEONGSOO S, XU W, TEO YY, COOKE BAILEY JN, KANG JH, HAINES JL, CHENG CY, SAW SM, TAI ES; ICAARE-GLAUCOMA CONSORTIUM; NEIGHBORHOOD CONSORTIUM, RICHARDS JE, RITCH R, GAASTERLAND DE, PASQUALE LR, LIU J, JONAS JB, MILEA D, GEORGE R, AL-OBEIDAN SA, MORI K, MACGREGOR S, HEWITT AW, GIRKIN CA, ZHANG M, SUNDARESAN P, VIJAYA L, MACKAY DA, WONG TY, CRAIG JE, SUN X, KINOSHITA S, WIGGS JL, KHOR CC, YANG Z, PANG CP, WANG N, HAUSER MA, TASHIRO K, AUNG T, VITHANA EN.

- *A common variant near TGFB3 is associated with primary open angle glaucoma*

Hum Mol Genet. 2015 Jul 1;24(13):3880-92.

BHARANIDHARAN DEVARAJAN, LOGAMBIGA PRAKASH, THIRUMALAI RAJ KANNAN, ALOYSIUS A ABRAHAM, USHA KIM, VEERAPPAN MUTHUKARUPPAN AND AYYASAMY VANNIARAJAN

- *Targeted next generation sequencing of RB1 gene for the molecular diagnosis of Retinoblastoma*

BMC Cancer. 2015 Apr 28;15(1):320.

KANNAN THIRUMALAIRAJ, ALOYSIUS ABRAHAM, BHARANIDHARAN DEVARAJAN, NAMRATA GAIKWAD, USHA KIM, VEERAPPAN MUTHUKARUPPAN AND AYYASAMY VANNIARAJAN

- *A stepwise strategy for rapid and cost-effective RB1 screening in Indian retinoblastoma patients*

Journal of Human Genetics 2015; 1-6

GOWRI PRIYA CHIDAMBARANATHAN, SAUMI MATHEWS, ARUN KUMAR PANIGRAHI, JEENA MASCARENHAS, VENKATESH PRAJNA NAMPERUMALSAMY, MUTHUKARUPPAN VEERAPPAN,

- *In vivo confocal microscopic analysis of limbal stroma in limbal stem cell deficient patients*

CORNEA 2015; August 26 (E Pub ahead of print)

KATHRYN P. BURDON, RHYS D. FOGARTY, WEIYONG SHEN, SOTOODEH ABHARY, GEORGIA KADONIS, BINOY APPUKUTTAN, ALEX W. HEWITT, SHIWANI SHARMA, MARK DANIELL, ROHAN W. ESSEX, JOHN H. CHANG, SONJA KLEBE, STEWART R. LAKE, BISHWANATH PAL, ALICIA JENKINS, GOWTHAMAN GOVINDARJAN, PERIASAMY SUNDARESAN, ECOSSE L. LAMOUREUX, KIM RAMASAMY, MARIA PEFKIANAKI, PHILIP G. HYKIN, NIKOLAI PETROVSKY, MATTHEW A. BROWN, MARK C. GILLIES, JAMIE E. CRAIG

- *Genome-wide association study for sight-threatening diabetic retinopathy reveals association with genetic variation near the GRB2 gene*

Diabetologia 2015; 19th July (E Pub ahead of print)

YU ZHOU, BIBHUTI B SAIKIA, ZHILIN JIANG, XIONG ZHU, YUQING LIU, LULIN HUANG, RAMASAMY KIM, YIN YANG, CHAO QU, FANG HAO, BO GONG, ZHENG FU TAI, LIHONG NIU, ZHENGLIN YANG, PERIASAMY SUNDARESAN AND XIANJUN ZHU

- *Whole-exome sequencing reveals a novel frameshift mutation in the FAM161A gene causing autosomal recessive retinitis pigmentosa in the Indian population.*

Journal of J Hum Genet. 2015 Aug 6. (Epub ahead of print)

NAMRATA G, VANNIARAJAN A ,AKRAM HUSAIN, JEYARAM I, THIRUMALAIRAJ K SANTHI,R, MUTHUKKARUPPAN V, USHA KIM

- *Knudson's hypothesis revisited in Indian retinoblastoma patients*

Asia-Pac J Clin Oncol 2015 (Epub ahead of print)

SRINIVASAN SENTHILKUMARI, MOHAN NEETHU, RADHAKRISHNAN SANTHI, SUBBIAH RAMASWAMI KRISHNADAS, VEERAPPAN MUTHUKKARUPPAN.

