Details of the research work duly signed by the applicant, for which the Sun Pharma Science Foundation Research Fellowship is claimed, including references and illustrations (not to exceed 6000 words).

Title: ROP Prevention Research program @ Prof Brien Holden Eye Research Centre, LV Prasad Eye Institute, KAR campus Hyderabad

ROP is a global problem and remains a major cause of childhood blindness in both developed as well as developing countries. Despite advanced treatment modalities, preterm infants face lifelong learning, hearing and visual disabilities due to incomplete development. Every year about 32000 neonates develop partial/complete blindness due to ROP. According to different reports, incidence of ROP varies from 38-51.9% and is the reason of blindness in 1 out of 1000 low birth preterm infants. Out of 26 million live low birth weight (<2000g) two million infants are at increased risk for developing ROP. A report from South-India has found that compromised pulmonary functions along with oxygen, surfactant therapy and hypoxic shock are important risk factors for severe ROP. Development of ROP is due to multifactorial interplay of which premature gestational age and low birth weight are the two most important risk factors. However, for some preterm infants, ROP develops even in the absence of these identified risk factors. While it's a major ocular complication of preterm birth, the exact mechanism and key events leading to pathogenesis of ROP are yet to be identified. Our group at L V Prasad Eye Institute is involved in active state-wide ROP screening and research program for identifying the risk factors contributing to the susceptibility to ROP.

Our major focus has been on identifying novel biological factors in disease pathogenesis which could have a key role in ROP prevention and to aid in the existing clinical management. We have employed a comprehensive strategy to achieve our mission as shown in figure 1. Our research program envisions to expands the existing knowledge on the biological mechanisms underlying disease, early identification of disease including pre- and post-pregnancy and changes that might occur *in utero* and post birth and disease monitoring and developing newer drug targets/combinations for an effective management of disease for preventing visual loss among children and improving their quality of life.

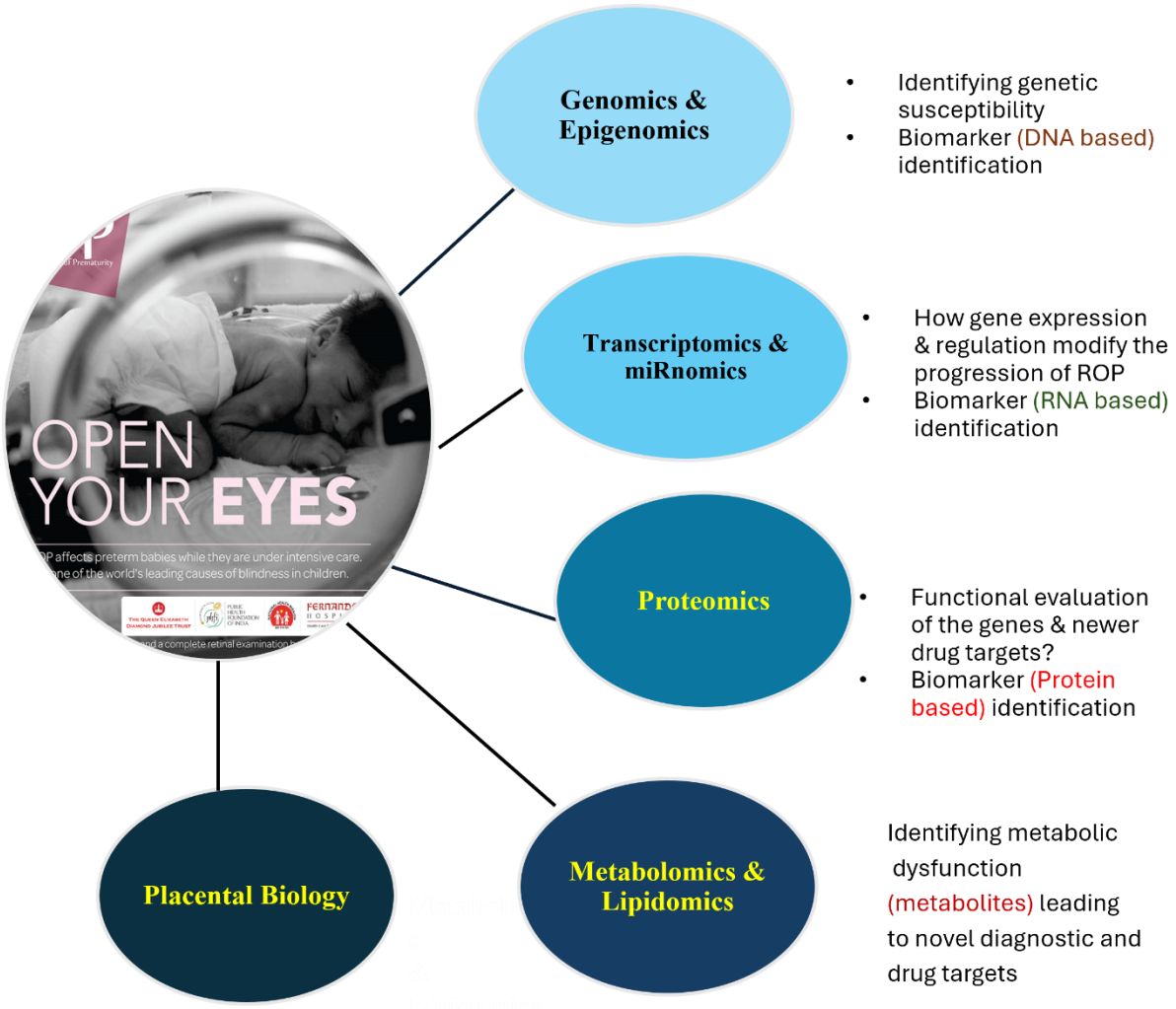

Figure 1: A comprehensive strategy for the ROP prevention research program

Basic science research on ROP pathogenesis

Genetic susceptibility for ROP:

ROP has been extensively studied as an oxygen induced retinopathy in past while the underlying genetic predisposition and molecular mechanisms in ROP have not been understood very well. Several studies in past analyzed SNPs in candidate genes for their association to disease development, however these could not generate any better understanding due to study design issues primarily. A genomic analysis conducted by our team for preterm born babies with and without ROP using single nucleotide polymorphism (SNP)-based targeted microarrays revealed a strong role for the genes involved in angiogenesis, growth and development of the fetal retina, trans-endothelial migration, oxidative stress, inflammation, cholesterol metabolism and neurodegenerative processes in ROP pathogenesis (Rathi et al. 2011). We observed strong associations of ROP with increased levels of proteins in the

extracellular matrix (ECM) and complement pathways in the vitreous humor samples of ROP patients (figure 2).

