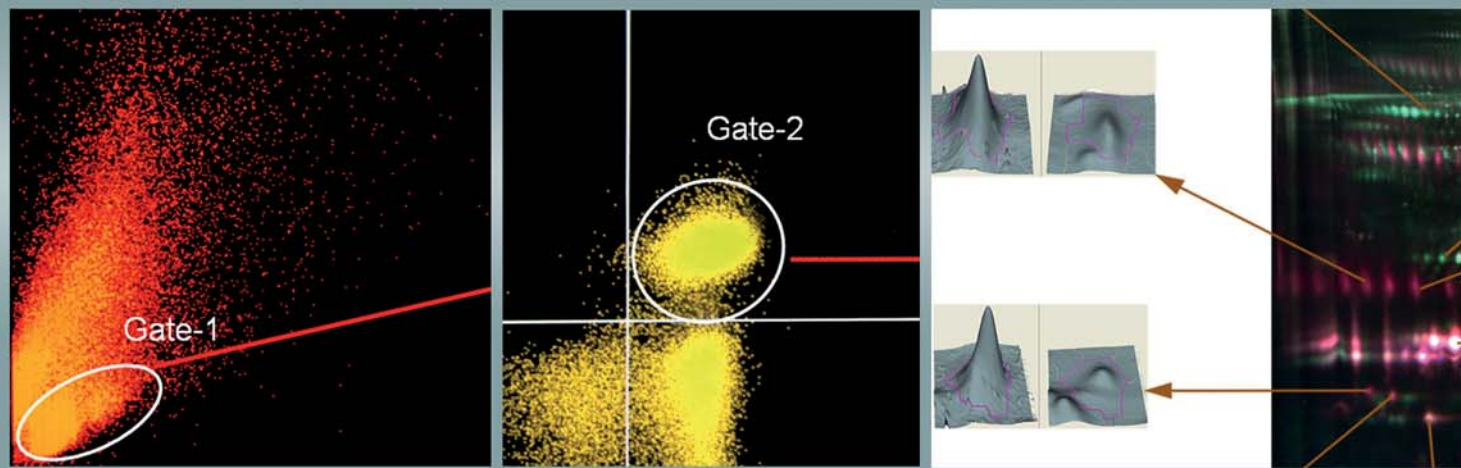


Basic Research in Ophthalmic Sciences



Annual Report 2010-2011

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Aravind Medical Research Foundation

Annual Report 2010 - 2011



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

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INTRODUCTION

Fundamental research in Ophthalmology opens up possibilities to improve existing treatment options by a better understanding of the disease processes. Eye research, particularly in the Indian context, is imperative to optimize treatment modalities for better treatment outcome. The infection load and pathogen profile are different in the agrarian society of India and in addition it is well known that the immunological profile of the Indian population is different when compared to the western population, consequently large scale studies using appropriate patient groups are of paramount importance. Blind extrapolation of studies done in other populations is incompatible when applied to patient groups in India.

Examining disease processes at all levels of genomic transactions is important. Large cohort studies already provided enough evidence that genome variation at individual level are very revealing when examined at the level of transcripts and proteins. At AMRF fundamental aspects of selected diseases are being examined at the level of genome, transcriptome and proteome. The approaches are designed to further the knowledge as well as to apply the research findings in clinical practice.

Molecular genetics group has been focusing on the analysis of associative Single Nucleotide Polymorphisms (SNPs) and the primary mutations that could be responsible for the disease. They examine several inherited eye diseases such as ocular cutaneous albinism, Leber's Hereditary Optic Neuropathy (LHON), Duane Retraction Syndrome (DRS), Leber's Congenital Amaurosis (LCA), diabetic retinopathy etc. They discovered an interesting polymorphism in the intronic region of myocilin gene in primary open angle glaucoma. They also examine the functions of selected genes in eye diseases.

Microbiology group is actively involved in the understanding of the immune response to fungal infection. Unlike other tissues, eye is immunologically distinct. The corneal epithelium, neutrophils, macrophages and dendritic cells are the cells that are involved in innate immune response against fungi. The main receptor that recognizes the fungal components is Dectin-1. The objective of the study is to show the events leading to the development of innate immune defense, and the subsequent adaptive response. It is increasingly evident from the work of this group that adaptive response also plays a role in antifungal defense in the infected eye. Microbiology group is also involved in the development of new diagnostic tools for early detection of causative agent for Uveitis. Infective Uveitis is due to several different ocular and systemic infections. The disease mechanism behind trematode induced Uveitis is another area of study by this group.

Immunology group is also a referral center for systemic and ocular Leptospirosis Uveitis. The group's main focus is the exploration of Leptospirosis Uveitis and to understand the mechanism of development of acute cataract in Leptospirosis infections. They are focusing on the autoimmune component in this infection.

Stem cell biology group has developed a new two parameter approach for the identification of stem cells among limbal epithelial cells generated in vitro. They have used this approach to identify buccal epithelial stem cells as well. They have developed a simple culture method to generate stem cell -rich epithelium for transplantation in a small group of patients. The results are very encouraging and further studies are in progress to develop xeno-free, simple and cost effective method in the GMP facility.

Ocular pharmacology group is interested in the pharmacokinetics of voriconazole used for treating fungal keratitis patients. This group is also interested in the study of disease mechanism in the case of diabetic retinopathy and Age-related macular degeneration (AMD).

Proteomics group has been added recently to AMRF. Proteomics of ophthalmic diseases is emerging fast as an area of study and there are groups examining eye diseases from different populations across the globe using proteomics. Proteomics group is examining the proteome profile of the easily accessible tissues in infections. Tear is being chosen for examining the disease specific changes in Mycotic keratitis. It has been shown that there is significant difference in the proteome profile of male and female tears. It has also been shown that very early in fungal infection one could see differences in the proteome profile of tears. Other diseases being studied are primary open angle glaucoma and diabetic retinopathy. Aqueous humor proteome is used as target tissue to examine the proteome level alterations in these diseases. Apart from identifying early events in infection, proteomics will also allow us to examine the disease mechanism.

CONTENTS

Department of Molecular Genetics	1
Department of Ocular Microbiology	9
Department of Proteomics	14
Department of Immunology	20
Department of Stem Cell Biology	22
Department of Ocular Pharmacology	26
Conferences Attended	31
Training	33
Visitors	34
Workshop	35
Publications in Peer Reviewed Journals	36
Research Scholars at AMRF	38

DEPARTMENT OF MOLECULAR GENETICS

Molecular Genetics Laboratory at Aravind Medical Research Foundation is focusing on the genetics of various inherited eye diseases in Indian population. The overall goals of our research program are to identify the candidate genes for eye diseases and to identify the genetic variations in the known candidate genes especially for aniridia, primary open angle glaucoma, albinism, diabetic retinopathy, age related cataract, retinitis pigmentosa, keratoconus, globe anomalies, familial exudative vitreoretinopathy, blepharophimosis-ptosis-epicanthus inversus syndrome, Leber's hereditary optic neuropathy, X-linked juvenile retinoschisis and Leber congenital amaurosis in Indian population. Phenotype-genotype correlation, understanding the gene expression in relation to pathogenesis and developing molecular diagnosis for early detection of the eye disease are also our interest.

Spectrum of candidate gene mutations associated with Indian familial oculocutaneous and ocular albinism

Investigators	:	P. Sundaresan (Aravind Medical Research Foundation, Madurai) P. Vijayalakshmi (Aravind Eye Hospital, Madurai) Asim Kumar Sil (Vivekananda Mission Ashram Netra Niramay Niketan, West Bengal)
Research scholar	:	Ms. K. Renugadevi
Funding agency	:	Department of Biotechnology and Department of Science and Technology

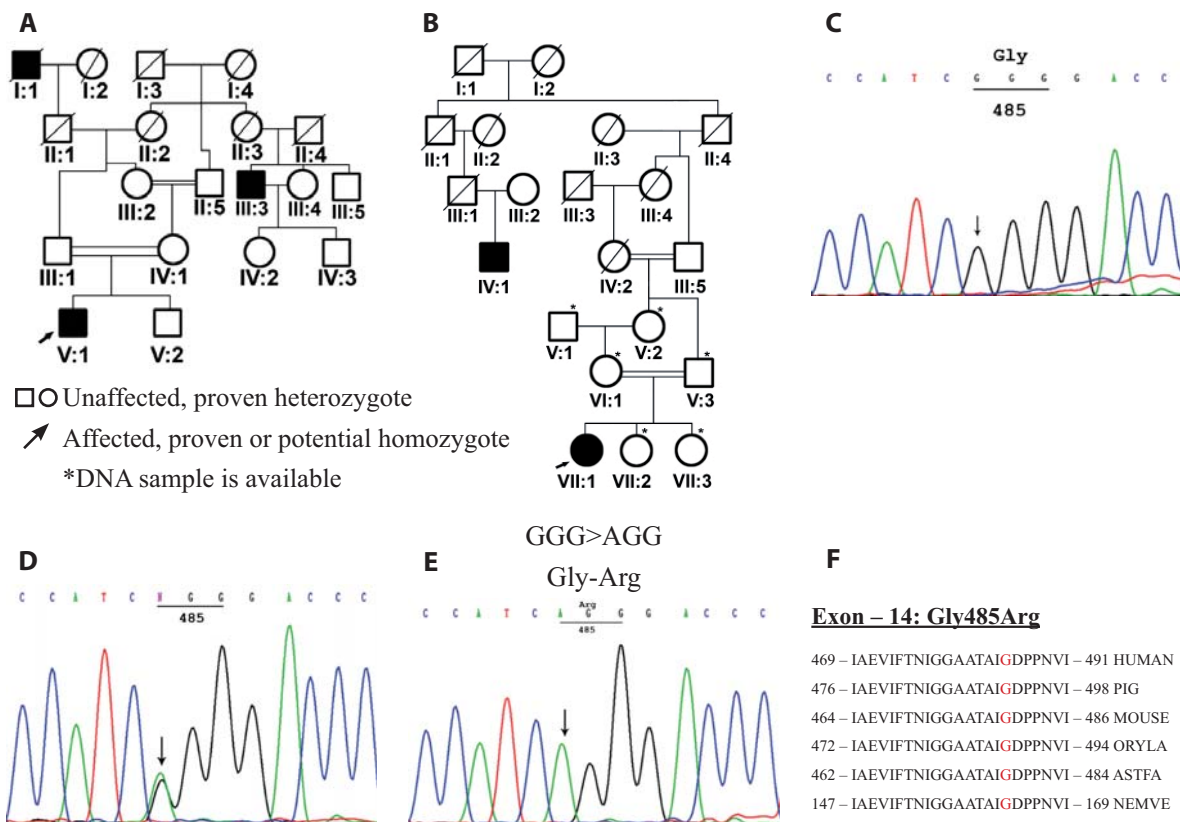
Background and aim

Albinism is a very rare congenital defect and the disease refers to a group of inherited conditions of melanin biosynthesis or distribution. There is no treatment available for this hereditary disorder and its frequency across the human population was estimated to be about 1 in 20,000 in worldwide. Albinism is an autosomal recessive disorder and the phenotype ranges from a complete or partial lack of melanin in ocular and cutaneous regions due to mutations in TYR, P, MC1R, TYRP1 and MATP genes or in ocular region alone due to GPR143 gene.

In 2003, incidence of albinism in eastern part of India was observed and the research team started working on the genetics of albinism in Indian population. The aim of the study was to identify the spectrum of genetic variations in Indian population and identify the genetic markers of albinism for molecular diagnosis.

A total of 141 patients from southern and eastern parts of India was recruited. The common clinical problems in these patients include, severe photosensitivity, reduced vision, nystagmus, macular/foveal hypoplasia, misrouting of optic fibers at the chiasm, and greatly decreased visual acuity. Bi-directional DNA sequencing of these Oculocutaneous (TYR, P, TYRP1, MATP) and Ocular Albinism (GPR143) candidate genes were performed. Among these candidate genes, 17.39% of variations in TYR gene were observed from most of the affected individuals. Therefore, the results suggest that prevalence of OCA type 1 is higher than the other OCA types in Indian cohort.

Recently, five generations of two different families (Fig-1 A and B) phenotypically confirmed as Oculocutaneous Albinism type II (OCA-II) were recruited. Based on the sequencing analysis of P gene, one novel missense mutation c.1453G>A (G485R) was observed in these two probands. In one of the family (Fig-1 B) the proband's parents, maternal grandmother and first younger sister were carriers for G485R mutation. The finding sheds new light on the P gene mutation and highlights the importance of analyzing this P gene along with TYR gene in Indian patients.



Pedigree (A,B) and chromatogram of two albinism families harboring novel mutation Gly485Arg (C-F)

Implication of the project

The spectrum of genetic variations in the candidate genes of albinism in Indian population has been identified and molecular diagnosis for albinism is being performed.

Publications

1. Kathirvel Renugadevi, Asim Kumar Sil, Vijayalakshmi Perumalsamy, Periasamy Sundaresan. "Spectrum of candidate gene mutations associated with Indian familial oculocutaneous and ocular albinism". *Molecular Vision* 2010; 16:1514-1524.

Presentations

1. P. Sundaresan, K. Renugadevi, A.K. Sil, P. Vijayalakshmi. "Spectrum of candidate genes mutation associated with Indian familial oculocutaneous albinism patients" US-ARVO-2010 Annual meeting, Fort Lauderdale, Florida, USA (2605/A394)

Reduced frequency of known mutations in a cohort of LHON patients from India

Investigators : P. Sundaresan (Aravind Medical Research Foundation, Madurai)
 S. Mahesh Kumar (Aravind Eye Hospital, Madurai)
 Stewart Thompson (University of IOWA, USA)
 John H. Fingert (University of IOWA, USA)

Funding agency : Department of Science and Technology

Background and aim

Leber's Hereditary Optic Neuropathy (LHON) is characterized by bilateral acute or sub acute, painless, loss of central vision typically presenting between 26 and 45 years old. LHON is caused by mutations in mitochondrial DNA (mtDNA) that are passed down to 100% of offspring through maternal lineages, but has incomplete penetrance and is more common in males. Recent estimates of the prevalence of LHON in various populations have ranged from 1/30,000 to 1/53,000. The mutations G11778A, T14484C, and G3460A contribute to >95% of LHON in populations. The prevalence of LHON and the relative frequency of LHON mutations in India are unknown. Here the frequencies of the three most common known LHON mutations in a population of well-characterized south Indian patients have been assessed.

Screening of LHON primary mutations

In order to determine these three LHON mutations, a total of 90 unrelated individuals with LHON; 20 family members and 50 normal controls were recruited for the study at the Aravind Eye Hospital, Madurai, Tamilnadu. Peripheral blood (5ml) was collected and total genomic and mitochondrial DNA was extracted by salt precipitation. ARMS (Amplification–Refractory Mutation System) PCR assays were used to screen DNA samples from the 90 LHON probands, 20 family members and 50 control subjects for G3460A, G11778A and T14484C. For each sample and amplicon, PCR reactions were made with one common primer and with one allele-specific primer (either specific for normal or mutant DNA sequence). Internal control primers were included in each reaction. PCR products were analyzed using 2% agarose gel electrophoresis and confirmation of mutations by direct sequence analysis.

In this study, a total of 11 of 90 (12%) of the LHON probands had positive test results for these common mutations. Eight of 91 (8.9%) had a G11778A mutation, three of 90 (3.3%) had a T14484C mutation, and none had a G3460A mutation. No mutations were detected in 79 (88%) of 90 probands. Eight probands in our study had a positive screening test for the G11778A mutation using the ARMS PCR assay. We reported an unexpected low frequency of known mitochondrial mutations in a cohort of LHON patients from south India.

Implication of the project

These results suggest that a unique set of mutations may be responsible for LHON in this patient population. Future mutation detection studies of our cohort of LHON patients from south India may help to identify such novel disease causing mutations.

Publications

1. Sundaresan P, Kumar SM, Thompson S, Fingert JH. "Reduced frequency of known mutations in a cohort of LHON patients from India" *Ophthalmic Genetics* 2010; 31 (4) 196-199.

Association analysis of nine candidate gene polymorphisms in Indian patients with type 2 diabetic retinopathy

Investigators : P. Sundaresan, Dr. B. Suganthalakshmi (Aravind Medical Research Foundation, Madurai)
R. Kim, Dr. Anand Rajendran, Dr. P. Namperumalsamy (Aravind Eye Hospital, Madurai)
Dr. J Fielding Hejtmancik, (National Eye Hospital, National Institute of Health, Bethesda, USA)
Research Scholar : Mr. G. Gowthaman
Funding Agency : TIFAC- CORE

Background and aim

Diabetic Retinopathy (DR) is classically defined as a microvasculopathy that primarily affects the small blood vessels of the inner retina as a complication of Diabetes Mellitus (DM). It is a complex disease with a strong genetic component. Several genes are involved in the pathogenesis of DR. The main aim of this

study was to investigate the association of a set of nine candidate genes with the development of diabetic retinopathy in south Indian population with type 2 diabetes mellitus (T2DM).

