

DR. G.VENKATASWAMY EYE RESEARCH INSTITUTE

Aravind Medical Research Foundation

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

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

BASIC RESEARCH IN OPHTHALMIC SCIENCES Annual Report 2012 - 2013

DR. G. VENKATASWAMY EYE RESEARCH INSTITUTE
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.*

G. Venkataswamy



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INTRODUCTION

Effort to understand eye diseases and application of the research findings at the patient care level has been our major focus this year also. Apart from the continuation of the ongoing activities AMRF embarked on a major research program on fungal keratitis. This group has received major funding in the form of a program support from the Department of Biotechnology, Government of India, for fungal keratitis research. This support allowed the institute to strengthen the Proteomics research area. The grant made it possible for the institute to procure a state of art Mass spectrometer, Orbitrap Velos Pro, for the study of proteomics of eye diseases. This will allow the proteomics group to do the deep analysis of expression and quantitation of proteins and their isoforms. Having this grant allowed the proteomics group to successfully attract funding from Cognizant industry also. This group, with faculty and senior investigators with Ph.D degree, will be a model for other groups to consolidate and attract major funding.

Molecular genetics group continues to explore the genetic organization and disease specific markers for various eye diseases. Apart from looking at known and new markers and mutations this group also looks at the whole genome using genome wide association approach for the discovery of population specific mutations. They have also tried to extend their findings to clinical use with close collaboration with clinicians.

Stem cell biology group is undertaking major efforts to understand the biology of buccal stem cells and also keeps in focus the application aspects. The new area of focus is role of micro RNAs in stem cells. This is an active area of research and hopefully some novel finding will emerge from this analysis.

Ocular pharmacology group is continuing its efforts to bring about drugs based on the findings in diabetic retinopathy and age related macular degeneration. The Human Organ Culture Anterior Segment System is now optimized and new results are emerging from this novel experimental system, since there is no in vivo system available for glaucoma studies.

Microbiology group has done significant work in bacterial keratitis, particularly in the determination of virulence factors in *Pseudomonas* keratitis. This group is also exploring the role of micro RNAs in pathogenic process.

The new group on Bioinformatics has started its activities in the structural biology of single nucleotide variants. This group also helps in setting up various analysis platforms for NGS data. The analysis of whole genome sequence data on pathogenic bacteria by this group has helped in the identification novel factors involved in pathogenesis.

Faculty of the institute also has taken up various teaching activities in the form of workshops and short term hands on training activities.

MOLECULAR GENETICS

Ongoing research programmes of this group are related to commonly occurring eye diseases in Indian population like Diabetic retinopathy, Glaucoma, Cataract, Corneal dystrophies and Retinal dystrophies.

Currently gene therapy is available for Lebers Congenital Amaurosis (LCA). To implement gene therapy in Indian population, the department has been screening RPE65 genes mutations in LCA patients who can be benefited from this therapy in future. The department has recently initiated studies to explore mitochondrial involvement in Primary Open Angle Glaucoma and association of microRNA with keratoconus in collaboration with Queens University, Belfast, UK. The department has identified a candidate gene for ocular coloboma in collaboration with Center for Human Molecular Biology and Genetics, Sichuan Academy of Medical Sciences and Sichuan Provincial People's Hospital, Sichuan, China. Recently it has also initiated the study to characterize the gene expression profile of normal, diabetic and diabetic retinopathy human donor eye balls obtained from Rotary Aravind International Eye Bank. The Department has also analysed several families with Retinoblastoma and made significant contributions in the management of Retinoblastoma by identifying RB1 mutations in new borns and treating the disease at an early stage. The department organized a workshop entitled "Vision Genomics" to motivate young researchers to involve in basic/ophthalmic research. Many of the study designs of the department emphasize the molecular mechanisms of various eye diseases at the gene expression level.



Molecular genetics of Leber Congenital Amaurosis in South Indian population

Investigators : P. Sundaresan, Aravind Medical Research Foundation, Madurai
P. Vijayalakshmi, Aravind Eye Hospital, Madurai
Ph.D. Scholar : Anshuman Verma
Funding agency : Indian Council of Medical Research (ICMR), University Grant Commission (UGC) Fellowship

Background and aim

Leber congenital amaurosis (LCA) is the most severe form of visual impairment found in children. At present, more than twenty genes have been reported for LCA, and among them RPE65 is a likely candidate for gene therapy. The aim of this study is to perform a comprehensive screening of LCA genes emphasizing RPE65. In this study, three sequencing technologies were applied - (a) Direct sequencing to screen RPE65 mutations in 30 LCA probands (b) Microarray based comprehensive detection of all known LCA pathogenic variations in 25 LCA patients excluded for RPE65 mutations (c) Next generation sequencing (NGS) based targeted enrichment in a separate set of 25 LCA pooled DNA samples was carried out to screen coding regions of 56 genes, including 15 known and 41 predicted LCA genes. In addition, in silico characterization of the identified mutations and related clinical phenotype of patients were studied.

The combined approach of RPE65 mutational screening with Asper chip analysis revealed ten different disease causing variations in 6 LCA genes from 11 LCA patients. The targeted resequencing of 56 genes uncovered total 97 variations, mostly in known LCA genes and a few predicted genes. However, most of the variations were in flanking intronic regions and found to be as polymorphism.

Implication of the data

This is the first study, which applies advanced techniques for a comprehensive mutational analysis of LCA genes in South Indian cohort. The combined approach of direct sequencing and Asper chip analysis was instrumental to identify pathogenic mutations in 36.6 % of the patients with the major involvement of RPE65 (16.6%), GUCY2D (10%), RPGRIP1 (3.3%), AIPL1 (3.3%), CRX and IQCB1 (3.3%). In this study, RPE65 mutations were found to be the main cause for LCA followed by GUCY2D. The NGS based targeted resequencing can be productive for the comprehensive screening of a large number of genes but are unfavourably influenced by various sequencing steps in pooled samples, resulting in its limitation to identify rare pathogenic variations.

Molecular genetics and functional analysis of candidate genes associated with microphthalmia, anophthalmia and coloboma

Investigators : Dr. P. Sundaresan (Aravind Medical Research Foundation, Madurai)
Dr. P. Vijayalakshmi (Aravind Eye Hospital, Madurai)
Dr. S. K. Kedia (Sadar Hospital, Ara, Bihar)
Collaborator : Dr. Yang Zhenglin, Sichuan Academy of Medical Sciences, China
PhD Scholar : Mr. Sushil Kumar Dubey
Funded Agency : ICMR

Background and aim

Microphthalmia, anophthalmia and coloboma (MAC) are a group of congenital eye diseases that represent important causes of paediatric blindness. These ocular disorders have variable and poorly understood effects that extend all the way to total blindness. Extensive studies of these ocular disorders have reported mutations in a large number of genes and also several chromosomal aberrations in various populations. Lack of similar studies for Indian population limits the knowledge regarding the genetics of these globe anomalies. This study aims to find out the spectrum of genetic variations in candidate genes and their role in causing disease.

DNA samples of 65 MAC patients were evaluated for mutations and sequence variants in the candidate genes by direct sequencing approach. All the exons along with flanking exon- introns boundaries of RAX, OTX2, SOX2 and ABCB6 genes were screened in all MAC samples. Hundred ethnically-matched healthy control samples were also sequenced to confirm the identified variants as patient specific. Comparative sequence analysis of RAX, OTX2, SOX2 and ABCB6 genes with their homologs across different species was done to determine whether the mutations are present in the conserved domains. Mutation identified in ABCB6 gene at AMRF was functionally characterized in Zebrafish by Sichuan Provincial Key Laboratory for Human Disease Gene, Sichuan Academy of Medical Sciences Affiliated Hospital, Sichuan, China.

Five novel mutations were identified in RAX, OTX2, SOX2 and ABCB6 genes in this cohort. A homozygous substitution mutation p. Arg179Trp, found in one bilateral anophthalmia patient, was identified in RAX gene. Three heterozygous mutations p. Pro142Fs [c. 426insC (in a proband with bilateral anophthalmia)], p. Thr186Fs [c. 558delC (in one proband with microphthalmia in one eye and anophthalmia in other)] and a compound heterozygote p. Gln104X, p. Gln106 His (in one patient with microphthalmia) were identified in OTX2. These three mutations in OTX2 gene cause premature termination of the coding sequence. Mutation p. Val303Val was identified in SOX2 gene in only one patient with microphthalmia and iris coloboma. Screening of ABCB6 gene identified a heterozygous mutation (p. Ala57Thr) in exon1. Comparative sequence analyses of RAX, OTX2, SOX2 and ABCB6 genes with their homologs showed that mutation sites are highly conserved across various species. To test the hypothesis that disruption of the normal function of ABCB6 can cause coloboma, zebrafish embryos treated with ABCB6 morpholinos (MOs) showed coloboma phenotype and retarded development. These phenotypes can be rescued by

the coinjection of WT ABCB6 mRNA but not by the coinjection of human mRNA containing Ala57Thr mutation (Figure 1).

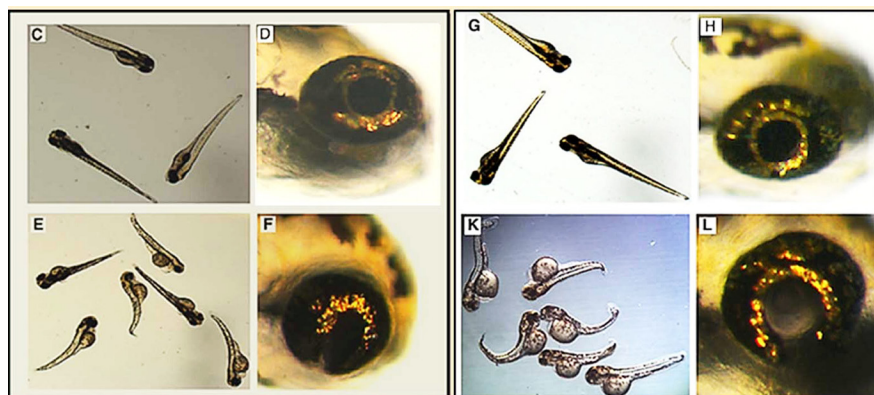


Figure 1. Zebrafish eye development disturbed by knockdown of abc b6 expression and rescue of abc b6 morphants

Compared with zebrafish embryos treated with standard control MOs (C and D), zebrafish embryos treated with abc b6 MO show coloboma and retarded development in (E and F). These phenotypes can be rescued by the coinjection of WT ABCB6 mRNA (G and H), but the phenotypes could not be rescued by coinjection of the human mRNA containing Ala57Thr (K and L). This is the result of the work carried out by collaborator Sichuan Academy of Medical Sciences, China

Implication of the project

Five novel mutations identified in RAX, OTX2, SOX2 and ABCB6 suggest the role of these genes in ocular development. Three mutations in OTX2 gene cause premature termination of the coding sequence. These can be a pathogenic change as the protein conformation may get altered leading to loss of function. Morpholino knockdown and rescue studies of ABCB6 in Zebrafish demonstrated that mutations in ABCB6 gene might cause coloboma. Further in silico and in vitro studies of the normal and mutant protein will unravel the role of the mutations in disease development.

Molecular genetic studies on different subtypes of Primary Angle Closure (PAC) patients in South Indian Population

Investigators	: Dr. P. Sundaresan (Aravind Medical Research Foundation, Madurai) Dr. R. Venkatesh (Aravind Eye Hospital, Pondicherry) Dr. Kavitha Srinivasan (Aravind Eye Hospital, Pondicherry) Dr. Pradeep Ramulu (Johns Hopkins Hospital, USA) Dr. Robert Wojciechowski (Johns Hopkins Bloomberg School of Public Health, Wilmer Eye Institute, Johns Hopkins, School of Medicine, USA)
PhD scholar	: Roopam Duvesh
Funding agency	: Aravind Eye Care System & Wilmer Pooled Professorial Grant UGC (Fellowship)

Background and aim

Primary angle closure glaucoma (PACG) is a complex disorder and a leading cause of blindness worldwide, especially in Asians. Being a complex disease, several genetic, environmental, anatomical and physiological factors are involved in the pathogenesis of PACG. Several studies have suggested a genetic basis for

PACG. But, still no specific gene for PACG has yet been identified. Recently, the association of three new loci, including SNP markers rs11024102 in PLEKHA7, rs3753841 in COL11A1 and rs1015213 located intergenically between PCMTD1 and ST18 genes has been reported with PACG. The purpose of this study is to evaluate the genetic association of these three markers with different subtypes of primary angle closure patients in a South Indian population.

A total of 351 patients were recruited from South India and divided into three groups: Primary Angle Closure Suspect (PACS; N=171), Primary Angle Closure/ Primary Angle Closure Glaucoma (PAC/PACG; N=180) and a combined any angle closure group (AAC). The control group consisted of 411 ethnic and age-matched individuals. Genotyping of cases and control groups for three SNPs was performed by Taqman real time allelic discrimination assay (Fig.1). The association of each of these SNPs was determined by chi-square test and logistic regression using STATA-11 software.

In this study, significant genetic association was identified for rs1015213 (PCMTD1-ST18) in the PAC/PACG ($p=0.004$) and AAC ($p=0.006$) group. However, no significant association was seen in PACS subjects ($p=0.103$). The other two SNPs, rs3753841 and rs11024102 were not found to be associated with any of the case groups ($p>0.05$) in South Indian population. Further work is necessary to confirm the importance of COL11A1 and PLEKHA7 in the pathogenesis of glaucoma.

Implication of the project

There are a few studies present that show the involvement/or association of genes and polymorphisms with PACG in the Indian population. So, this study will help to understand the involvement of genetic components in PACG susceptibility and hence their role in pathogenesis of angle closure glaucoma.

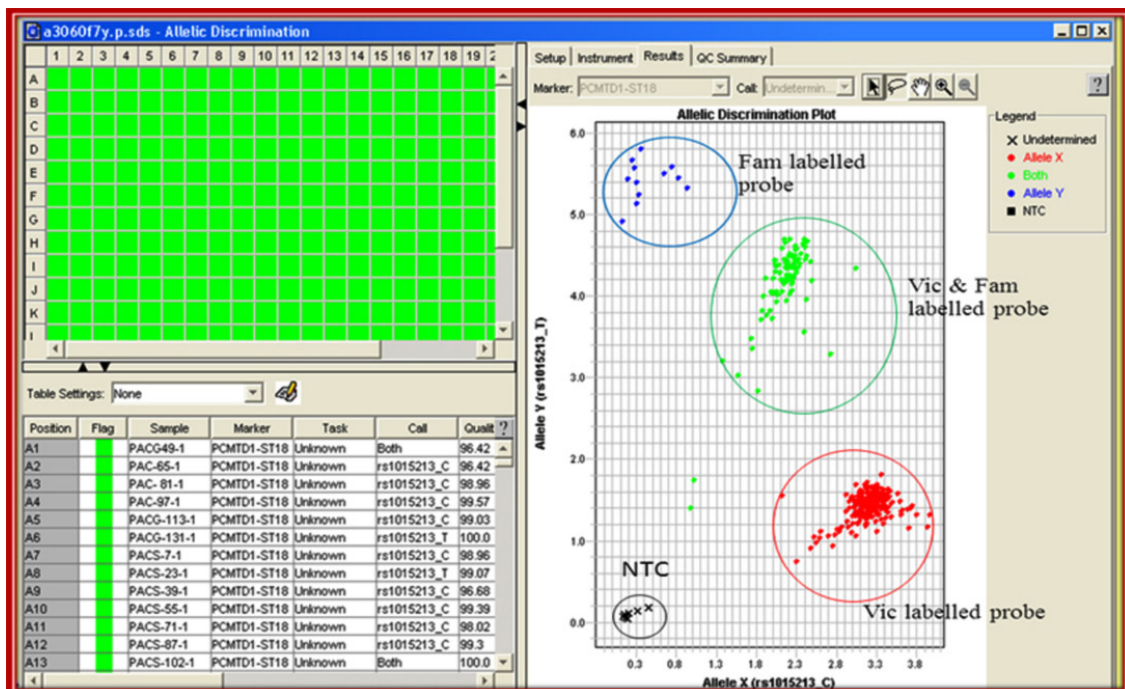


Fig.1 Real Time Taqman SNP Assay for rs1015213 (PCMTD1-ST18)

Genetic evaluation of Myocilin and Optineurin Genes in Familial Primary Open Angle Glaucoma

Investigators	:	Dr. P. Sundaresan (Aravind Medical Research Foundation, Madurai) Dr. R. Ramakrishnan (Aravind Eye Hospital, Tirunelveli) Dr. Mohideen Abdul Kadeer (Aravind Eye Hospital, Tirunelveli) Dr. S.R.Krishnadas (Aravind Eye Hospital, Madurai)
Collaborators	:	Dr. Alan L. Robin (Johns Hopkins University Baltimore, USA) Dr. Pradeep Ramulu (Wilmer Eye Institute, USA) Dr. John Fingert (University of IOWA, USA)
Ph.D Scholar	:	Ms. Prasanthi Namburi
Funding Agency	:	Proposal submitted to ICMR

Background and aim

Glaucoma is a heterogeneous group of optic neuropathies with a complex genetic basis and is the second-leading cause of blindness in the world. Based on blindness prevalence survey, 12.8% of blindness in India is caused by glaucoma. Primary Open Angle Glaucoma (POAG) is one of the most prevalent types of glaucoma affecting over 33.15 million people in the world. It is estimated to affect 6.48 million people in India.

Until date, three candidate genes (Myocilin, Optineurin and WDR36) were identified from the 25 identified loci linked with POAG. However, the known glaucoma-causing genes are together responsible for only 4-5% of POAG cases. The remaining percentages of POAG cases are likely to be due to the combined action of unknown candidate genes, the environmental and other risk factors. The present study was designed to determine the role of myocilin and optineurin genes in the pathogenesis of primary open angle glaucoma in a large pedigree with autosomal dominant disease from South India.

Family history is one of the risk factors for glaucoma. Family-based studies have been successful in discovering candidate genes. Therefore, 240 participants from a single large four generation family (Figure-1) were recruited for this study. Among them 22 are POAG and 20 members are POAG suspect cases. Each member of this pedigree received comprehensive ophthalmic examinations. The DNA samples collected from the family members were analyzed for disease-causing mutations in the myocilin and optineurin genes using a combination of restriction fragment length polymorphism (RFLP) analysis and bi-directional DNA sequencing.

Among the 240 members studied, twenty two members were diagnosed with POAG, twenty were judged to be POAG suspects and 198 did not meet criteria for either diagnosis. Screening of all the study participants identified the absence of disease-causing mutations in Myocilin and Optineurin genes.

Implication of the data

The absence of mutations in these two genes that are currently known to cause POAG, strongly suggests that the disease in this pedigree may be caused by a novel glaucoma gene. The team is in the process of investigating the new candidate gene using linkage analysis and next generation DNA sequencing.