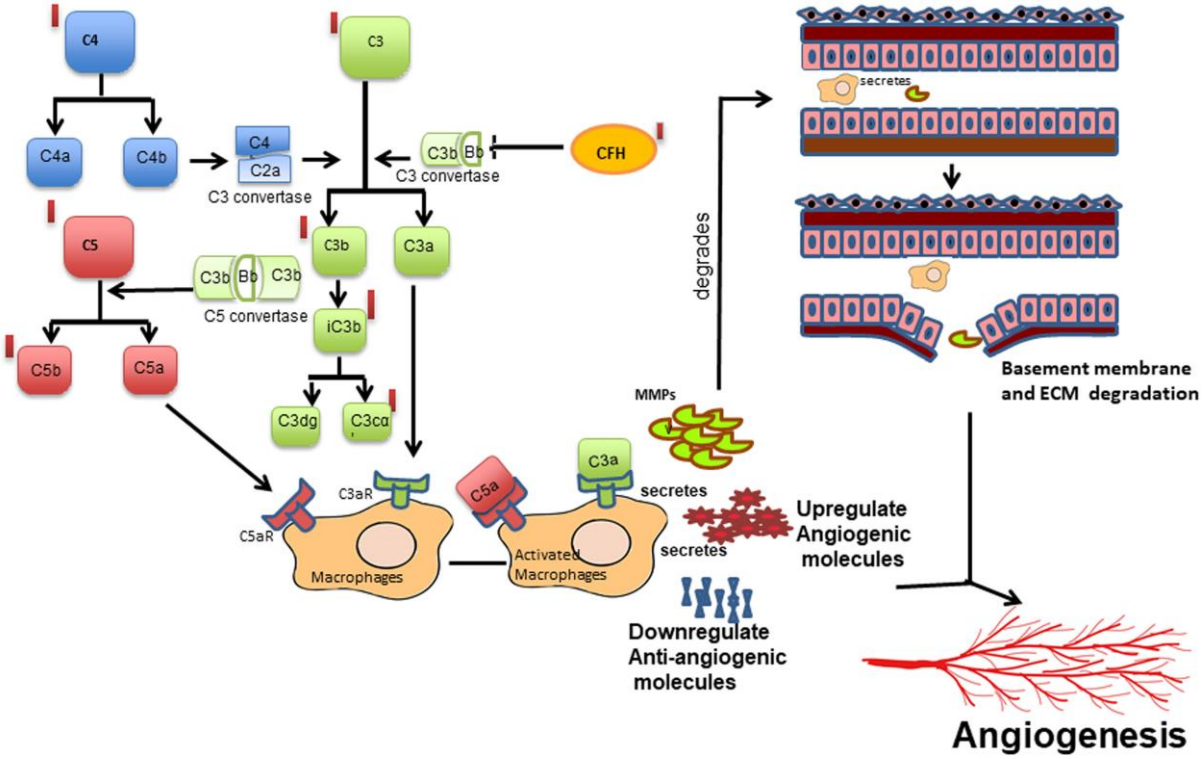

Figure 2: A proposed mechanism of complement activation and its potential effect on macrophage-mediated angiogenesis in retinopathy of prematurity.

It was interesting to note that the levels of matrix metalloproteases enzymes MMPs (known to induce inflammation and angiogenesis) in tears were significantly higher in severe ROP compared to no-ROP and mild ROP eyes that underscored its potential use as a biomarker for an early prediction of the condition (Patnaik et al. 2020) (figure 3). We recently performed an Illumina based global gene expression arrays for 24 infants (with and without ROP) to check the gene expression at different stages of ROP. Several genes were differentially expressed (115 up-regulated and 27 down-regulated) among different stages of ROP and no-ROP subjects. Inflammation mediated by chemokine and cytokine was found to be the major pathway (33%) along with minor pathways such as endothelial signalling, toll receptor signalling, CCKR signalling and gonadotropin releasing hormone receptor pathway (data under publication).

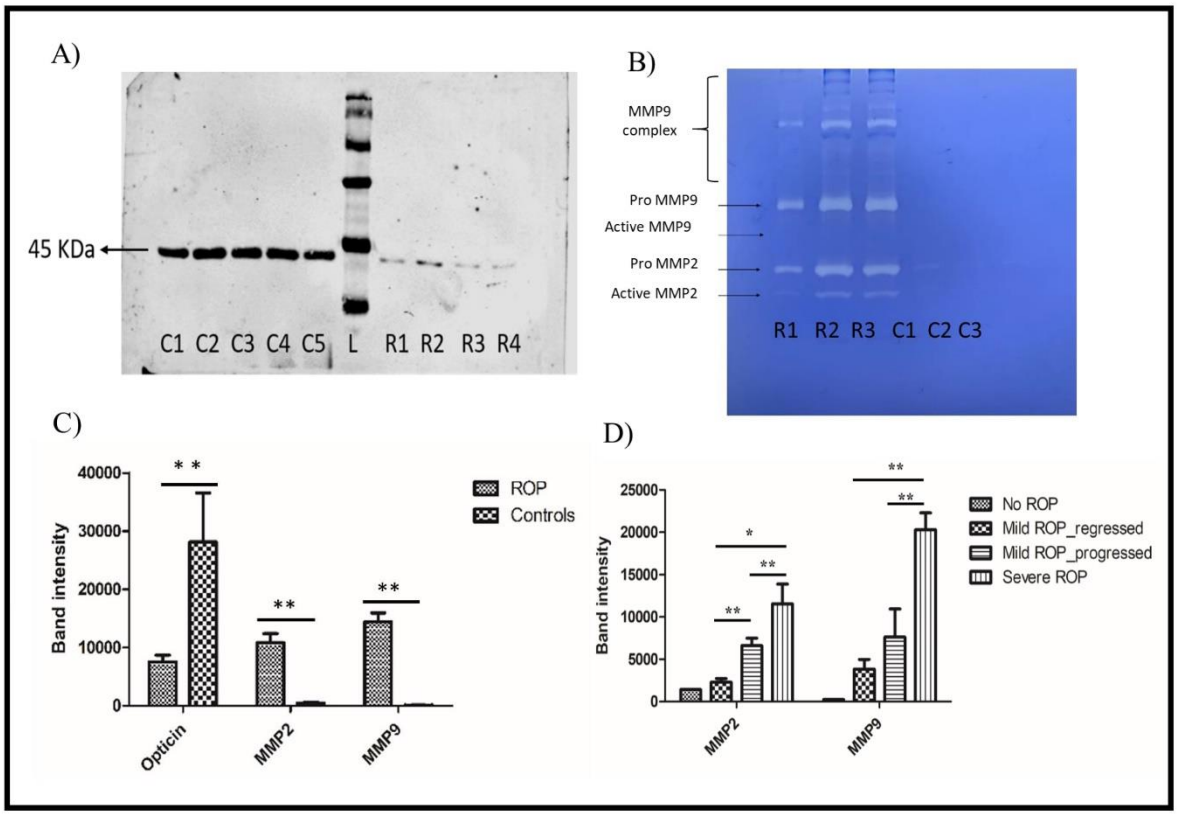

Figure 3: Representative image of (A) western blot of opticin levels in ROP patients and controls (B) representative gelatin zymography of vitreous from ROP and congenital cataract (controls) (C) quantification of opticin (45K Da) levels in vitreous samples of ROP (n=30) and control (n=30) and MMPs in ROP (n=11) and control (n=11) (D) MMPs levels estimated in ROP tears samples by zymography in extended cohort, quantification of MMP9 and MMP2 estimated for severe ROP (n=16), mild progressed to progressed ROP (n=12), mild ROP to regressed ROP (n =12), and no ROP premature controls (n=18), **p = 0.001, *p = 0.05; data represented as mean ± SEM, C, control vitreous; R, ROP vitreous; L, protein ladder (Patnaik *et al*. 2020 ***Scientific reports,*** *11*(1), p.7444.)