Nine candidate genes (RAGE, PEDF, AKR1B1, EPO, HTRA1, ICAM, HFE, CFH and ARMS2) were chosen based on their roles in biological pathways implicated in DR. A total of 14 single nucleotide polymorphisms (SNPs) and one dinucleotide repeat polymorphism in nine candidate gene regions were genotyped in 345 Diabetic Retinopathy (DR) and 356 diabetic patients without retinopathy (DNR). The genes which showed positive association in this screening set were tested further in additional sets of 100 DR and 90 DNR patients from the Aravind Eye Hospital. Genotypes were evaluated using a combination of direct sequencing, TaqMan SNP genotyping, RFLP analysis, and SNaPshot PCR assays. Among the 15 SNPs studied, only one SNP rs2070600 (G>A) in exon 3 of RAGE, which leads to a change of amino acid glycine to serine at codon 82 displays significant association with DR in this study population.

Implication of the project

Screening of large number of samples will confirm the disease causing gene variations and invariably act as universal marker for different ethnic groups.

Publications

Suganthalakshmi Balasubbu, Periasamy Sundaresan, Anand Rajendran, Kim Ramasamy, Gowthaman Govindarajan, Namperumalsamy Perumalsamy, J Fielding Hejtmancik. "Association analysis of nine candidate gene polymorphisms in Indian patients with type 2 Diabetic retinopathy" BMC Medical Genetics 2010, 11:158

Analysis of the SALL4 gene in patients with duane retraction syndrome in a south Indian population

Investigators : P. Sundaresan (Aravind Medical Research Foundation, Madurai)
P.Vijayalakshmi, Shashikant Shetty (Aravind Eye Hospital, Madurai)
Research Scholar : Mr. Lalan Kumar Arya

Background and aim

Duane Retraction Syndrome (DRS) is a congenital eye movement disorder characterized most typically by partial or, complete failure of abduction and narrowing of palpebral fissure with globe retraction on adduction. The SALL genes encode zinc finger transcription factors which presumably have large influence during human embryogenesis. The SALL4 gene (20q13.13-q13.2) is found to be associated with DRS. Mutations of the SALL4 gene on chromosome 20 have been linked to DRS associated with radial forearm malformations (Okihiro syndrome) and also association with other nonocular anomalies, such as Klippel-Feil anomaly, Wildervanck syndrome, and Goldenhar syndrome. The aim of this study was to screen the mutations in SALL4 gene of patients with isolated DRS and DRS-associated syndromes in south Indian population.

In this prospective, non-interventional study screening was done for SALL4 mutations in 72 patients clinically diagnosed as having isolated DRS or DRS associated syndromes. All four exonic and the neighboring intronic regions of SALL4 gene were amplified by sixteen sets of primers using polymerase chain reaction and were subjected to bi-directional sequencing and BLAST analysis. None of the patients and control samples revealed any mutations in the coding region or the neighboring intronic regions of the SALL4 gene. No genetic variations were detected in the coding region and in the neighboring intronic regions of the SALL4 gene suggesting an alternative mechanism in the pathogenesis of these disorders in the south Indian population.

Implication of the project

SALL4 gene mutation frequency is low in patients with duane retraction syndrome in a south Indian population.

Publications

1. Lalan Kumar Arya, Anand B. Kumar, Shashikant Shetty, Vijayalakshmi Perumalsamy and Periasamy Sundaresan “Analysis of the SALL4 gene in patients with Duane Retraction syndrome in a south Indian population” *Ophthalmic Genetics*, March 15, (Online, 2011).

Glaucoma database

Investigators	:	S. Krishnaswamy, (Madurai Kamaraj University, Madurai) S.R. Krishnadas (Aravind Eye Hospital, Madurai) P. Sundaresan (Aravind Medical Research Foundation, Madurai)
Research Scholars	:	Mr. K. Rangachari, Ms. M. Dhivya, Ms. Eswari Pandaranayaka Ms. N. Prasanthi
Funding Agency	:	Department of Biotechnology

Background and aim

Glaucoma is a complex disease that comprises a group of heterogeneous optic neuropathies characterized by a progressive degeneration of the optic nerve head and visual field defects. Primary Open Angle Glaucoma (POAG) is the most common subset of glaucoma. MYOC, OPTN, CYP1B1 and WDR36 are the important candidate genes. Nearly 4% of the glaucoma patients have mutation in any one of these genes. Mutation in any of these genes causes disease either directly or indirectly. The specific aim of this study is to develop a glaucoma database.



Snapshot of the Database

Data collection

A literature search was conducted using PubMed, from that all the available data was collected till date. Search terms included are myocilin, MYOC, CYP1B1, OPTN, WDR36, glaucoma. The information has also been collected from the gene cards database and myocilin database (www.myocilin.com). All the related mutations and SNPs in the above genes have been compiled and developed a database, to help access statistical and clinical information of particular mutation. This database is available online at <http://bicmku.in:8081/glaucoma>. The database was constructed using SQL, contains data pertaining to the SNPs and mutation information involved in the above genes and relevant study data.

Implication of the project

Developed a comprehensive database to understand the genetics of Glaucoma

Publication

Rangachari K, Dhivya M , Eswari Pandaranayaka PJ , Prasanthi N , Sundaresan P, Krishnadas SR, Krishnaswamy S. “Glaucoma Database.” *Bioinformation* 5(9): 398-399 (2011).

Presentation

1. K. Rangachari, M Dhivya, Pandaranayaka PJ, N Prasanthi Eswari, P Sundaresan, S.R Krishnadas, S Krishnaswamy. “Glaucoma Database” Indian Eye Research Group 18th Annual Meeting, LV Prasad Eye Institute, Hyderabad (2010), Abstract No: ICP 012.

Polymorphisms in an intronic region of the myocilin gene associated with primary open-angle glaucoma - a possible role for alternate splicing

Investigators : S. Krishnaswamy (Madurai Kamaraj University, Madurai)
 S.R. Krishnadas (Aravind Eye Hospital, Madurai)
 P. Sundaresan (Aravind Medical Research Foundation, Madurai)

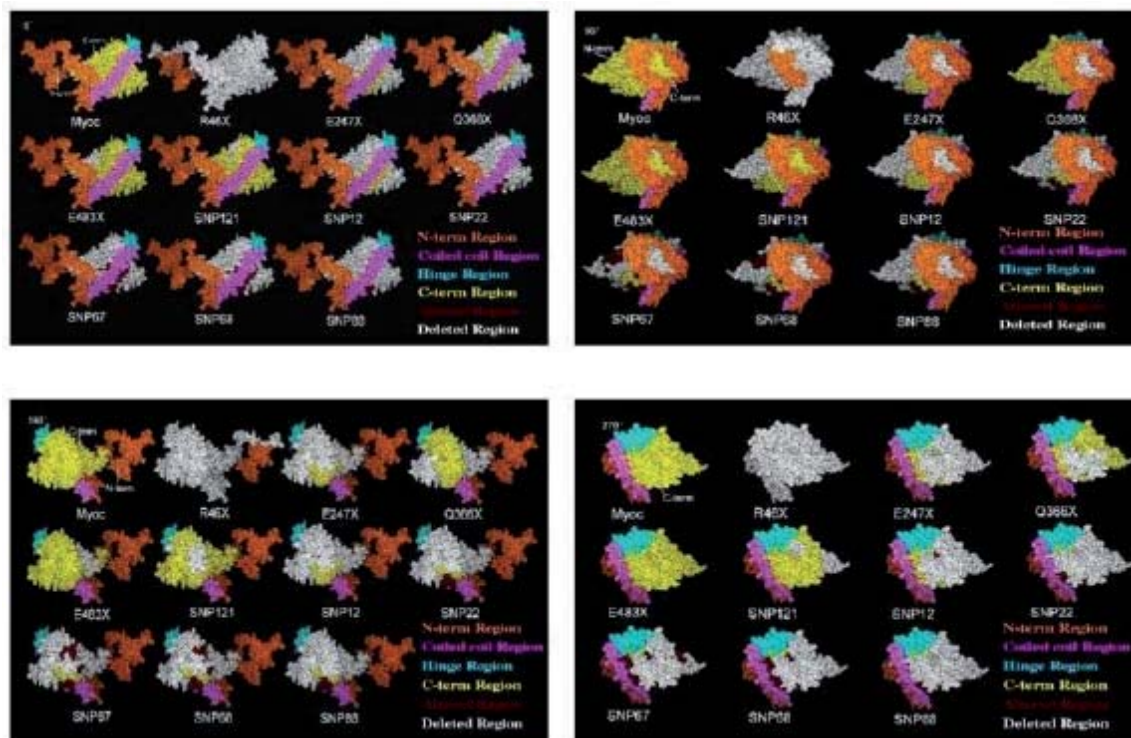
Research scholars : Ms. Eswari Pandaranayaka, Ms. N.Prasanthi, Mr. N. Kannabiran,
 Mr. K. Rangachari, Ms. M. Dhivya

Funding agency : Indian Council of Medical Research and Department of Biotechnology

Background and aim

Glaucoma is a heterogeneous group of optic neuropathies which a complex genetic basis. Primary Open Angle Glaucoma (POAG) is one of the subtypes of glaucoma occurs most frequently. It has been reported that mutations in the myocilin (MYOC) gene cause POAG, which implies a possible role for the product of this gene in the intraocular pressure (IOP) elevation. Approximately 2% of Indian POAG patients harbored MYOC mutations. The MYOC gene consists of three exons that together encode for 55–57 kDa myocilin protein with 504 amino acids.

A plausible model has been built for myocilin protein by the research team, to understand the structural basis of the protein and the mutations. To understand the association of mutations with POAG, the mutations were mapped onto the structural model. Though synonymous or silent mutations do not cause any amino acid change, such mutations have been reported to be associated with POAG. Although environmentally induced conformational changes remain a distinct possibility, since these mutations cannot be directly correlated with possible protein conformational changes, they become attractive candidates for investigating other possible mechanisms. Single base changes could lead to activation of cryptic splice sites, resulting in



Space-filling model in four orientations for modified myocilin proteins. Full length myocilin, deletions due to presence of stop codon and deletions/modifications due to possible alternative splicing caused by the single nucleotide polymorphism shown in the model. Different regions in the model are colored as follows; NH2-terminal region (Orange), coiled coil region (Pink), hinge region (Cyan), COOH-terminal region (Yellow), regions predicted to be deleted (White) due to stop codon mutation or possible alternative splicing, regions predicted to be modified due to possible alternative splicing

aberrant splicing. The possibility of such an alternative splicing mechanism induced by genomic variations in the human myocilin gene was explored using sequence analysis tools.

The specific aim of this study was to examine the possible role of alternate splicing leading to aggregation of myocilin in primary open-angle glaucoma.

The model for myocilin was built using a knowledge-based consensus-modeling approach. Several single nucleotide variations found in the myocilin (MYOC) genomic region were collected and examined for their possible role in causing splice-site alterations. These variants were mapped onto the model built to show the possible altered, or truncated region.

A total of 124 genomic variations were screened, and six polymorphisms that lead to altered protein products were detected as possible candidates for the alternative splicing mechanism. Five of these lie in the intronic regions, and the one that lie in the exon region corresponded to the previously identified polymorphism (Tyr347Tyr) implicated in primary open-angle glaucoma. Experimentally screening of the predicted splice site variants of intronic region of MYOC gene in 150 open-angle glaucoma patients and 50 normal age-matched control subjects showed the presence of predicted g.14072G>A polymorphism, g.1293C/T heterozygous polymorphism, instead of our predicted g.1293C/- polymorphism. Other than the prediction, two novel SNPs (g.1295G>T and g.1299T>G) and two reported SNPs (g.1284G>T and g.1286G>T) were also identified. Cluster analysis showed the g.14072G>A homozygous condition was more common in this cohort than the heterozygous condition.

Implication of the project

This study suggests that polymorphisms in the myocilin genomic region that causes synonymous codon changes or those that occur in the intron regions can possibly lead to altered myocilin protein products through altered intron–exon splicing.

Publication

P.J. Eswari Pandaranayaka, N. Prasanthi, N. Kannabiran, K. Rangachari, M. Dhivya, Subbiah R. Krishnadas, P. Sundaresan, S. Krishnaswamy “Polymorphisms in an intronic region of the myocilin gene associated with primary open- angle glaucoma—a possible role for alternate splicing.” *Molecular Vision* 2010; 16:2891- 2902.

Presentation

Namburi Prasanthi, P J Eswari Pandaranayaka, K Rangachari, Subbiah R Krishnadas, S Krishnaswamy, P Sundaresan. “Myocilin Gene Splice Site Variants Role in POAG”. Indian Eye Research Group 18th Annual Meeting, LV Prasad Eye Institute, Hyderabad (2010); Abstract No: IBP016.

Molecular genetics of leber congenital amaurosis in south Indian population

Investigators : P. Sundaresan (Aravind Medical Research Foundation, Madurai)
P. Vijayalakshmi (Aravind Eye Hospital, Madurai)
Research scholar : Anshuman Verma
Funding agency : Indian Council of Medical Research and University Grant Commission

Background and aim

Leber’s Congenital Amaurosis (LCA) is a genetic retinal disorder causing congenital blindness. 15 genes involved in various functional pathways have been identified for disease causing mutations. The aim of the study is to identify the spectrum of genetic variations in these candidate genes. The genetic spectrum of RPE65 mutation is essentially required in support of emerging gene therapy treatment for LCA. Therefore, screening of RPE65 gene in 15 clinically well diagnosed LCA cases was initially started through direct sequencing. In addition 50 ethnically matched healthy control samples were sequenced to validate the pathogenic nature of variations.

A novel homozygous thymine insertional mutation at c.361 (c.361insT) was identified in one patient. This insertion was not detected in 50 control samples. The same patient also harbors an intronic polymorphism G/C (rs1925955). Screening of other LCA genes with aid of high throughput techniques in 50 LCA patients is under investigation.

Implication of the project

These results show that RPE65 accounts for 6-10% of LCA cases. The screening of other LCA genes with high throughput technique will help to know the genetic mutational spectrum of LCA in south Indian population which will aid in genetic diagnosis of the patients eligible for possible gene therapy in future.

Presentations

Anshuman Verma, Sundaresan Periasamy, Vijayalakshmi Perumalsamy “A Novel Insertional RPE65 Mutation in Southern Indian Cohort of Leber Congenital Amaurosis (LCA) Patients” US ARVO 2011 Annual Meeting, Fort Lauderdale, Florida, USA (2382/D645)

Involvement of mitochondrial genes in Leber’s Hereditary Optic Neuropathy (LHON)

Investigators : P. Sundaresan (Aravind Medical Research Foundation, Madurai)
S.R. Krishnadas, S. Mahesh Kumar (Aravind Eye Hospital, Madurai)
Funding agency : Department of Science and Technology

Background and aim

Leber’s Hereditary Optic Neuropathy (LHON) is a maternally inherited neurodegenerative disorder which results in blindness in young adults due to degeneration of Retinal Ganglion Cells (RGC). LHON is due to one of the three primary point mutations: m.3460G>A, m.11778G>A and m.14484T>C, all of which involve genes encoding complex I subunits of the mitochondrial respiratory chain. The factors influencing vision loss in an individual include heteroplasmy, haplogroup and tissue specific threshold effect. The prevalence of the primary mutations is less in south Indian population. The aim of the study is to screen the entire mitochondrial genome to identify the variations and specific mutation associated with LHON patients in south India.

Screening of mitochondrial genome

15 LHON affected individuals and 15 age matched controls were recruited for the study. 24 primers were used to screen the entire mitochondria of the individuals. The sequences are analyzed against rCRS (revised Cambridge Reference Sequence). The analysis revealed two primary mutations (m.3460G>A & m.11778G>A). Three new variations were identified in LHON patients, mutation in t-RNA serine (m.7448A>G), t-RNA leucine (m.12308A>G) and ND3 (m.10158T>G). In case of controls only haplogroup specific mutations were identified.

Implication of the project

This result suggests that the mutations occurring in t-RNA may result in change in the clover leaf structure. Mutation in ND3 (NADH dehydrogenase 3) (p.S35A), may change the protein conformation. These pathogenic mitochondrial variations can cause mitochondrial dysfunction and lead to apoptosis in Retinal Ganglion Cells (RGC).

Presentations

1. C.Jayashree, Dr. S. Mahesh Kumar, Dr. P. Sundaresan. “Involvement of mitochondrial genes in Leber’s Hereditary Optic Neuropathy (LHON).” International conference on genetic eye diseases and retinoblastoma, Narayana Nethralaya, Bangalore (2011). Abstract number: 115.

DEPARTMENT OF OCULAR MICROBIOLOGY

Infectious agents as a cause of ocular morbidity are well recognized area of concern in most of the developing world. The department of Ocular Microbiology at AMRF is routinely involved in understanding the nature of these infectious agents. In addition, with the advantage of having one of the largest repository of ocular isolates, the department is poised to design and conduct basic research which will specifically address the needs of the region.