Presentation

Prasanthi Namburi, R. Ramakrishnan, Mohideen Abdul Kadeer, S.R.Krishnadas, Alan L. Robin, Pradeep Ramulu, John Fingert and P.Sundaresan. – Presented a poster entitled “Evaluating the Role of Myocilin and Optineurin Genes in Familial Primary Open Angle Glaucoma” at ARVO 2012 Annual Meeting, Visionary Genomics, in Fort Lauderdale, Florida, USA

Association analysis of nuclear encoded ARHGAP22, PLXDC2 and MnSOD genes Polymorphisms in patients with Type-2 Diabetic Retinopathy in Indian Population

Investigators : Dr. P. Sundaresan (Aravind Medical Research Foundation, Madurai)
Dr. Kim Ramasamy (Aravind Eye Hospital, Madurai)
Collaborators : Dr. G. Banuprakash Reddy, Dr. Nasreen Z.Ethesam (National Institute of Nutrition, Hyderabad)
PhD Scholar : Mr. Gowthaman.G
Funding Agency : Department of Biotechnology

Background and aim

Diabetes mellitus is a common disease with serious complications. One of the most devastating microvascular complications of Diabetes mellitus is Diabetic Retinopathy (DR). In most developing countries, DR remains the single major cause of blindness. DR is multifactorial and its pathogenic mechanism remains unclear. Several genetic, environmental and biochemical, growth factors are involved in DR. Recently, Rho GTPase – activating protein 22 (ARHGAP22), Plexin Domain Containing 2 (PLXDC2) and Manganese Superoxide Dismutase (MnSOD) were reported as the candidate genes associated with DR.

The aim of this study is to investigate the possible genetic association of nuclear encoded ARHGAP22, PLXDC2 and MnSOD genes polymorphisms with Type-2 Diabetic Retinopathy in Indian population.

A case-control association study was performed using 700 unrelated patients with type 2 diabetes. Type II DM patients with and without retinopathy were enrolled through the Aravind Eye Hospital in Madurai. DR consists of 350 patients with proliferative diabetic retinopathy confirmed by fundoscopy and the control group NDR consists of 350 subjects with more than 10 years duration of type-2 diabetes with no signs of DR. Clinical documentation like seven field Fundus photography and biochemical tests have been carried out for all recruited patients.

Two single-nucleotide polymorphisms (SNPs) in ARHGAP22 (rs11101355 and rs11101357), one SNP in PLXDC2 (rs1571942) and one in MnSOD (rs4880) were evaluated in these two groups and genotyped using Taq Man SNP genotyping assay. Statistical analysis was performed using the STATA-statistical software version 8.1 (StataCorp, College Station, TX) to identify the distributions of allele, genotype frequencies, fisher exact test p-values, odds ratios and Hardy Weinberg equilibrium. The p values < 0.05 were considered to be statistically significant.

Among the four SNPs screened, SNP rs11101357 of ARHGAP22, showed a marginal association with $p=0.017$ for the genotypic frequency test. The remaining three SNPs ARHGAP22 (rs11101355), PLXDC2 (rs1571942) and MnSOD (rs4880) showed insignificant association between cases and the controls.

Implication of the data

This is the first study to demonstrate the association of these nuclear encoded gene polymorphisms in patients with type 2 DR in the Indian cohort. The data suggests that the genetic variants of ARHGAP22, PLXDC2 and MnSOD did not differ significantly between the DR and NDR group. However, one genetic variant in ARHGAP22 (rs11101357) shows marginal association with DR in this study population.

Presentation

Gowthaman Govindarajan, Suganthalakshmi Balasubbu, Vignesh Thoguluva Prabhakaran, Kim Ramasamy, Sundaresan Periasamy – Presented a poster entitled “Association of ARHGAP22, PLXDC2 Genes Polymorphisms with Type-2 Diabetic Retinopathy in Indian Population” at ARVO 2012 Annual Meeting, Visionary Genomics, in Fort Lauderdale, Florida, USA

Mitochondrial genes involvement in Leber's Hereditary Optic Neuropathy (LHON)

Investigators : Dr. P. Sundaresan (Aravind Medical Research Foundation, Madurai)
Dr. S. Mahesh Kumar (Aravind Eye Hospital, Madurai)
PhD Scholar : Mr. Bibhuti Ballav Saikia
Funding Agency : DST, CSIR (JRF)

Background and aim

Leber's Hereditary Optic Neuropathy (LHON) is primarily the result of three primary point mutations: m.3460G>A, m.11778G>A and m.14484T>C and affects complex I subunit of the Electron Transport chain (ETC) in mitochondria. Though LHON is maternally inherited, the risk of vision loss across all mutations is about 46% for men and 11% for women. LHON patients can be analyzed for the presence of three primary mutations along with other secondary mutation. Therefore, the investigation of mitochondrial abnormalities in LHON patients can be done by whole mitochondrial genome screening. The variations occurring in mitochondrial genome of LHON patients will be classified as changes corresponding to a haplogroups and pathogenic mutations.

In the present study, forty LHON affected individuals and forty age matched controls were recruited. Twenty four sets of primers were used to screen whole mitochondrial genome. The sequences were analyzed against rCRS (revised Cambridge Reference Sequence). Two primary mutations: m.3460G>A, m.11778G>A were identified in LHON patients. Apart from primary mutations, 13 disease associated known mutations were also identified in protein coding genes and tRNA gene. In addition, the team has identified private 23 non synonymous variants other than the haplogroup-defined variants in 13 LHON families.

The phylogenetic analysis of mtDNA based haplogrouping of the LHON patients revealed that they belonged to different haplogroup. The M derived haplogroup was observed in 62.5% (25/40) and other haplogroup in 37.5% (25/40) of the individual analyzed.

Implication of the data

Primary mutations along with other mitochondrial DNA point mutations may have significant role in the pathogenesis of the disease. Haplogroups have influence in the clinical expression of LHON harboring primary mutations. This study could not find the influence of haplogroup in the disease. Further study is required to know the role of mitochondrial DNA mutations and haplogroup in the pathogenesis of LHON.

A genetic component to the INDEYE study of cataract and age-related macular degeneration (AMD) in India

Investigators : Astrid E Fletcher, London School of Hygiene and Tropical Medicine, London
P.Sundaresan, Aravind Medical Research Foundation, Madurai
R.D.Ravindran, Aravind Eye Hospital, Madurai
D. Nitsch, L Smeeth, London School of Hygiene and Tropical Medicine, London
Research Scholars : V.Saravanan, J.Radha
Funding Agency : Wellcome Trust

Background and aim

INDEYE Study focuses on epidemiology of age-related eye diseases (Cataract & Age related Macular Degeneration-AMD) in India. Cataract is the leading cause of adult blindness in India. Out of world's 38 million cases of cataract, India has 9 million people suffering from this devastating disease. The prevalence and risk factors for earlier stages and for different types of cataract have not been estimated.

AMD is a disease of the retina and the macula. Late stage AMD may result in severe and irreparable loss of central vision. So far there is no therapy available for this disease. Very few studies have been

conducted in developing countries such as India to assess the prevalence and risk factors for this disease. The present study was initiated

- To investigate genetic variants as possible contributors to high rates of cataract in India, complementing the ongoing research on environmental factors being undertaken in the INDEYE study.
- To analyze the genes associated with age related macular degeneration using the samples acquired from INDEYE study.

Progress on the INDEYE genetic study

Cataract (3984 from North India and 4215 from South India) and AMD (519) blood samples from the population based study have been processed in timely manner and DNA was extracted. DNA stock was diluted by Semi Automatic Robotic Machine –epMotion5070 and genotyping was carried out in Real time PCR. So far, large scale genotyping for CFH, EPHA2, LOC387715 / ARMS2, HTRA1, CFB, APOE, MIER1, SLC family, CRYGB, TLR3, TLR7, TIMP3, CETP, VEGFA, FRK, HPR, genes have been performed. Analyses are still ongoing

Cataract genetics–role of SLC35D1

In the INDEYE population based study, persons aged 40 years and over were randomly selected and enrolled. Participants underwent lens photography and provided blood for genetic analysis. The Lens Opacities Classification System III (LOCS III) was used for grading cataract: cortical ≥ 3 , posterior subcapsular cataract (PSC) ≥ 2 , nuclear cataract ≥ 4 . Any cataract was defined as any nuclear, cortical or PSC cataract or dense opacities or operated cataract. Controls were defined as the absence of any of the above. Genotyping of SLC35D1 SNPs (rs2755250, rs2208577) was carried out with Real Time PCR. Analysis was by logistic regression adjusted for age, sex, location and survey design. Of 7354 participants, 538 had cortical cataract, 1,101 PSC, 2461 nuclear, 4159 any type of cataract and 3195 were controls. rs2755250 and rs2208577 were correlated ($r=0.6$), the control MAFs were 11% and 24% respectively and similar to those reported in HapMed, The genotype frequencies were in Hardy-Weinberg equilibrium for rs2208577 ($p=0.4$) but not for rs2755250. Results therefore are reported only for rs2208577.

Implication of the Data

This is the first study to demonstrate the association of SLC35D1 gene polymorphisms in patients with cataract in the Indian population. The SNP rs2208577 showed a significant association with the rare genotype and cortical and PSC cataract. These findings require replication in other study populations.

Presentation

- Radha Jeyaraman¹, Sundaresan Periasamy¹, Ravilla D Ravindran², Praveen Vashist³, Dorothea Nitsch⁴, Giovanni Maraini⁵, Monica Camparini⁵, Usha Chakravarthy⁶, James F. Hejtmancik⁷, Astrid E Fletcher MIER¹ Polymorphisms and Age-Related Cataract in India. US ARVO-2012 Annual meeting, Fortlauderdale, USA (Programme Number: 1548/D769)

Publication

- Periasamy Sundaresan, Praveen Vashist, Ravilla D. Ravindran, Ashwini Shanker, Dorothea Nitsch, Bareng A. S. Nonyane, Liam Smeeth, Usha Chakravarthy, Astrid E Fletcher “*Polymorphisms in ARMS2/HTRA1 and complement genes and age related macular degeneration in India: findings from the INDEYE study*” IOVS 2012 Nov 1;53(12):7492-7497

Genetic analysis of RB1 in South Indian Retinoblastoma patients

Investigators : A. Vanniarajan, VR. Muthukkaruppan - Aravind Medical Research Foundation, Madurai
Usha Kim, R. Santhi, G. Namrata - Aravind Eye Hospital, Madurai
Research Scholars : R.S. Akram Husain, K. Thirumalai Raj, A. Aloysius Abraham
Funded by : Aravind Eye Foundation

Background and aim

India has the highest incidence of retinoblastoma (RB) among the developing countries. The inheritance of RB is largely dependent on mutation pattern of RB1 gene. Hence, genetic analysis of RB patients will be helpful in predicting the risk of RB in siblings and offsprings. The research focus here is to understand the RB1 mutations and inheritance pattern in South Indian population.

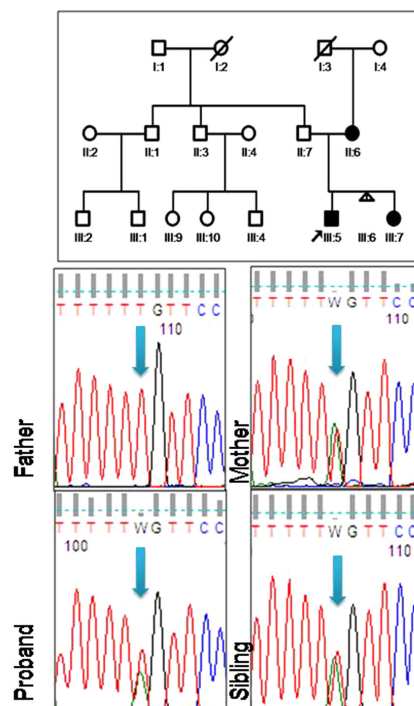
Complete case histories of 73 (31 bilateral & 42 unilateral) RB patients had been analyzed. Pedigree showed positive family history in 14 patients (19%) that include 12 bilateral and 2 unilateral RB. Bilateral RB occurred much earlier (9.82 ± 11.52 months) than unilateral RB (24.02 ± 15.11 months), in accordance with Knudson's hypothesis. Genetic analysis of 7 patients showed a spectrum of RB1 mutations. In 2 unilateral cases, analysis of tumor samples showed somatic, nonsense mutations that were present only in tumor but not in blood samples of probands and parents and hence the risk of RB was predicted to be <1% in siblings.

In the 3 bilateral sporadic cases, parents did not show any mutations but the patients had (1) deletion of complete RB1, (2) deletion of exons 4-6 and (3) a truncation mutation in their blood samples. These 3 mutations could be post-zygotic de novo mutations which will be passed on to next generation and hence children of patients had to be checked for RB1 mutations. Out of 2 cases with positive family history, the deletions of exons affecting the pocket domain of RB1 was observed in proband and affected father and hence further siblings are at increased risk of getting retinoblastoma.

A splice site mutation likely to cause truncated RB1 protein was noted in proband and mother of another family and hence the next sibling was predicted to have 50% risk of getting RB based on the pedigree and mutation pattern (Figure). Since the predisposition of RB in the family was explained, the next sibling was brought to the clinic as early as 12 days after birth and diagnosed with RB by clinical examination and genetic testing. By the early detection of RB, the child was treated successfully and vision was preserved.

Implication of the project

Genetic analysis confirmed the Knudson's two-hit hypothesis in Indian population for the first time. Detection of somatic mutations in sporadic unilateral cases predicted a lesser chance of RB in future generation. The identification of heritable mutation in bilateral cases predicted an increased risk of RB in siblings and next generation, thereby providing early diagnosis and treatment.



OCULAR MICROBIOLOGY

Ocular infections pose a major challenge to the community with a high morbidity rate and threat of vision loss. Rapid diagnosis and early treatment are mandatory for the effective management of ocular infections. The thrust area of research in the department of ocular microbiology is to identify and characterize the ocular pathogens like Methicillin resistant *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Acanthamoeba* and Trematodes by phenotypic and genotypic approaches. Studies are aimed at understanding the pathogenesis of ocular infections and the host – pathogen interactions. The innate and adaptive immune response mechanisms and the inflammatory mediators are being studied in bacterial and fungal corneal ulcers. The possible involvement of immunomodulators like vitamin D is being analyzed in corneal inflammation and further studies will be carried out to assess the antimicrobial/anti-inflammatory functions. The knowledge gained from these studies may help to improvise the existing therapeutic interventions and to identify potential new drug targets. Large repository of ocular isolates available was used to characterize the virulence mechanisms and antibiotic resistance patterns of ocular pathogens. Also newer molecular methods are being adapted for the rapid identification of known and emerging ocular pathogens and for the characterization of virulence factors with a view to improve the current therapeutic approaches for ocular diseases.

Microbiological clearance time and sensitivity assay for *Acanthamoeba* keratitis

Principal Investigator : Dr.Jeena Mascarenhas, Aravind Eye Hospital, Madurai
Co-Investigator : Dr.Lalitha Prajna, Aravind Eye Hospital, Madurai
Research scholar : Fathima Sulthana.K
Funded By : University of California, San Francisco

Acanthamoeba is a ubiquitous, free living amoeba which can be found in air, soil, dust and water. It exists in two stages during its life cycle: a dormant resistant cyst and a mobile vegetative trophozoite stage. Under adverse conditions, *Acanthamoeba* possess a double cell wall and pores called ostioles and remains as a cyst for years. However, in favourable conditions such as abundant nutrients (including *E.coli*, *Aerobacter aerogenes* Gram negative bacteria), neutral pH, appropriate temperature (30o C), and osmolarity (50-80 mOsmol), the cyst reverts back to a trophic form (trophozoite stage) by dislodging the operculum which covers the pores and exhibit distinct spike shaped pseudopodia (acanthopodia) and food vacuoles. The acanthopodia helps in adhesion to surfaces, cellular movements and in capturing the prey.

Acanthamoeba keratitis is a vision threatening ocular infection which leads to corneal ulceration, loss of visual acuity, corneal scarring and mono-ocular blindness. If not diagnosed early and treated aggressively, the infection may spread to deep stroma and other ocular tissues. Numerous recent studies have reported incidences of *acanthamoeba* keratitis in contact lens wearers but a six years prevalence study, from a tertiary eye hospital in Coimbatore, South India suggests that the incidence may be increasing in non-contact lens wearers mainly due to corneal trauma and contaminated water.

The incidence rate of *Acanthamoeba* is 1.8% in the year 2012, at Aravind Eye Hospital, Madurai, India. *Acanthamoeba* keratitis is difficult to treat and there is minimal evidence on which to base treatment decisions. The main objectives of this study are to estimate the microbiological clearance time for

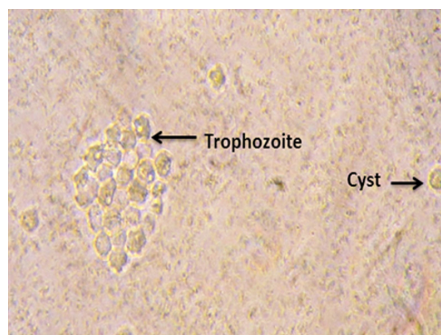
Acanthamoeba, determination of Minimum Cysticidal Concentration (MCC) of anti-microbial drugs and to evaluate combination of anti-microbial drugs by invitro synergy assay.

Corneal swabs from patients who presented with keratitis were subjected to conventional microbiological culture methods and molecular identification using Real Time PCR targeting 18s rDNA. For the invitro estimation of Minimum Cysticidal Concentration (MCC), 20 Acanthamoeba isolates were randomly selected from the year 2006 to 2011 and their sensitivity was tested against eight different drugs (0.02% polyhexamethylenebiguanide (PHMB) 0.02% chlorhexadine, 1% voriconazole, 3.3% neomycin, 0.1% hexamidine, 5% amikacin, 1% itraconazole, 0.1% propamidine). The MCC reading will correspond to the concentration of the drug which inhibits the formation of trophozoite as observed under phase contrast microscope.

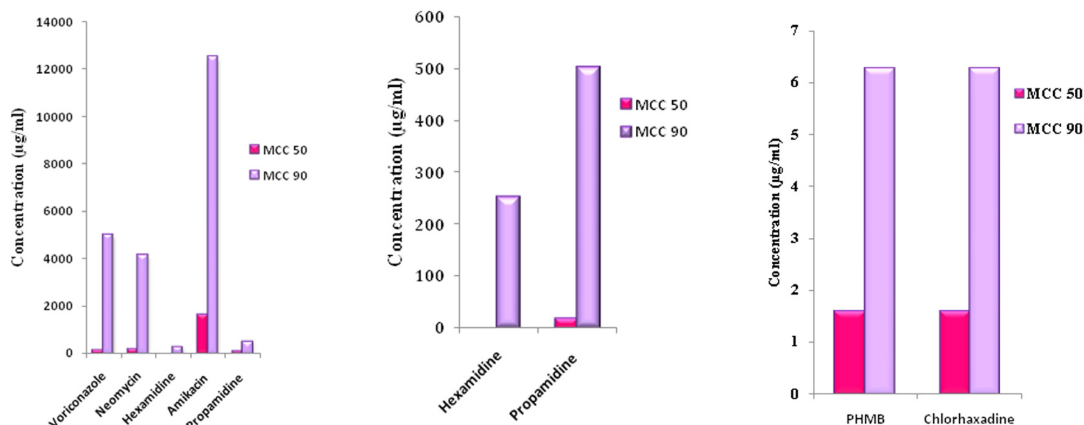
As per the results, PHMB and Chlorhexadine had lower MCC50/MCC90 when compared to other drugs. Ulcers in the cornea are painful and are often resistant to treatment with usual antimicrobial agents. Hence, a combination of drugs may prove to be more effective than a single agent at lower concentrations. To check this possibility, a synergy assay will be performed based on the MCC values obtained with the ocular isolates.