- *Identification of glaucomatous optic nerve head changes in Indian donor eyes without clinical history*

Indian J Ophthalmol 2015;63:600-5.

SHIRISH DONGARE, SHARMILA RAJENDRAN, S SENTHILKUMARI, SURESH K GUPTA, RAJANI MATHUR, ROHIT SAXENA, SUSHMA SRIVASTAVA.

- *Genistein Alleviates high glucose induced toxicity and angiogenesis in cultured human RPE cells.*

Int J Pharm Pharm Sci, Vol 7, Issue 8, 294-298

HEMADEVI BOOMIRAJ, VIDYARANI MOHANKUMAR, LALITHA PRAJNA. BHARANIDHARAN DEVARAJAN

- *Human corneal microRNA expression profile in fungal keratitis*

Invest Ophthalmol Vis Sci.2015;56:7939–7946.

LALAN KUMAR ARYA, SIVAKUMAR R. RATHINAM, PRAJNA LALITHA, USHA R. KIM, SUDEEP GHATANI, VEENA TANDON

- *Trematode Fluke Procerovum varium as Cause of Ocular Inflammation in Children, South India*

Emerging Infectious Diseases Vol. 22, No. 2, February 2016; 192-199

YANAN DI, LULIN HUANG, PERIASAMY SUNDARESAN, SHUJIN LI, RAMASAMY KIM, BIBHUTI BALLAV SAIKIA, CHAO QU, XIONG ZHU, YU ZHOU, ZHILIN JIANG, LIN ZHANG, YING LIN, DINGDING ZHANG, YUANFEN LI, HOUBIN ZHANG, YIBING YIN, FANG LU, XIANJUN ZHU & ZHENGLIN YANG

- *Whole-exome Sequencing Analysis Identifies Mutations in the EYS Gene in Retinitis Pigmentosa in the Indian Population*

Scientific Reports 2016 | 6:19432 | DOI: 10.1038/srep19432

ONGOING RESEARCH PROJECTS

No	Projects	Funded by	Investigators	Research Scholar
MICROBIOLOGY				
1.	Etiology and Immunopathogenesis of Trematode induced Uveitis in children of South India	AMRF	Dr. SR. Rathinam Dr. Lalitha Prajna	Lalan Kumar Arya
2.	Characterization of the virulence determinants of Pseudomonas aeruginosa causing ocular infections using genomic and proteomic approaches	AMRF	Dr. M. Vidyarani	J.Lakshmipriya
3.	Genotypic characterization and analysis of virulence factors in Methicilin resistant staphylococcus aureus (MRSA) causing ocular infections in South India	AMRF	Dr.Lalitha Prajna	V.Nithya
4.	Elucidating the role of microRNAs in microbial keratitis	CSIR- Research Associate (From Oct 2014- March 31,2016)	Dr.Lalitha Prajna	Dr.B.Hemadevi
5.	Analysis of bacterial persistence mechanisms in recalcitrant ocular Pseudomonas aeruginosa infections	SERB	Dr.M.Vidyarani	
PROTEOMICS				
6.	CoE – Human Mycotic Keratitis	DBT	Dr.N.Venkatesh Prajna Dr.K,Dharmalingam Dr.Lalitha Prajna Dr.J.Jeya Maheshwari	S.Mohammed Razeeth; A.Dhivya K.R.P.Niranjana Naveen Luke Demonte; C.Sathya Priya; R.Nithya; Swati Krishnan N. Sudha
7.	Proteomics and peptidomics of human infectious diseases and biomarker discovery	DBT - Distinguished Biotechnology professorship award	Dr.K.Dharmalingam	K.Sandhya
8.	Predictive biomarkers for diabetic retinopathy among diabetics and stage specific biomarkers for NPDR and PDR.	Bagchi grant	Dr.K.Dharmalingam Dr.R.Kim Dr.J.Jeya Maheshwari Dr.S.Senthilkumari	Roopesh R.Pai R.Sharmila Naveen Luke Demonte A.Divya K.Sandhya