The thrust areas of research in the Department of Ocular Microbiology are detection and discovery of novel pathogens causing various eye infections using both conventional and newer molecular technologies. The laboratory undertakes investigation, epidemiology, and in vitro susceptibility studies of ocular pathogens that can help to improve patient outcomes. The other areas of interest are in the understanding of the pathogenesis of ocular infectious diseases. Some of the common organisms that cause sight threatening eye diseases are fungus like *Aspergillus* and *Fusarium* and bacteria like *Pseudomonas* and *Staphylococcus*. Our main focus of research is on understanding the immune response in fungal and bacterial corneal ulcers with the aim of identifying potential targets that can be used in the treatment. The pathogenesis and virulence mechanisms of other common ocular infections-like mycobacterial, Leptospiral and Trematode are also being studied. Several laboratory methods to elucidate the mechanism of how systemic infection results in ocular inflammation are also applied. The laboratory has received grants from various government and non--governmental agencies. It is envisaged that the research work done in the department of Ocular Microbiology will help ophthalmologists and vision scientists working with infectious eye diseases to devise newer strategies to combat and prevent blindness due to infections.

The ongoing Projects are as follows

Host immune response in fungal and bacterial corneal ulcers in humans

Investigators : Lalitha Prajna, Dr. Venkatesh Prajna (AMRF, Madurai)
K. Dharmalingam (Madurai Kamaraj University, Madurai)
Eric Pearlman (Case Western Reserve University, Cleveland, USA)

Research scholar : Siva Ganesa Karthikeyan.

Funding agency : Indian Council of Medical Research.

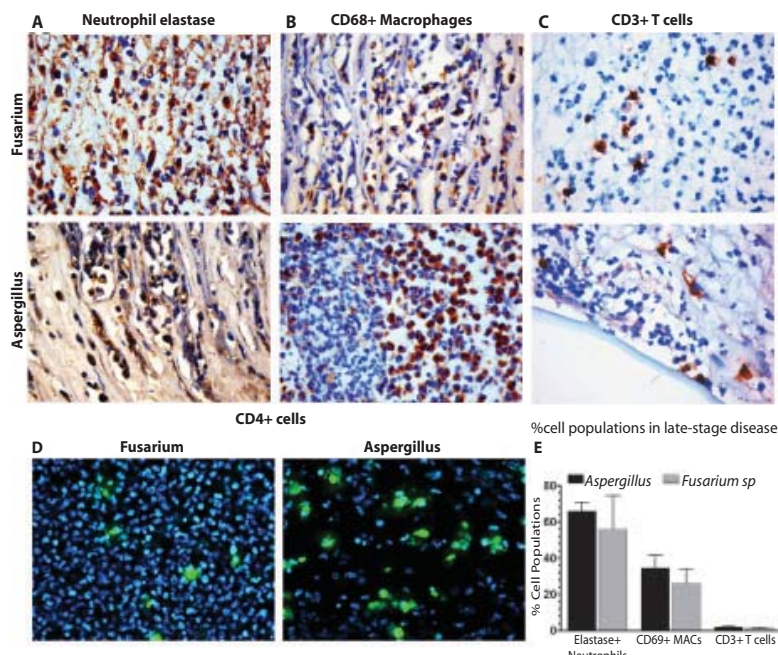
Infective corneal ulcers due to fungus or bacteria are a major cause of blindness in India and it occurs in healthy individuals engaged in outdoor activities. A corneal ulcer occurs following trauma with plant/vegetative matter or soil. The infective agent either bacteria or fungus that enters the eye along with the foreign body causes the ulcer and the infection if not treated promptly and correctly, results in blindness. Host response is also responsible for the potential damage caused by the invading organism. The aim of this study is to understand the mechanisms of how these organisms establish the infections and how the host responds to the infections. This study mainly concentrates on studying the innate immunity of fungal infections as well as the adaptive immune response. This study will also involve bacterial infections to understand the specificity of the response.

A. Host immune response against fungal keratitis

Filamentous fungi of the genus *Aspergillus* and *Fusarium* are major causes of fungal corneal ulcers in the world, resulting in significant visual impairment and blindness. Fungal spores are ubiquitous in the environment, which can gain its entry to corneal stroma following trauma. Once the corneal epithelial barrier is breached, fungal spores germinate and penetrate throughout the corneal stroma and require topical anti-fungal treatment. However, depending on the inoculum and the time between infection and the treatment,

fungal growth can be arrested or in very severe cases the infected individuals have to undergo corneal transplantation or even enucleation of the affected eye. The epithelium of the cornea forms a physical barrier and prevents microorganisms from entering the other layers of the cornea. Surface of the cornea expresses certain receptors that are very important in the reorganization of the invading pathogen and this is called “pathogen pattern recognizing receptors” such as Toll Like Receptors (TLR). Once the spores enter the corneal stroma, the resident macrophages in the cornea detect the presence of germinating conidia leading to proinflammatory cytokine production followed by cellular recruitment into the cornea which lead to the elimination of the offending pathogen. However, immune response in humans during fungal keratitis is not well understood. In this study, The host response to these organisms in corneal tissue within three to seven days following infection was characterized. RNA was extracted from 110 corneal ulcers infected with either *F. solani* or *A. flavus*, and gene expression was analyzed by quantitative PCR.

During the infection in the human cornea by the fungus *Aspergillus* sp and *Fusarium* sp, they initiate a cascade of events leading to the activation of immune cells. The cornea had only a few immune cells mainly the macrophage and the dendritic cells. The entry of the fungal pathogens are recognized by these immune cells through Toll family Receptors (TLR2, and TLR4) and C-type lectin receptors (Dectin-1) which leads to the release of the various pro inflammatory cytokine (IL-1 α , IL-1 β , IFN γ , IL-17, and TNF α) as well as the chemokine (IL-8). The chemokines IL-8 plays a crucial role in recruiting the new immune cells from the neighboring blood vessels to the infection site for killing the fungus. Neutrophils are the major type of cells found to be involved in this mechanism.



Immunostained Corneas with antibodies to (A) neutrophils (elastase), (B) macrophages (CD68), (C) CD3 and (D) CD4, and counterstained by hematoxylin (A-C), and DAPI (D). (E) Percent cell type in *Fusarium* and *Aspergillus* infected corneas

Study also showed the possibility of involvement of the T cells during the later stage of this disease. Current study is in progress to understand the role of these T cells against the fungal pathogens.

B. Host immune response against the bacterial infection

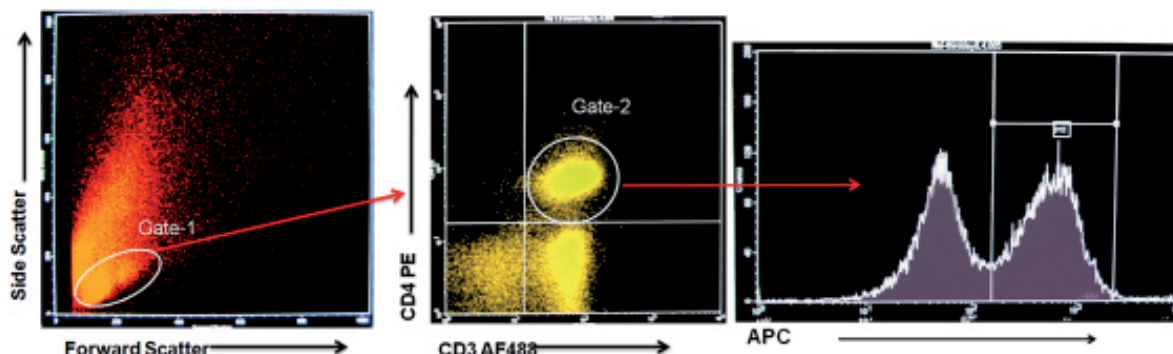
A similar study as described above is being undertaken to study the host immune response in case of bacterial corneal ulcers. Among the bacterial pathogens, *Pseudomonas aeruginosa* and *Streptococcus pneumonia* are the major causative agents. In the developed countries bacterial keratitis are mainly associated with contact lens wearing, but in the developing countries corneal trauma predispose to the development of this disease. Once the pathogen enters the cornea, Toll like Receptors interact with the bacterial components such as flagellin and respond by activating signaling pathways in immune cells, resulting in the production of an array of cytokines and chemokines that in turn lead to the recruitment of cells like neutrophils and macrophages to the cornea. Immune response in humans during bacterial keratitis is not well understood and the host immune response may also contribute to the outcome of the disease and animal model studies demonstrate the importance of host immune response during the bacterial corneal infection, This study aims to understand the role of host immune mediators during *Pseudomonas* and *Streptococcus* infections in the

human cornea and to understand the extent of tissue destruction contributed by the host. This study also aims to characterize the virulence factors of the *Pseudomonas aeruginosa* and *Streptococcus pneumoniae* that cause corneal ulcers.

The findings so far show that in the bacterial infected corneal tissues TLR2, TLR4, TLR5, TLR9 and pro-inflammatory cytokines - IL-1 α , and IL-1 β and also the Inflammasome components ASC and NALP3 were found to be elevated. These immune mediators play a crucial role in recognition of bacterial components. This study is in progress to understand the role of other immune mediators possibly involved in this disease pathogenesis and the interaction of the various bacterial virulence factors

C. Study of the role of adaptive immune response in patients with fungal corneal ulcer

Fungal corneal ulcers occur most frequently after trauma to the cornea in people engaged in outdoor work. Fungal spores are present in abundance in the atmosphere in both tropical and temperate climates. Majority of people developing fungal corneal ulcers live in tropical climates and may have constant exposure to these fungal spores present in the environment. It is estimated that a normal individual in temperate regions of industrialized nations inhales 200 to 300 fungal spores daily. First exposure to foreign antigens typically leads to the development of memory T and B cells, which persists beyond the initial infection and respond more rapidly during a second infection. It is likely that fungal keratitis patients may harbor such memory T and B cell responses to both *Aspergillus* and *Fusarium* species. In the previous studies, both CD3/ CD4⁺ T-cells as well as elevated IL-17 and IFN γ in fungal-infected corneas were identified but it is unclear whether these T cells were pre-existing prior to corneal infection, or developed following fungal infection in the cornea.



Flowcytometry: Lymphocyte population is gated based on the cell size (forward scatter) and granularity (Side scatter) from (A) scattered plot and the CD3⁺/CD4⁺ double positive T cells were gated and used to plot the histogram (C) to estimate the specific cytokines (IFN- γ , IL-4 and IL-17) which is labeled with APC.

This work aims to test the hypothesis that normal healthy individuals as well as patients with fungal keratitis exhibit memory T cell responses to *Aspergillus* and *Fusarium* species by analyzing peripheral blood T cell specificity to *Aspergillus* and *Fusarium* antigens. The PBMC's are stimulated in the in vitro condition with the crude hyphal extract of the clinical fungal isolates and the specific T cell activation are examined using the Flow cytometry and ELISA.

This study is in progress and will examine T-Cell response in the peripheral blood mononuclear cells to fungal antigens (mainly *Aspergillus flavus*, *Aspergillus fumigatus* and *Fusarium*) by characterization of T-cell activation markers, and expression of T cell cytokines. Specific response to fungal antigens will also be studied and knowledge obtained from this study will enhance the current understanding of adaptive responses during fungal keratitis.

Publication

- Siva Ganesa Karthikeyan, Sixto M. Leal, Jr, Venkatesh Prajna, Kuppamuthu Dharmalingam, David M. Geiser, Eric Pearlman, Prajna Lalitha "Expression of innate and adaptive immune mediators in human corneal tissue infected with aspergillus or rusarium". Journal of infectious diseases 2011;204(6):942-50

Presentation

- “Elevated expression of TLRs, Dectin-1, IL-17, and ISN- γ but not Dectin-1 or IL-4 in human corneas infected with *Aspergillus* or *Fusarium*” Gordon Brown Conference – Immunity to fungal infection, Galveston, Texas, USA (2011).
- “Innate immunity in *Aspergillus* and *Fusarium* keratitis in Tamil Nadu, India”, US ARVO 2010 Annual meeting, Fort Lauderdale, Florida, USA.

Molecular insights into the aetiology of infectious uveitis

Investigators : Lalitha Prajna, Rathinam Sivakumar (Aravind Eye Hospital, Madurai)
Research scholar : Reena Joseph
Funding agency : Department of Biotechnology

Uveitis, which involves infection and inflammation of the iris, ciliary body and the choroid of the eye, can cause debilitating pain and can lead to serious and permanent visual loss due to complication like cataract and glaucoma. Causes of infective Uveitis include bacterial causes like *Mycobacterium tuberculosis*, *Leptospirosis*, etc. Viral causes like Herpes, Cytomegaloviruses and emerging viral infections like West Nile, parasites and fungus can also cause infective Uveitis. Clinical confirmation of Uveitis due to these different agents is very difficult and conventional microbiology techniques are not sensitive for detection. Molecular techniques offer more sensitive, specific and more rapid diagnosis of infectious Uveitis which can aid in specific treatment for the patients.

Real Time PCR is one such technology which can be used for the specific diagnosis of the Uveitis and which is more sensitive than the conventional PCR. Real Time can be used for determining the pathogenic load, gene expression and regulation and allelic discrimination in the samples. The purpose of this study was to investigate the cause of infectious uveitis and to confirm the etiology by molecular techniques like Real Time PCR and sequencing for identifying the organism and strain variations for bacterial, viral and parasitic causes.

The techniques have been standardized for *Mycobacterium tuberculosis*, *Leptospira*, bacteria, Herpes simplex, Cytomegalovirus, Varicella Zoster, *Toxoplasma* and other emerging infectious agents like West Nile Virus and *Rickettsiae*. Ocular samples like vitreous and aqueous humor were collected from patients suspected with infectious Uveitis and Real time PCR was run for the respective infectious agents. The sensitivity and specificity was established. The sensitivity as compared to conventional PCR was found to be more in identifying infectious agents. This technique is being used as a routine in patient care.

Publications

Molecular detection and characterization of West Nile Virus associated with multifocal retinitis in human patient from Southern India. *International Journal of Infectious diseases* (In press).

Presentation

Real Time PCR in the diagnosis of Postoperative Endophthalmitis, Indian Eye Research Group, 18th Annual meeting, LV Prasad Eye Institute, Hyderabad (2010).

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

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

Background and aim

Uveitis due to trematode infection was identified by the presence of tegument of trematode in the Sub-conjunctival scleral nodule and anterior chamber granulomas in children who were exposed to village pond or river water in various districts of Tamilnadu and Kerala (Am Academy of Ophthalmology, 2001 and Am J Ophthalmology 2002).

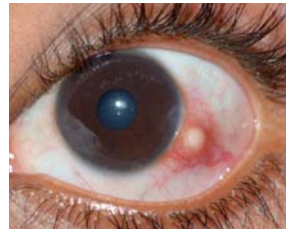
In order to confirm the etiology of the disease in relation to environmental source, present study aims to confirm the causative agent, their source and to understand the pathogenesis of disease using molecular techniques. Anterior chamber granuloma and sub-conjunctival granuloma were collected from 24 clinically suspected patients treated in Aravind Eye Hospital, Madurai. Snails were collected from the 14 different village ponds to obtain cercaria on the basis of patient's history of taking bath. DNA was extracted from the subconjunctival and anterior chamber granulomas and environmental cercaria. Conventional and Real-Time polymerase chain reaction assays were carried out targeting rDNA region spanning the ITS2 sequence of trematode using universal primer. The trematode DNA found in the 7 human ocular granulomatous samples (four subconjunctival and three anterior chamber granuloma) out of 24 was matched with the environmental cercariae DNA which were harvested from the snail (*Melanoistheria*) from the disease locality. The present study gives strong evidence of trematode etiology in parasitic granulomatous uveitis in children of south India.

Implication of the project

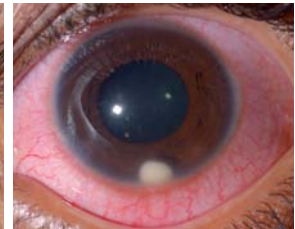
Once the etiology is confirmed, preventive measures can be planned to protect the children in this geographic region.

Presentations

1. Lalan Kumar Arya, SR. Rathinam, Lalitha Prajna, Usha Kim, Veena Tandon "Presumed trematode induced granulomatous Uveitis in south India" 18th Indian Eye Research Group Meeting, LV Prasad Eye Institute, Hyderabad (2010).
2. Lalan Kumar Arya, SR. Rathinam, Lalitha Prajna, Usha Kim, Veena Tandon "Presumed trematode induced subconjunctival and anterior chamber granulomatous Uveitis in south India in 22nd National Congress of Parasitology, University of Kalyani, Kalyani, West Bengal (2010).