The major challenge of ROP management is to distinguish the subgroup of infants may progress to severe ROP stage. To address this, we performed gene identification by whole exome sequencing of twin ROP pairs (concordant and discordant ROP profile) unique variation in 84, 20 and 11 genes in severe ROP, mild ROP and aggressive posterior ROP(APROP), respectively have been found with highest predictive damage score (Figure 4). On pathway analysis, genes are associated with metallopeptidase activity, lipid transport and metabolism, developmental processes, neurogenesis, calcium ion homeostasis, nervous system development and neuron development were found to be significantly associated.

Both exome sequencing and global gene expression array has shown an increased expression of the early growth response (EGR) factor gene in ROP and AOROP infants compared to controls (Figure 2). The EGR factor encoded by this gene is a zinc finger transcription factor that is highly expressed in T-cells in hypoxic conditions and control their proliferation and activation. It also triggers the angiogenesis process via c-FOS.

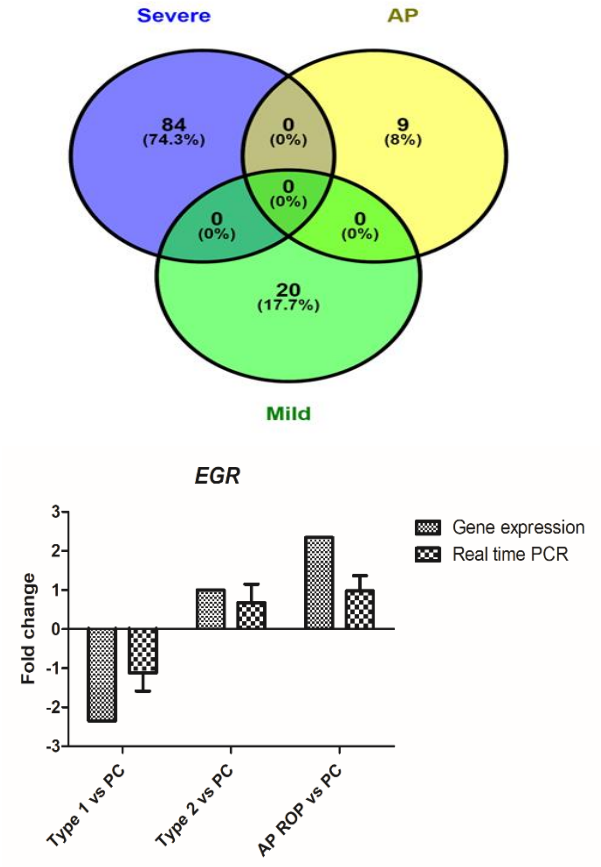

Figure:4 Venn diagram showing the number of unique genes found in Severe ROP, AP ROP and Mild ROP from whole exome sequencing on twin pairs

Link between inflammation, oxidative stress and altered lipid metaboloism in ROP pathogenesis:

Previously, our genome-wide association study (GWAS) studies implicated single nucleotide polymorphism (SNPs) in lipid metabolizing genes such as DHCR7, CYP1B1 and complement pathway genes in ROP pathogenesis. Subsequent studies in our lab demonstrated abnormal complement activation in the ROP eyes leading to inflammation and abnormal angiogenesis. Several independent clinical studies have attempted supplementation of omega-fatty acids to reduce the risk of ROP yielding variable responses. However, the underlying mechanism of genetic regulation of lipid metabolism has not been explored. Lipids are important structural,

signalling, and metabolic biomolecules with high abundance in photoreceptors and different retinal layers. Purpose: Based on the significant association of alternate complement pathway and fatty acid metabolism genes with ROP, we proposed that an interplay of complement activation and altered lipid metabolism, disrupts the retinal homeostasis leading to inflammation, abnormal angiogenesis and neurodegeneration leading to blindness.

Macular layer in retina is rich in long-chain polyunsaturated fatty acids (PUFAs)* such as arachidonic acids (AA), docosahexaenoic acid (DHA), Eicosapentaenoic acid (EPA) etc. which are prone to frequent lipid peroxidation and structural modification under the oxidative stress. These PUFAs are hydrolysed by Cytochrome P450 enzymes such as CYP1B1 and CYP2C8 to yield epoxy fatty acids. Epoxy fatty acid PUFAs such as epoxyeicosatrienoic acids (EETs), epoxyeicostetraenoic acid (EEQs), epoxydocosapentaenoic acids (EDPs) and eicosapentaenoic acid (EPA) are catalysed by soluble epoxide hydrolase (sEH) into their respective diols. These fatty acids are important structural and signalling molecules in endothelial layer* and maintain optimal retinal physiology and anatomy. These diols further regulate angiogenesis via Notch signalling pathway*. Diols produced from the enzymatic activity of sEH acts on presinilin I protein which couples with gamma-secretase to regulate the Notch signalling pathway. If notch regulation is overexpressed, it leads to irregular neovascularization of blood vessels, leading to severe ROP stages. Preliminary data from our lab on the metabolic profiling of ROP cases revealed suggested an increased expression these fatty acids with a concurrent increase in severity (abnormal angiogenesis) of the disease. It would be interesting to study the role of altered lipid metabolism with increased inflammation and abnormal angiogenesis. Identification of novel mediators of abnormal lipid metabolism and their genetic regulation can then be tested as novel drug targets for checking inflammation and thus neovascularization in the retina.

We performed an integrative retinal transcriptomics, proteomics and lipidomics analysis of retinal sections and vitreous humor (VH) from ROP patients and age-matched controls. The blood (ROP=56, Control=70), VH (ROP=25, Control=24), retina, and FVM samples were collected from ROP-diagnosed infants and controls. Retinal transcriptomics (ROP=3, Control=3) using microarray was performed and targeted screening of key lipid metabolizing enzymes, angiogenesis, and apoptotic genes were performed using semi-quantitative PCR in blood. Untargeted global proteome (ROP=4, Control=3) and lipid metabolites profile (ROP=19, Control=19) were identified and quantified by LC-MS in VH. GeneSpring GX, Proteome Discoverer 4.1 and Metaboanalyst 5.0 were used for intuitive data analysis,

pathways, and visualization for gene expression, proteomics, and lipidomics analysis respectively. The overlapping network analysis was performed among gene expression, proteome and lipidome dataset to identify key genetic regulators. Significant identified VH proteins were validated using multiplex ELISA. Further functional assessment of the selected proteins was done by analyzing changes in FVM and retina by immunohistochemistry. Oxidative stress and lipid expression was assessed by DCFDA and Nile Red Staining.