Clinical Picture of Acanthamoeba keratitis sharing corneal ulcer and hypopyon



Cyst and Trophozoite forms of acanthamoeba



MCC50 / MCC90 values of Anti-microbial drugs

Minimum cysticidal concentration of antimicrobial drugs against ocular isolates of Acanthamoeba. The MCC50 and MCC90 values are the concentrations that inhibit formation of trophozoites by 50% and 90% respectively.

Genotypic characterization and analysis of virulence factors in methicillin resistant staphylococcus aureus causing ocular infections in south India

Principal investigator : Dr. Lalitha Prajna

Research scholar : Nithya.V

Funding agency : ICMR

Methicillin Resistant Staphylococcus aureus (MRSA) is known for causing high mortality all over the world due to multi drug resistance and rapid progression. Methicillin resistance in *S. aureus* is encoded by the *mecA* gene located in the staphylococcal cassette chromosome *mec* (SCCmec) region. There are two major types of MRSA, hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA), which is further differentiated into SCCmec type I, II, III, IIIA, IV, V according to their evolutionary basis and genetic variations in the SCCmec cassette. Type I, II, III, IIIA are grouped as hospital acquired MRSA and type IV, V are grouped as community acquired MRSA. CA-MRSA is more virulent than hospital acquired MRSA, due to the secretion of cytotoxin Pantone-valentine Leucocidin (PVL). There are other toxins like exotoxins and exfoliative toxins known to be involved in MRSA pathogenesis. The purpose of this study is to perform molecular characterization and epidemiological analysis of MRSA causing ocular infections in South India.

Between January 2012 and December 2012, 36 MRSA isolates were collected at Aravind Eye Hospital, Madurai, India, from the patients who presented with various ocular infections like orbital infections, infective keratitis and lacrimal sac abscess. Preliminary screening was performed by antibiotic susceptibility method using oxacillin and cefoxitin discs and further confirmed by *mecA* screening. Staphylococcal chromosome *mec* typing, Multi locus sequence typing (MLST), Staphylococcal protein A typing and detection of Pantone-Valentine leucocidine toxin were done for 17 isolates by PCR and sequencing.

A total of 136 Staphylococcus aureus were isolated, among which 36 (26%) were confirmed as MRSA. 17 of 36 MRSA isolates were screened, in which 10 were SCCmec type V, five were SCCmec type IV and two were not typeable. Most of the SCCmec type V isolates belonged to ST772 with *spa* type t657 (7/10) and 3/5 of SCCmec IV isolates had ST1037, ST30 (1/5) and ST2124 (1/5) with *spa* type t852 and t11714. The two non-typeable strains had ST672 and ST2066. 82% (14/17) isolates were found to be positive for the toxin Pantone-Valentine leucocidine.

Community acquired MRSA strains (SCCmec type IV and V) were more prevalent than hospital acquired MRSA in causing ocular infections. The *spa* type t657 with correspondence to ST772 was the most predominant. Most of the community acquired MRSA isolates harbor the PVL gene which may be responsible for the pathogenicity and ocular morbidity.

Further studies will be done to analyze the expression of toxin genes in association with various ocular infections using Real Time PCR and their accessory gene regulator (*Agr*) type.



Fig 1: An eleven month old baby had suture infection caused by MRSA after lower lid slit correction. Swelling over the lower lid with pus formation was observed due to prolonged infection.

Characterization of the host immune response during corneal infection with pathogenic fungi and bacteria

Principal investigator : Dr. Lalitha Prajna, Aravind Eye Hospital, Madurai
Co-investigators : Dr. Venkatesh Prajna, Aravind Eye Hospital, Madurai
Dr. K. Dharmalingam, Madurai Kamaraj University, Madurai
Collaborator : Dr. Eric Pearlman, Case Western University, USA
Research scholar : R. Siva Ganesa Karthikeyan
Funding agency : ICMR

Human fungal infections are predominantly either pulmonary or systemic, which are mainly associated with the immunocompromised status of the individual. In contrast, patients with fungal keratitis are fully immunocompetent. Study on human healthy individuals demonstrated a significant lymphoproliferative response to *A. fumigatus* proteins which may be due to a constant exposure to fungal antigens. Fungal conidia are ubiquitous and airborne, and our respiratory tract is in constant exposure to these spores. It was estimated that humans inhale several hundred to thousands of *A. fumigatus* spores per day, but the development of invasive fungal disease is rare because of efficient innate and adaptive immune responses against the fungal pathogen.

The conidia inhaled are inert, until it germinates into germ tube and hyphae resulting in exposure of β -glucan on the cell wall. The β -glucan is recognized by the macrophages in the lung through the surface pathogen recognition receptor dectin-1, resulting in release of inflammatory cytokines and activation of dendritic cells (DC). Once the DC gets activated in lungs they travel to lymph nodes and present the fungal antigens to naive T cells and thus activate the adaptive system. The development of the T-helper 1 (Th1) and Th17 phenotypes, will be protective and also facilitate the killing of the pathogens.

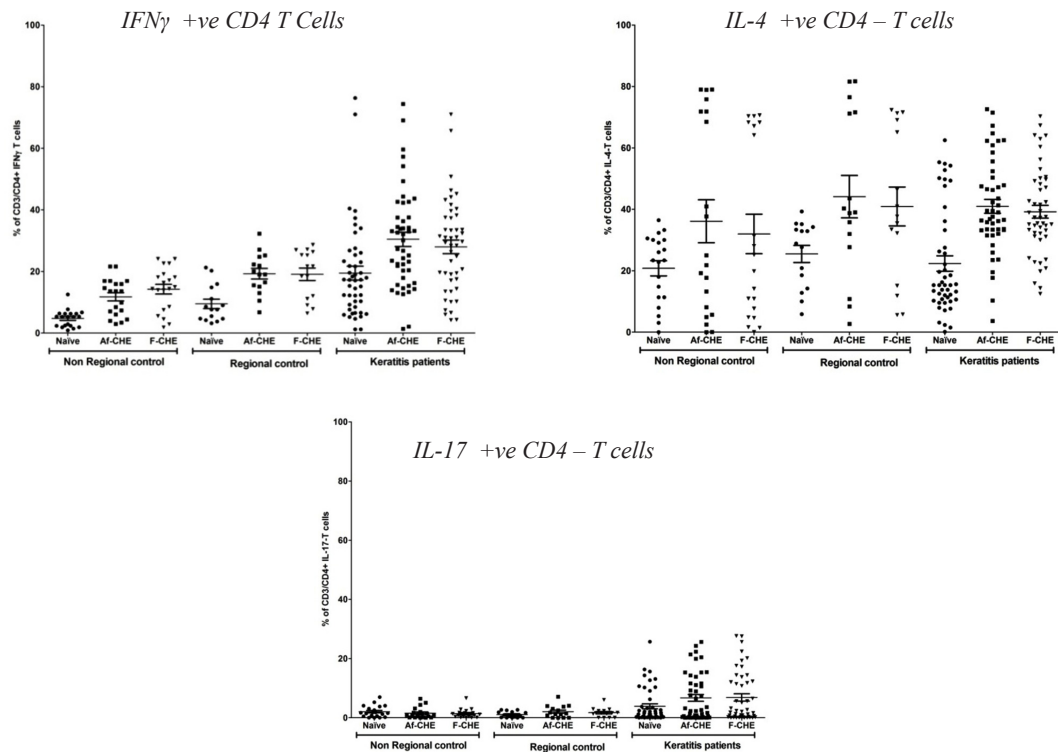
Animal model and the team's previous study on fungal corneal infections revealed that the neutrophils are the main population of cells responding to the fungal infection in the cornea. Upon encountering hyphae, both TLR4 and Dectin-1 on the neutrophil surface engage their respective ligands on the hyphal cell wall, activate NADPH-oxidase, and initiate ROS-dependent killing of fungal hyphae. Simultaneously, infiltration of Th1 cells and IFN γ production during corneal infection enhances the phagocytic potential of infiltrating macrophages, which mediate killing of conidia and hyphae. Along with neutrophils, infiltrating Th17 cells also secrete IL-17 which binds to the IL-17R on many cell types in the cornea and induces the synthesis of chemokines, which recruits more. Thus the production of the IL-17A seems to have a central role by enhancing the immune response and in clearing the pathogen from the cornea.

Previous studies at the department with fungal infected corneal tissue revealed an elevated expression of PRR's Dectin-1, TLR2 and TLR4 which may lead to the production of the chemokine IL-8, which helps in recruiting the PMNs into the corneal stroma for killing of the pathogen. During the later stage of the disease, presence of CD4⁺ T cells along with the increase in the cytokines IFN- γ , and IL-17A suggests that there may be an involvement of Th1 or Th17 cell response respectively. But the cellular analysis revealed that, the majority of cell population during early and late stage of the disease consists of PMN and it suggests that it may also be an active source for IL-17A. But for the production of IL-17A, the T cells or neutrophils require additional signal through other cytokines like IL-6/23 which may be secreted from the macrophages or DC. It is hypothesized that the constant exposure to the fungal spores in healthy individuals through the respiratory tract will prime the immune system to the fungal antigen. Thus fungal antigen specific immune cells (DC, Macrophage, Neutrophils, and T cells) may exist even before the corneal infection.

Increased Th1 and Th17 response in keratitis patients

The PBMC from patients and controls were stimulated overnight with crude hyphal extract of *A. flavus* and *Fusarium* separately. After stimulation, the lymphocytes in the PBMC population were labeled with CD3/CD4 surface marker and intracellularly labeled for either IFN γ or IL-4 or IL-17 (Fig.1).

Figure 1:



Intracellular cytokine staining of CD4+ T cells isolated from keratitis patients and controls upon stimulation with fungal antigens

Each data point in the scatter plot, represent individuals from non regional controls, regional controls and patients infected with *Aspergillus flavus* or *Fusarium* sp. Percentage of A) CD3+/CD4+/IFN γ + T cell (Th1), B) CD3+/CD4+/IL-4+ T cell (Th2), C) CD3+/CD4+/IL-17+ T cell (Th17). (Af- *A. flavus*; F- *Fusarium*; CHE- Crude Hyphal Extract).

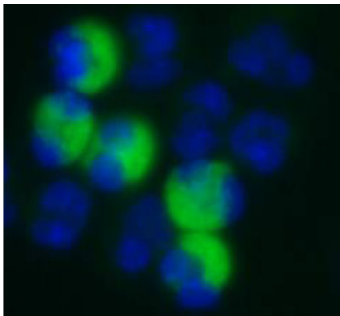
A predominant Th1 and Th17 response was seen in keratitis patients with both *Aspergillus* and *Fusarium* crude hyphal extracts.

Intracellular IL-6 and IL-23 positive dendritic cells and macrophages were determined using PBMC's isolated from keratitis patients and controls. An increased percentage of IL-6 and IL-23 positive cells were seen in naive and fungal antigen stimulated PBMC from regional controls and patients.

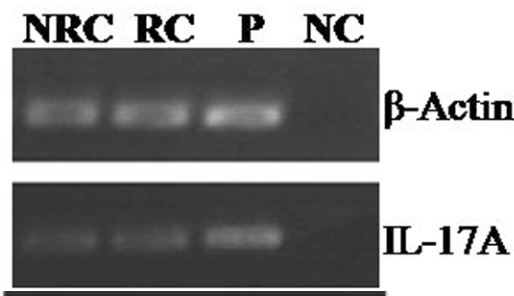
Intra cellular IL-17 expression in neutrophils

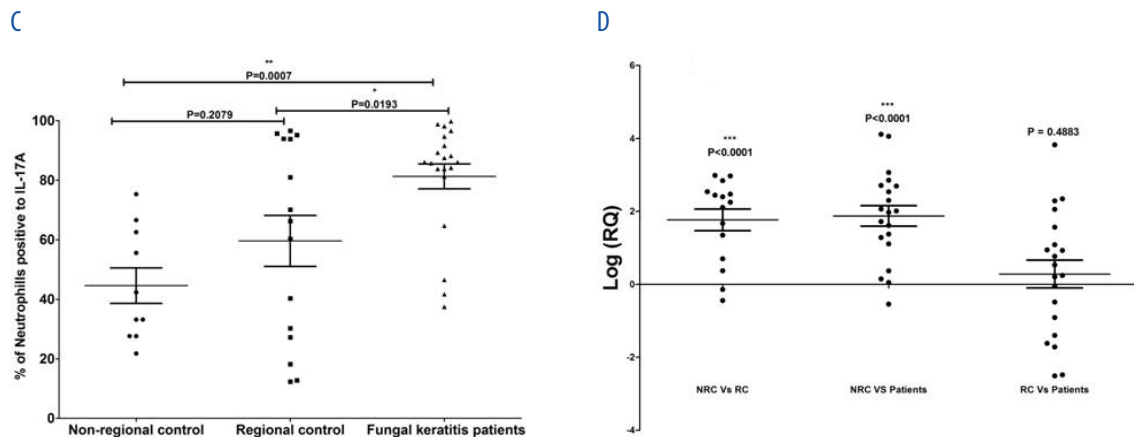
Figure 2:

A. IL-17 +ve Neutrophils



B. IL-17A +ve mRNA expression in Neutrophils





Intra cellular IL-17 staining in neutrophils

The neutrophils were separated from the blood and stained for the intra cellular IL-17A. The cells were imaged under fluorescent microscope at 40X objective A) merged representative image from one of the keratitis patients; (DAPI – Blue nucleus; Green – IL-17A FITC; B) Agarose gel showing the amplified product of the endogenous control β -actin and IL-17A mRNA expression. C). The percentage of neutrophils positive to IL-17A was analyzed by *Quanti quest* software; D) IL-17A mRNA transcript expression in neutrophils was compared among the different groups by $\Delta\Delta$ ct method. (NRC- Non Regional control; RC- Regional control; P-Fungal keratitis patient; NC – Negative control; RQ- Relative Quantification)

In vitro stimulation of neutrophils from non regional controls

The total PBMC was stimulated overnight with crude hyphal extract of *A. flavus* and *Fusarium* separately. The PBMC culture supernatant of patients was used to stimulate the neutrophils of non regional controls for two hours.

IL-17A mRNA expression was increased in neutrophils treated with supernatants of PBMCs stimulated with crude hyphal extracts.

To conclude, the constant exposure of the fungal spores from the environment activates the DC and macrophages in the lungs. The activated DC migrates to the neighboring lymph node and activates the naïve T cell to become TH1 or TH17 subset. The DC and macrophages help in the priming and development of the PMN17 subtype. Thus higher percentage of PMN-17 was present in the regional controls and patients due to high exposure to the fungal spores from environment.

In case of traumatized cornea, the spore/hyphae can penetrate the corneal epithelium and enter the corneal stroma. The resident macrophages or dendritic cells in the corneal stroma recognize the germinating spore or hyphae through PRRs, TLR4 and Dectin-1, leading to proinflammatory cytokine and chemokine production. The chemokine IL-8 mediate the recruitment of inflammatory cells into the cornea (blood neutrophils-PMN17) from the nearby blood vessels in the limbal region. Neutrophils (PMN-17) express surface PRR like Dectin-1, which helps in the recognition of the fungal pathogen, and since the neutrophils (PMN-17) were already primed they will get activated immediately which results in effective killing of fungal pathogen by the release of antimicrobial components in their granules. These effective killing mechanisms cause extensive damage to the corneal tissue that result in the development of corneal opacity leading to vision loss.

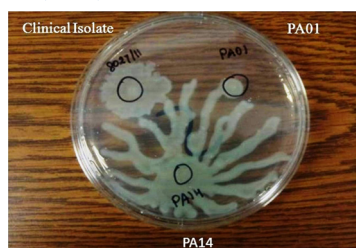
In combination with effective antifungal treatment and targeted immune suppression, inflammation related corneal tissue destruction needs to be controlled to prevent vision loss. Even though this study revealed some of the key steps in the immunopathogenesis of fungal keratitis, still further studies need to be done to understand the disease fully. The information obtained from this study as well as from future works will translate into advancement in therapeutic approach thus saving the vision of fungal keratitis patients.

Characterization of the virulence determinants of *Pseudomonas aeruginosa* causing ocular infections using genomic and proteomic approaches

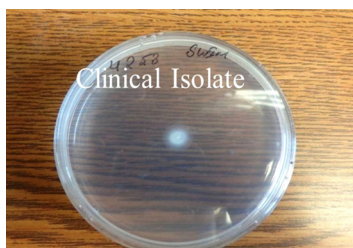
Principal investigator : Dr. Lalitha Prajna
Co-investigator : Dr. Vidyarani Mohankumar
Research scholar : J. Lakshmi Priya
Funding agency : Submitted to DST

Bacterial corneal infection is an important cause of keratitis, which constitutes 34% of all corneal ulcers in South India, among which *Pseudomonas aeruginosa* (62%), is the predominant causative agent. The pathogenesis of *P. aeruginosa* is mediated through the production of several cell-associated and extracellular virulence factors. The virulence factors most associated with ocular damage in *P. aeruginosa* include Type III secretion system (T3SS), factors involved in bio film formation and other virulence factors like elastase B, alkaline protease, protease IV etc. The formation of biofilm by *P. aeruginosa* during infections facilitates microbial survival in hostile environments, protects the bacteria from the host immune response and enhances their resistance to antibiotics. The flagellar motility and type IV pilus of the bacteria help in the initial attachment to the host which leads to the formation of a mature biofilm. The purpose of this study is to evaluate the ability of the ocular isolates of *P. aeruginosa* to form biofilm on a surface. 50 *Pseudomonas aeruginosa* isolates from patients with corneal ulcer and two reference strains PA01 and PA14 were used for this study. The organisms were confirmed by sequencing the 16s rRNA and phenotypic assays for swarming, swimming and twitching motilities were performed. Quantitative biofilm assay was done using microtitre plates. Among the 50 *P. aeruginosa* isolates analyzed, 80% were positive for the motility assays. 47% of the isolates had multi drug resistance and also showed strong biofilm formation. The results suggest that the ocular clinical isolates of *P. aeruginosa* establish initial attachment with the host using flagellum and type IV pili. Also formation of a strong biofilm may be a property associated with Multi Drug resistant (MDR) strains. Future studies will involve genome and transcriptome analysis of the ocular isolates to identify and characterize the factors contributing to virulence and antibiotic resistance in ocular infections.