Clinical photograph of anterior chamber granulomatous uveitis

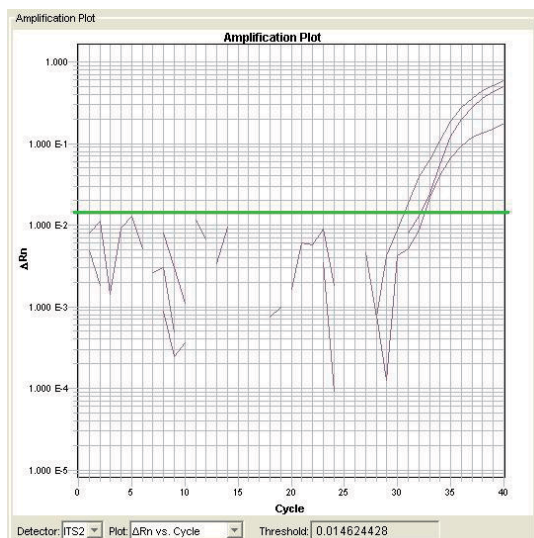


Clinical photograph of subconjunctival granulomatous uveitis

Amplification plot of environmental trematode DNA



Amplification plot of sub conjunctival granulomas DNA of patients



DEPARTMENT OF PROTEOMICS

The study of the protein levels and interactions at the cellular level helps in understanding the disease process and progression better. The interaction and localization of proteins are important assessors for the proper functioning of the cells. The study of these proteins and their interactions, collectively called as proteomics is gaining prominence in recent days. The proteomics at AMRF laboratory is engaged in using this nascent technology to understand ocular diseases such as fungal keratitis, diabetic retinopathy and glaucoma which are widely prevalent in Indian population. Understanding the manifestation and the progress of the disease at the protein level help us assess the host response to the pathology as well. Such knowledge gained will ultimately personalize the treatment strategies for individual patients. The diagnosis of potential biomarkers may help understand the population at risk, so that effective and early treatment strategies can be planned in a cost effective manner.

At present, the laboratory focuses on three specific areas namely, fungal keratitis, diabetic retinopathy and primary open angle glaucoma. Fungal keratitis is a disease, more common in developing countries like India and hence knowledge gained will immensely benefit people from the lower socio economic group. The sheer magnitude of diabetic retinopathy and the lack of effective screening mechanisms for primary open angle glaucoma have prompted us to concentrate on these two common eye diseases. The aim of the research carried out is to apply a variety of proteomic tools to understanding the pathobiological mechanisms and to identifying new diagnostic, prognostic and therapeutic biomarkers for these clinical conditions.

Host pathogen interaction in human mycotic keratitis

Investigators : N. Lini, Lalitha Prajna, N. Venkatesh Prajna (Aravind Medical Research Foundation, Madurai)
K. Dharmalingam (Madurai Kamaraj University, Madurai)

Research scholar : Ms. S. Ananthi

Funding agency : Department of Biotechnology

Fungal keratitis is an important cause of corneal disease and is reported to constitute up to two-third of all cases of suppurative keratitis in developing countries like India. It is an enormous public health problem in India affecting young-adults in their most economically productive period. The mainstay of treatment of this disease is topical antifungal medications and in advanced cases, surgery in the form of penetrating keratoplasty. A population-based study in the Madurai district of southern Tamil Nadu, India, performed by Aravind Eye Hospital had estimated an annual incidence of corneal ulceration of 11.3 per 10,000 populations, which is 10 times that of the United States. The Cornea department in Aravind Eye Hospital sees an annual average of around 1200 patients with fungal keratitis. This is probably the largest number of patients seen in our country in a single institution. The poor visual results often obtained in these patients, even after treating them with the appropriate, available antifungal agents, clearly emphasize the need to develop newer therapeutic modalities to combat fungal keratitis, so that the need to operate on this condition decreases. A study conducted by Srinivasan and his group showed *Fusarium* (47%) and *Aspergillus* (16%), were the predominant etiological agents responsible for 44% of all corneal ulcers. To understand the mechanism of pathogenesis of fungal keratitis, the protein specific alterations in the tear as well as infected cornea from keratitis infected patients were examined and compared with healthy control samples.

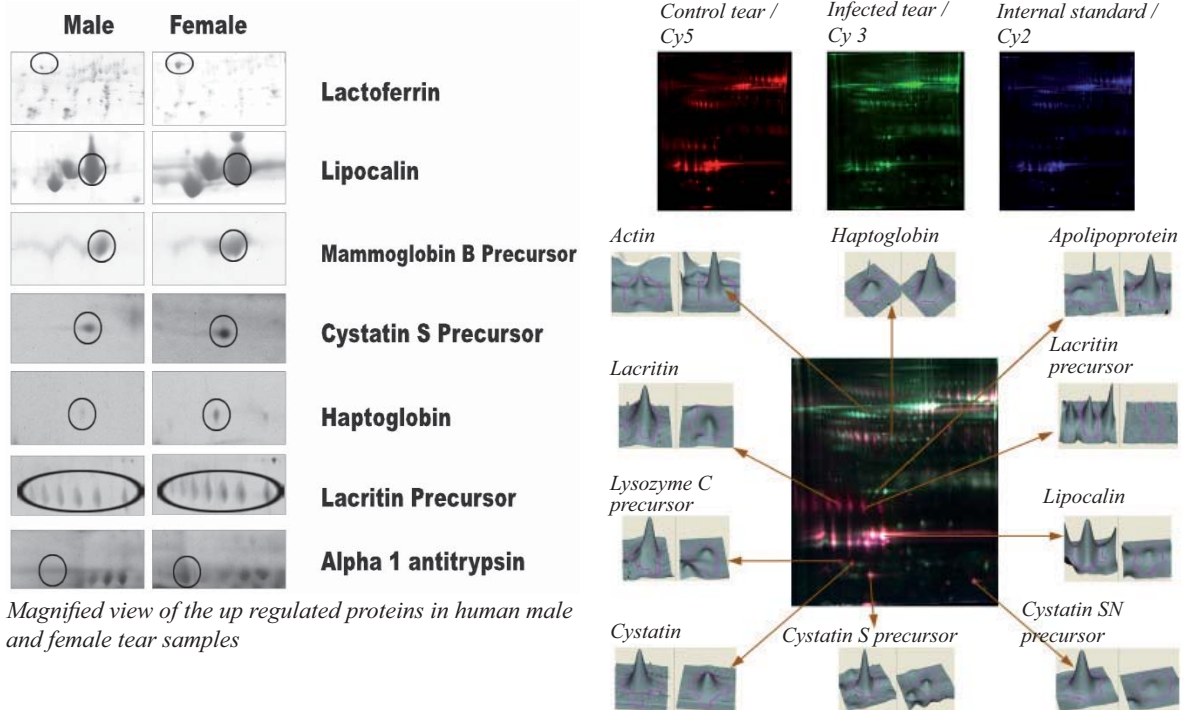
The proteomic profile of tear fluid is of fundamental interest in eye research. Tear has critical roles in the optical system, lubricates the eye, provides nutrients and growth factors to the epithelium and serves as a barrier to the outside environment. Human tear fluid is shown to have more than 600 proteins and a

Progression of fungal keratitis



recent report identified 491 proteins. Tear sample preparation method for two-dimensional (2D) analysis was optimised and determined the protein profile of tear fluid from healthy males and females was determined.

To find the most efficient method for tear sample preparation, four widely applied precipitation methods and ultrafiltration were compared. Of these, TCA precipitation and ultra filtration resulted in efficient sample concentration and desalting. Using this optimized method, tear protein profile was analyzed from healthy males and females. Of the thirty six tear proteins identified by LC-MS/MS, seven tear proteins (mammoglobin B precursor, cystatin S precursor, lipocalin, haptoglobin, lacritin precursor, alpha 1 antitrypsin, and lactoferrin) in the female tear sample were found to be significantly up regulated in the healthy female tear samples when compared to the male tear samples. These results indicate that the tear protein profile differs with respect to the sex. Mostly, the up regulated proteins are involved in the local immune defense; implying that there may be a sex difference in the ability to defend against infection at the anterior segment of the eyes of normal individuals. These observations imply the importance of gender difference in the understanding the efficacy of the drug treatment.



Magnified view of the up regulated proteins in human male and female tear samples

2D- DIGE analysis showing the differentially regulated proteins in 3D view between control and infected tears

Complex patterns of tear and corneal proteins were detected from fungal keratitis patients. Among 37 tear proteins identified, 2D-DIGE analysis showed down regulation of 12 proteins including lipocalin precursors, Lysozyme-C precursor, Cystatin precursors, Lacritin precursor, Mammaglobin-B precursor, Lactoferrin, Prolactin inducible protein and up regulation of 13 proteins including Serum albumin, Haptoglobin, Apolipoprotein E, Actin in the infected tear samples. Among 30 corneal proteins identified, Transforming growth factor precursor, Ferritin heavy chain, Glutathione S-transferase, Peroxiredoxin 2, Chloride intracellular channel proteins were down regulated and Transthyretin precursor. Ferritin light chain were up regulated in the *Fusarium, A. flavus, A. fumigatus* infected corneas when compared to normal corneas with respect to 3 biological replicates in each group.

Comparing Tear and Cornea 2D profile, it was found that 16 proteins were identical and among them, 14 have been identified. The presence of identical proteins between tear and cornea gives possible clues about the migration of the proteins. The down regulated tear and corneal proteins are known to be involved in local immune function and oxidative stress related gene expression respectively. Thus the study reveals the significance of tear and corneal proteomic responses in fungal keratitis with respect to early and late stage of infection, which can give further insights for better treatment.

Publications

- Ananthi S, Santhosh RS, Valarnila M, Prajna NV, Lalitha P, Dharmalingam K., “Comparative proteomics of human male and female tears by Two-dimensional electrophoresis”. *Exp Eye Res.* March 2011 online, 92; 454-463.
- Ananthi S, H. R. Krithiga, P. Lalitha, N. V. Prajna, and K. Dharmalingam. “Proteomic analysis of tear and cornea in patients with fungal keratitis”. *Invest. Ophthalmol. Vis. Sci.* 2010 51: E-Abstract 2893. (US ARVO 2010, Fortlauderdale, Florida, USA)

Presentations

- Ananthi S, Venkatesh Prajna N, Lalitha P, Dharmalingam K “Comparative analysis of the tear and cornea protein profile in mycotic keratitis patients” at 5th AOHUPO congress on new perspectives in proteome research, 2010, CCMB, Hyderabad
- Ananthi S, Venkatesh Prajna N, Lalitha P, Dharmalingam K. “Corneal proteome profile in fungal keratitis patients” at National conference, Biotech 2010, Madurai Kamaraj University, Madurai

Characterization of serum proteome in proliferative diabetic retinopathy

Investigators : N. Lini, VR. Muthukkaruppan (Aravind Medical Research Foundation, Madurai)
T.P. Vignesh (Aravind Eye Hospital, Madurai)
K. Dharmalingam (Madurai Kamaraj University, Madurai)

Research Scholar : M. Valarnila

Funding agency : Champalimaud –AMRF Research Grant

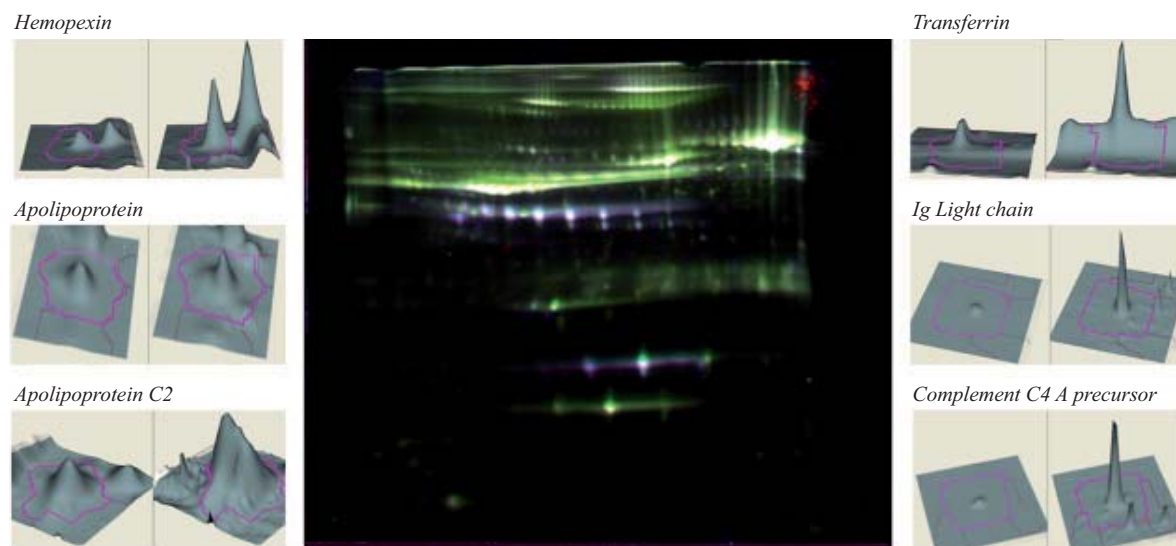
Diabetic Retinopathy (DR) is one of the most common micro vascular complications caused by Diabetes Mellitus (DM) and is a leading cause of vision loss among working-age adults in both the developing and developed countries. It has been suggested that good diabetes control is important to prevent development of retinopathy in diabetes patients. However some diabetes patients develop DR and others escape retinopathy irrespective of the blood sugar levels and sugar control measures. Thus we find diabetes patients without retinopathy even for 10 years and others develop DR within a short period. Therefore, it is required to identify serum biomarkers to distinguish those diabetes patients who are at risk of developing retinopathy and progression.

A comprehensive proteomic analysis and comparison of serum samples from individuals with type 2 diabetes mellitus (T2DM) but without Diabetic Retinopathy (NDR), with Proliferative Diabetic Retinopathy

(PDR) and non-diabetic individuals (Controls) was performed. Neat/ albumin and IgG depleted sera and immunodepleted samples were analyzed using SDS-PAGE and two-dimensional gel electrophoresis (2DE). The protein spots/bands were excised and digested using trypsin and the dried peptides were analysed using Liquid Chromatography-Tandem Mass Spectrometry (LC-MS/MS). Further, proteins were identified using MASCOT (matrix science) search engine.

Proteins involved in binding, catalytic activity, regulatory activity, inhibition activity, transport activity, cellular and sub cellular localization and proteins involved in various other functions have been identified. Although the pattern of protein expression profile of the serum was identical in-between the study groups, the volume of some protein spots in PDR gels were increased when compared with the gels of NDR and control. Among the identified protein spots the levels of 12 proteins were increased in PDR serum when compared with NDR samples. Apolipoprotein E and C2 were increased in PDR when compared with NDR and control. The most abundant protein in serum is albumin, which is 60% of total protein and increased 4.0-fold in PDR patients. Proteins involved in binding, catalytic activity, transport activity and others functions such as haptoglobin β chain, haptoglobin α 2 chains, apolipoprotein J, hemopexin, complement C3, ceruloplasmin, alpha-1B-glycoprotein precursor, apolipoprotein A-I and alpha 1 anti-trypsin were increased in the PDR group when compared with the NDR group

Haptoglobin shows significant level of upregulation in PDR. Apart from haptoglobin many acute phase response proteins including ceruloplasmin, Hemopexin and complement factors were detected in this study. Differentially expressed proteins such as Ig mu chain C region, Apolipoprotein J, Transferrin, Hemopexin were screened and quantified using Differential In-Gel Electrophoresis (DIGE).



Differential regulation of serum proteins and their quantification using DIGE (PDR vs control)

Acute phase response is believed to contribute to defensive or adaptive capabilities, although the excessive overproduction of acute-phase proteins may result in tissue damage. Thus, it is possible that increased level of these acute phase response proteins in PDR are related to inflammation and subsequent retinal damage in DR. The data provides insight on the extent of serum protein relation with PDR, thereby giving fundamental information for further research. Based on the results, we proposed that the analysis of haptoglobin expression and other protein changes could be used as a predictive biomarker for early diagnosis and disease progression.

Publications

- M. Valar Nila, T. P. Vignesh, V. R. Muthukkaruppan, and K. Dharmalingam. "Evaluation of haptoglobin and its isoforms as plasma/serum biomarker for diabetic retinopathy". Invest. Ophthalmol. Vis. Sci. 2010 51: E-Abstract 5088. (US ARVO 2010, Fortlauderdale, Florida, USA).