The gene expression of retina showed 981 genes (fold change: >1.5; p-value: <0.05). Heatmap and PCA plot showed distinct expression profiles among ROP and controls. Retinal gene expression showed some of the key immune regulating complement genes, C3 (fold change: 7.17; p-value: 0.001) and C4A (fold change: 3.54; p-value: 0.01); arachidonic acid pathway genes, ALOX5 (fold change: 3.25; p-value: 0.03) and PTGS1 ((fold change: 9.19; p-value: 0.0004). Top significant pathways included Human Complement System (p-value: 7.25E-08); Prostaglandin Synthesis and Regulation (p-value: 0.00098); Oxidative Stress (p-value: 0.001) Arachidonate-epoxygenase (p-value: 0.01) pathways.

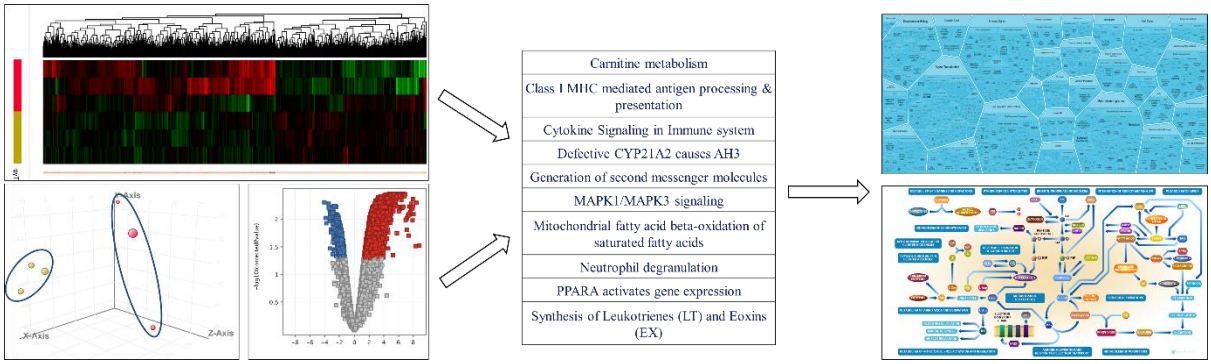

Figure 5: A representative image of global gene expression among ROP and control retina showing distinct gene expression-based signature for the two categories; significant pathways involved in the ROP pathogenesis and their interactions

QT-PCR was performed in blood for genes of arachidonic acid metabolism showing significant upregulation of ALOX5 (fold change: 6.73; p-value-0.03), COX2 (fold change: 3.85; p-value-0.05), CYP1B1 (fold change: 4.9; p-value-0.001), CYP2C8 (fold change: 3.1; p-value-0.05) while significant downregulation of EPHX2 (fold change: -1.6; p-value-0.04) (Figure 6).

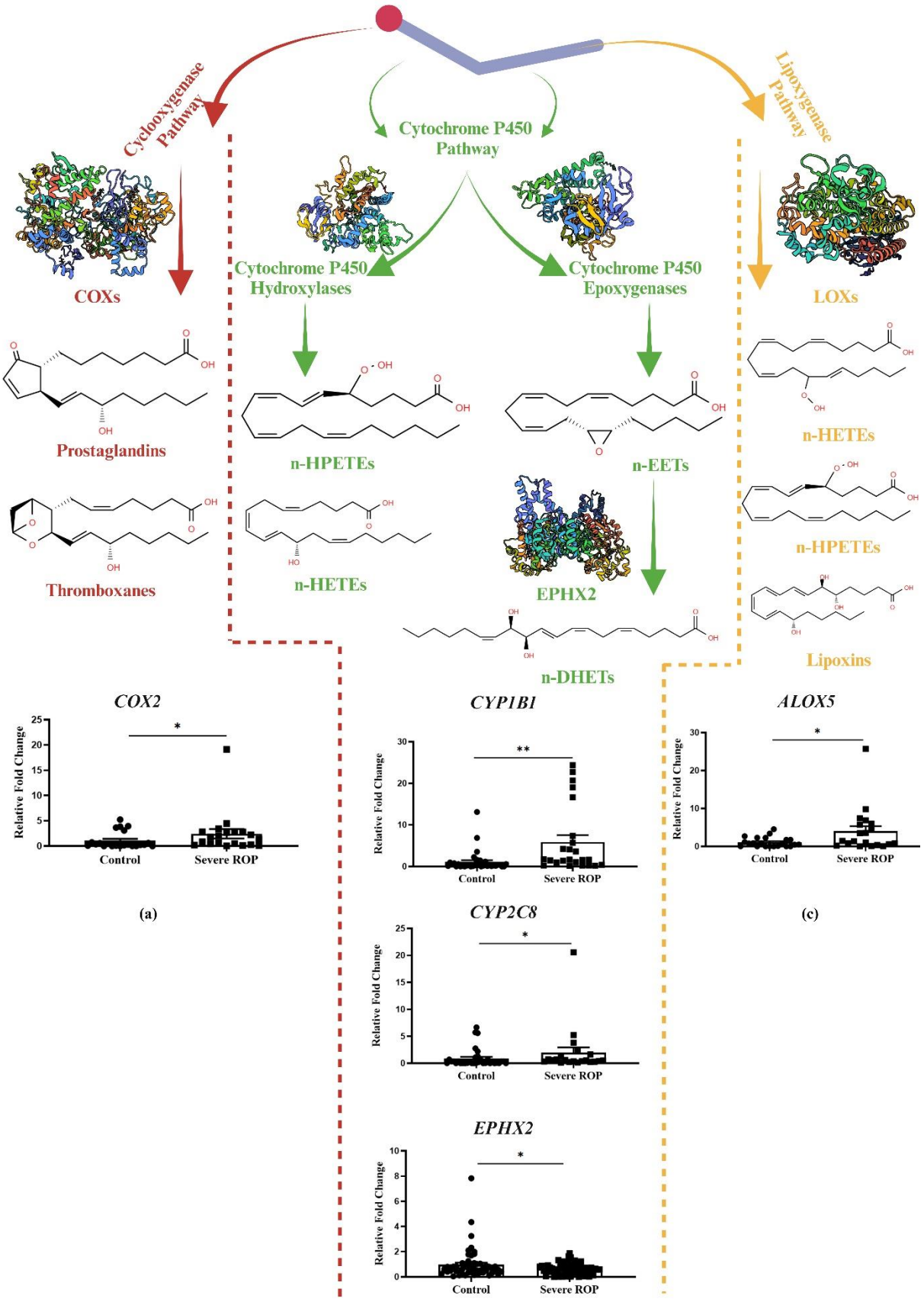
Cyclooxygenase Pathway
Cytochrome P450 Pathway
Lipoxygenase Pathway
COXs
Cytochrome P450 Hydroxylases
Cytochrome P450 Epoxygenases
LOXs
Prostaglandins
n-HPETEs
n-EETs
n-HETEs
Thromboxanes
n-HETEs
EPHX2
n-HPETEs
n-DHETs
Lipoxins
COX2
CYP1B1
ALOX5
CYP2C8
EPHX2
Relative Fold Change
Control
Severe ROP
(a)
(b)
(c)

Figure 6: Differential activity and expression of major enzymes involved in PUFA (arachidonic acid) metabolism among ROP and controls.