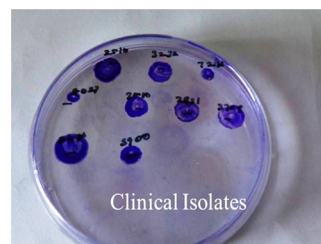
Motility assay



Swarming Plate

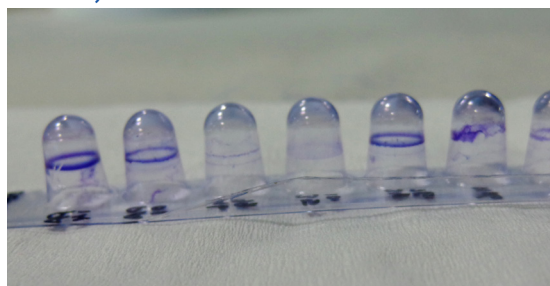


Swimming Plate



Twitching Plate

Bio film assay



PA14 & PA01 – Reference strains

CI 1 - Clinical Isolate 1

CI 2 – Clinical isolate 2

CI 3 – Clinical Isolate 3

CI 4 – Clinical Isolate 4

Presentation

Jeganathan lakshmi priya, Rajapandian Sivaganesa Karthikeyan, Namperumalsamy Venkatesh Prajna, Eric Pearlman, Arne Rietsch, Prajna Lalitha “*Characterization of pseudomonas aeruginosa type three secretory system (TTSS) effector molecules (ExoU/S/T) from human corneal ulcer*” ARVO 2012, Fort Lauderdale, Florida, USA

Etiology and immunopathogenesis of subconjunctival and anterior chamber granulomatous uveitis in children

Investigators : Dr. S.R Rathinam - Aravind Eye Hospital, Madurai
Co-investigators : Dr. Lalitha Prajna - Aravind Medical Research Foundation, Madurai
Dr. Veena Tandon - North Eastern Hill University, Shillong
Research scholar : Mr. Lalan Kumar Arya
Funding agency : Indian Council of Medical Research

Pediatric parasitic ocular inflammation is one of the common clinical conditions in South India. Histopathological analysis and molecular methods confirmed it as trematode infection (Am Academy of Ophthalmology, 2001 and Am J Ophthalmology 2002 & Archives of ophthalmology 2012). Using Real-time PCR and molecular sequencing in the present study, 38 patients' samples were analysed in which 10 (26%) were found to be positive for the trematode *Procerovum varium*. The age range of children was 6 to 17 years, with a mean and standard deviation of 11.32 ± 2.69 years. Six of them were females (16%) and 32 were males (84%). The study proved the etiology as *Procerovum varium*. However only 26% of the clinically diagnosed samples were positive for *Procerovum varium* and the remaining samples were negative. It is assumed that it may be because of complete degeneration of parasite in the granuloma or, possibility of other species of trematode.

Residential address of the patients was collected and a team from the hospital visited the village pond where these patients got exposed to water prior to developing the infection. A total of five species of snails were found in the same geographical region. On the basis of shell morphology, snails collected from the 52 different village ponds of nine different districts of Tamil Nadu were identified as *Bellamya dissimilis*, *Pila virens*, *Melanoides tuberculata*, *Stenomelania torulosa* and *Lamellidens marginalis* by the National Zoological Survey of India. It was found that among five species of snails, only *Melanoides tuberculata* released cercariae in the laboratory when they were provided with the natural environmental condition. The released cercariae were analyzed using PCR and molecular sequencing and found to be *Procerovum varium*. The trematode *P. varium* sequence was very similar to those identified in the patients' granuloma samples.

Immunohistochemistry studies are now being done to analyze the inflammatory cellular pattern to understand the pathogenesis.

Implication of the Data

In this study, only 26% cases were found to be positive for trematode *Procerovum varium* and a large number of cases were negative for either this trematode species or, any other entity. To increase the possibility of identifying these trematode species from the granulomatous tissue, we need to design specific set of primers and antibodies..

Presentation

- Lalan Kumar Arya, SR. Rathinam, Prajna Lalitha, Kim R. Usha, Veena Tandon *Molecular based identification of Procerovum varium (Trematode: Heterophyidae) in human eye causing granulomatous uveitis in children of South India* at ARVO, S 2012 Annual Meeting, Vision Genomics, in Fort Lauderdale, Florida, USA.

Publication

- SR. Rathinam, Lalan Kumar Arya, Kim R. Usha, Prajna Lalitha, Veena Tandon "Novel etiological agent - Molecular evidence for trematode induced anterior uveitis in children" ARCH OPHTHALMOL/ VOL 130 (NO. 11), NOV 2012.

STEM CELL BIOLOGY



Use of stem cells in regenerative medicine is gaining more prominence now. The focus of research here is on adult stem cells- specifically corneal epithelial stem cells. Research at the department has earlier established a two parameter concept for identifying putative stem cells, and developed a simple method for ex-vivo expansion of the limbal/buccal epithelial stem cells for corneal surface reconstruction in limbal stem cell deficient patients in compliance with good manufacturing practice. With the objective of improving stem cell expansion and transplantation outcome, the current focus is to understand the

mechanism involved in the maintenance of stemness at (i) molecular level - the role of specific genes, regulatory elements and associated signaling pathways and (ii) cellular level - the microenvironment or niche in the limbal stroma.

Studies on the characterization of limbal niche - their role in maintenance and ex vivo expansion of human corneal epithelial stem cells

Investigators : C. Gowri Priya, VR. Muthukkaruppan - Aravind Medical Research Foundation
N. Venkatesh Prajna, Usha Kim - Aravind Eye Hospital
Research fellow : Saumi Mathews
Funding agency : Defence Research Development Organisation

Background and aims

Corneal epithelium is a rapidly regenerating squamous epithelium. During homeostasis and following injury the corneal epithelium is regenerated from a distinct population of corneal epithelial stem cells (CESCs), residing in the basal epithelial layer of the corneo-scleral limbus. A deficiency of CESCs occurs due to genetic disorder like aniridia or due to acquired factors such as chemical or thermal injury, ultraviolet or ionizing radiation, drug toxicity, ocular cicatricial pemphigoid and Steven-Johnson's syndrome. As a result of this limbal stem cell deficiency (LSCD), the limbal barrier effect may be lost and the adjacent conjunctival epithelium migrates over the corneal surface, resulting in persistent epithelial breakdown and vascularization leading to impaired vision.

Ex-vivo expansion of CESCs/ buccal mucosal epithelial stem cells (BMESCs) is a valuable alternate to allogenic transplantation in patients with unilateral/ bilateral LSCD respectively. To prevent the potential transmission of adventitious agents such as prions and animal viruses, the team has established a xenobiotic-free culture method in compliance with Good Manufacturing Practice (GMP).

It is known that the limbal microenvironment is different from that of cornea. The team has identified CD90 and CD105 positive mesenchymal stem cells (MSC) in the anterior limbal stroma and not in cornea. Therefore it is essential to elucidate key structural and functional components of the limbal SC niche that control the SC behavior for efficient expansion of SCs. The aim of the current study is to develop limbal fibroblast cell line and characterize limbal stroma specific factors that are associated with the expansion of SCs, which will aid in establishing a better method for culturing limbal/buccal epithelium for corneal surface reconstruction.

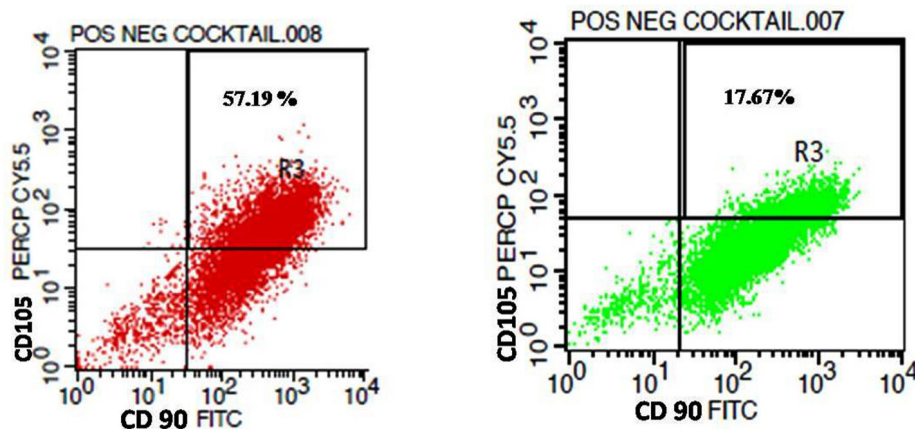
To identify the limbal niche associated factors that can enrich the SC content in culture

In order to identify the limbal niche associated factors, limbal (LF) and corneal fibroblasts (CF) were isolated from limbal and corneal stroma using donor eyes (Rotary Aravind International Eye Bank). After deepithelialization using dispase II for 45 minutes, the limbal/corneal stroma was minced to less than 1 mm pieces and digested using collagenase treatment overnight. Isolated LF and CF were then cultured in DMEM with 10 % FBS in CO2 incubator for 8 to 12 days. Upon reaching 80% confluency, LF/CF were harvested by trypsinization and population doubling was calculated from P2 to P6. The cumulative population doubling level was 14.71 for LF, while was 4.71 for CFs. They were non-tumorigenic, maintaining their signature karyotype (46, XX) upto P6 studied.

LFs and CFs were characterized for the MSC markers specified in international society for cellular therapy criteria till P6 using flow cytometry. LFs found to be positive for MSC markers CD 90, CD105, CD73, CD44 and negative for CD34, CD45, CD11b, CD19, HLA-DR. Contradicting to the findings of native tissue, CFs expressed MSC markers at P2 and P3, and not at the later passages.

Markers	Limbal Fibroblasts (mean $\pm$ SD)		Corneal Fibroblasts (mean $\pm$ SD)	
	P2	P6	P2	P6
CD90+	98.7 $\pm$ 0.9	97.1 $\pm$ 0	98.1 $\pm$ 2.0	93.9 $\pm$ 4.4
CD105+	99.3 $\pm$ 0.9	87.3 $\pm$ 3.0	94.8 $\pm$ 5.4	27.8 $\pm$ 23.4
CD73+	99.6 $\pm$ 0.4	95.4 $\pm$ 2.7	99.8 $\pm$ 0.1	97.9 $\pm$ 0.1
CD90+,CD105+	93.2 $\pm$ 3.9	73.8 $\pm$ 23.4	64.6 $\pm$ 25.9	8.9 $\pm$ 12.4
CD90+,CD105+,CD73+, CD45-, CD34-,CD11b-, CD19- and HLA-DR-	99.4 $\pm$ 0.4	87.7 $\pm$ 14.9	99.1 $\pm$ 0.9	99.8 $\pm$ 0.2

Flowcytometry analysis of limbal and corneal fibroblasts showing expressionm of MSC markers:limbal fibroblasts showed positive expression of CD90,CD105,CD73 from P2 through P6 while corneal fibroblasts lost expression of CD105 as passage increased



Flow cytometric analysis showing higher percent CD90 and CD105 positivity in limbal fibroblasts (passage 6) compared to corneal fibroblasts

Further analysis of its efficiency to replace mouse fibroblast feeder layer has to be carried out. An understanding of the secreted factors present in the limbal niche will also help to improve the culture conditions of C ESCs and BM ESCs for ocular surface reconstruction.

Presentations

- Gowri Priya Chidambaranathan, Saumi Mathews, N Venkatesh Prajna, VR. Muthukkaruppan *Identification Of Mesenchymal Stem Cells In The Anterior Limbal Stroma*. Poster presentation in Indian Eye Research Group 20th Annual Meeting on July 28-29, 2012 at LV Prasad Eye Institute Hyderabad.
- Saumi Mathews, Gowri Priya Chidambaranathan, VR. Muthukkaruppan *Identification of CD 90 And CD 105 Positive Mesenchymal Stem Cells In The Anterior Human Limbal Stroma*. Poster presentation in International society for Stem cell Research 10th annual meeting, 13-16th June 2012, Pacifico Yokohoma, Japan.

Enrichment of human corneal epithelial stem cells (CESCs) to elucidate the expression of Δ Np63 isoforms in relation to stemness

Investigators : C. Gowri Priya, Aravind Medical Research Foundation, Madurai
VR.Muthukkaruppan, Aravind Medical Research Foundation, Madurai
Research scholar : Jhansi Rani Kasinathan
Funding agency : Department of Biotechnology, New Delhi

Background and aims

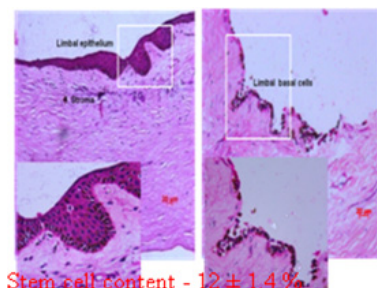
Understanding the molecular signature of CESC has been a challenge till date, mainly due to the lack of a specific marker for isolation and low stem cell (SC) content in the human limbal epithelial cell population analyzed. Though there is no known exclusive marker to CESC, a high expression of nuclear transcription factor - p63, specifically the Δ Np63 α isoform has been reported. By combining high expression of p63 in small cells with large N/C ratio, the team has established a specific method to identify and quantify CESC (Arpitha et al., 2005; 2008 a,b). Hence, to understand the molecular mechanisms associated with the maintenance of stemness, it is essential to have an enriched population of CESC. The current study aims to:

1. Establish a better method to enrich CESC from human donor limbal epithelium and
2. Elucidate the expression pattern of Δ Np63 isoforms in relation to stemness.

Enrichment of CESC to 80%

The corneal/limbal tissue from donor eyes (<65 years) were obtained from Rotary Aravind International Eye Bank, Madurai. A two stage enrichment of SCs was carried out (Figure). The limbal basal epithelial cells were isolated by differential enzymatic treatment as described by Arpitha et al. (2008a). Two parameter analysis using confocal microscopy identified $12 \pm 1.4\%$ of isolated limbal basal epithelial cells as SCs. Along with these SCs (negative for differentiation marker connexin 43) $3 \pm 1.4\%$ cells in the lower right quadrant (positive for Cx 43) also had high N/C ratio. Based on these data, selection of small cells with

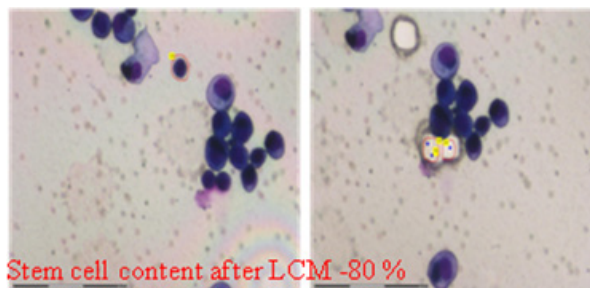
Step 1: Basal cell isolation



Step 2: LCM of cell > 0.7 N/C ratio

Before catapulation

After catapulation



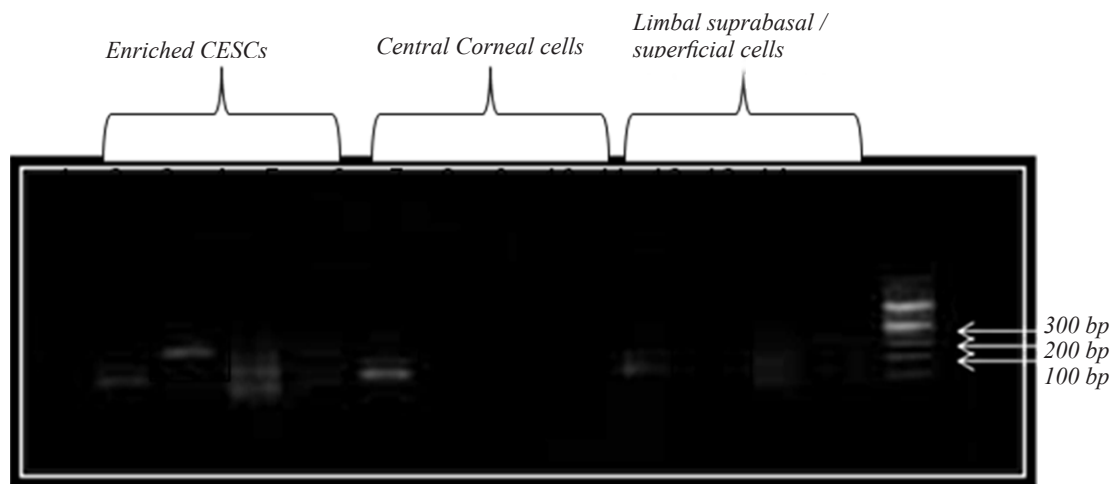
Enrichment of CESC. The CESC were enriched to 80% by isolation of limbal basal epithelial cells followed by laser capture microdissection of cells with N/C ratio >0.7.

N/C ratio >0.7 may yield a highly enriched population of CESC_s (80%). Hence, for the second stage of enrichment, cytopins of basal cells in a PEN membrane slide were Giemsa stained and cells with N/C ratio > 0.7 were catapulted using laser capture microdissection (LCM).

Unique expression of Δ Np63 α isoform in enriched CESC_s

RT-PCR analysis showed expression of Δ Np63 α only in enriched population of CESC_s but not in controls (differentiated epithelial cells of limbal suprabasal / superficial layers and central cornea). In contrast, beta and gamma isoforms were expressed both in enriched CESC_s and suprabasal/superficial cells but not in central corneal epithelial cells. The unique expression of Δ Np63 α in the enriched population of SC_s indicates that it may be implicated in the maintenance of stemness. Further studies are being carried out to understand the associated molecular mechanisms.

Semi-quantitative RT-PCR analysis for the expression of Δ Np63 isoforms in enriched CESC_s in comparison to differentiated cells. Expression of Δ Np63 α was observed only in enriched CESC_s while the Δ Np63 β and γ isoforms were expressed both in enriched CESC_s and suprabasal/superficial cells. None of isoforms were expressed in central corneal cells.



*L 1-Non Template Control; L 2, 6, 10 - β - actin - 180bp;
L 3, 7, 11 - Δ Np63 α - 299bp; L 4, 8, 12 - Δ Np63 - 100bp;
L 5, 9, 13 - Δ Np63 - γ - 290bp; L 14 - 100bp ladder*

TRANSLATIONAL RESEARCH

Knowledge gained through performing basic research becomes useful when it has practical applications that enhance human health and well-being. Based on the basic research on stem cells, transplantation of bioengineered stem cell rich autologous limbal/buccal epithelium for corneal surface reconstruction in unilateral/bilateral limbal stem cell deficient patients respectively is being carried out since 2007. A state-of-the-art class 1000 good manufacturing facility is being used for such ex-vivo expansion, the first of its kind in India for ophthalmic research. Recently, Aurolab launched Aurolab Aqueous Drainage Implant (AADI), a cost effective implant for developing countries, to divert the aqueous humor from the anterior chamber to an external reservoir for glaucoma patients. The current research is focused on (1) evaluation of AADI, for its surface free energy and cell adhesion property and (2) to evaluate whether there is an association between VEGF and outcome of glaucoma surgery.

Effect of VEGF levels in Tenon tissue on the outcome of glaucoma surgery

Investigators : C. Gowri Priya, Manju Pillai, SR. Krishnadas, VR. Muthukkaruppan
Research scholar : Ms. Sonam Lata
Funding agency : AMRF-Aurolab Research Grant

Background

One of the major causes for increased IOP even after trabeculectomy is excessive conjunctival scarring. Failure of the immune system to deactivate itself could play an important role in the development of chronic inflammation, where persistence of inflammatory cells continue to secrete cytokines and growth factors stimulating fibroblasts to produce scar tissue. In addition, vascular endothelial growth factor (VEGF) has been implicated with poor outcome of glaucoma surgery, particularly when its level was higher. Since Tenon fibroblasts are the main effector cells in the initiation and mediation of wound healing and fibrotic scar formation after glaucoma surgery, level of cytokines in the subconjunctival space or Tenon tissue may be more important in the wound-modulating process. In a recent study, the level of VEGF in Tenon's capsule was significantly higher in tissue of the failure group compared to the success group (Lopilly Park et al., 2012). Hence, the objectives of the current study are:

1. To estimate the levels of VEGF in Tenon tissue and aqueous humor of glaucoma patients in comparison with controls and
2. To check whether there is any correlation between VEGF levels and surgical outcome.