Presentations

- Valar Nila. M, Vignesh.T.P, Muthukkaruppan.VR, Dharmalingam. K (2010) "Proteomic analysis of serum in patients with diabetic retinopathy" at 5th AOHUPO Congress on new perspectives in proteome research, CCMB, Hyderabad, India
- Valar Nila. M, Vignesh. T.P, Muthukkaruppan.VR, Dharmalingam. K (2010) "Proteomic profiling of serum in proliferative diabetic retinopathy" at Biotech 2010 conference, Fusion of Advanced Research and Teaching, MKU, Madurai, India

Towards the identification of Biomarkers for primary open angle glaucoma

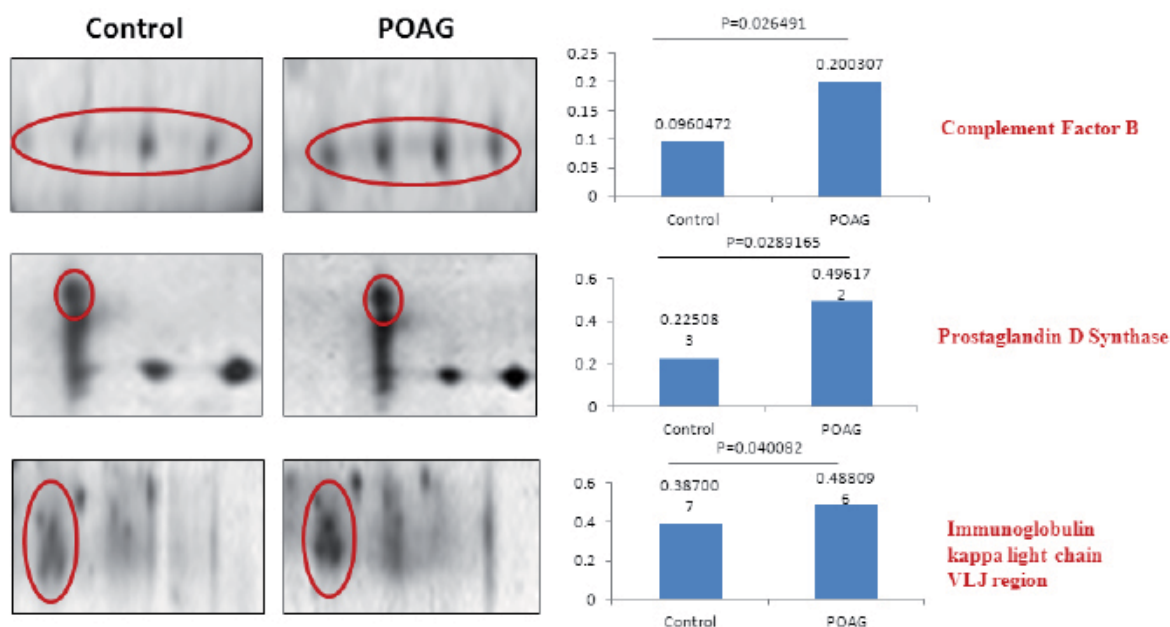
Investigators : S.R. Krishnadas (Aravind Eye Hospital, Madurai)
 N. Lini, P. Sundaresan (Aravind Medical Research Foundation, Madurai)
 K. Dharmalingam (Madurai Kamaraj University, Madurai)

Research Scholar : I. Paul PownRaj

Funding agency : Department of Biotechnology

Primary Open Angle Glaucoma (POAG) is one of the leading causes of blindness in the elderly population worldwide. It is asymptomatic and the loss of vision is irreversible. It is characterized by the excavation of optic nerve head, apoptotic death of retinal ganglion cells and visual field loss. However, the pathogenesis and the mechanisms of glaucoma manifestations are still not fully explained. Aqueous Humor (AH) is closer to the site of damage and it is believed to be involved in the pathogenesis of POAG. After carefully

Differential regulation of proteins in POAG AH:



Comparative protein profile of Aqueous humor of control and POAG pools : 40 µg of aqueous humor proteins were loaded for first dimension (pH 4-7, 24cm IPG strips were used) and 12.5% SDS-PAGE followed by glutaraldehyde silver staining. Marked are the spots showing differential expression. The protein names are indicated along the sides.

assessing patient medical profile, more than 350 AH samples (165 POAG & 185 Control) were collected during anterior chamber surgery for this study. Two dimensional gel electrophoresis was optimized by standardizing with various sample preparation techniques and Ultra-filtration followed by acetone precipitation was found to be the best method for protein concentration and desalting and is adopted as the standard methodology for the analysis.

AH proteins were identified from 2DE gels using nano LC-MS/MS. In total, we have identified 58 spots corresponding to 21 different proteins from 2DE gels representing a 2DE map of AH. We have already examined three biological replicates and one experimental replicate to study the differential regulation of proteins in the POAG samples compared with the control. 2DE data were analyzed using Image Master Platinum 7 software (GE Healthcare, Hongkong, China). The differentially regulated proteins were identified as prostaglandin D synthases, immunoglobulin kappa light chain VLJ region, and complement factor B. Depletion of high abundant proteins would give more insight into the aqueous humor proteome to look for more specific differences in POAG. 2D-DIGE will be employed to validate the regulation differences observed in the low abundant proteins between the control and POAG groups.

Presentations

- Paul Pown Raj. I, Krishnadas. SR, Sundaresan P, Dharmalingam.K (2011). “Understanding primary open angle glaucoma using aqueous humor: a proteomic approach” at The Indian Proteomics Conference, IPCON 2011, New Delhi, India

DEPARTMENT OF IMMUNOLOGY

The main focus of research in this department is to understand the pathogenic mechanism associated with development of infectious uveitis. Extensive studies were carried out on leptospiral uveitis which occurs weeks to months after systemic infection and leptospiral lipopolysaccharide was demonstrated to be a cause for the pathogenesis of the disease. With the establishment of the Microscopic Agglutination Test (MAT), the gold standard test for diagnosing leptospiral infection, the department is also a reference centre for serodiagnosis of both systemic and ocular leptospiral infection. It has been observed that the leptospiral uveitis patients developed cataract within a few months. The current thrust of research is to understand whether the acute development of cataract, in the leptospiral uveitis patients is due to an autoimmune mechanism.

Antigenic mimicry between leptospiral and human lens proteins

Investigators : Gowri Priya Chidambaranathan, VR. Muthukkaruppan
(Aravind Medical Research Foundation, Madurai)
SR. Rathinam (Aravind Eye Hospital, Madurai)
Research Scholar : Arun Kannan
Funding Agency : ALCON Anterior Segment Research Grant

Background and aims

The first epidemic outbreak of leptospiral uveitis after systemic infection following heavy monsoon rains and the largest case series was reported from Aravind Eye Hospital in 1994. It can be differentiated from other forms of uveitis on the basis of a specific combination of clinical features with development of early cataract in 12.9% of these patients. Similar to leptospiral uveitis in humans, *Leptospira* sp. is the causative factor for Equine Recurrent Uveitis (ERU) in horses. Antigenic mimicry between leptospiral proteins and horse ocular antigens (equine cornea and lens) has been suggested as a possible cause for recurrent uveitis in horses. Interestingly, a significant number of patients diagnosed as leptospiral uveitis and autoimmune uveitis (Fuch's and Behcet's uveitis with cataract as one of the complications) had antibodies to these leptospiral lipoproteins, indicating the possibility of autoimmune mechanism in the pathogenesis of uveitis in humans also. Hence the purpose of the current study is to experimentally confirm the antigenic mimicry between these proteins.

For this study, serum samples were collected after getting informed consent from patients diagnosed clinically as leptospiral uveitis, non-leptospiral uveitis, age related cataract and healthy population controls. Western blot analysis identified presence of antibodies to a 21kDa lens protein and a 36kDa leptospiral protein in sera of leptospiral uveitis patients while the control sera showed minimal reaction with these proteins. Further studies are being carried out to identify and characterize the proteins using LC-MS/MS and also to verify whether they are cross-reactive.

Thus this study will help us to understand the pathogenic mechanism associated with the development of early cataract in these uveitis patients with infective aetiology.

International leptospirosis MAT proficiency testing scheme

Investigators : Gowri Priya Chidambaranathan (Aravind Medical Research Foundation, Madurai)
SR. Rathinam (Aravind Eye Hospital, Madurai)
Research Scholar : Arun Kannan
Funding Agency : Aravind Medical Research Foundation

Microscopic Agglutination Test (MAT) is the gold standard test for serodiagnosis of leptospiral infection. The International Leptospirosis Society encourages all laboratories to participate in the International Leptospirosis MAT proficiency testing scheme every year, to validate the quality of the test. AMRF has been participating in this test from 2005 (Round 4) till 2011 (Round 9). Lyophilized coded serum samples are sent to the participants every year. These samples were analysed using the panel of live leptospiral serovars available in Aravind Eye Hospital and the results were submitted for evaluation by the international society. This validation is more important for periodical quality testing of the test since it is used not only for research purpose but also used as a service to other hospitals in and around Madurai, and Aravind satellite hospitals for serodiagnosis of leptospiral infection – both systemic and ocular.

DEPARTMENT OF STEM CELL BIOLOGY

The concept of adult stem cells (SC) in regenerative medicine is gaining prominence in recent times. Research at AMRF is focused on understanding the identification and potential use of corneal epithelial stem cells. A two parameter concept for identifying stem cells located in limbus – the cornea scleral junction has already been postulated and published. The current work focuses on developing a simpler and specific method for identification and quantification of limbal epithelial stem cells. The limbal niche that is thought to be responsible for the maintenance and expansion of stem cells is also being characterizing.

The limbal epithelial stem cells play a significant role in maintaining a steady supply of corneal epithelial cells, thereby maintaining corneal transparency. Damages to these cells occur due to thermal and chemical injuries. In cases of unilateral injury, limbal biopsy from the uninvolved fellow eye can be cultured and the epithelial sheet containing known amount of stem cells can be transplanted to the injured eye. In cases of bilateral injuries, the stem cells are acquired from non ocular sources such as buccal mucosa. In both cases, the department has been successful in the recovery of useful vision. At present, the team is developing simple, reproducible, cost-effective and xenobiotic-free culture condition to generate stem cell rich epithelium.

Developing xenobiotic-free culture conditions to generate stem-cell rich epithelium for corneal surface reconstruction

Investigators : C. Gowri Priya, VR. Muthukkaruppan (Aravind Medical Research Foundation, Madurai)
N. Venkatesh Prajna, M. Srinivasan (Aravind Eye Hospital, Madurai)
Research Fellows : T. Lalitha, Minu Jennifer, Saumi Mathews, Tilak Prasad
Funding Agency : ALCON Anterior Segment Research Grant, Defence Research Development Organisation, Aravind Medical Research Foundation

Background and aims

Transplantation of cultured autologous limbal/buccal epithelium is the current choice of treatment for patients with unilateral/bilateral limbal stem cell deficiency (LSCD) respectively. The research group has established a simple method of explant culture for expansion of limbal epithelial cells and insert culture using isolated buccal epithelial cells with growth arrested mouse fibroblast 3T3 as feeder layer for expansion of buccal epithelial cells. But the SC content in the cultured epithelium has not been reported so far since there is no single marker for both limbal/buccal mucosal epithelial stem cells (LESCs/BMESC). They have earlier demonstrated the importance of combining two parameters (high p63 expression in cells with large nucleus/cytoplasmic (N/C) ratio) and established it as a specific method for the identification and quantification of LESCs (Arpitha et al., IOVS 2005; Cornea 2008; Microscopy Res. Tech. 2008) and BMESCs (Priya et al., Accepted in Eye). In continuation, studies have been carried out to confirm this two parameter analysis and to develop simpler methods for SC identification. This method will be useful to validate the cultured limbal/buccal epithelium for its SC content. Further, with growing concerns regarding the potential transmission of infectious agents like prions and viruses from culture components of animal origin, it is essential to develop a xeno-free culture condition for the expansion of limbal/buccal epithelial cells.

Hence the aims of this study are

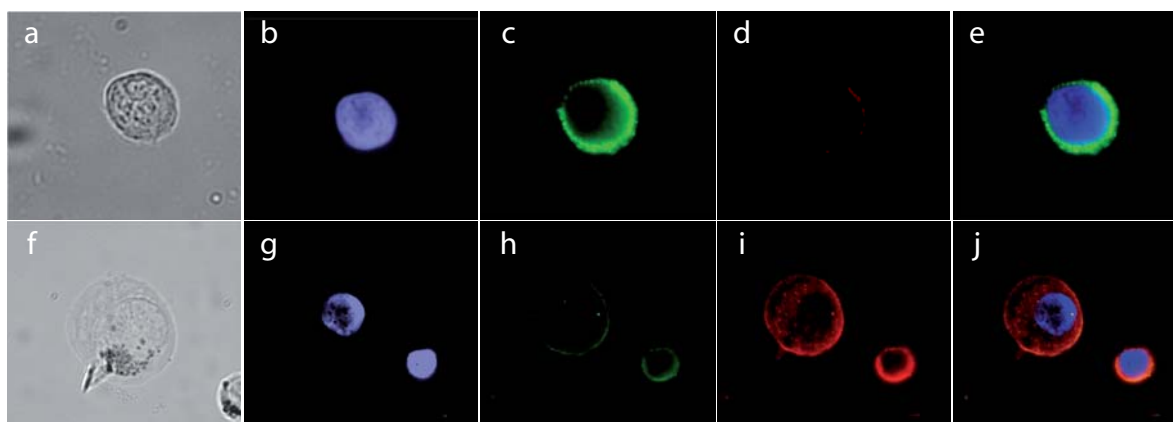
1. To demonstrate the presence of stem cells in the cultured limbal/buccal epithelium and to evaluate the cultured epithelium for its stem cell content.

- To develop a xeno-free culture condition for the ex-vivo expansion of limbal/buccal epithelial stem cells.
- To evaluate the efficacy of such cultured limbal/buccal epithelium in corneal surface reconstruction in patients with unilateral/bilateral LSCD.

The limbal tissues for this study were obtained from Rotary Aravind International Eye Bank and buccal biopsies from bilateral LSCD patients undergoing buccal epithelial cell transplantation, after getting informed consent.

Simpler and specific method for limbal epithelial stem cell identification and quantification

The two parameter analysis was further confirmed to be specific for SC identification by the high expression of the ATP binding cassette protein – ABCG2, in high p63 positive cells with high N/C ratio using confocal microscope. Based on this, a simpler method of high ABCG2 expression in cells with high N/C ratio was established to identify SCs using fluorescence microscope with ISIS V 5.4.2 software. The validity of this method was confirmed by the presence of small cells with high N/C ratio, highly positive for ABCG2 but negative for connexin 43, a gap junction protein (negative marker for SCs) only in the limbal epithelial cells and not in central corneal cells.



Limbal epithelial cells double immunostained for ABCG2 (FITC) and Connexin 43 (Alexa 594): a & f: phase contrast image, b and g: DAPI, c and h: ABCG2, d and i: Connexin and e and j: fluorescence merged image.

Upper panel showing a small cell highly positive for ABCG2 and negative for connexin – this cell represents Limbal epithelial stem cells.

Lower panel showing a large cell negative for ABCG2 and positive of connexin – a differentiated cell in the limbal epithelium.

Ex-vivo expansion of limbal/buccal epithelial stem cells

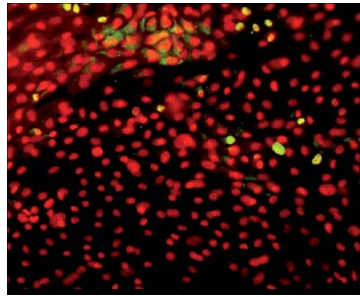
The presence of SCs in the cultured limbal epithelium was demonstrated functionally by the presence of slow-cycling, BrdU label retaining cells in the outgrowth. Such slow cycling cells were found only in the region of outgrowth closer to the explant, indicating the importance of the limbal stroma/niche in the maintenance of SCs (Figure). Further, analysis of cultured limbal epithelium by two parameter analysis using confocal microscope revealed a ten-fold increase in the SC content. Similar analysis of the cultured buccal epithelium revealed a six-fold increase in the SC content. Thus, for the first time we demonstrate that there is an increase in SC content while expanding the epithelial cells.

Region of Outgrowth

Periphery

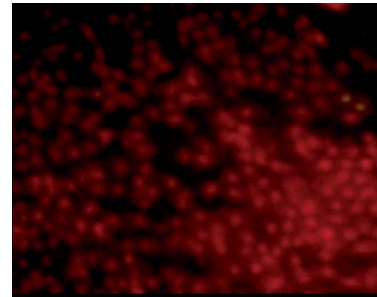
Proliferating Cells

20%



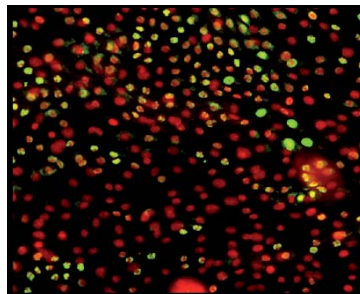
Label retaining cells

0.3%

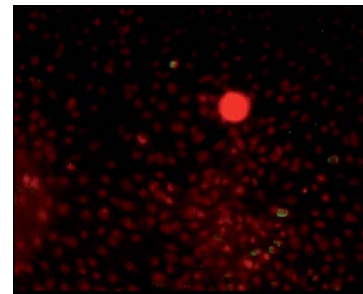


Intermediary

21%

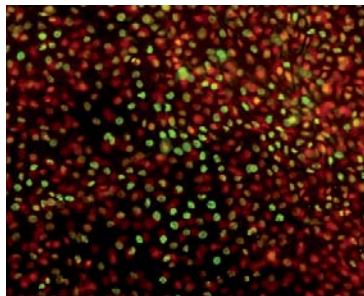


1.3%

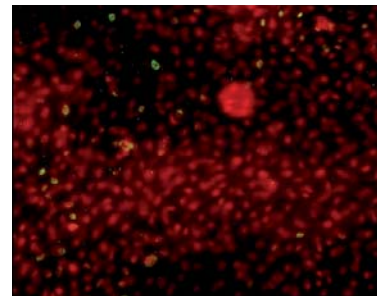


Near Explant

40%



4%



Presence of BrdU Label retaining cells in the outgrowth near the limbal explant in comparison to the periphery.