In this figure we have shown details on the Arachidonic acid metabolism: Classification of different metabolites formed from AA by different enzymatic pathways. The cyclooxygenase and lipoxygenase pathways are mediated by the activity of COXs forming prostaglandins and thromboxanes and LOXs forming HETEs, HPETEs, and lipoxins, respectively. Epoxygenase and ω- hydroxylase pathways are mediated by the activity of CYPs, forming classes of EETs, HETEs, and HPETEs, respectively. EPHX2 acts on the epoxygenase- generated metabolites to form dihydroxy alcohols (n- DHETs). (a) qRT- PCR analysis of transcripts for *COX2* (*D*f = 42; *t*- value = 2.01; *n* = 21 preterm babies with severe ROP; *n* = 23 preterm babies with no/mild ROP; *p* = 0.0503) (b) qRT- PCR analysis of transcripts for *CYP2C8* (*D*f = 59; *t*- value = 2.01; *n* = 25 preterm babies with severe ROP; *n* = 36 preterm babies with no/mild ROP; *p* = 0.0504); *CYP1B1* (*D*f = 60; *t*- value = 2.80; *n* = 26 preterm babies with severe ROP; *n* = 36 preterm babies with no/mild ROP; *p* = 0.0072); and qRT- PCR analysis of transcripts for *EPHX2* (*D*f = 124; *t*- value = 2.07; *n* = 70 preterm babies with severe ROP; *n* = 56 preterm babies with no/mild ROP; *p* = 0.0415). (c) *ALOX15* expression (*D*f = 42; *t*- value = 2.16; *n* = 21 preterm babies with severe ROP; *n* = 23 preterm babies with no/mild ROP; *p* = 0.0503). All the graphs are representative of experimental triplicates. β- Actin was used as the normalization control for qRT- PCR. Statistical significance is represented as * and ** for $p \leq 0.05$ and $p \leq 0.01$, respectively.

Subsequently, the proteome analysis showed confirmed high expression level for complement C3 (FC: $\log_2$1.99) and C4a (FC: $\log_2$1.45), along with the other cleaved fragment of complement proteins. Major differentially regulated proteins included: ALOX12, PTGDS, lipocalin1, Cathepsin D, LRG2, Endothelial Protein C, Vitamin D 25-hydroxylase, and Vitamin-D binding protein. High abundance of serum proteins in VH such as classes of apolipoproteins, ECM proteins, complement etc. reflect the damage in blood-retinal barrier. Further, lipid metabolites activity from arachidonic acid pathway showed similar trend to gene expression profile. Lipids such as HETEs, HpETEs, EETs (from CYPs), Prostaglandins, Thromboxanes (from COXs and PTGS1), and leukotrienes, lipoxins (from ALOXs) showed high abundance, which are also known to inflammatory metabolites. DHETs, anti-inflammatory metabolites from EPHX2 activity showed low abundance. Complement components C3a, C4a, and others attract neutrophils and macrophages, which, in the process of ingesting complexes, arachidonic acid metabolites, release mitochondrial enzymes, and oxygen-derived free radicals, causing retinal damages (figure 6).

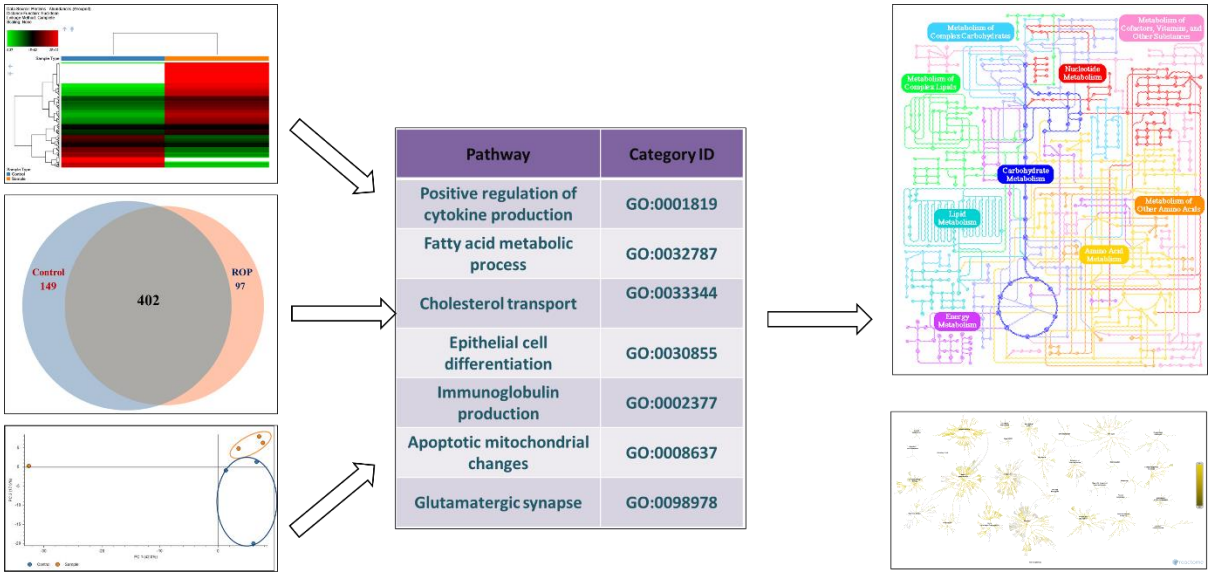

Figure 6: A representative picture of comparative vitreous humor proteomics analysis among ROP and control confirming the global gene expression profiling data.

Genes for angiogenesis (VEGF165 (FC: 7.24), VEGF189 (FC: 2.74), NOTCH1 (FC: 6.6)) and apoptosis (CASP3 (FC: 3.82), CASP8 (FC: 3.86)) were significantly upregulated, stating abnormal angiogenesis and cell death. Notch is regulated by component of γ-secretases genes, significantly upregulated APH1B1 and significantly upregulated PSEN1. Together, these genes activate Notch1 leading to VEGF upregulation causing an increase in angiogenesis (Figure 7).