Sample Collection

Aqueous humor and Tenon tissue (4mm x 4mm) were obtained from 22 POAG patients during glaucoma surgery and from 19 cataract patients at the time of surgery. Informed consent was obtained from the recruited patients and the study was carried out after getting the approval of the Institutional Ethical Committee.

VEGF levels in aqueous humor and outcome of surgery

The VEGF concentrations in the aqueous humor samples were determined using an enzyme-linked immunosorbent assay (Quantikine ELISA Kit; R&D Systems) with appropriate standards. Detectable level of VEGF was observed in 7 POAG cases and 5 cataract controls. No correlation was observed between the VEGF levels and the outcome of surgery after a maximum of one year follow-up. Standardization of the protein extraction from Tenon tissue is underway to estimate the VEGF levels.

Evaluation of surface free energy of Aurolab Aqueous Drainage Implant (AADI) and its influence on cell adhesion property, in comparison with Baerveldt Implant

Investigators : C. Gowri Priya, Manju Pillai, K. Sivakumar,
VR. Muthukkaruppan, RD. Sriram, S.R. Krishnadas
Funding agency : AMRF-Aurolab Research Grant

Background

Glaucoma drainage implants have been used conventionally to drain the aqueous humor from anterior chamber to an external reservoir in glaucoma patients and long term success is limited by fibrous encapsulation of the base plate. Recently, Aurolab launched Aurolab Aqueous Drainage Implant (AADI), a cost effective non-valved glaucoma drainage device for developing countries. In a recent report, the surface topography of the drainage implants available in the market were compared with the outcome and increased roughness was associated with poor clinical outcome. Based on this, the current study was carried out to evaluate both the surface free energy of AADI and its influence on the adhesion of human Tenon fibroblast, and to compare it with Baerveldt implants.

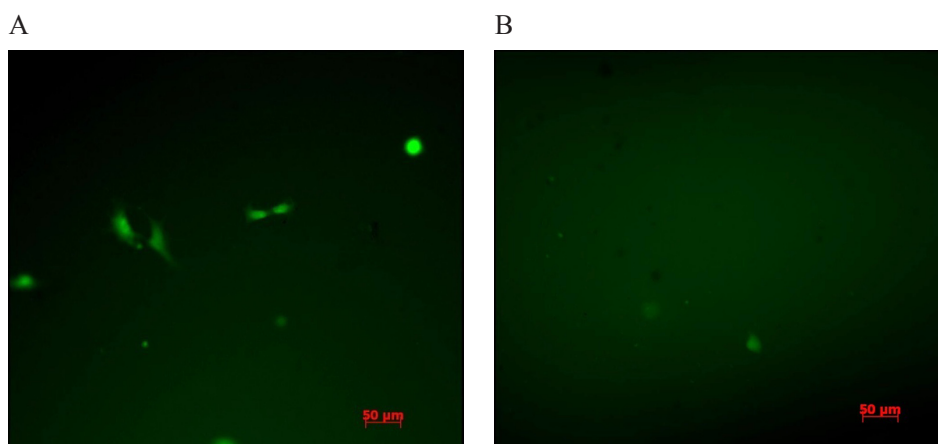
High surface free energy for AADI

The surface free energy of AADI and Baerveldt implants were measured using a contact angle meter with five different standards –water, formamide, diiodomethane, ethylene glycol and 1-bromonaphthalene. The surface free energy of AADI (15.8 mJ/m^2) was found to be higher than that of Baerveldt (13.2 mJ/m^2).

Higher cell adhesion property of AADI

The human Tenon fibroblast cultures were established using the Tenon's capsule obtained during routine cataract surgery from three donors, who had given prior written consent. The implants were attached to the culture dishes using single component silicone and tested for toxicity. After growing the fibroblasts in these dishes for 72 hours and staining with cell tracker green, their adhesion onto the implants was quantified in fluorescent microscope. Adhesion of Tenon fibroblasts corresponded well to the surface free energy and was more profound on AADI ($18 \pm 13 \text{ cells/ } 35 \text{ mm}^2$) while it was less on Baerveldt implants ($5 \pm 4 \text{ cells/ } 35 \text{ mm}^2$).

The above results indicate that due to higher surface energy and cell adhesion property, AADI might attract more fibroblasts and fibrous encapsulation compared to Baerveldt. However, the clinical outcome of using AADI had been successful (30 patients) with significant IOP reduction after 6 months of surgery. A long term follow-up of these patients is essential for further studies.



Fluorescence microscopic images of Tenon fibroblasts (cell tracker green stained) adhered onto (A) AADI and (B) Baerveldt implant. The number of Tenon fibroblasts adhered to AADI is more compared to Baerveldt ($p < 0.05$)

PROTEOMICS



The proteomics group at Aravind Medical Research Foundation (AMRF) laboratory is engaged in using proteomics to understand ocular diseases such as fungal keratitis, diabetic retinopathy and glaucoma which are widely prevalent in Indian population. Expression levels of proteins and the interaction of proteins at the cellular level helps in understanding the normal as well as disease process and progression. Understanding of the disease at the protein level will help assess the host response to the pathogen as well. Such knowledge will ultimately allow to optimize the treatment strategies for individual patient. The diagnosis of potential biomarkers may help in

understanding the population at risk, so that effective and early treatment strategies can be planned.

The laboratory focused on three specific areas namely fungal keratitis, diabetic retinopathy and primary open angle glaucoma. Fungal keratitis is a disease, most common in developing countries like India, Bangladesh, Nepal and China; hence knowledge gained will immensely benefit people from the lower socio economic group. The sheer importance of diabetic retinopathy and the lack of effective screening mechanisms for primary open angle glaucoma have prompted the department to concentrate on these two common eye diseases. The aim of the research is to apply a variety of proteomic tools to understand the host pathogen mechanisms and to identify new diagnostic, prognostic and therapeutic biomarkers for these diseases. Recently substantial grant was given by DBT-Govt. of India to establish a center of excellence in fungal keratitis, a programme that supports the study on human mycotic keratitis and the causative fungi and bacteria. The mechanism of pathogenesis would be studied using proteomics of both the host and the pathogen in the coming years.

Host pathogen interaction in human fungal keratitis

Investigators : N. Venkatesh Prajna, Dr. Lalitha Prajna, Chitra Thangavel & K.Dharmalingam
Research associates: Jeyalakshmi K, Jeya Maheshwari J, Partho Chatteraj, R.Sivasamy
Research scholars : S. Ananthi, R. Muthuselvam, R.Nithya, K.R.P. Niranjana, Charan Konakalla, M. Niveditha, Agalya Priyadharsini
Funding agency : 1. Department of Biotechnology. Govt. of India,
2. Indian Council of Medical Research, Govt. of India
3. AMRF

Suppurative keratitis is an important cause of ocular morbidity in India and other developing countries. The prevalence of fungi as etiological organism in causing this suppuration is steadily increasing. It is estimated that more than 50% of these suppurations are caused by fungi. The most common fungi implicated are *Fusarium* and *Aspergillus* species. The treatment outcome following fungal keratitis is highly variable due to host and pathogen factors. The thrust of the research here is to study the host factors such as tear proteins and infected corneal buttons, as well as the virulence and secretome profile of the pathogenic fungi. In order to understand the mechanism of pathogenesis of the fungi in the human cornea, host pathogen interaction using in vitro model cell lines has been initiated. HeLa cell lines have been used as a model to optimize all the culture and infection conditions which would be extended to HCE cell lines.

FUNGAL PATHOGENS AND PATHOGENESIS

Part A

Comparative analysis of the exoproteome profile of *aspergillus flavus* clinical isolates (CI) and ATCC strains

The secreted proteins of three different clinical isolates and an ATCC strain were analyzed using high resolution two-dimensional polyacrylamide gels electrophoresis (2D-PAGE) on 24 cm IEF gels in the pH range of 4-7, followed by second dimension in a 24 cm SDS-PAGE. Strains were selected based on their in vivo virulence assay using *Galleria mellonella*, and samples were prepared as per the standardized protocol mentioned in the earlier report.

Proteomic profile of various CIs and ATCC were compared with each other using Image Master Platinum software (GE Health Care) which revealed the differential regulation of proteins (Fig 1). Experiments done in duplicates were compared. Among the identified hundred spots, data pertaining to significantly regulated seventeen protein spots are given below.

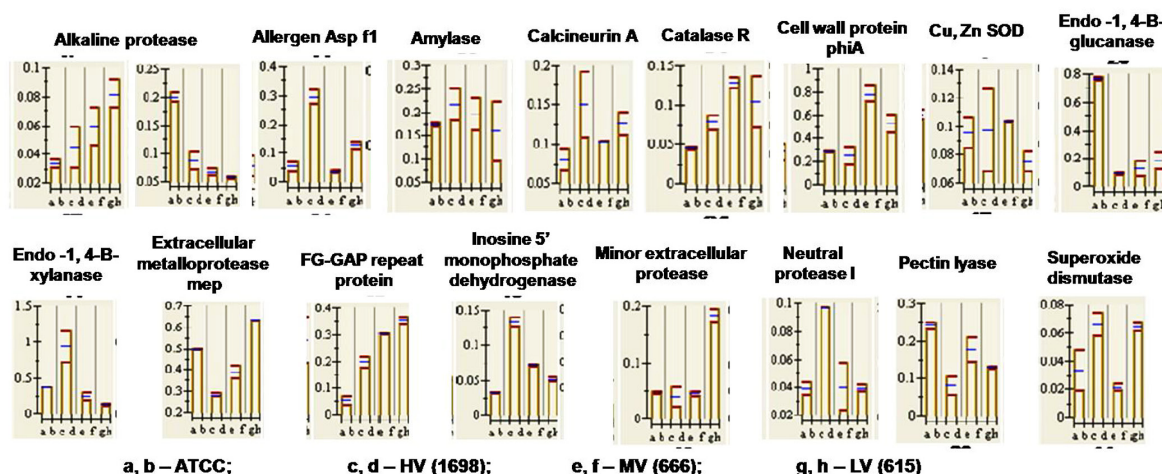


Figure 1: Histograms of differentially regulated secreted proteins of *A. flavus* isolates. Histograms showing expression level of proteins in different isolates of *A. flavus*. Along with the ATCC (a, b), clinical isolates were classified as HV- high virulent (c, d), MV- Moderate virulent (e, f) and LV- Low virulent (g, h) based on the in vivo assay. Experiments were done in duplicates and the bars were depicted with error bars.

Isoforms of secreted alkaline protease

Majority of the secreted proteins were extracellular proteases, and this was also confirmed by quantitative analysis using azocaseinase assay. Among the secreted proteases, alkaline protease constitutes 14% of the total secreted proteins of *A. flavus*. Alkaline protease was identified using LC-MS/MS analysis. More than one protein spot was identified as alkaline protease and there are isoforms (spots, 19, 20 and 21) that differ only in their pI (Fig 3). These will be validated using 2D western analysis.

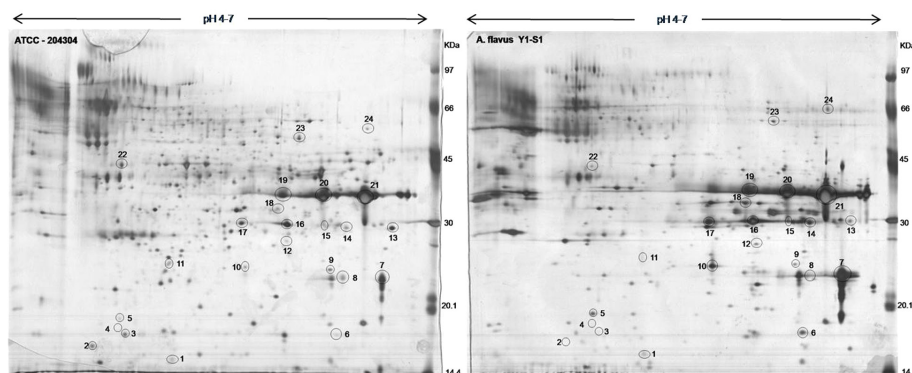


Figure 2: Identification of the multiple forms of alkaline protease of *A. flavus* isolates

The secreted samples (40µg) of *A. flavus* ATCC and Clinical Isolate Y1-S1 was electrophoresed in a 24cm linear IPG strip of pH 4-7 for 1D separation and in 12.5% polyacrylamide gels for 2D separation. Gels were stained with glutaraldehyde silver and the identified alkaline protease spots were assigned with number. Marker shows molecular weight of proteins in kDa. Numbered spots were identified using Mass spectrometry.

Part - B

Analysis of aspergillus fumigatus atcc and clinical isolates(cis)

Identification of the A.fumigatus species

A.fumigatus isolated from fungal keratitis infections are identified mainly by microscopy and by examination of the growth characteristic features in solid media in clinical labs. In this study further verification of fungal strains could be done by using molecular identification methods such as fungal specific PCR amplification targeting ITS region and rodA gene polymorphism by gene sequencing. *A.fumigatus* strains used in this study were obtained from the keratitis patients attending Aravind Eye Hospital, Madurai. American Type Culture Collection (ATCC-204305) of *A. fumigatus* was used as control.

Identification of *A.fumigatus* was carried out using the single nucleotide polymorphism specific for the *A.fumigatus*.

12 clinical isolates were identified as *Aspergillus fumigatus*. Clinical isolate 8147, was found to be *Aspergillus lentulus*.

I Analysis of the exoproteome profile of aspergillus fumigatus

SDS-PAGE analysis of secreted proteins revealed differential expression of 14 proteins among the clinical isolates, as well as between ATCC and clinical isolates. These proteins were further subjected to tryptic digestion and mass spectrometry. Among the fourteen differentially expressed proteins, the following proteins catalase, alkaline protease, α-glucosidase, FAD-Dependent oxygenase, Dipeptidylpeptidase, FG-GAP repeat protein, Mn superoxide dismutase and cell wall PhiA were taken for further analysis. Out of 14 proteins, two proteins Mn-SOD and FGAP proteins were found to be significantly upregulated in the clinical isolates compared to ATCC.

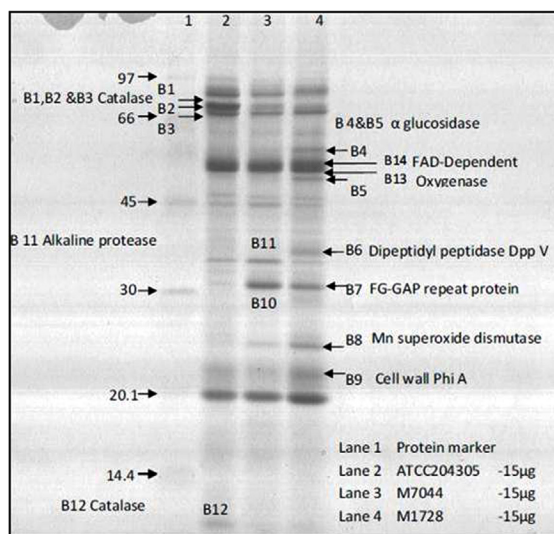


Fig 3: 1D PAGE analysis of secreted proteins of *A.fumigatus*: Proteins showing differential expression are indicated in the figure

STUDY OF HOST RESPONSE TO THE FUNGAL INFECTION

Part A.

Characterization of Tear Proteome

Tear protein profile of *Fusarium* keratitis patients were examined during 2012-13. *Fusarium* is the major causative agent of fungal infections leading to corneal ulcer (keratitis) in India and other tropical countries. Keratitis caused by *Fusarium* is a difficult disease to treat unless antifungal therapy is initiated during the early stages of infection. In this study, tear proteins prepared from keratitis patients were classified based on the duration of infection. Proteins were separated by differential gel electrophoresis (DIGE) and the differentially expressed proteins were quantified using DeCydersoftware analysis (Fig 4). The following

differentially expressed (PlosOne.Volume8 | Issue 1 | e53018) proteins namely alpha-1-antitrypsin, haptoglobin a2 chain, zincalpha- 2-glycoprotein, apolipoprotein, albumin, haptoglobin precursor - b chain, lactoferrin, lacrimal lipocalin precursor, cystatin SA III precursor, lacritin precursor were identified using mass spectrometry. Variation in the expression level of some of the proteins was confirmed using western blot analysis. This is the first report to show stage specific tear protein profile in fungal keratitis patients. Validation of this data using a much larger sample set could lead to clinical application of these findings.

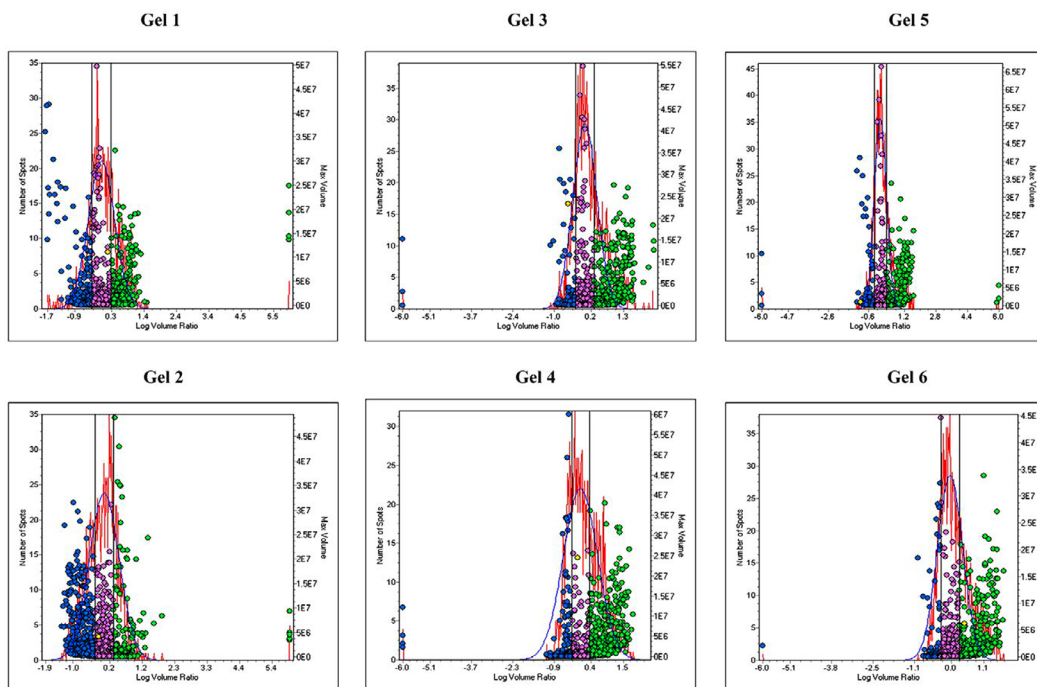


Fig 4. Discrimination of differentially expressed proteins in six gels. The histograms were generated by DeCyder DIA software, plotting spot frequency (left y-axis) against log volume ratio (x-axis), and a normal distribution model (red line) was fitted to the main peak of the frequency histogram. A 2 fold variation in expression level was set as threshold. The threshold values are represented by vertical black lines in the histogram, and the differentially expressed spot features are shown as blue spots (volume decreased) and green spots (volume increased).

This study has been published in Plos One (January 2013 | Volume 8 | Issue 1 | e53018).

Characterization of corneal proteome

Diseases of the cornea are extremely common and cause severe visual impairment worldwide. The present study characterizes the proteome of the diseased cornea obtained from fungal keratitis patients and compared them with normal human cadaver corneas which served as controls.