The proliferating cells in the outgrowth after 8 days was determined by pulse labeling of the cells with BrdU and the label retaining cells after a chase of 10 days (on 18th day).

Development of xeno-free culture condition

The limbal/buccal culture components of animal origin are fetal bovine serum (FBS) and mouse fibroblast 3T3. By two parameter analysis, it was established that use of autologous serum from patients to be equivalent to FBS in the maintenance of SCs in culture. Preliminary studies using adult human limbal fibroblast cell lines (hALF1,2) established at AMRF laboratory revealed its efficacy in supporting the SC content to be equivalent to 3T3. Further studies are being carried out to characterize these cell lines for human application.

Corneal surface reconstruction using cultured limbal/buccal epithelium

During 2010-2011, a class 1000 GMP (Good Manufacturing Practice) facility was established, in which, seven limbal and four buccal mucosal biopsies from LSCD patients were cultured. All patients who underwent limbal (30)/buccal (14) transplantation from 2007 onwards were followed up for a maximum of three years for anatomical and visual improvement. In both, the success of transplantation was 23-30%.

Anatomical and visual Improvement in a patient with unilateral LSCD after transplantation of *ex vivo* expanded autologous limbal epithelium

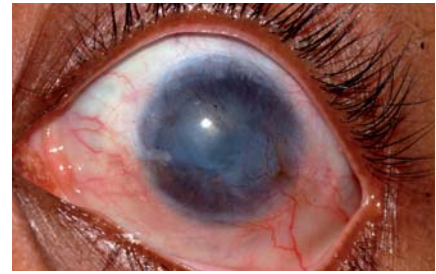
Studies are now being carried out to understand the basis for this sub-optimal success, to identify the niche factors associated with the maintenance of SCs and to identify better culture conditions for enrichment of SCs. Thus, this basic research on limbal/buccal epithelial stem cells will help establish a better method for expanding epithelial cells in compliance with GMP for clinical application, while preserving the proliferative potential and SC characteristics.

Publication

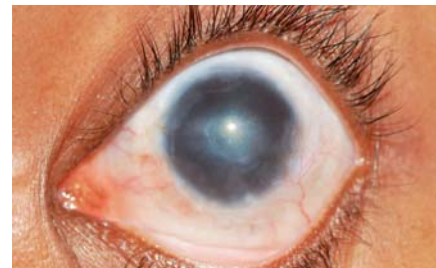
- Chidambaranathan Gowri Priya, Parthasarathy Arpitha, Sivaramakrishnan Vaishali, Namperumalsamy V. Prajna, Kim Usha, Kamdar Sheetal, Veerappan Muthukkaruppan “Adult human buccal epithelial stem cells: Identification, *ex-vivo* expansion and transplantation for corneal surface reconstruction” – accepted for publication in EYE.

Presentations

- Gowri Priya C, Arpitha P, Lalitha T, Jeyalakshmi S, Prabhu P, Prajna NV, Muthukkaruppan VR. “A specific marker for corneal epithelial stem cells and a simple method for their *ex-vivo* expansion under xenobiotic-free condition. Oral presentation in Asia ARVO, January 20-22, 2011 held at Singapore.
- Gowri Priya C, Arpitha P, Lalitha T, Vaishali S, Prajna NV, Usha Kim, Muthukkaruppan VR. “Characterization of buccal mucosal epithelial stem cells and evaluation of its efficacy in corneal surface reconstruction”. Oral presentation in Indian Eye Research Group 19th Annual Meeting 31 July -1 August, 2010 held at Hyderabad.



View of the left eye of a patient with unilateral limbal stem cell deficiency due to chemical injury (Grade IV) showing vascularisation, no distinct limbal area and with a vision of 1/60.



View of the same eye three years after transplantation of bioengineered limbal epithelial sheet showing both anatomical (establishment of limbal barrier effect) and visual improvement (6/36).

DEPARTMENT OF OCULAR PHARMACOLOGY

The department of Ocular Pharmacology aims to focus on, the pathogenic mechanisms, newer drug development and suitable ocular delivery for the major disease conditions like ocular infections, Diabetic Retinopathy (DR), age-related macular degeneration (AMD) and Glaucoma.

Role of aldose reductase in the pathogenesis of diabetic retinopathy

Diabetic Retinopathy (DR) is an important cause of blindness and it is estimated that approximately 2% of people become blind worldwide and about 10% develop severe visual impairment after 15 years of diabetes. Hyperglycemia is reported to be a major causative factor in the development of diabetic retinopathy that increases morbidity and mortality. Aldose Reductase, an enzyme plays a central role in the pathogenesis of DR which is accelerated during hyperglycemia.

The severe microvascular complications of retina are manifested as the abnormal formation of blood vessels which is the leading cause of blindness in diabetic susceptible patients. This is due to the imbalance between the secretion of pro-angiogenic and anti-angiogenic factor by retinal pigment epithelium. High levels of VEGF have been well documented in the vitreous of proliferative diabetic retinopathy patients. However, the secretory profile of angiogenic and anti-angiogenic factor and its relation to polyol pathway (Aldose Reductase) is poorly understood. Therefore, studies are being undertaken to evaluate the effect of high glucose on the expression of aldose reductase and secretion of vascular endothelial factor in retinal pigment epithelium in vitro. This study is further proposed to evaluate the effect of aldose reductase inhibition in the secretion of VEGF by RPE cells.

Role of macular carotenoids in inhibiting the accumulation of A2E, a major fluorophore in the pathogenesis of age related macular degeneration

Age-related macular degeneration (AMD), a major cause of blindness for which no satisfactory treatments exist, leads to a gradual decrease in central high acuity vision. The accumulation of fluorescent materials, called lipofuscin, in retinal pigment epithelial cells of the aging retina is most pronounced in the macula as a side effect of visual cycle. One of the fluorophore of retinal pigment epithelial lipofuscin has been characterized as A2E, a pyridinium bis-retinoid, which is derived from two molecules of vitamin A aldehyde and one molecule of ethanolamine. Increased levels of A2E have been confirmed biochemically in autopsy eyes of elderly individuals and in donor eyes from individuals with macular dystrophies.

It is demonstrated in vitro that the macular carotenoids may inhibit A2E mediated oxidative damage either by direct quenching or by screening phototoxic blue light. Many studies demonstrated that people with low intake of fruits and vegetables rich in macular carotenoids had a significantly higher risk for age-related macular degeneration compared with those whose consumption was high. However, the diverse food habits among the people of different ethnic group as well as exposure levels of ultra violet radiation and genetic variation may also play a major role in the macular uptake of carotenoids for their beneficial role in preventing the accumulation of A2E, a major fluorophore in the pathogenesis of AMD. Therefore, the present study is proposed to evaluate the levels of macular carotenoids and A2E accumulation in Indian donor eyes.

The other area of interest is on ocular disposition of drugs meant for ocular use (ocular pharmacokinetics). The ocular delivery of drugs is often challenged with the presence of well guarding mechanisms present in the eye. The availability of drugs inside the ocular structures determines the efficacy of ocular therapy. Therefore, understanding the ocular disposition of drugs meant for ocular use is of prime importance for the effective management during sight threatening ocular infections.

Topical kinetics of voriconazole (1% and 0.1%) in humans

Investigators : S.Senthilkumari (Aravind Medical Research Foundation, Madurai)
N.Venkatesh Prajna, Lalitha Prajna, Haripriya Aravind (Aravind Eye Hospital, Madurai)
T. Velpandian (All India Institute of Medical Sciences, New Delhi)

Funding source : Champalimaud Research Grant

Background and aims

Voriconazole (VZ) is a broad spectrum newer anti-fungal agent with good intraocular penetration following oral, systemic and topical administration. The oral voriconazole is approved by US FDA for systemic aspergillosis. Due to the emergence of resistant strains against the existing antifungals, ophthalmologists worldwide tried to prove the efficacy of topical voriconazole in the management of fungal keratitis. However, the dosing regimen was instituted based on the other azole anti-fungal drugs like fluconazole without knowing the ocular kinetics of voriconazole following topical instillation. Therefore, an attempt was made to design a suitable dosing regimen in human for better clinical outcome with the following objectives.

1. To evaluate the single and multidose kinetics of topical voriconazole in humans
2. Stability and efficacy analysis of topical formulation of voriconazole

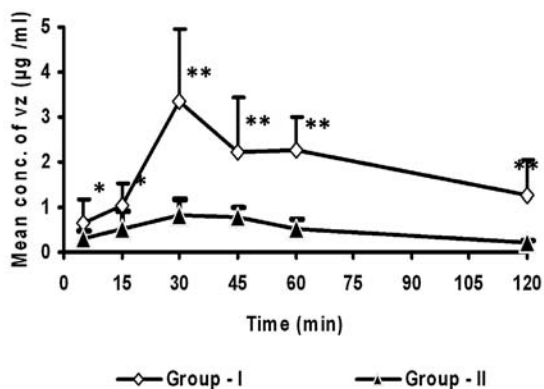
This will give information for designing the topical dosing regimen for the effective management of infections caused by the causative fungal pathogen in the clinical setup.

1. Single dose and multidose kinetics of topical voriconazole in patients undergoing cataract surgery

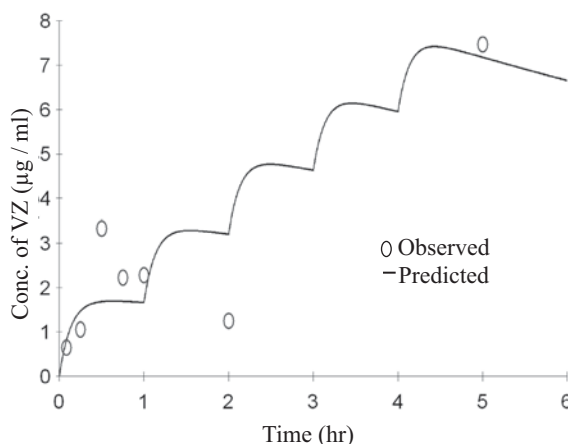
For achieving the above said objectives, patients undergoing cataract surgery were recruited and allowed to receive single drop of 1% (Group I) or 0.1% (Group II) topical voriconazole before the surgery. In addition to single dose instillation, multiple instillations of topical voriconazole every 1 hr or every 2 hrs was also carried out to ensure which dosing regimen best suited for effective infections management. Aqueous humor was collected in an operation suite and quantified for voriconazole concentration by High performance Liquid Chromatography (HPLC).

Following single instillation, both 1% and 0.1% reached maximum concentration at 30 minutes with mean concentration of $3.33 \pm 1.61 \mu\text{g/ml}$ and $0.817 \pm 0.36 \mu\text{g/ml}$ respectively. The mean concentration–time profile for both groups is represented in the figure.

In case of multidose kinetics study, the 1hr multidosing group showed mean aqueous concentration $7.47 \pm 2.14 \mu\text{g/ml}$ 5 hr after initiating VZ instillation and $4.69 \pm 2.7 \mu\text{g/ml}$ 9 hr after starting voriconazole dosing in the 2hr group. As guided by the compartmental analysis, the 2 hr dosing regimen



The mean aqueous concentration–time profile of VZ following a single topical instillation in human eyes. Note: The values are shown as mean \pm SD. **Represents statistical significance of $p \leq 0.005$; *Represents statistical significance of $p \leq 0.05$.



Predicted one compartmental model for multiple dosing of VZ at hourly intervals in human eyes.

may be suitable for maintaining the MIC90 of the causative fungal organisms for successful therapy.

Note

These compartmental simulations were done using WinNonlin 5.1 after subjecting the estimated mean aqueous concentration to one compartment with inputs multidosing, 1st order lag time and 1st order elimination.

2. Stability and efficacy study of topical voriconazole

Topical voriconazole is not available commercially and in the clinical set up, topical formulation was reconstituted from voriconazole power for intravenous use. The reconstituted formulations are usually instructed to store at 4°C and discarded after a week based on the poor stability of voriconazole in solution. This study was undertaken to evaluate the stability and efficacy of the formulation (formulation I (1%) and formulation II (0.1%) after reconstitution which would benefit the patients to avoid affording the reconstituted formulation for every week.

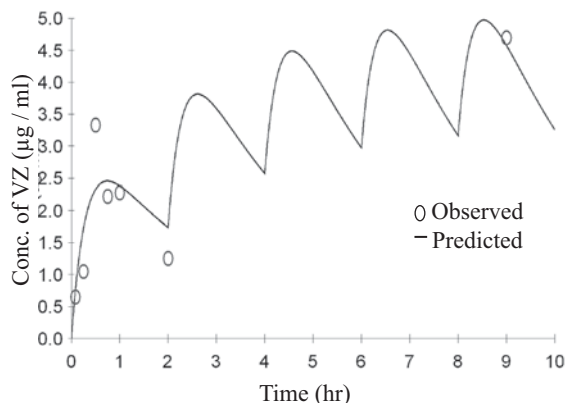
The study demonstrated that, the formulations kept at three different temperatures (-86, 37 and 4°C) were stable for 30 days after reconstitution. A maximum degradation of 3% was observed in formulation II incubated at 37°C during the study period. The effect of temperature on the stability of voriconazole is shown in figure. Even after storing the reconstituted formulation (II) at 37°C, more than 95% of voriconazole was found to be stable at the end of 30 days. However, formulation I was stable throughout the time period and did not show any remarkable degradation at studied temperatures.

Publication

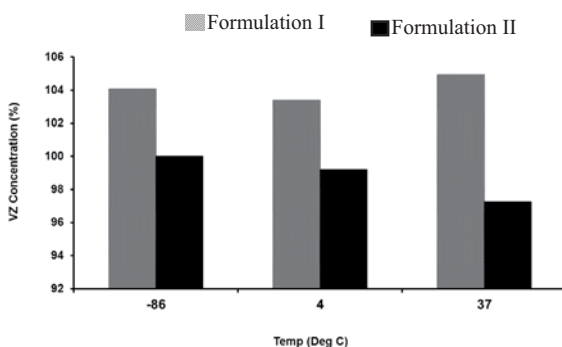
Senthilkumari S, Lalitha P, Venkatesh Prajna N, Haripriya A, Nirmal J, Pankaj G, Velpandian T. "Evaluation of single dose ocular pharmacokinetics of topical VZ in human, its stability and multidose kinetics in rabbits". Current Eye Research 2010; 35(11): 953 – 960.

Presentations

Senthilkumari Srinivasan, Lalitha Prajna, Venkatesh Prajna Namperumalsamy, Haripriya Aravind, Nirmal Jayabalan, Pankaj Gupta, Thirumurthy Velpandian. "Ocular kinetics of topical voriconazole in human & its stability". 18th Annual meeting of Indian Eye Research group, 2010 at Hyderabad, India.



Predicted one compartmental model for multiple dosing of VZ at two hourly intervals in human eyes.



Effect of temperature on the stability of reconstituted voriconazole formulations.

Note: Formulation I was stable at all studied temperatures after 30 days. However, only 3% degradation was observed in formulation II stored at 37°C.

Role of macular carotenoids in Age-related Macular Degeneration (AMD)

Investigators : Dr. S. Senthilkumari (Aravind Medical Research Foundation, Madurai)
*Dr. T. Velpandian ((All India Institute of Medical Sciences, New Delhi)
Funding Source : Department of Science & Technology (DST, New Delhi - *PI)

Background and aims

Age-related macular degeneration (AMD) is the leading cause of blindness that causes severe visual impairment in the elderly population (above 60 yrs). Xanthophylls especially lutein (L) and zeaxanthin (Z) are reported to accumulate in the macular region of the human retina, where oxidative damage of blue light is prevented by their absorption. Unlike green plants and fungi, humans cannot synthesize carotenoids and hence the retinal uptake is only through diet. It is demonstrated that adults in the highest quantity of carotenoids intake had a 43% lower risk of age-related macular degeneration, compared with adults in the lowest quantity of intake. Multiple epidemiological studies also encouraged the intake of dark green leafy vegetables rich in xanthophylls in reducing the risk of developing AMD. Moreover the reported dietary sources for macular xanthophylls are derived from the fruits and vegetables of western origin that may not be applicable to our commonly affordable Indian vegetables. Therefore a preliminary screening was undertaken with the following objectives:

- a. To estimate the total xanthophylls, betacarotene and lycopene in commonly available fruits and vegetables in north and southern part of India.
- b. Lutein and zeaxanthin were quantified from macula collected from both southern (SI) and northern (NI) regions of India.

a. Estimation of total xanthophylls, Betacarotene & lycopene in Fruits & Vegetables

Fresh fruits (N=20) and vegetables (N=51) were collected from two zones of India viz. north India (New Delhi) and south India (Madurai). The carotenoids were extracted from the vegetable/fruit matrices and quantified for total xanthophylls (L+Z), betacarotene and lycopenene using High performance Liquid Chromatography (HPLC). The selected fruits and vegetables showing high content of total xanthophylls were subjected for the quantification of lutein and zeaxanthin alone.