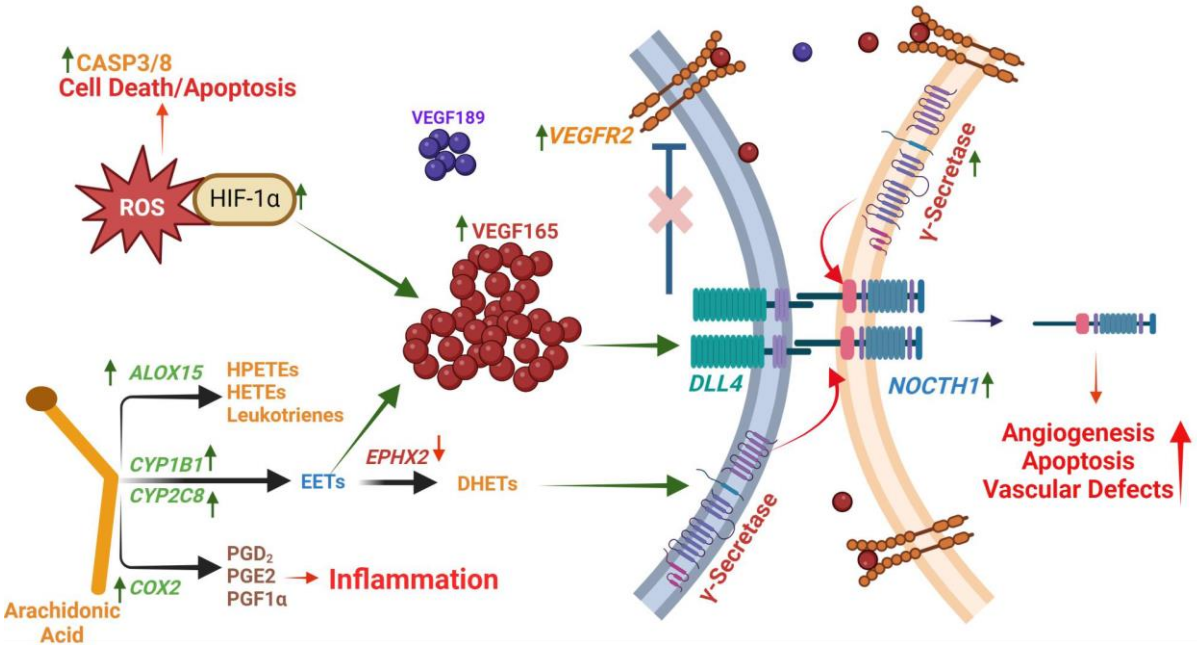

Figure 7: Schematic diagram of the mechanism of the proposed hypothesis of AA-metabolizing enzymes and its potential effect on angiogenesis, cell death, and inflammation.

Oxidative damage attributed by changing oxygen concentration and complement activation could impair the mitochondrial activity and initiates an early apoptosis of neurons. DCFDA staining showed high reactive oxygen species (ROS) expression in ROP retina. High retinal cell death could be involved in lipid accumulation, which was confirmed by Nile Red staining and TEM in ROP retina and FVMs (figure 8).