The corneal sample preparation and protein profiling were optimized using cadaver corneas. The corneal protein extraction was performed with liquid nitrogen chilled mortar and pestle which was reported for rat corneal samples but found to be ineffective to human corneas. Among the various procedures used for corneal sample preparation, protein extraction in presence of liquid nitrogen was observed to be efficient. Similarly protein precipitation techniques using solvents and cut off membranes was found to be ineffective as it accompanied with protein loss, hence neat corneal proteins without any preprocessing was found to be the best method for corneal proteomic analysis. 2D map of human corneal proteins is shown in the figure below.

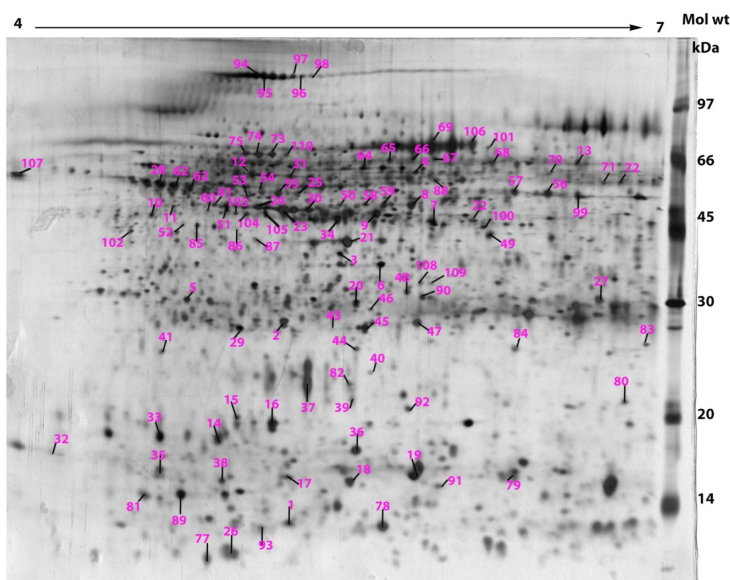


Fig 5. Cornea from a *Fusarium* keratitis patient was analyzed on 2D PAGE and the identified spots were labeled.

In this study EASY-nLCmicroTOF-Q was used for corneal protein identification from 2D PAGE analysis. A total of 110 corneal proteins could be identified with respect to *Homo sapiens* taxonomy and with the fungal taxonomy though three proteins were identified experimental pI and molecular weight were not matching for protein identity and so they were not considered as significant identification. As fungal proteins were not identified, it gives the clue that active fungal components might be absent in the late stage of the infection.

STUDY OF HOST PATHOGEN INTERACTION IN INVITRO MODEL CELL LINE

HeLa is a non-phagocytic (epithelial) cancerous cell line of the human cervix. It was chosen as a model cell line for studying infectomics of host-pathogen interaction, for (a) optimizing cell culture conditions, (b) optimizing mass spec platform and (c) comparing analytical sensitivity with published literature.

HeLa cells were grown in DMEM growth medium supplemented with 10% heat-inactivated fetal bovine serum. A total of 5×10^6 cells were plated and infected with 1×10^8 germinating spores of *Aspergillus flavus* Y1S1 (clinical strain) at a moi of 20. Both infected and uninfected cultures were incubated for 24 hours at 37°C, 5% CO₂ culture conditions. Cells were then scraped out of culture flasks, washed several times with phosphate buffered saline and used for 1D or 2D gel electrophoresis.

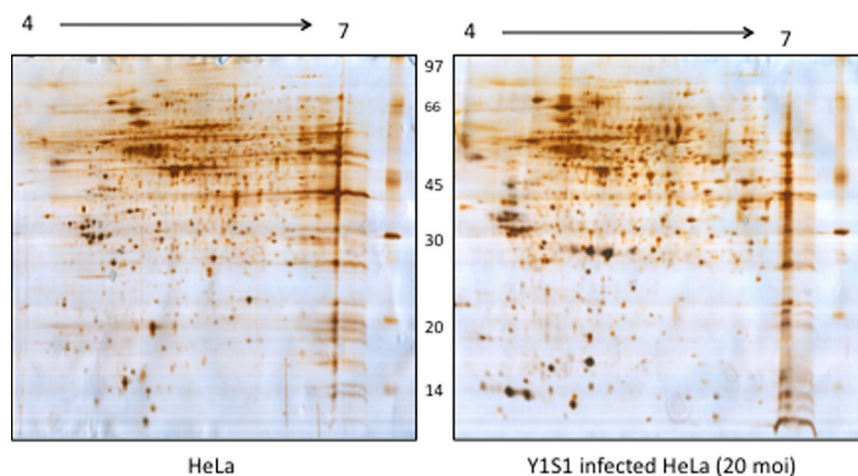


Figure 6: 2D protein profile of HeLa and Y1S1 infected HeLa samples

2D protein profile of infected HeLa is significantly different from the control HeLa as well with the exo-proteome of *A. flavus* Y1S1 when the latter is cultured in *Aspergillus* minimal medium. A detailed study is planned to understand the significance of this differences.

Publications

- Sivagnanam Ananthi, Namperumalsamy Venkatesh Prajna, Prajna Lalitha, Murugesan Valarnila, Kupppamuthu Dharmalingam “Pathogen Induced Changes in the Protein Profile of Human Tears from Fusarium Keratitis Patients” PLoS one 2013;8 (1):e53018. Epub 2013 Jan 8.

The Identification of Biomarkers for Primary Open Angle Glaucoma

Investigators : S.R. Krishnadas, P.Sundaresan, C. Thangavel and K.Dharmalingam,
Research associate : Dr. Jeya Maheshwari
Research scholar : R. Sugumar
Funding agency : Department of Biotechnology, Govt. of India

Primary Open Angle Glaucoma (POAG) is the second leading cause of blindness worldwide, contributing to 12% of world blindness and its prevalence in South India is around 1.6% in rural and 3.5% in the urban population. It is asymptomatic and the loss of vision is irreversible. Primary open angle glaucoma refers to a chronic optic neuropathy with no known ocular or systemic disorder. An elevated intraocular pressure owing to resistance to outflow of aqueous through the trabecular meshwork is believed to be the most significant causative risk factor in the pathogenesis of the disease. Aqueous humor (AH) contains proteins secreted from anterior segment tissues and is closer to the site of damage in glaucoma and it is believed to play a vital role in the pathogenesis of POAG. Identification of abnormal aqueous proteins could aid in understanding the patho-physiology of glaucoma, and facilitate evolution of better therapeutic agents in management of glaucoma. Age matched (Male > 50) AH samples were collected during anterior chamber surgery for this study.

Proteome analysis of aqueous humor (AH) in the case of POAG shows differential regulation of proteins across different stages such as mild, moderate and severe when compared to control cataract. Among these two proteins such as Transferrin and Prostaglandin, D synthase shows distinct variation in AH of POAG. Interestingly the alterations in the expression of isoforms - transferrin were observed in POAG samples when compared to that of control, which is taken for validation in large samples by western blot using transferrin specific antibody.

OCULAR PHARMACOLOGY

The main focus of research in this department is on age-related ocular diseases such as Diabetic Retinopathy, Age-related Macular Degeneration, Glaucoma and cataract.

India being the diabetes capital, the ocular complications related to this disease is of major health threats to the country. Diabetic Retinopathy (DR) is a micro vascular complication of retina leading to vision loss. Understanding and identifying newer drug targets would improve the treatment modalities for the blinding disorder as a consequence of diabetes. The department carries out research to understand the interplay between polyol pathway and angiogenic factor and its regulation in retinal pigment epithelium (RPE) using in vitro model system. This study will give insight into the factors that perturb the balance between angiogenic and anti-angiogenic factor in the susceptible eye tissues.

Age-related macular degeneration

Accumulation of A2E, a major fluorophore of lipofuscin in human retinal pigment epithelium is reported to be one of the causative factors in the pathogenesis of AMD. The accumulated A2E acts as photo-sensitizer causing damage and cell death in the macula leading to vision loss. Rich intake of eye anti-oxidants (lutein and zeaxanthin) is reported to protect macula from blue light induced oxidative damage. Therefore, the department is interested to investigate the levels of a2e and macular xanthophylls in Indian Eyes using LC/MS/MS. This will give insight into the understanding of the normogram of macular carotenoids and its protective role in preventing the accumulation of A2E in the pathogenesis of AMD.

Glaucoma

Currently available anti-glaucoma drugs are being targeted to lower IOP and do not act on the affected tissue, the trabecular meshwork. The department has recently established an ex vivo model system “Human Organ culture Anterior Segment (HOCAS)” for screening drugs with potential anti-glaucoma property using human donor eyes which is beneficial to investigate new class of drugs for glaucoma.

Cataract

India accounts for 20% of global burden of blindness due to cataract. Earlier studies of the department demonstrated that the low plasma ascorbate and other eye antioxidants may be associated with high prevalence of age-related cataract in Indian population. At present, the department is interested to study the relationship between haptoglobin genotype and low vitamin C in South Indian cataract patients. This will give insight into whether oral supplementation of vitamin C would be beneficial in preventing/delaying the progression of cataract.

Profiling of ALR expression in retinal tissues from human diabetic donor eyes

Investigator	:	Dr. S. Senthil Kumari
Senior research fellow	:	R. Sharmila
Funding Source	:	Department of Biotechnology (DBT) – RGYI (2011 -2013)

Background and Aims

Diabetic retinopathy (DR) is a vascular disorder affecting the microvasculature of the retina. In India with the epidemic increase in type 2 diabetes mellitus as reported by the World Health Organization (WHO), Diabetic retinopathy is fast becoming an important cause of visual disability. Among the various pathogenic mechanisms reported for DR, the polyol pathway is believed to play a central role.

In cellular glucose metabolism, a small percentage (3%) of glucose is metabolized through the polyol pathway whereas in diabetes, more than 30% of glucose fluxes through this pathway. Aldose reductase

(ALR) is the first and the rate limiting enzyme of polyol pathway which reduces glucose to sorbitol, which is then converted to fructose by sorbitol dehydrogenase (SDH). The excessive build-up of sorbitol in cells is thought to cause osmotic damage to the retinal vascular cells leading to DR. Despite three decades of intense investigations, including some clinical studies, the details of ALR mediated hyperglycemic injury remain unclear. In particular, the mechanism which controls and regulates the expression of ALR gene and the catalytic activity of ALR protein is poorly understood.

Most of the research on the pathophysiology of DR has been focused on the impairment of neural retina (Inner Retinal Barrier), whereas the effect of diabetes on the Retinal Pigment Epithelium (Outer Retinal Barrier) is poorly understood. RPE secretes many of the growth factors which are responsible for

the structural and functional integrity of neural retina. Therefore understanding the role of ALR in RPE is essential to find the interplay between polyol pathway and secretion of VEGF.

Studies at the department have previously reported that ARPE-19 cells incubated with high glucose (25mM) showed 50 fold increases in ALR expression as compared to 5mM glucose. However, no significant change was observed in VEGF secretion at 24hrs.

To validate the previous findings, the team hypothesized whether hyperglycemia induces high ALR expression in human diabetic retinal tissues. So the objective framed was,

- To quantify the ALR expression in Diabetic Neural Retina (N. Retina) and Retinal Pigment Epithelium (RPE) from human donor eyes using qPCR

Methods

Donor eyes (N=24; 12 - Diabetic with known diabetic history and 12- Non-Diabetic as control) were collected from Rotary Aravind International Eye Bank, Aravind Eye Hospital, Madurai. Eyes with visible ocular pathology were excluded from the study. The mean age for non diabetic and diabetic donor is 72.83 ± 10.26 and 73.16 ± 9.94 (Mean + S.D) respectively with mean diabetic duration of 14.5 ± 9.5 years.

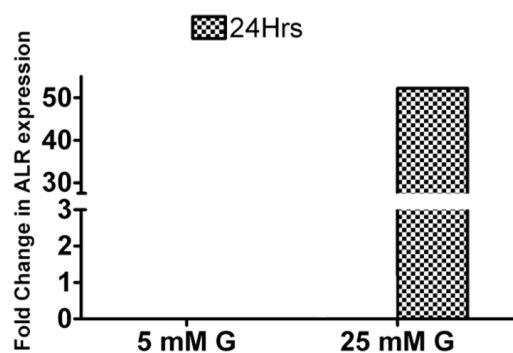
The neural retina and RPE from same donor were collected. All the samples were stored at -80°C till analysis. Total RNA was isolated from N. Retina using TRIzol Reagent (Sigma, USA) and RPE using RNeasy Mini kit. cDNA template from total RNA was synthesized using cDNA Reverse Transcription Kit. ALR expression was quantified by SYBR green chemistry. The cycle parameters consisted of an initial denature step of 95°C for 10 minutes followed by 40 cycles of 95°C for 15 seconds and 60°C for 60 seconds.

Results

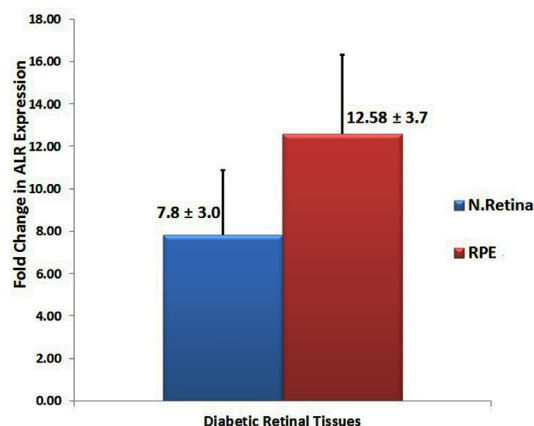
Time between enucleation and sample collection for non diabetic and diabetic cadaver eyes is 17.37 ± 8.48 and 11.76 ± 5.12 (Mean + S.D) in Hrs respectively.

Quantification of ALR expression in diabetic retinal tissues

Diabetic neural tissue and retinal pigment epithelial tissue showed 7.8 fold and 12.58 fold increase in ALR Expression respectively when compared with control.



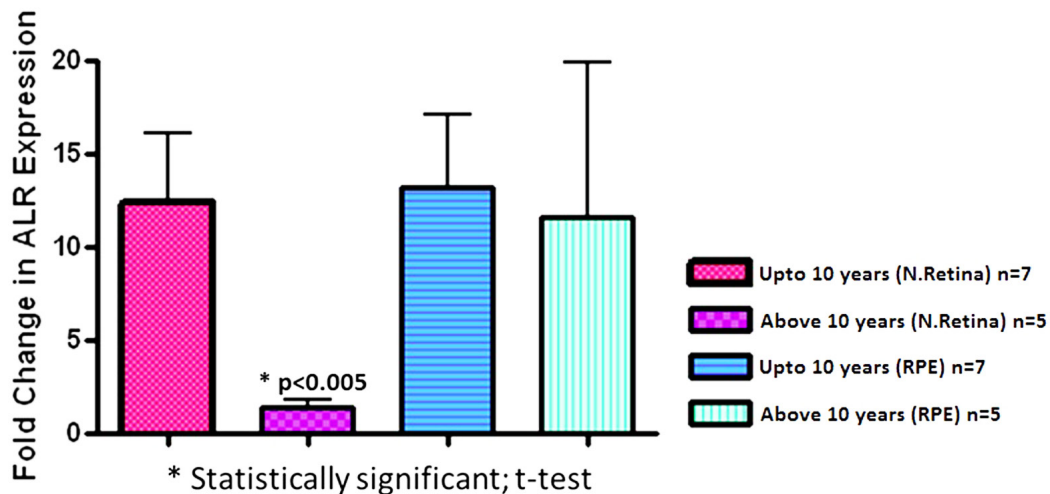
ALR Expression in ARPE19 Cells Challenged with Normal (5mM) & High Glucose (25mM)



Quantification of ALR expression in diabetic retinal tissues

Profiling of ALR Expression based on Diabetes duration

- Donor tissues with 10 years diabetic duration showed high ALR expression in N.Retina as compared to donor tissues more than 10 years duration.
- ALR expression in RPE did not show any significance based on diabetic duration.



Profiling of ALR Expression based on Diabetes duration

Summary

High ALR expression was observed in both diabetic N. Retina and RPE as compared to Non-diabetic control tissues. Donors with 10 years diabetic duration showed significantly high ALR expression in N. Retina as compared to donors with more than 10 years of diabetes. This study further confirms that polyol pathway may contribute to the pathogenesis of Diabetic Retinopathy. Further studies are underway to investigate the cross-talk between polyol pathway and VEGF up regulation.

A pilot study to understand the relationship between vitreous and plasma ascorbate in non-diabetic patients undergoing vitrectomy

PI : Dr. S. Senthilkumari, Scientist, Dept. of Ocular Pharmacology, AMRF
Co-investigators : Dr. P. Sundaresan, Senior Scientist, Dept. of Genetics, AMRF
Dr. Naresh Babu, Retina Clinic, AEH, Madurai
International : Prof. David Beebe, Dept. of Cell Biology & Physiology, Washington University,
Collaborators St.Louis, Missouri
Prof. Astrid Fletcher, London School of Hygiene & Tropical Medicine, London.
Funding agency : Aurolab Research Grant

Background

Under normal physiologic conditions; the human lens exists in an environment with very low oxygen partial pressure. The oxygen diffuses from the retinal vasculature into the vitreous gel near the surface of the retina. The main functions of the vitreous gel are to maintain the volume of the globe and provide optically clear media. It also consumes oxygen in an ascorbate dependent manner and thus serves to maintain lower levels of molecular oxygen near the lens. This is how the vitreous offers protection against oxidative stress.

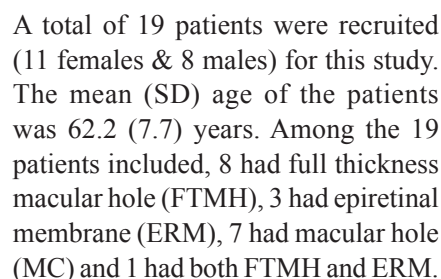
The consumption of oxygen by vitreous gel is altered due to liquefaction of the vitreous as it can occur as a result of aging or due to vitrectomy surgery. When the vitreous liquefies, oxygen diffusing from the retinal vessels is readily carried away and distributed throughout the eye by fluid currents generated by convection and movement of the eyes or head. The more that oxygen is mixed with the vitreous fluid, the

Objectives

- ## Methods

In an operation suite, 0.2-0.3 ml vitreous fluid was collected at the time of vitrectomy and 3-5 ml blood was also collected for plasma. The collected samples were kept on ice and brought to the laboratory and stabilized with 10% metaphosphoric acid (MPA). The MPA stabilized samples were stored at -80° C till analysis by HPLC.

Results



Of the 19 patients, 15 had data on both plasma and vitreous ascorbate, 4 had data only on vitreous ascorbate. Of the 15, the mean (SD) of plasma and vitreous ascorbate were 32 (17) $\mu\text{mol/L}$ and 1448 (653) $\mu\text{mol/L}$ respectively.