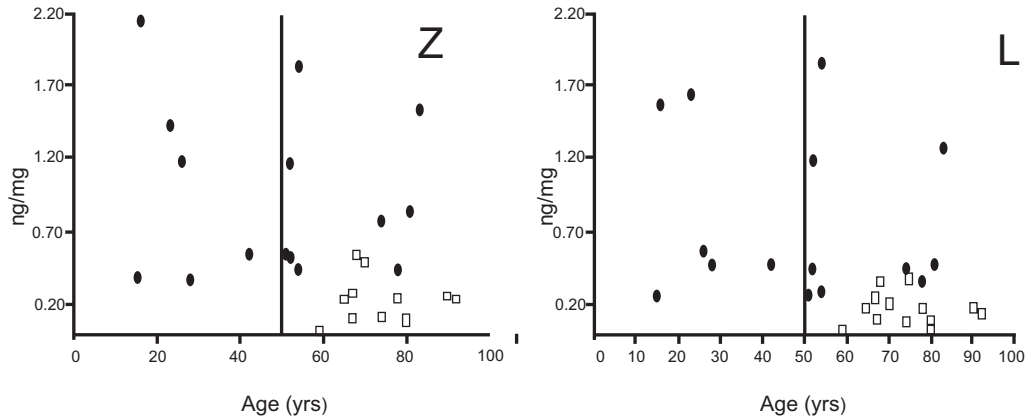
The study demonstrated that high content (more than 10µg/g) of total xanthophylls in fully ripened guava and black grapes. The vegetables such as beans, broad beans, carrot, green chilli, cluster beans, coriander leaves, curry leaves, drumstick leaves, edible amaranth, fenugreek leaves, ginger, guard bean, hummingbird tree leaves, mint leaves, pumpkin, sorrel leaves, tinda, Turkey berry, bitter guard, gherkin, kundru and snake guard showed high total xanthophylls. B-carotene content was found to be more than 10µg/gm in carrots, coriander leaves, and dwarf copperleaves, guardbean, humming bird tree leaves, sorrelleaves, snake guard and mango. Lycopene content was found to be more then than 10µg/gm in red carrots, snake guard and guard beans.

Those fruits and vegetables showed higher amount (>10µg/gm) of total xanthophylls were further quantified for lutein and zeaxanthin. The green colored vegetables such as spinach, coriander leaves, mint leaves, bitter guard, snake guard and green chilli showed high content of lutein. Zeaxanthin was found to be high (>10µg/gm) on snake guard, mint leaves and pumpkin.

This study confirmed that higher content of lutein and zeaxanthin was present in many economic leafy vegetables and fruits.

b. Macular levels of Lutein and zeaxanthin from N. Indian & S. Indian Donors

The aim of the study was to understand the macular levels of lutein and zeaxanthin in relation to dietary intake of colored fruits and vegetables. Therefore, maculae were collected from the donor eyes (from respective centres) and quantified the levels of L and Z by HPLC. The mean macular levels of lutein and zeaxanthin from northern part of India were found to be 0.8ng/mg and 1ng/mg wet weight of macula respectively.



The age distribution of zeaxanthin (Z) and lutein (L) in macula from human donors of northern India (●) and southern India (□) is shown in the picture.

These values were derived from the cadaveric eyes of 8 males and 7 females having the mean age of 50 years (median 52 yrs, range 15-83 yrs). The mean macular levels of lutein and zeaxanthin from southern part of India was found to be 0.2 and 0.2ng/mg wet weight of macula and these values were obtained from the cadaveric eyes of 4 males and 9 females having the mean age of 74 years (Median 74 yrs (range: 59-92). Surprisingly, the mean macular levels of lutein and zeaxanthin of SI donor eyes (n=13) were found to be significantly ($P<0.001$) four times lesser than NI donor eyes (n=15) and the macular levels of northern India were comparable with reported levels in western population.

In order to investigate the relevance of these findings with prevalence of AMD in south India, the macular levels in Indian donor eyes are being undertaken with increased sample size. This study might shed light onto the bioavailability of these xanthophylls in their blood in relation to their food intake.

Implication of the findings

This study is of great help in understanding the correlation existing between the macular health and food intake. This type of information is very essential to understand the importance of dietary intake of fruits and vegetables rich in macular xanthophylls available in India and also to establish a dietary supplements for the older people (>50 yrs) in the prevention/delay the progression of AMD.

Publications

Velpandian T, Arora B, Senthilkumari S, Ravi AK, Ghose S. “Regional variation in the levels of macular xanthophylls and carotenoids in dietary components: Comparing north and south India”. J Nut Sci Vit. 2010; 56(6):411-20.

CONFERENCES ATTENDED

ARVO 2010 annual meeting

Fort Lauderdale, Florida, USA, May 2-6

DR. P. SUNDARESAN

- *Spectrum of candidate genes mutation associated with Indian familial Oculocutaneous Albinism patients*

P. MURUGESWARI

- *Angiogenesis induced by proliferative diabetic Retinopathy and eales' disease vitreous is mediated by a common pro-inflammatory mechanism*

B. HEMADEVI

- *Protein profile of autosomal recessive Congenital Hereditary Endothelial Dystrophy (CHED2) and Fuchs Endothelial Corneal Dystrophy (FECD)*

S. ANANTHI

- *Proteomic analysis of tear and cornea in patients with fungal keratitis*

M. VALARNILA

- *Evaluation of Haptoglobin and its isoforms as plasma/serum biomarker for diabetic retinopathy*

R. SIVA GANESA KARTHIKEYAN

- *Innate immunity in aspergillus and fusarium keratitis in Tamil Nadu, India*

ASHWINI SHANKER

- *EPHA2 polymorphisms and age related cataract in India-findings from the INDEYE genetics study*



M. Valarnila, Ashwini Shanker, P. Murugeswari, Dr. P. Sundaresan, S. Ananthi, R. Siva Ganesa Karthikeyan, B. Hemadevi at ARVO 2010, Florida, USA

Indian Eye Research Group

LV Prasad Eye Institute, Hyderabad, July 31 - August 1

SUSHIL KUMAR DUBEY

- *Involvement of LOXLI gene variations in south Indian patients with Exfoliation syndrome and exfoliation glaucoma*

N. PRASANTHI

- *Myocilin gene splice site variants role in POAG*

S. SUDHA PRIYA

- *Characterization of the age related macular degeneration in donor eyes*

T. MERLIN PREMALATHA

- *Cytokine profile in aqueous humor of parasitic granuloma*

J. CORNELIA REENA

- *Real time PCR in the diagnosis of postoperative endophthalmitis*

J. LAKSHMI PRIYA

- *Identification of fusarium species by molecular methods and their antifungal susceptibility from patient's with corneal ulcer*

LALAN KUMAR ARYA

- *Presumed trematode induced granulomatous Uveitis in South India*

B. SUGANTHALAKSHMI

- *AMD genes and its association with DR in South Indian Population*

DR. P. SUNDARESAN

- *Spectrum of candidate genes mutation associated with Indian familial oculocutaneous albinism patients*

DR. C. GOWRI PRIYA

- *Characterization of buccal mucosal epithelial stem cells and evaluation of its efficacy in corneal surface reconstruction*

DR. S. SENTHILKUMARI

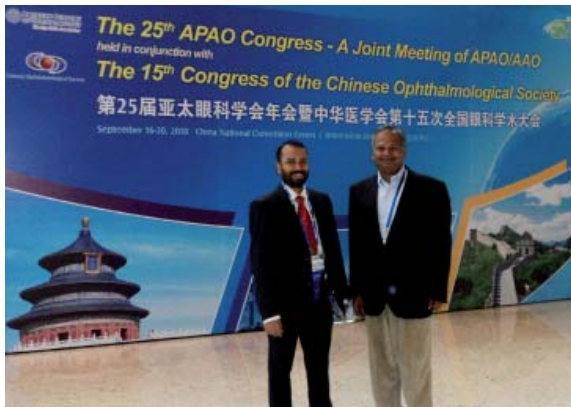
- *Ocular kinetics of topical voriconazole in human and its stability*

Asia Pacific Academy of Ophthalmology (Asia Pacific Society of Eye Genetics (APSEG) symposium)

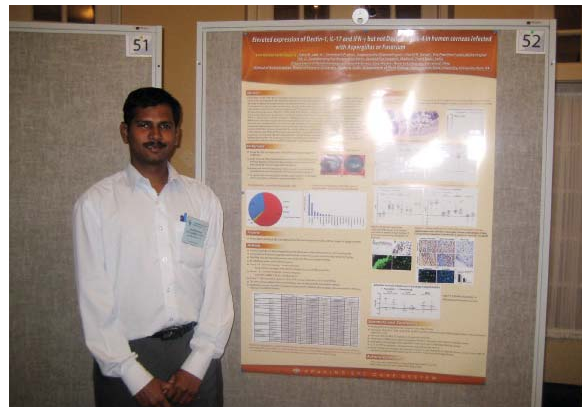
Beijing, China, September 16–20, 2010

DR. P. SUNDARESAN

- *Molecular genetics of ocular anomalies in Indian population*



Dr. P. Sundaresan, APAO meeting at Beijing, China



Mr. Siva Ganesa Karthikeyan, Gordon Brown-immunity to fungal infection at Texas, USA

22nd National Congress of Parasitology

University of Kalyani, Kalyani, West Bengal
Oct. 30 – Nov 1, 2010

MR. LALAN KUMAR ARYA

- Presumed trematode induced subconjunctival & anterior chamber granulomatous uveitis in south India

Gordon Brown - Immunity to fungal infection

Galveston, Texas, USA, January 16-21, 2011

MR. SIVA GANESA KARTHIKEYAN

- Elevated expression of Dectin-1, IL-17, and IFN-gamma but not Dectin-2 and IL-4 in human corneas infected with *Aspergillus* or *Fusarium*.

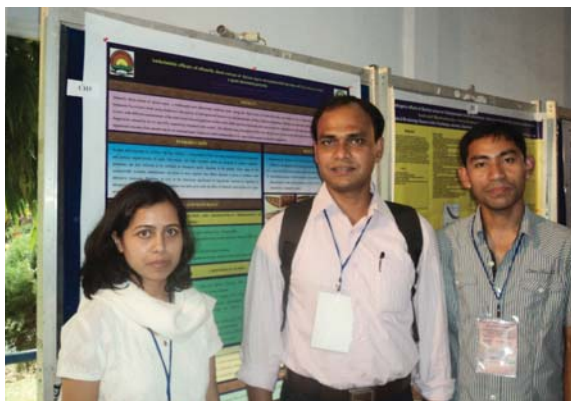
ASIA ARVO 2011

Singapore, January 20-22, 2011

DR. C. GOWRI PRIYA

- A specific marker for corneal epithelial stem cells and a simple method for their ex vivo expansion under xenobiotic-free condition

Mr. Lalan Kumar Arya at University of Kalyani, Kalyani, West Bengal



DR. P. SUNDARESAN

- Genome and proteome wide approach towards corneal endothelial dystrophies

MS. K. RENUKADEVI

- Molecular genetic analysis of Tyrosinase (TYR) gene with Oculocutaneous Albinism (OCA) patients in Indian population

MR. SUSHIL KUMAR DUBEY

- Evaluation of Lysyl Oxidase – Like 1 and Clusterin genes polymorphisms in south Indian population with pseudoexfoliation syndrome and pseudoexfoliation glaucoma

MR. G. GOWTHAMAN

- Screening of Aldose Reductase gene promoter region with Type-2 diabetic retinopathy in south Indian population.

AMRF faculty members and students in Asia ARVO-2011 at Singapore



TRAINING

Ms. K. RENUGADEVI, Senior Research Fellow received training on the expression of novel mutations using DNA samples from Aravind Eye Hospital under the guidance of Dr. Markus Preising, Department of Ophthalmology, Laboratory for Molecular Ophthalmology, JUSTUS-LIEBIG-UNIVERSITY, Giessen, GERMANY from 2nd - 22nd July 2010. She is working in DST-DAAD Indo-German collaborative project to understand the “Molecular Genetics of Albinism in the Indian population”.



MR. LALAN KUMAR ARYA, Junior Research Fellow visited Department of Zoology (Parasitology section), North Eastern Hill University, Meghalaya, Shillong under the supervision of Prof. Veena Tandon to learn the basic parasitological techniques such as mounting assay, separation of parasites from their intermediate host like snails, fish and phylogenetic tree analysis. PCR and molecular sequencing, targeting internal transcribed space (ITS) ribosomal gene of trematode using various set of primers such as ITS1, ITS2 and Mito Co1 for identification and speciation of trematode.



DR. S. SENTHIL KUMARI, Scientist, Department of Ocular Pharmacology spent 3 months (Nov 1, 2010 to Jan 31, 2011) at Dr. Paul Kaufman’s Laboratory, Department of Ophthalmology and Visual Sciences, University of Wisconsin, Madison to receive hands on- training on “Anterior segment organ culture techniques”. The training was supported by Division of International Ophthalmology, University Wisconsin, Madison.



R. SIVA GANESA KARTHIKEYAN, Junior Research Fellow, Department of Microbiology undergone training in immunological techniques in the laboratory of Dr. Eric Pearlman, Ph.D, Professor and Director of Research, Department of Ophthalmology and Visual Sciences, Institute of Pathology, Case Western Reserve University, Cleveland, Ohio, USA from 22nd, January to 26th, February 2011.

Ph.D Programme in Queen’s University, Belfast

Ms. S. SUDHA PRIYA, Research scholar, Aravind Medical Research Foundation has been awarded grant for carrying out further studies for PhD in Queen’s university, Belfast in the scheme of University Studentship. She is working on “MicroRNA in retinal ageing and age related Macular degeneration” during the period of 1st October 2010 to 30th September 2013.



Ph.D awarded by Madurai Kamaraj University

Ms. B. SUGANTHALAKSHMI, Research Scholar from 2003-2010 has worked on “Molecular genetics of diabetic retinopathy” under the guidance of Dr.P.Sundaresan, Senior Scientist, Department of Genetics, Aravind Medical Research Foundation. She was awarded Doctor of Philosophy (Ph.D) by Madurai Kamaraj University, Madurai in October 2010.



VISITORS

DR. ERIC PEARLMAN, Case Western Reserve University, Cleveland visited AMRF and gave lecture on “Innate Immunity in Pseudomonas aeruginosa keratitis”. He also had a discussion on the collaboration on fungal keratitis with Dr. K. Dharmalingam, DBT Distinguished Professor, Madurai Kamaraj University, Dr. NV. Prajna, Chief –Cornea Services, and Dr. Lalitha Prajna, Chief Microbiologist, Aravind Eye Hospital.



Under the Indo-German collaboration (DST-DAAD) Project Based Personnel Exchange Programme (PPP), **DR. MARKUS PREISING**, Head of Laboratory for Molecular Ophthalmology, Justus-Liebig University of Giessen, University Eye Hospital Giessen Marburg GmbH, Dept. Giessen, Friedrichstrasse 18, Germany visited Department of Genetics, AMRF from 2nd to 13th January 2011. Dr. Markus met all the faculty members and interacted with all the research scholars at AMRF and gave a guest lecture on “Combined Mutations in CRX, PRPH2, and RHO - Chance Association or Modifier”. This visit was specially targeted to study the expression pattern of novel mutations identified in the candidate genes of Oculocutaneous Albinism.



DR. JOHN FINGERT, MD, Ph.D, Department of Ophthalmology and Visual Sciences, Glaucoma Services, University of Iowa, Iowa city, Iowa, USA visited Aravind Eye Care System from November 15-20, 2010. The purpose of his visit was to develop a collaborative glaucoma research project and strengthen our long term relationship with University of Iowa. He interacted with all the senior

Dr. John Fingert with research scholars and faculty at AMRF



faculty members at AECS and students at Aravind Medical Research Foundation. He gave a guest lecture on “New Directions in Glaucoma Genetics”. He visited Aravind Eye Hospital-Tirunelveli and Pondicherry and also participated in glaucoma camp at Kayalpattam.

DR. BRENDA GALLIE, Professor, Campbell Family Institute for Cancer Research, Ontario Cancer Institute/Princess Margaret Hospital, University Health Network, Department of Ophthalmology, University of Toronto, Toronto, Ontario, Canada visited AMRF and gave a seminar to the students.

Dr. APJ. Abdul Kalam visits AMRF

6th January 2011

Dr. APJ. Abdul Kalam visited Aravind Medical Research Foundation on 6th January 2011 and he was briefed about the various facilities available at the institute. After visiting the laboratories, he had interactions with the faculty and research scholars. He motivated the students and appreciated the recent achievements of Aravind faculty in the field of fungal keratitis, diabetic retinopathy and telemedicine. He stressed the need to focus research on prevention of glaucoma.



WORKSHOP

Workshop on “Stem Cells for Vision”

Oct 25-30, 2010

A one-week workshop to provide hands-on experience to young scientists was organized by Dr. G.Venkataswamy Eye Research Institute on 25th October 2010, sponsored by ICMR and DRDO, New Delhi. This training course was unique in several respects. It is the first of its kind in India to teach

a variety of modern laboratory techniques to show how stem cells could bring back vision to patients who are blind for several months to years. This is the only method to benefit these patients who became blind due to thermal or chemical injuries in one or both eyes.