9.	Mycotic Ulcer Treatment	AEH	Dr.K.Dharmalingam Dr.N.Venkatesh Prajna	M.Muthukumar R.V.Angela Asir
10.	Pathogenic Aspergillus interaction with Innate Immune cells	CEFIPRA	Dr.Lalitha Prajna	P.M.Vaishali
11.	Functional analysis of circulating microRNAs and their regulatory role in Diabetic Retinopathy	SERB	Dr.O.G.Ram Prasad, Prof. K. Dharmalingam Dr. Bharanidharan Dr. Kim Ramasamy	B.Muthu Meenakshi
MOLECULAR GENETICS				
12.	Molecular genetics of Albinism in the Indian population	AMRF Post-Doctoral Fellow	Dr.P.Sundaresan Dr.P.Vijayalakshmi	K.Renugadevi
13.	Molecular genetics studies of Primary Angle closure Glaucoma (PACG) in South Indian Population	UGC Fellowship	Dr.P.Sundaresan	Roopam Duvesh
14.	Genetic and functional approaches to understand the pathogenicity of Primary Open Angle Glaucoma (POAG)	ICMR-SRF	Dr.P.Sundaresan	Moh'd Husain Shah
15.	Genetic and transcript analysis of RB1 gene in South Indian Retinoblastoma Patients	ICMR	Dr.A.Vanniarajan Dr.Usha Kim Dr.R.Santhi	K.Thirumalai Raj
16.	Establishing the genetic testing centre for childhood ocular cancer (retinoblastoma) in Aravind Medical Research Foundation	Aravind Eye Foundation	Dr.A.Vanniarajan Dr.Usha Kim Dr.R.Santhi Prof. VR.Muthukkaruppan Dr.D.Bharanidharan	A.Aloysius Abraham
17.	Understanding the molecular mechanisms of chemoresistance in retinoblastoma	CSIR-NET JRF	Dr.A.Vanniarajan	T.S.Balaji
18.	Genetic evaluation of genes involved in homocysteine metabolism and hyperhomocysteinemia with Pseudoexfoliation syndrome in South Indian population	AEH	Dr.P.Sundaresan	G.Prakadeeswari
19.	Molecular genetics of macular corneal dystrophy (MCD) in Indian population	DST INSPIRE Fellowship	Dr.P.Sundaresan Dr.N.Venkatesh Prajna	M.Durga

	Whole-exome Sequencing Analysis Identifies Mutations in the FAM161A and EYS Gene in Retinitis Pigmentosa in Indian Population	CSIR-SRF	Dr.P.Sundaresan	Bibbuti Ballav Saikia
IMMUNOLOGY AND STEM CELL BIOLOGY				
20.	Limbal miRNAs and their potential targets associated with the maintenance of stemness	DBT	Dr.C.Gowri Priya Dr.Venkatesh Prajna Prof. VR.Muthukkaruppan Dr.D.Bharanidharan	K.Lavanya
21.	Molecular signature of Corneal Epithelial Stem Cells (CESCs)	ICMR-SRF	Dr.C.Gowri Priya Dr.Venkatesh Prajna Prof. VR.Muthukkaruppan	M.K.Jhansirani
22.	Structural and functional integrity of corneal endothelium after storage in Cornisol, an indigenous intermediate stage corneal storage medium	AEH	Dr. N. Venkatesh Prajna Dr.C.Gowri Priya	S.Yogapriya
OCULAR PHARMACOLOGY				
23.	Studying the Role of Rho A – Rock Signalling in conventional outflow pathway using Human Organ Culture Anterior Segment (HOCAS)	SERB	Dr.S.Senthilkumari Dr.SR.Krishnadas Dr.C.Gowri Priya	S.Ashwin Balaji
24.	Indian Macular Carotenoids Research (INDMACARE) – A Feasibility study	ICMR	Dr.S.Senthilkumari Dr.Anand Rajendran Dr.Venkatesh Prajna	Yelchuri Madhavi Latha
25.	Is Human Amniotic Membrane (HAM) a Suitable Reservoir System for the Release of Drugs in Ocular use?	AEH	Dr.S.Senthilkumari Dr.Venkatesh Prajna	Yelchuri Madhavi Latha
BIO INFORMATICS				
26.	Clinical exome analysis pipeline for eye disease next-generation sequencing panel	AMRF	Dr.D.Bharanidharan	K.Manojkumar
27.	Comparative genome analysis to identify genomic variants and genes associated with drug resistance in Methicillin-Resistant Staphylococcus aureus (MRSA) and Pseudomonas aeruginosa ocular strains	AEH	Dr.D.Bharanidharan Dr. M. Vidyarani Dr. Lalitha Prajna	K.Kathirvel



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