Summary

33

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

Indian Investigators	: Dr. S .Senthilkumari, Scientist II, Department of Ocular Pharmacology, AMRF, Madurai, Dr. R Krishnadas, Consultant, Glaucoma Clinic, Aravind Eye Care System, Madurai
US Collaborators	: Paul Kaufman, Professor & Chair, Department of Ophthalmology & Visual Sciences, University of Wisconsin, Madison B'Ann Gabelt, Distinguished Scientist, Department of Ophthalmology & Visual Sciences, University of Wisconsin, Madison
Funding Agency	: Aravind Eye Foundation (AEF), New York, USA
Project Fellow	: K. Cholaraja

Background

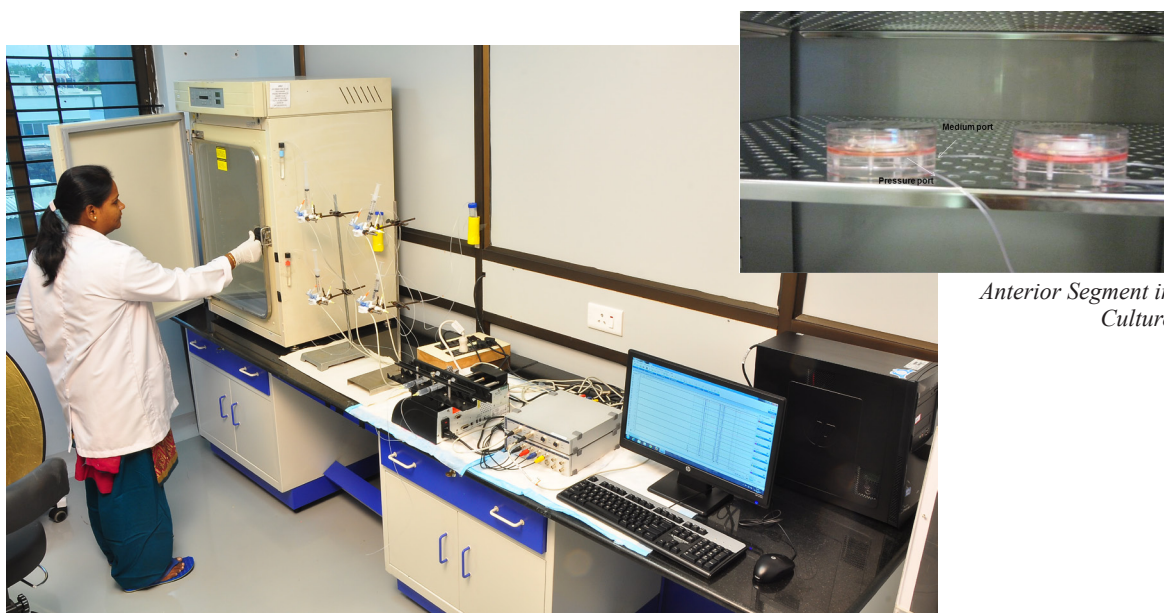
The organ culture anterior segment system has been developed as an intermediate step between cell culture and whole animal studies for modulating trabecular meshwork (TM) outflow facility (OF) using investigational drugs. This system allows direct experimentation of candidate drugs on human/bovine/pig and monkey eyes and thus provides direct and meaningful information about the candidate drugs for glaucoma. Therefore, the purpose of the present study was to establish HOCAS and its responsiveness to actin monomer-binding cytoskeletal drug, Latrunculin B (Lat B).

Specific Aims

1. To establish the Human Organ Perfusion Anterior System
2. To determine the IOP and outflow facility responses to Lat B

The entire globes of human donor eyes not suitable for corneal transplantation were obtained from the Rotary Aravind International Eye Bank, Aravind Eye Hospital, Madurai and were handled in accordance with the Declaration of Helsinki. The donor eyes were enucleated within 2hrs of death (Mean elapsed time between death and enucleation was 1.3 ± 0 hrs), kept at 4°C and cultured within 30hrs (Mean elapsed time between enucleation and culture 27.8 ± 0.7 hrs) for the study.

Baseline outflow facility (OF) was measured in paired eyes mounted onto a specially designed Petri dish. Pressure within the organ culture was monitored with pressure transducers (Isotec; Harvard Apparatus, Holliston, MA) connected to amplifiers (Quad Bridge Amplifier; AD Instruments) and the resulting data transferred to a computer as shown in the figure below.



Anterior Segment in Culture

After baseline equilibration (~2 days), one eye of each pair received 2 μ M Lat B and the contra lateral eyes received vehicle. Perfusion was continued with medium containing Lat B or vehicle after anterior chamber exchange. The intraocular pressure (IOP) was monitored for 24-48 hrs post treatment and the eyes were then fixed by perfusion using 4% para formaldehyde for assessing the aqueous outflow of tissues using light microscopy.

Effect of Lat B on Aqueous Outflow in HOCAS

A total of 9 pairs of human eyes were used to study the effect of Lat B in HOCAS. Out of 9 pairs, six pairs of eyes were discarded due to unstable or asymmetric baseline, unacceptable tissue morphology and hence 3 pairs were used to study the effect of Lat B on HOCAS.

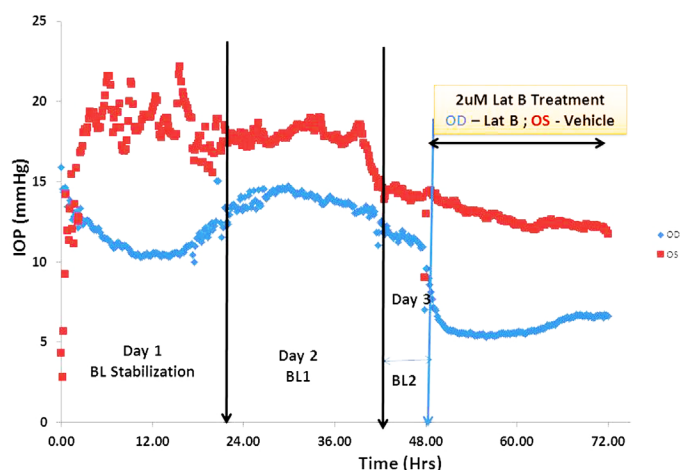
Perfusion with 2 μ M LatB showed a rapid and significant increase in outflow facility in human eyes and its net drug outflow is given in the table below.

Effect of 2 μ M lat B on human aqueous outflow facility

Drug	Outflow Facility (μ l/min/mmHg) (N=3)		OF Ratio	% Δ OF
	(Treated)	(Control)		
BL	0.21 $\pm$ 0.07	0.21 $\pm$ 0.02	0.96 $\pm$ 0.30	-4.0
Rx-3hrs/BL	1.71 $\pm$ 0.44	1.16 $\pm$ 0.15	1.46 $\pm$ 0.24	46.1
Rx-12hrs/BL	1.86 $\pm$ 0.63	1.07 $\pm$ 0.11	1.72 $\pm$ 0.42	71.7
Rx-24hrs/BL	1.81 $\pm$ 0.68	1.04 $\pm$ 0.34	1.70 $\pm$ 0.48	70.3

In HOCAS, 2 μ M Lat B caused 46% , 72% and 70% increase in outflow facility as compared with baseline at 3hrs, 12 hrs and 24 hrs post exchange (n=3; p<0.01; Table 2) respectively. There was a slight increase in the outflow facility in control eyes that was not statistically significant at 12 hrs and 24 hrs. Representative IOP profile after Lat B treatment is shown in the figure below:

IOP Profile after 2 μ M Lat B Treatment



IOP profile after 2 μ M Lat B Treatment: The anterior segments were allowed for stabilization until stable baseline was obtained. After that, all right eyes received drug treatment and left eyes received vehicle. A significant reduction of IOP was observed after Lat B treatment.

Summary

HOCAS facility was successfully established at AMRF and validated with known F- actin disruptor, Lat B. Lat B treatment showed 70% reduction in outflow facility at the studied concentration.

Work in progress

Morphological Analysis of anterior segment after Lat B treatment is being analyzed using Transmission Electron Microscopy (TEM). The effect of Y27632 (Rho kinase inhibitor) on human aqueous humor outflow facility is under investigation.

BIO INFORMATICS

The Bioinformatics Centre has been established at Aravind Medical Research Foundation (AMRF) in 2012 to carry out research in bioinformatics and computational biology and to aid translational vision research. Bioinformatics is an emerging field between biological and computational sciences, which has become essential part in vision research today. The necessity of Bioinformatics in medical research is to understand the biological processes at the molecular level.

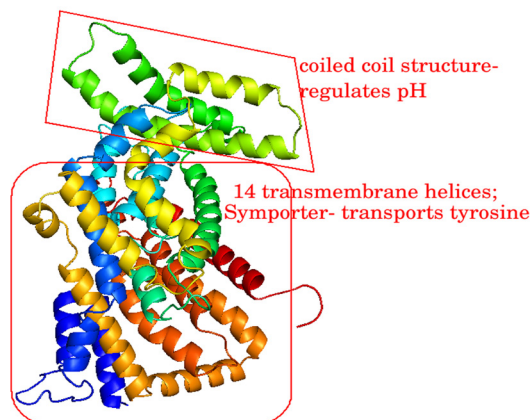
The main focus of the Bioinformatics centre is to provide state-of-the-art computational methods involving genome analysis, mutational analysis, protein structure-function analysis, protein-protein interactions, evolutionary analysis and data analysis of next-generation sequence data, which would help to understand the pathogenesis of eye diseases. Currently the centre has been developing a structure-based bioinformatics approach to understand the molecular mechanism by which the mutations lead to genetic eye diseases such as Oculocutaneous Albinism Type II and Retinitis Pigmentosa. This analytical approach will help to create a platform to understand the pathogenesis of all other genetic eye diseases that are common in India.

Theoretical model building and molecular analysis of OCA2 gene mutations

Investigator : Dr. D. Bharanidharan
Research scholar : P. Logambika
Funding source : Aravind Medical Research Foundation (AMRF)

OCA2 gene encodes the human homologue of the mouse p (pink-eyed dilution) gene. The encoded protein is believed to be an integral membrane protein involved in small molecule transport, specifically tyrosine - a precursor of melanin. It has also been suggested that it might regulate melanosomal pH, or regulate glutathione metabolism. Mutations in this gene results in type 2 oculocutaneous albinism. Several recent studies have applied Bioinformatics methods to predict potentially deleterious effects of missense mutations. Many of them are based on protein sequence, but several are structure-based, as the latter are more reliable and provide more information. A model of the so far elusive three-dimensional structure of oca2 provides insight into its molecular functions. Further the structure can be used to study the effect of disease-related mutations and to predict their potential molecular pathogenic effect.

As there is no significant template by sequence alignment through BLAST search, an alternative approach of ab initio method that uses fragment libraries and threading method that helps in identifying the correct three dimensional fold were chosen. Rosetta 3.4 - membrane ab initio module was used for ab initio modelling. Using threading meta servers I-Tasser, Genesilico and RaptorX, threading based models



3D structure of OCA2 by ab initio modelling

were generated. Structures with best scores from different threading servers were selected as templates for comparative modelling. ModellerV9.11 was used for comparative structure modelling. The structures from ab initio modelling and comparative modelling were superimposed. Structures with lowest RMSD values were considered as the best model of OCA2. The best ab initio model is shown in the figure, functional parts are highlighted in Red.

This study gives a preliminary insight into the structure of OCA2 and into the structural effects of associated mutations. This can be useful to predict the potential effect of each single mutation, to devise new biological experiments and to interpret the biological significance of new mutations.

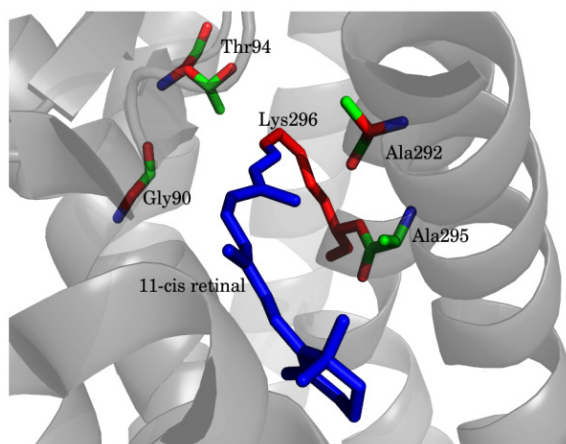
Structure-Based Bioinformatics approach to the analysis of nsSNVs in RHO and RPE65 genes, and Prediction of their associations with disease phenotypes

Investigator : Dr. D. Bharanidharan
 Research scholar : P. Logambika
 Funding source : Aravind Medical Research Foundation (AMRF)

Retinitis pigmentosa (RP) is an eye related disorder that causes progressive vision loss. It affects the retina, which is a layer of light-sensitive tissue at the back of the inner eye. Retina converts light images to nerve signals and sends them to the brain. Mutation at RHO and RPE65 may cause RP. Apart from RP, mutation in RHO and RPE65 may also lead to Congenital Stationary Night Blindness (CSNB) and Lebers Congenital Amaurosis (LCA) respectively. Since there is no clear genotype-phenotype correlations for RP, Bioinformatics approaches have been widely used to understand the disease mechanism.

List of missense mutations related to RP were collected from Swiss-var and dbSNP database. For performing molecular level analysis the 3D structural information is necessary. Since, there is no experimental structure for human RHO and RPE65, the comparative modelling approach has been used to generate model structures. The changes in protein stability and functional sites upon mutation were analysed by various structure-based bioinformatics tools. In addition, the conservational score has been included in the analysis to increase the prediction accuracy.

The bioinformatics approach to the analysis of patient-specific missense mutations in RHO gene has shown the molecular causes of CSNB and RP. The patient-specific missense mutations (G90D, T94I, A292E, A295V) identified in CSNB may affect the 11-cis retinal binding with RHO (as shown in the figure). And these mutations are less likely to affect the protein stability whereas the missense mutations which affects the protein stability and functional sites of RHO may cause RP. The molecular analysis of missense mutations that affect the structural stability and function of RPE65 is being studied for its associations with disease phenotypes.



11-cis retinal (blue) in the ligand binding pocket of RHO linked through Schiff base with Lys 296 (red) and surrounding residness

CONFERENCES ATTENDED

US ARVO 2012

Fort Lauderdale, Florida, USA, May 6-10

Dr.P.Namperumalsamy, Chairman-Emeritus led a group of young Scientists to attend US ARVO meeting held in Fort Lauderdale, Florida, USA from May 6-10, 2012. He presented the overall research activities at Aravind at the Indo-US collaborative vision research program.

Lalan Kumar Arya, Prasanthi Namburi received the 2012 ARVO Foundation Developing Country Eye Researcher (AFER) Travel Fellowship. Dr.Senthilkumari, J.Lakshmi priya, K.Renukadevi and P.Murugeswari have received the support from Indian Council of Medical Research, Department of Biotechnology, IERG-ARVO India Chapter award and AMRF.

Poster presentations

DR. P.SUNDARESAN

- *Genetic carrier screening for oculocutaneous Albinism in India*

DR. S.SENTHILKUMARI

- *Elucidating the correlation between the levels of Macular Xanthophylls and A2E in normal Indian donor eyes*

N. PRASANTHI

- *Evaluating the role of myocilin and optineurin genes in familial primary open angle glaucoma*

K. RENUKADEVI

- *Prenatal molecular diagnosis of fetal sample*

LALAN KUMAR ARYA

- *Molecular based-identification of procerovum varium (trematode: Heterophyidae) in human eye causing granulomatous uveitis in children of South India*

LAKSHMI PRIYA

- *Characterization of pseudomonas aeruginosa type three secretory system (TTSS) effector molecules (Exo U/S/T) from human corneal ulcer*

P. MURUGESWARI

- *Secretion of vascular growth factors/inflammatory cytokines by Rpe is inhibited by Ranibizumab*

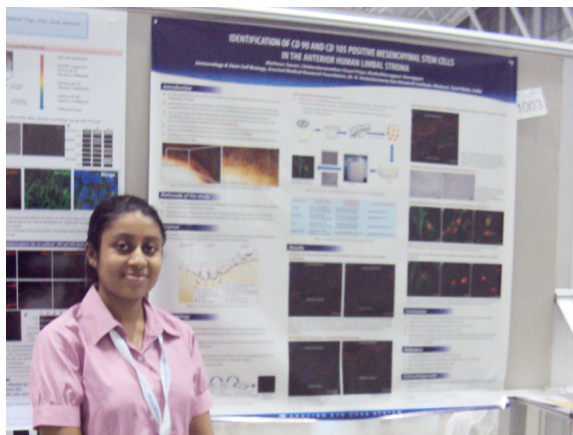
10th Annual Meeting of International Society for Stem Cell Research (ISSCR)

Pacifico Yokohama, Yokohama, Japan, June 13-16, 2012, Ms Saumi Mathews, Junior Research Fellow working under Dr. C. Gowri Priya, Stem Cell Biology Department in Aravind Medical Research Foundation attended 10th annual Meeting of ISSCR held during June 13 - 16, 2012, at Pacifico Yokohama in Yokohama, Japan and presented a poster entitled "Identification of CD 90 & CD 105 positive mesenchymal stem cells in the anterior human limbal stroma". She was awarded travel fellowship from the Indian Council of Medical Research.

Lalan kumar Arya, Prasanthi Namburi, Dr.S.Senthil Kumari, Dr.P.Sundaresan, Dr.P.Namperumalsamy, J.Lakshmi priya, K.Renukadevi, P.Murugeswari at ARVO meeting, Fort Lauderdale, Florida, USA



Saumi Mathews at 10th Annual Meeting of International Society for Stem Cell Research (ISSCR), Japan



20th Annual Meeting of Indian Eye Research Group (IERG) - ARVO - India Chapter

LV Prasad Eye Institute, Hyderabad - July 29-30

Faculty members and research scholars from Aravind Medical Research Foundation participated and presented their work at the 20th IERG-ARVO-IC Meeting at LV Prasad Eye Institute, Hyderabad.

Presentations

Dr. P. Sundaresan chaired the session on Retina, Glaucoma and Oncology and delivered an invited talk on “Carrier Detection and Prenatal Diagnosis of Oculocutaneous Albinism”.

DR. LALITHA PRAJNA

- *TLR and cytokine expression in human corneas infected with pseudomonas aeruginosa or streptococcus pneumonia (paper)*

DR. C. GOWRI PRIYA

- *Identification of mesenchymal stem cells in the anterior limbal stroma (poster)*

MS. ROOPAM DHUVESH

- *Mitochondrial Genes Involvement in Leber's Hereditary Optic Neuropathy (poater)*
Dr. A. Vanniarajan and Mr. R.Muthuselvam participated in the meeting.

Annual conference on mitochondria in health and disease

Gandhi Nagar, Gujarat, November 2-3 November

Dr. P.Sundaresan, Senior Scientist and Mr. Bibhuti Ballav Saikia, Research scholar from Dept. of Genetics, attended Society for Mitochondrial Research and Medicine-India - 2nd Annual conference entitled “Mitochondria in Health and Disease” organized by School of Life Sciences,



Central University of Gujarat, Gandhinagar on 2-3 November 2012. Dr. P.Sundaresan gave talks on “Mitochondrial Involvement in Ocular Disorders and Emphasizing Leber's Hereditary Optic Neuropathy in Indian Scenario”. Mr. Bibhuti Ballav Saikia presented his poster entitled “Mitochondrial DNA variants associated with Leber's Hereditary Optic Neuropathy”.

Biology 2012 and Beyond

Centre for Cellular and Molecular Biology, Hyderabad, November 25-27

Dr. P. Sundaresan, Senior Scientist and Dr. A. Vanniarajan, Scientist were invited to participate in the symposium on “Biology 2012 and Beyond” as part of the 25th Foundation Day celebrations of Centre for Cellular and Molecular Biology, Hyderabad.