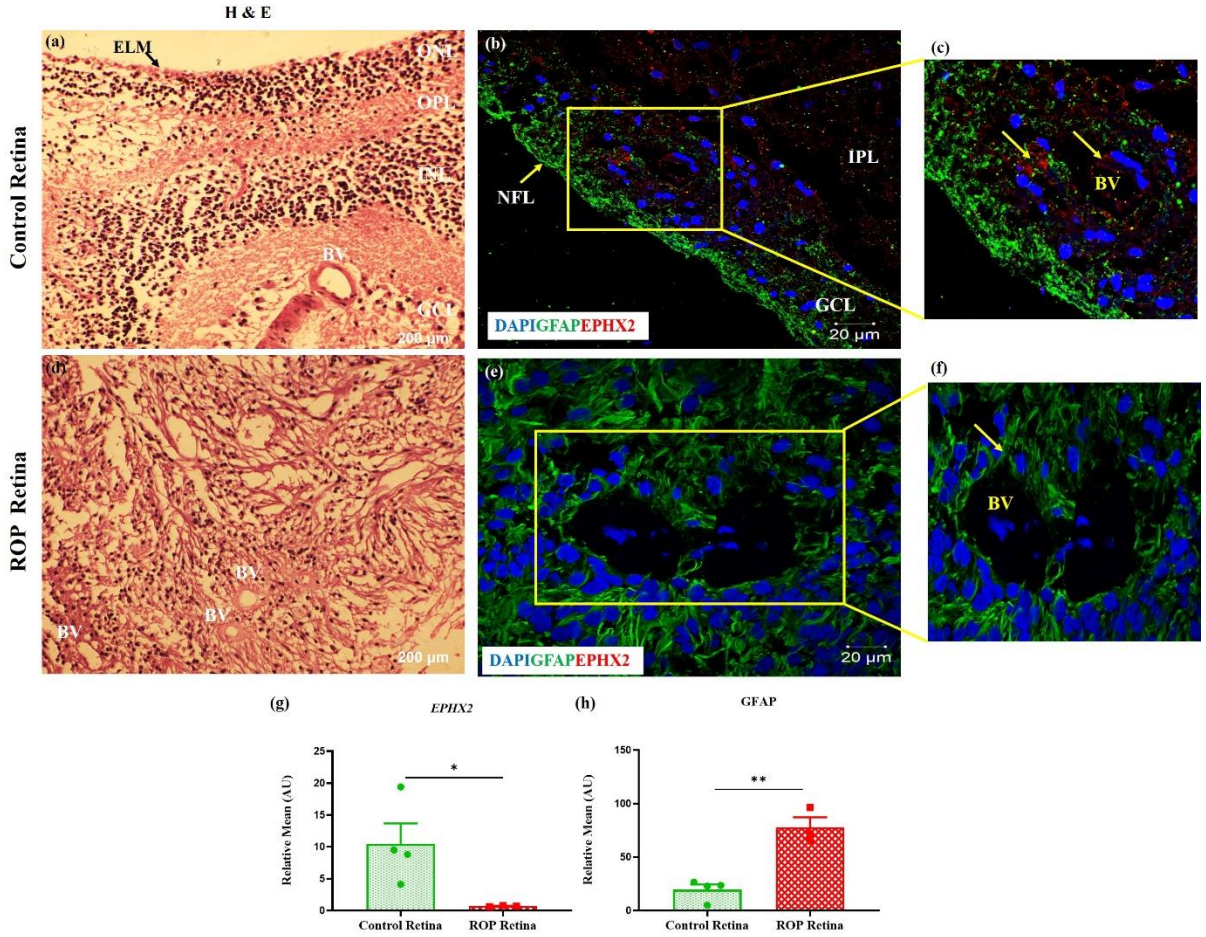

Figure 8: (a) H&E staining of the control retinal panel showing different retinal layers and blood vessels. (Scale: 200 µm; Magnification: 20×; Microscope: EVOS M5000 Imaging System) (b) EPHX2 and GFAP expression in the control retina showing expression of GFAP in the blood vessels lining, near the NFL and GCL toward the VH, while EPHX2 expression is observed at the IPL and blood vessels lining. (c) Zoom- out section of the control retina showing the expression of EPHX2 colocalized with GFAP in the blood vessels lining. (d) H&E staining of the ROP retina shows no distinct retinal layer and loss of different retinal cells. (Scale: 200 µm; Magnification: 20×; Microscope: EVOS M5000 Imaging System) (e) EPHX2 and GFAP expression in the retina of ROP infants. The image shows an almost lack of expression of EPHX2, while significantly higher expression of GFAP across the retina. (f) Zoom- out section of the ROP retina showing very high expression of GFAP in the blood vessels lining but no expression of EPHX2 (g) Quantification of EPHX2 (*D*f = 5; *t*- value = 2.56; $n = 3$ for ROP retina; $n = 4$ for control retina; $p = 0.05005$) (h) Quantification of GFAP (*D*f = 5; *t*- value = 5.91; $n = 3$ for ROP retina; $n = 4$ for control retina; $p = 0.0101$). ELM – External Limiting Membrane; ONL – Outer Nuclear Layer; OPL – Outer Plexiform Layer; INL – Inner Nuclear Layer; IPL – Inner Plexiform Layer; GCL – Ganglion Cell Layer; NFL – Nerve Fiber Layer; BV– Blood Vessels. The arrow shows blood vessels lining. The experiments were performed thrice with a similar protocol. A retinal section with no primary antibody was used as a negative control for normalization and human skin as a positive control for EPHX2 (Figure SF2). Scale: 20 µm; Magnification: 64X; Microscope: Carl Zeiss AG. Statistical significance is represented as * and ** for $p \leq 0.05$ and $p \leq 0.01$, respectively.

Dysregulated genes, proteins, and metabolites of arachidonic acid and complement pathways lead to aberrant retinal angiogenesis, inflammation, and cell death in ROP. C3 in association with other complement proteins is required for mobilization of the arachidonic acid leading to enhanced production of TXB2, prostaglandins and other metabolites. Changes in the interplay of genes and proteins of arachidonic acid metabolizing enzymes could dysregulate its metabolism leading high inflammatory lipid metabolite synthesis, aberrant neovascularization, and cell death under oxidative imbalance (figure 9).

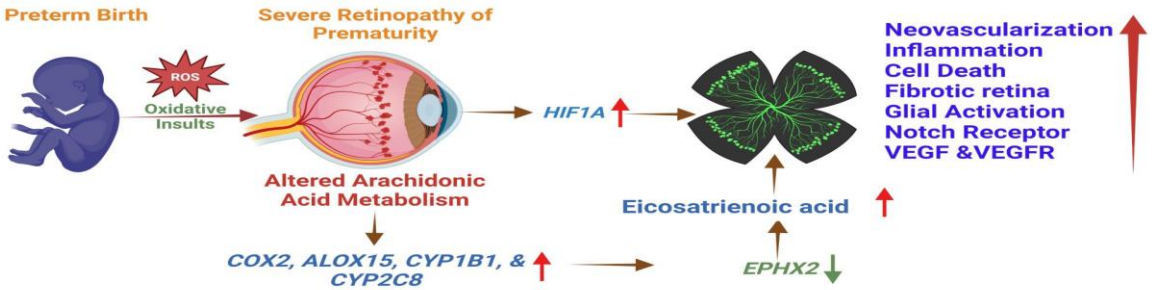

Figure 9: Overall summary of how an interplay of hypoxia and lipid metabolism could regulate retinal vascularization and in leading to ROP among preterm infants

<u>Clinical translation of ROP research findings:</u>

Tear markers for early ROP risk prediction:

A comprehensive genomic and proteomic analysis of ROP revealed a strong association of markers of inflammation namely: Complement component C3 and MMP9 levels in vitreous humor with disease progression. We further observed that these could be detected in the tear samples of ROP babies and thus could be used as markers for ROP progression (Rathi et al. 2017). The tear samples were an obvious choice for this study as obtaining tears from preterm infants is non-invasive, safe and convenient. Under this project, premature infants who were screened for ROP at the NICUs of Nilofur hospital and those who are referred to L V Prasad Eye Institute were recruited in the study after obtaining due consent from their parents. Initially the levels of MMPs (2 & 9) and C3 in tears were validated among the preterm babies with and without ROP (n=100 in each category) by multiplex ELISA. A subsequent validation of ELISA

results and assessment of MMP activity was performed by Zymography. We have identified matrix metalloproteinases (MMPs) to be potential tear biomarkers, the increased levels of MMP-9 in tears are associated with ROP progression.

Though an IMPRINT grant from Ministry of Human Resource and Development (MHRD) and Department of Science and Technology (DST) we worked in collaboration with scientists of Indian Institute of Technology Hyderabad develop a simple and rapid device for the detection of tear inflammatory markers for community level screening of preterm infants. The vanadium disulfide nanowires were hydrothermally synthesized and utilized as a sensing platform for the fabrication of chemiresistive biosensors. The VS_2NWs deposition on interdigitated microelectrodes was followed by a four-step process for the complete bio fabrication of tear MMP-2 and MMP-9 detection. The biosensor fabrication process was optimized using a hybrid central composite design (CCD)- support vector regression (SVR)- genetic algorithm (GA) demonstrating in an enhanced signal amplification of the sensor output ($\Delta R/R$). The optimized biosensor was calibrated for MMP-9, and MMP-2 tear proteins, and the lower limit of detection (LOD) was found to be 0.556 pg/mL and 6.05 pg/mL respectively. The biosensor showed a similar inclination for the expression of MMP-2 and MMP-9 with the commercial ELISA kits. Further, the sensitivity and specificity of MMP-9 using the biosensor compared with commercially available ELISA showed better results to predict the ROP disease and progression (work under submission for publication).

Development of intraocular implant providing controlled and localized combination-drug-therapy: towards efficacious treatment of retinal vascular diseases (RVD).

Retinal vascular diseases including retinopathy of prematurity (ROP), age-related macular degeneration (AMD), diabetic retinopathy (DR), diabetic macular edema (DME), and branch/central retinal vein occlusion (RVO) associated with pathological neovascularization and neurodegeneration, are leading causes of blindness worldwide. Despite their wide occurrence, it is unclear how despite different onset ages and patient histories, these diseases share similar clinical features including neo-vascularization, edema and chronic inflammation. Although increased expression of vascular endothelial growth factor (VEGF) – a key regulator of vascular permeability has been implicated in the pathogenesis of these diseases, a systematic understanding of the molecular pathways involved in driving inflammation-mediated neo-angiogenesis and neurodegeneration in the retina has not been studied. Further challenges in disease management include lack of adequate methods for the prediction of disease risk, time to intervene, and robust therapeutic interventions that ensure effective delivery of drugs.