Totally 10 research students/young scientists from Iladevi Cataract IOL Research Centre,

Participants at the Stem Cells workshop



Ahmedabad, Sankara Nethralaya, Chennai, Sri Chitra Tirunal Institute of Medical Sciences and Technology and Kerala University, Thiruvananthapuram had the opportunity to learn methods of identifying and growing patient's stem cells under specific culture conditions. In addition to Aravind faculty, the experts from LV Prasad Eye Institute, Hyderabad, Sankara Nethralaya, Chennai, Sri Chitra Tirunal Institute of Medical Sciences and Technology, Thiruvananthapuram, Invitrogen Bioservices India, Bangalore gave invited lectures during the morning session. All the basic techniques and culture methods for ex-vivo expansion of corneal/buccal epithelial stem cells for human application were carried out in the afternoon laboratory session using human cadaver globes. The students had hands-on experience in these techniques.

The workshop was conducted as part of October Summit, a series of programmes held in honour of the Founder Chairman of Aravind Eye Care System. The course was organized by Dr. C. Gowri Priya, scientist in collaboration with the Cornea clinic of Aravind Eye Hospital.

PUBLICATIONS IN PEER REVIEWED JOURNALS

MOLECULAR VISION

2010; 16:1514-1524

KATHIRVEL RENUGADEVI, ASIM KUMAR SIL, VIJAYALAKSHMI PERUMALSAMY, PERIASAMY SUNDARESAN
- *Spectrum of candidate gene mutations associated with Indian familial oculocutaneous and ocular albinism patients.*

2010; 16:2891-2902

J. ESWARI PANDARANAYAKA, N. PRASANTHI, N. KANNABIRAN, K. RANGACHARI, M. DHIVYA, SUBBIAH R. KRISHNADAS, P. SUNDARESAN, S. KRISHNASWAMY
- *Polymorphisms in an intronic region of myocilin gene associated with primary open-angle glaucoma – a possible role for alternate splicing.*

OPHTHALMIC GENETICS

2010;31(4): 196-199

PERIASAMY SUNDARESAN, S. MAHESH KUMAR, STEWART THOMPSON, AND JOHN H. FINGERT
- *Reduced frequency of known mutations in a cohort of LHON patients from India.*

CURRENT EYE RESEARCH

2010; 35(11), 953-960

SENTHILKUMARI S, LALITHA P, VENKATESH PRAJNA N, HARIPRIYA A, NIRMAL J, PANKAJ G, VELPANDIAN T.
- *Single and multidose ocular kinetics of extemporaneous formulation of topical voriconazole in human and evaluation of its stability*

BMC MEDICAL GENETICS

2010;11:158

SUGANTHALAKSHMI BALASUBBU, SUNDARESAN PERIASAMY, ANAND RAJENDRAN, KIM RAMASAMY, NAMPERUMALSAMY PERUMALSAMY, J. FIELDING HEJTMANCIK
- *Association analysis of nine candidate gene polymorphisms in Indian patients with type 2 diabetic retinopathy*

JOURNAL OF NUTRITIONAL SCIENCE AND VITAMINOLOGY

2010; 56:411-420

VELPANDIAN T, ARORA B, SENTHILKUMARI S, RAVI AK, GAYATHRI C, AZAD R AND GHOSE S

- *Regional variation in the levels of macular xanthophylls and carotenoids in dietary components: Comparing north and south India.*

MICROBIOLOGY

2011; 157(pt-2) 430-437

CADY KC, WHITE AS, HAMMOND JH, ABENDROTH MD, KARTHIKEYAN RS, PRAJNA L, ZEGANS ME, O' TOOLE GA

- *Prevalence, conservation, and functional analysis of Yersinia and Escherichia CRISPR regions in clinical pseudomonas aeruginosa isolates.*

EXP EYE RES

2011; 92 (2011) 454-463

ANANTHI S, SANTHOSH RS, VALARNILA M, PRAJNA NV, LALITHA P, DHARMALINGAM K.

- *Comparative proteomics of human male and female tears by two-dimensional electrophoresis*

BIOINFORMATION

2011; 5(9): 398-399

RANGACHARI K, DHIVYA M, ESWARI PANDARANAYAKA PJ, PRASANTHI N, SUNDARESAN P, KRISHNADAS SR, KRISHNASWAMY S

- *Glaucoma Database*

OPHTHALMIC GENETICS (IN PRESS)

LALAN KUMAR ARYA, ANAND B. KUMAR, SHASHIKANT SHETTY, VIJAYALAKSHMI PERUMALSAMY, PERIASAMY SUNDARESAN

- *Analysis of the SALL4 gene in patients with duane retraction syndrome in a south Indian population 2011.*

JOURNAL OF INFECTION DISEASES

2011 Sep; 204(6): 942-50

SIVA GANESA KARTHIKEYAN, SIXTO M. LEAL JR, VENKATESH PRAJNA, KUPPAMUTHU DHARMALINGAM, DAVID M.GEISER, ERIC PEARLMAN, LALITHA PRAJNA

- *Expression of innate and adaptive immune mediators in human corneal tissue infected with aspergillus or fusarium.*

EYE (EPUB AHEAD OF PRINT)

CHIDAMBARANATHAN GOWRI PRIYA, PARTHASARATHY ARPITHA, SIVARAMAKRISHNAN VAISHALI, NAMPERUMALSAMY V. PRAJNA, KIM USHA, KAMDAR SHEETAL, VEERAPPAN MUTHUKKARUPPAN

- *Adult human buccal epithelial stem cells: Identification, ex-vivo expansion and transplantation for corneal surface reconstruction.*

GRAEFER'S ARCHIVE FOR CLINICAL AND EXPERIMENTAL OPHTHALMOLOGY (SUBMITTED)

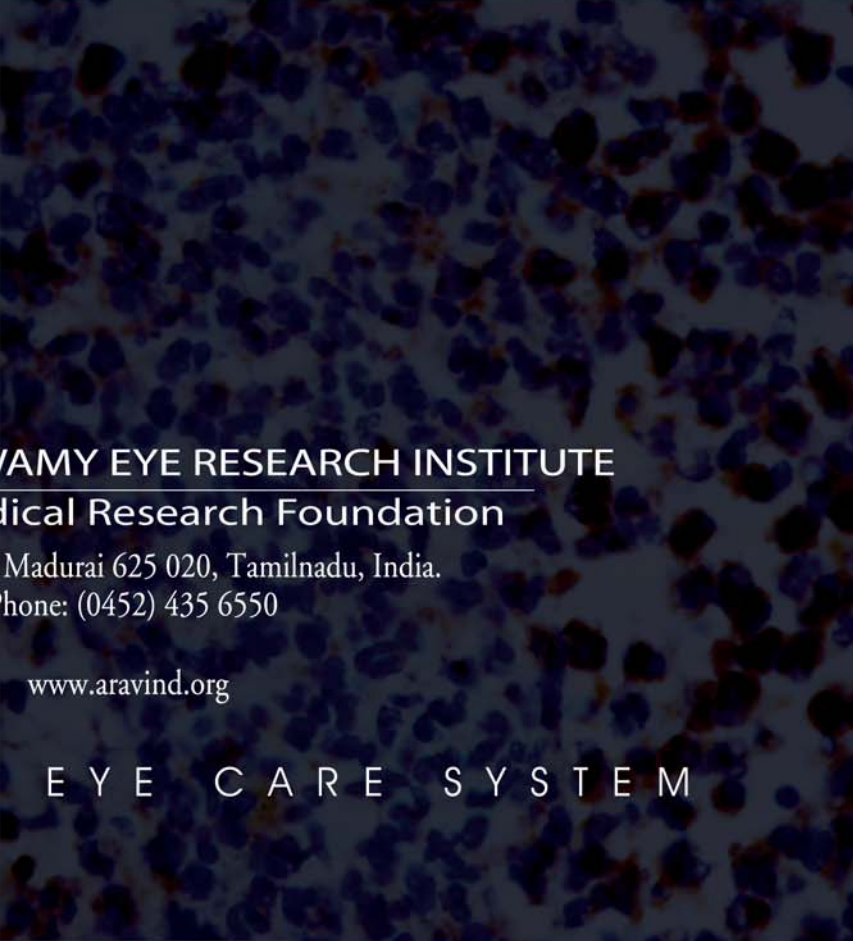
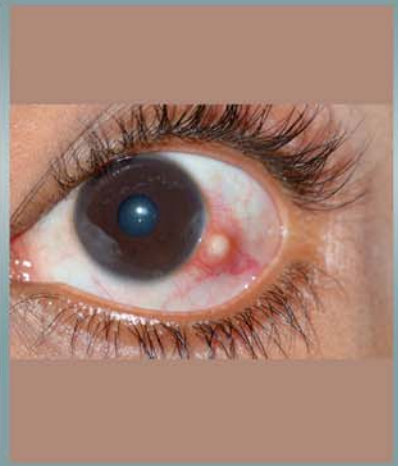
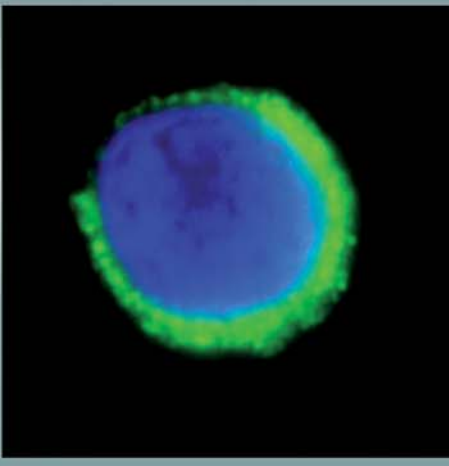
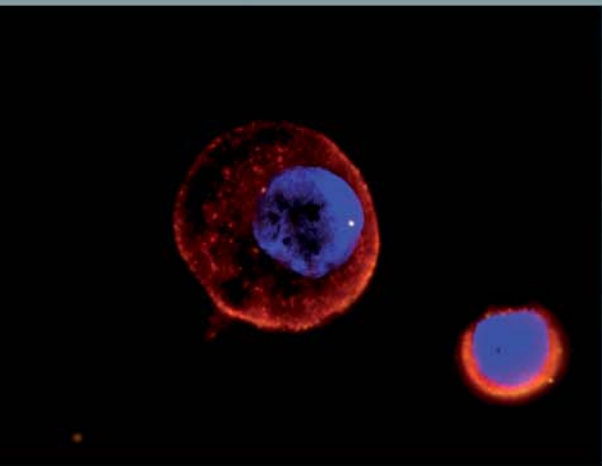
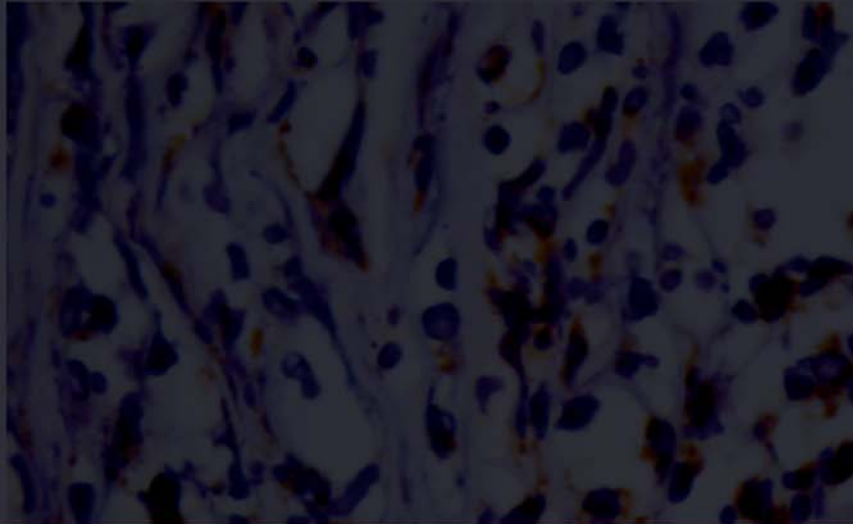
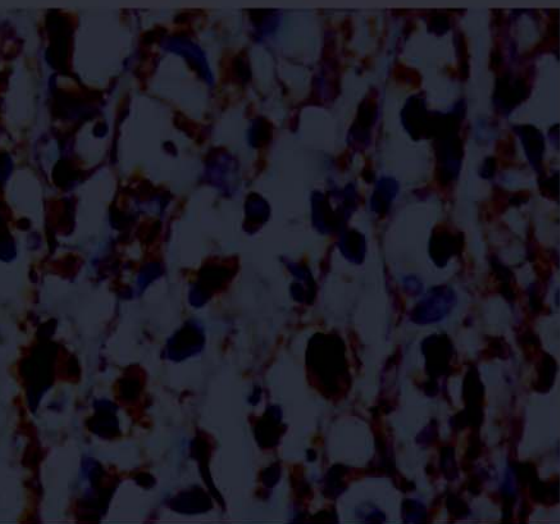
JEYALAKSHMI SURESHKUMAR, ARAVIND HARIPRIYA, BAOHE TIAN, VEERAPPAN MUTHUKKARUPPAN, PAUL L. KAUFMAN

- *Cytoskeletal drugs prevent posterior capsular opacification in human lens capsule in vitro.*

RESEARCH SCHOLARS AT AMRF

No	Scholar's Name	Investigators	Project
1.	M. Lalan kumar Arya	Dr. SR. Rathinam	Etiology and Immunopathogenesis of trematode induced Uveitis
2.	R. Siva Ganesha Karthikeyan	Dr. Lalitha Prajna Dr. K. Dharmalingam	Characterization of the host immune response during corneal infection with pathogenic fungi and bacteria
3.	J. Cornelia Reena	Dr. Lalitha Prajna Dr. SR. Rathinam	Molecular insights into the etiology of infectious uveitis
4.	J. Lakshmi Priya	Dr. Lalitha Prajna	Characterization and Speciation of Aspergillus and Fusarium Species from corneal ulcer
5.	S. Ananthi	Dr. N.V. Prajna Dr. Lalitha Prajna Dr. K. Dharmalingam Dr. N. Lini	Host pathogen interaction in human mycotic keratitis
6.	M. Valar Nila	Dr. K. Dharmalingam Dr.VR. Muthukkaruppan	Characterization of Serum Proteome in Proliferative Diabetic Retinopathy
7.	Paul Pown Raj	Dr. SR. Krishnadas Dr. P. Sundaresan Dr. K. Dharmalingam	Identification of Biomarkers for Primary Open Angle Glaucoma
8.	Ms. Minu Karthika	Dr. N.Venkatesh Prajna Prof. K. Dharmalingam Dr. Lalitha Prajna	Quantitative Proteomics of host pathogen interaction in human Aspergillus keratitis
9.	Mr. Muthu Selvam	Dr. N.Venkatesh Prajna Prof. K. Dharmalingam Dr. Lalitha Prajna	Quantitative Proteomics of host pathogen interaction in human Aspergillus keratitis
10.	K. Renukadevi	Dr. P. Sundaresan Dr. P. Vijayalakshmi	Molecular genetics of Albinism in the Indian population
11.	N. Prasanthi	Dr. P. Sundaresan Dr. SR. Krishnadas	Molecular Genetics and functional studies of genes associated with primary open angle glaucoma
12.	P. Mohanapriya	Dr. P. Sundaresan Dr. Manoranjan Das	Molecular genetics of Keratoconous

No	Scholar's Name	Investigators	Projec
13.	V. Saravanan (Sr. Technician)	Dr. P. Sundaresan Dr. R.D. Ravindran	A Genetic component to the INDEYE study of cataract and age related macular degeneration in India
14.	J. Radha (Sr. Technician)	Dr. P. Sundaresan Dr. R.D. Ravindran	A Genetic component to the INDEYE study of cataract and age related macular degeneration in India
15.	G. Gowthaman	Dr. P. Sundaresan Dr. R. Kim	Transcriptome and Proteome analyses of ALR2 and its involvement in the pathogenesis of Diabetic Retinopathy
16.	Sushil Kumar Dubey	Dr. P. Sundaresan Dr. P. Vijayalakshmi Dr. S.K. Kedia, Sadar Hospital, Ara, Bihar	Molecular genetic analysis of candidate genes associated with paediatric eye diseases: exclusively anophthalmia and microphthalmia in India
17.	Mr. Anhsuman Verma	Dr. P. Sundaresan Dr. P. Vijayalakshmi	Molecular genetics of leber congenital amaurosis in South Indian population
18.	Mr. Tilak Prasad	Dr. C. Gowri priya	Ex-vivo expansion of limbal / buccal epithelial stem cells for corneal surface reconstruction
19.	Ms. Saumi Mathews	Dr. C. Gowri Priya Dr. N.V. Prajna Dr. VR. Muthukkaruppan	Translational research to generate Corneal/ Buccal Epithelial stem cells with GMP compliance for corneal surface and socket reconstruction
20.	Mr. Arun Kannan	Dr. C. Gowri Priya Dr. SR. Rathinam	Antigenic Mimicry between Leptospiral and human lens proteins
21.	Mr. R. Ranjith Kumar	Dr. S. Senthilkumari	Role of Aldose Reductase in Retinal Pigment Epithelium – An understanding towards the pathogenesis of Diabetic Retinopathy



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