Indian eye research group meeting



TRAINING

J. Lakshmi Priya, Research Scholar visited Geisel school of Medicine, Department of Microbiology and Immunology, Hanover, USA, for 3 weeks in May 2012, to learn different types of assay in characterizing the phenotypes of *Pseudomonas aeruginosa* from ocular isolates, under the guidance of Dr. Michael Zegans. This training was useful to establish the techniques in AMRF.



Lalan Kumar Arya, Senior Research Fellow, visited University of Miami, Florida, USA from May 12th-21st 2012 to learn Granuloma histopathology in Bascom Palmer Eye Institute with Dr. Sander R. Dubovy, (Associate Professor of Pathology) and Granuloma immunohistochemistry, cell cytology and cell blocking in Jackson Memorial Hospital with Dr. Nadji Mehrdad, (Director, Immunohistochemistry Laboratory).



Dr. P. Sundaresan visited Centre for Vision and vascular Science, Royal Victoria Hospital, Queen's University, Belfast, UK from May 7-27, 2012. He worked on Molecular genetics of Primary Open Angle Glaucoma and interacted with Dr. Colin E Willoughby and Dr. David Simpson.



Dr. P. Sundaresan visited Centre for Human Molecular Biology and Genetics, Sichuan Academy of Medical Sciences & Sichuan Provincial People's Hospital, Chengdu, China during March 4-7, 2013. He met Dr. Zhenglin Yang Director – Research and Vice President of the hospital and discussed the future collaborative projects as well as interacted with eminent faculty such as Dr. Xianjun Zhu and Dr. Gong Bo as well as research scholars. He delivered a guest lecture titled The role of genes and inheritance of human eye diseases in Indian population.



VISITORS



DR. ERIC PEARLMAN, Research Director, Department of Ophthalmology and Visual Sciences, Case Western Reserve University, Institute of Pathology, Cleveland, Ohio, USA visited AMRF and gave a lecture on *“Recent advances in understanding the pathogenesis of fungal, bacterial and Acanthamoeba keratitis”*.



DR. MATTHEW BURTON, Senior Lecturer, International Centre for Eye Health, London School of Hygiene and Tropical Medicine, London visited AMRF and gave a lecture on *“The immunopathogenesis of scarring Trachoma”*.



DR. COLLIN WILLOUGHBY, Senior Lecturer/Consultant Ophthalmic Surgeon, School of Biomedical Sciences, Centre for Vision Science, Royal Victoria Hospital, Queen’s University, Belfast visited AMRF and gave a lecture on *“Molecular genetics of Keratoconus”*.



DR. APARNA LAKKARAJU, Assistant Professor, Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison, USA visited AMRF and gave a lecture on *“Insight into the intracellular trafficking in age – related macular degeneration (AMD)”*.



DR. K.V. VENKATACHALAM, Professor of Biochemistry, College of Medical Sciences, Nova Southeastern University, Fort Lauderdale, Florida , USA visited AMRF and gave the lecture on *“Methionine Gamma Lyase-2 Aminobutyrate Deaminase (MEGL-2ABD) and its Therapeutic uses in clinical Biology with special reference to cancer”*.



DR. C.N. PARAMASIVAN, Head of TB programme FIND (India) & South East Asia Foundation for Innovative New Diagnostics, New Delhi visited AMRF and gave lecture on *“Recent advances in the diagnosis of mycobacterium tuberculosis”*.



DR. K. VARADARAJ, Associate Professor, Department of Physiology & Biophysics, Health Sciences Center, State University of New York, New York visited AMRF and gave a lecture on *“Role of Aquaporins in lens and corneal transparency and homeostasis”*



DR. ARUP DAS, Chief and Professor of Ophthalmology, University of New Mexico, School of Medicine, Albuquerque, New Mexico visited AMRF and gave a lecture on *“Diabetic Macular Edema – VEGF and beyond VEGF: Future Targets”*.



DR. ASHISH BISWAS, Assistant Professor, Department of Biophysical Chemistry, Indian Institute of Technology, Bhubaneswar visited AMRF and gave a lecture on *“Effect of small molecules on the structure, stability and chaperone function of alpha crystallin – A Biophysical study”*.

AWARDS

DBT- CREST award

DBT CREST award aims to promote capacity building in cutting edge areas of biotechnology and life sciences. The Award will promote and support scientists of highest merit in their pursuit of skill enhancement in scientific research/training in Overseas laboratories.

Dr. S. Senthil Kumari, Scientist, department of Ocular Pharmacology received 2011-12 DBT CREST award (July - October 2012)

- She visited Dr. Paul Kaufman's Laboratory at the department of Ophthalmology and Visual sciences, University of Wisconsin (UW), Madison to carry out studies on *Understanding the role of endocannabinoids in the regulation of trabecular meshwork (conventional) outflow – implications in glaucoma therapy.*
- She visited Dr. Mike Fautsch's laboratory at the department of ophthalmology at Mayo Clinic, Rochester, Minnesota to understand *anterior segment organ culture system using human donor eyes.*



Best Scientific Poster Presentation award

Ms. Sudha Priya Soundara Pandi has been working in Aravind Medical Research Foundation as research scholar with Dr. VR. Muthukkaruppan during 2008-2010.

She is presently doing Ph.D at Queens University, Belfast, UK on *MicroRNA in retinal ageing and age-related macular degeneration* with Dr. Heping Xu and Dr David Simpson. She received Dr. Andrew Craig Award for Best Scientific poster presentation at the 9th alumni research meeting held on 28th of September 2012 in Belfast. The award contained a trophy and cash price of £500.



CONFERENCES / WORKSHOPS CONDUCTED

Vision Genomics – Hands on Training in Ocular Genetics

During the October Summit 2012, organized in honour of Founder-Chairman Dr.G.Venkataswamy, Department of Genetics, Aravind Medical Research Foundation conducted a workshop entitled “Vision Genomics” – Hands on training in Ocular genetics, from October 3-9, 2012. Twenty three participants from various parts of India including research scholars /post graduate students in the field of life sciences took part in this workshop. The programme involved individual hands on training on various molecular techniques and lectures by eminent faculty. Dr. Wallace Alward, Dr. John Fingert from University of Iowa, USA;

Dr.Colin Willoughby from Queens University ,Belfast, UK; Dr. G. Kumaramanickavel, Narayana Nethralaya, Bangalore; Dr. Kunalray, Indian Institute of Chemical Biology, Kolkata; Dr. Subhabrata chakravarti, LV Prasad Eye Institute, Hyderabad; Dr. Thangaraj, Centre for Cellular and Molecular Biology, Hyderabad; Dr. Krishnaswamy, Madurai Kamaraj University, Madurai; Dr. Karuthapandian, Alagappa University, Karaikudi; constituted the faculty for the workshop.

This programme was supported by Department of Biotechnology, Indian Council of Medical Research, Government of India, Genotypic Technology and Bioserve. The workshop was organized by Dr.P.Sundaresan, Molecular Genetics department.



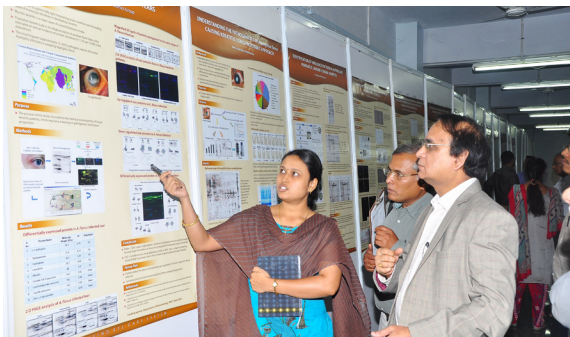
11TH RESEARCH ADVISORY COMMITTEE MEETING

The 11th Research Advisory Committee of Aravind Medical Research Foundation was held on 25th February 2013 at Dr.G.Venkataswamy Eye Research Institute.

All the research scholars presented their findings as posters, thus providing an opportunity for the members of research advisory committee to interact with them. The members also evaluated the posters. Among the 20 posters presented, three were selected for award.

The award winning posters are

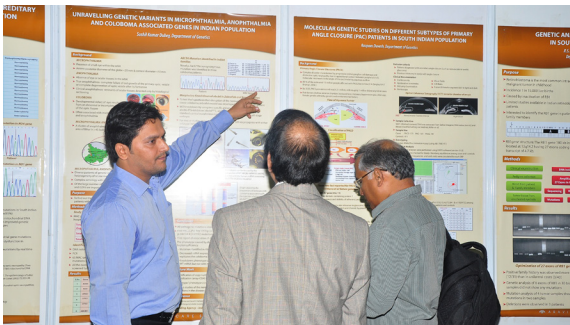
- Characterisation of PMN-17 cells from the blood of Patients with fungal keratitis by R.Siva Ganesa Karthikeyan, Department of Microbiology
- Identification of stage specific biomarkers for fungal keratitis in human tears by K.R.P.Niranjana, Department of Proteomics.
- Molecular genetics and functional analysis of candidate genes associated with microphthalmia, anophthalmia and coloboma by Sushil Kumar Dubey, Department of Molecular Genetics



Niranjana explaining her work to Dr.C.Mohan Rao.



Sivaganesa karthikeyan discussing with Dr.C.Mohan Rao and Dr. MRS. Rao



Sushil Kumar Dubey explaining his work



Lakshmi priya explaining her work to Dr.Kirushna Kumar



PUBLICATIONS 2012-2013

INDIAN JOURNAL OF MEDICAL MICROBIOLOGY

2012;30(4):418-22

ARUN KANNAN, GOWRI PRIYA CHIDAMBARANATHAN,
LALITHA PRAJNA, RATHINAM SIVAKUMAR

- *Efficiency of two commercial kits in serodiagnosis of leptospiral uveitis*

J OCUL BIOL JUN

2012;5;4(4):154-8

K.RANGACHARI, J. JEYALAXMI, P.J. ESWARI
PANDARANAYAKA, N.PRASANTHI, P.SUNDARESAN,
S.R.KRISHNADAS, S.KRISHNASWAMY

- *Significance of G-X-W motif in the myocilin olfactomedin domain*

ARCH OPHTHALMOL

2012; Vol 130 (11) p.1481-1484

RATHINAM SR, LALAN KUMAR ARYA, LALITHA.P, USHA
KIM, VEENA TANDON

- *Novel etiological agent – molecular evidence for trematode-induced anterior uveitis in children*

JOURNAL OF MEDICAL MICROBIOLOGY

2012; 61:1681-1687

SUBHA SIVAKOLUNDU, RATHINAM SR, GOWRI PRIYA
CHIDAMBARANATHAN, MANJULA SRITHARAN

- *Serological diagnosis of leptospiral uveitis by HbpA-IgG ELISA*

IOVS

2012 Nov 1;53(12):7492-7497

PERIASAMY SUNDARESAN, PRAVEEN VASHIST, RAVILLA
D. RAVINDRAN, ASHWINI SHANKER, DOROTHEA
NITSCH, BARENG A. S. NONYANE, LIAM SMEETH, USHA
CHAKRAVARTHY, ASTRID E FLETCHER

- *Polymorphisms in ARMS2/HTRA1 and complement genes and age related macular degeneration in India: findings from the INDEYE study*

THE AMERICAN JOURNAL OF HUMAN GENETICS

2012;90:1-9

LEJING WANG, FEI HE, JUAN BU, XIAQI LIU, WEI DU,
JIAMEI DONG, JEFFREY D. COONEY, SUSHIL KUMAR

DUBEY, YI SHI, BO GONG, JING LI, PAUL F. MCBRIDE,
YANLEI JIA, FANG LU, KATHLEEN A. SOLTIS, YING
LIN, PRASANTHI NAMBURI, CHEN LIANG, PERIASAMY
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PHILLIPS, AND ZHENGLIN YANG

- *ABCB6 mutations cause ocular coloboma*

MICROSCOPY RESEARCH AND TECHNIQUE

2013; 76:242-248

GOWRI PRIYA CHIDAMBARANATHAN, TILAK PRASAD,
NAMPERUMALSAMY VENKATESH PRAJNA, VEERAPPAN
MUTHUKKARUPPAN

- *Identification of human corneal epithelial stem cells on the basis of high ABCG2 expression combined with a large N/C ratio.*

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SIVAGNANAM ANANTHI, NAMPERUMALSAMY VENKATESH
PRAJNA, PRAJNA LALITHA, MURUGESAN VALARNILA,
KUPPAMUTHU DHARMALINGAM

- *Pathogen induced changes in the protein profile of human tears from Fusarium Keratitis patients*

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ANSHUMAN VERMA, MANORANJAN DAS, MUTHIAH
SRINIVASAN, NAMPERUMALSAMY V PRAJNA,
PERIASAMY SUNDARESAN

- *Investigation of VSX1 sequence variants in South Indian patients with sporadic cases of Keratoconus*

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ANSHUMAN VERMA, PERIYASAMY SUNDARESAN,
KARTHIKEYAN ARCOT SADAGOPAN, VIJAYALAKSHMI
PERUMALSAMY

- *“Gene therapy for leber congenital amaurosis” brings a hope to cure inherited childhood blindness*

RESEARCH SCHOLARS AT AMRF

No	Projects	Funded by	Investigators	Research Scholar
MICROBIOLOGY				
1.	Etiology and Immunopathogenesis of Trematode induced Uveitis in children of South India	ICMR	Dr. SR. Rathinam Dr. Lalitha Prajna Dr. Veena Tandon	Lalan Kumar Arya
2.	Characterization of the host immune response during corneal infection with pathogenic fungi and bacteria	ICMR	Dr. Lalitha Prajna Dr. K. Dharmalingam Dr. N. Venkatesh Prajna	R. Sivaganesa Karthikeyan
3.	Epidemiology, pathogenomics, and system biology of A. flavus infections in India - an integrative approach	DBT	Dr. Lalitha Prajna	J. Lakshmi Priya
4.	Genotypic characterization and analysis of virulence factors in Methicilin resistant staphylococcus aureus (MRSA) causing ocular infections in South India	ICMR	Dr. Lalitha Prajna	V. Nithya
5.	Microbiological clearance time and sensitivity assay for Acanthamoeba keratitis	AMRF	Dr. Lalitha Prajna Dr. M. Vidyarani	K. Fathima Sulthana
6.	Studies in the diagnosis and pathophysiology of corneal necrosis in severe microbial keratitis	Wellcome Trust, UK	Dr. N. Venkatesh Prajna Dr. Lalitha Prajna Dr. Mathew Burton	Jaya Chidambaram
PROTEOMICS				
7.	Quantitative Proteomics of host pathogen interaction in human Aspergillus Keratitis	DBT	Dr. N. Venkatesh Prajna Dr. K. Dharmalingam Dr. Lalitha Prajna	K. R. P. Niranjana R. MuthuSelvam
8.	Further experiments on MUTT study samples	AMRF	Dr. N. Venkatesh Prajna Dr. K. Dharmalingam Dr. Lalitha Prajna	R. Nithya
10.	CoE – Human mycotic Keratitis	DBT	Dr. N. Venkatesh Prajna Dr. K. Dharmalingam Dr. Lalitha Prajna	PROJECT FELLOWS: K. Agalya Priyadharsini M. Nivedhitha Naga Charan RESEARCH ASSOCIATE: Dr. Partho Chatteraj Dr. J. Jeya Maheswari Dr. Jeyalakshmi Kandhavel
11.	Identification of Biomarkers for Primary Open Angle Glaucoma	DBT	Dr. SR. Krishnadas Dr. P. Sundaresan Dr. K. Dharmalingam	R. Sugumar

MOLECULAR GENETICS				
12.	Molecular genetics of Albinism in the Indian population - Fellowship	CSIR	Dr. P. Sundaresan Dr. P. Vijayalakshmi	K. Renukadevi
13.	Genetic screening in a large family with Primary Open Angle Glaucoma - Fellowship	CSIR	Dr. P. Sundaresan Dr. SR. Krishnadas	N. Prasanthi
14.	Molecular genetics of leber congenital amaurosis in South Indian population	ICMR	Dr. P. Sundaresan Dr. P. Vijayalakshmi	Mr. Anshuman Verma
15.	Transcriptome and Proteome analyses of ALR2 and its involvement in the pathogenesis of Diabetic Retinopathy	DBT	Dr. P. Sundaresan Dr. R. Kim	G. Gowthaman
16.	Molecular genetic analysis of candidate genes associated with paediatric eye diseases: exclusively anophthalmia and microphthalmia in India	ICMR	Dr. P. Sundaresan Dr. P. Vijayalakshmi Dr. S.K. Kedia, Sadar Hospital, Ara, Bihar	Sushil Kumar Dubey
17.	Mitochondrial genes involvement in Leber's Hereditary Optic Neuropathy (LHON)	DST	Dr. P. Sundaresan, Dr. Mahesh Kumar	Bibhuti Saikia
18.	Molecular genetics studies of Primary Angle closure Glaucoma (PACG) in South Indian Population	UGC	Dr. P. Sundaresan, Dr. Mahesh Kumar	Roopam Duvesh
19.	A Genetic component to the INDEYE study of cataract and age related macular degeneration in India	Wellcome Trust, UK	Dr. P. Sundaresan Dr. R.D. Ravindran	V.Saravanan (Sr.Technician)
20.	Molecular Genetics of Retinitis Pigmentosa in Indian population	ICMR	Dr. P. Sundaresan	Harrison Immanuel
21.	Genetic and transcript analysis of RB1 gene in South Indian Retinoblastoma Patients	AMRF	Dr. A. Vanniarajan Dr. Usha Kim Dr. R. Santhi	K. Thirumalai Raj
22.	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	A. Alloysius Abraham
IMMUNOLOGY AND STEM CELL BIOLOGY				
23	Translational research to generate Corneal/ Buccal Epithelial stem cells with GMP compliance for corneal surface and socket reconstruction	DRDO	Dr. C. Gowri Priya Dr. N. Venkatesh Prajna Dr. VR. Muthukkaruppan	Saumi Mathews
24	Enrichment of human limbal epithelial stem cells to understand the stem cell to understand the stem cell related properties by whole genome analysis and p63 isoform expression profile	DBT	Dr. C. Gowri Priya, Dr. VR. Muthukkaruppan	M. K. Jhansirani

25	Antigenic Mimicry between Leptospiral and human lens proteins	AMRF	Dr. C. Gowri Priya Dr. SR. Rathinam	Arun Kannan
26	Effect of VEGF levels in Tenon's tissue on the outcome of glaucoma surgery	AMRF	Dr. C. Gowripriya	Sonam Lata
OCULAR PHARMACOLOGY				
27	Evaluating the Role of Macular Carotenoids in the accumulation of A2E, A fluorophore in the pathogenesis of Age related Macular Degeneration	DST	Dr. S. Senthilkumari	
28	Role of Aldose Reductase in Retinal pigment epithelium- An understanding towards the pathogenesis of Diabetic Retinopathy	DBT	Dr. S. Senthilkumari	R. Sharmila
29	Human Organ Culture Anterior Segment (HOCAS) for Trabecular Meshwork	Aravind Eye Foundation	Dr. S. Senthilkumari	K. Cholaraja
BIO INFORMATICS				
30.	1. Comprehensive Exome analysis pipeline using clinical next-generation sequencing data 2. Understanding the Molecular Mechanisms of Indian Genetic Eye Diseases: A Structure-Based Bioinformatics Approach and Database Development	AMRF	Dr. D. Bharanidharan	P. Logambika