Therefore, there is an urgent need to focus on developing robust strategies to help prevent vision loss thus offsetting the huge economic burden posed by RVDs nationally and globally.

Current clinical treatments in retinal vascular diseases include intravitreal administration of anti-VEGF agents to hinder new blood vessel growth and decrease swelling. While anti-VEGF injections have shown improved visual acuity, it shows variable response and is cumbersome and expensive as it requires monthly visits and frequent injections which can cause complications. A recent approach to the treatment of incomplete responders is to switch to corticosteroid administration or addition of corticosteroid (i.e., combination therapy) to address abnormal proliferation of cells and edema caused by chronic inflammation. However, high doses of corticosteroids (e.g., dexamethasone) over long periods can cause serious systemic side effects (which are of particular concern for pediatric patients), while topical applications do not penetrate adequately to the posterior segment. Further, the additional visual acuity gains from systemic corticosteroid administration are variable. Finally, current treatment options rely upon administration routes that neither provide precise control over drug delivery nor long-term sustained benefits when administered in isolation.

In this direction we are working with scientists at IITH to develop a unique biomaterial-based combination drug therapy to target chronic inflammation and neo-angiogenesis in retinal vascular diseases. We propose to develop and full characterize nanofibrous implants (prepared using a novel biomaterial fabrication approach) that can provide long-term spatio-temporal and independent control over the release of bevacizumab and dexamethasone. A preliminary dat on microglial cell grown on these nanfibre meshes coated with drugs in our lab has shown significant promise

Future directions of our ongoing research work on ROP

Placenta is a highly specialized temporary organ developed *in-utero* during pregnancy to maintain and regulate fetal well-being. Structurally, placental tissues have both maternal and fetal origins. Amnion, Chorion and the trophoblastic layer are of fetal origin whereas decidua is of maternal origin. The chorionic villi are formed out of both fetal and maternal origin. In toto, this complex organ performs circulatory, hormonal and immunological functions of mother and fetus. In the event of preterm birth, placental support to the developing fetus is not available and it acts as an independent risk factor for the neurological injury to the fetus which may affect the postnatal neurovascular development. *In-utero* fetal genetic programming is likely to be contributed by placental factors like the uteroplacental blood flow and transfer of

chemical signals through the placenta. The maternal malnutrition during pregnancy can also induce alterations in placental and fetal responses that leads to fetal reprogramming resulting in increased risk of metabolic and cardiovascular diseases in adulthood. As retina and brain are linked by optic nerve and corresponding neuro-vasculature, they share common risk factors for certain diseases. So, any developmental changes in the brain occurring due to utero-placental insufficiency can also affect the retinal structure/vasculature. The elevated levels of angiogenic and inflammatory proteins in the postnatal blood are associated with ROP are considered as reaction to prenatal/perinatal inflammatory responses which result in postnatal pathologies.[24] In fact, elevated cytokine levels in postnatal venous blood, cord blood and amniotic fluid are associated with many neonatal and postnatal pathologies. Severe ROP is associated with increased endoglin, endostatin and IGFBP-2 levels in amniotic fluid while vision threatening ROP is associated with high AF endoglin, IL-6 and IL-8 levels. The authors concluded that elevated levels of inflammatory and angiogenic mediators are independently associated with occurrence and progression of ROP suggesting prenatal pathogenic events that pre-dispose preterm infants to ROP in postnatal life.

A functional immune system is very crucial for providing protection to mother and fetus from potential pathogens while maintaining a healthy pregnancy. Also the maternal immune system should be able to distinguish pathogens from fetal tissues and avoid potential immune attacks at the fetal-maternal interface. Complement system recruits and immune cells that leads to opsonization, inflammation and killing of invading pathogens. Thus control of complement system activation at the fetal- maternal interface is also very important for avoiding adverse pregnancy outcomes including preeclampsia. For a healthy pregnancy, the fetal cells must avoid complement activation by inhibiting the activity of C3 and C5 convertase that further leads to membrane attack complex formation. Several studies have reported an association between deposition of complement factors in the placenta and placental dysfunction. There are reports of significantly higher levels of fragment Bb, a marker of alternate complement pathway activation in the preeclamptic maternal and cord blood plasma suggesting an association between alternative complement pathway and preeclampsia. Interestingly, our genetic study on ROP indicated a strong association with alternate complement pathway genes and an increased expression of complement proteins in the vitreous of preterm born infants with severe ROP, signifying an important role of complement activation in ROP pathogenesis.[33] Therefore, studying association between complement expression and deposition in the placenta particularly at the decidua, synctiotrophoblast and villus stromal

layers and in the ROP vitreous samples could provide a possible role of placental immune alterations with ROP susceptibility and adverse outcomes in ROP eyes despite of treatment.

In spite of the evidence for placental transcriptomic dysregulation in preterm labor, very few studies have evaluated the direct whole transcriptomic signature of placenta with preterm birth related co-morbidities including ROP. In a similar study, researchers found significant association of placental CpG methylation of inflammation, angiogenic, and neurotrophic genes with pre threshold ROP. Therefor epigentic signature of maternal and fetal portions of placenta needs to be explored to assess and quantify the activity of genes in different developmental stages that can further be correlated with neurovascular development in eyes and progression of ROP (this work has recently been started in our lab).

Impact of ROP Prevention Research

- Development of a simple and rapid nano biosensor-based device for the detection of tear inflammatory markers for community level screening of preterm infants. The biosensor showed an increased sensitivity and specificity of MMP-9 as compared with commercially available ELISA and great potential to predict the ROP disease and progression.
- Besides this, we are also developing newer drug delivery model for targeting inflammation and complement activation as an adjuvant to existing therapeutic options for ROP might lead to better management of the disease.
- Understanding the biological basis for inflammation and neo-angiogeneis in retinal vascular diseases through systematic identification of inflammatory mediators (genes/proteins/pathways) in ocular fluids/tissues will lead to identification of newer drug targets for inflammation and abnormal angiogenesis.
- Functional assessment of identified markers/mechanistic pathways involved in the development of abnormal vascularization via subjecting human *ex vivo* tissues to diseased conditions can aid development of timely diagnosis for better disease prognosis.
- Localized and controlled combination drug therapy using engineered biomaterials that avoids repeated anti-VEGF injections, significantly reduces adverse systemic effect and reduces the overall drug dosage of either drug while providing a synergistic effect.
- Quantitative assessment of indicators of inflammation and neovascularization in ocular fluids, *in vitro* cellular model and PVR animal model following targeted combination drug therapy will establish the therapeutic impact of our work and aid rapid translation of the proposed implants.

31/08/2024