Genetic Susceptibility to Retinopathy of Prematurity: A Paradigm for Preterm Disease Traits

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Retinopathy of prematurity (ROP) is a vasoproliferative retinal disorder in premature infants that can lead to blindness and the incidence is increasing with increased survival of infants born at very early gestational age and low birth weight. Majority of common diseases are complex and multifactorial resulting from the interplay of genetic components and environmental factors. As no single gene or genetic variant on its own can cause the disease pathology, complexities associated with common diseases present unique challenges for management and therapy. Genome wide association and resequencing projects together with gene environment interaction studies are expected to further define the causal relationships that connect genetic variants to ROP pathogenesis and should assist in better design of prevention and intervention. This review summarizes the recent literature on ROP with a special focus to recognize the genetic basis of the disease.

Keywords: • retinopathy of prematurity • premature infant • VEGF • eNOS

Retinopathy of prematurity is characterized by abnormal vascular development of retina in premature infants.1 Premature neonates have higher chance to develop ROP. Normally the retina becomes completely vascularized at full term (a full term pregnancy has a gestation of 38-42 weeks). ROP is characterized by abnormal neovascular development in the retina of premature infants. These abnormal blood vessels are fragile and can leak or bleed, scarring the retina and pulling it out of position. This causes a tractional retinal detachment which is the main cause of visual impairment and blindness in ROP.2 ROP progresses in two phases, the first phase begins with delayed retinal vascular growth after birth and partial regression of existing vessels, followed by a second phase of hypoxia-induced pathological vessel growth.3 Rather than in a healthy full term neonatal, vasculogenesis in the premature neonatal retina becomes disrupted. Abnormal new proliferating vessels develop at the juncture of vascularized and avascular retina. These abnormal new vessels grow from the retina into the vitreous, resulting in hemorrhage and tractional detachment of the retina. Since angiogenesis is interrupted the retina becomes hypoxic inducing the proliferation of new vessels between the vascularized and avascular retina; this is termed the vasoproliferative phase of ROP.4

International classification of retinopathy of prematurity have classified ROP using a clinical classification into 5 stages e.g. demarcation line, ridge, extraretinal fibro vascular proliferation, partial retinal detachment and total retinal detachment while other terminologies have used variants of the severity scale.

Incidence and Prevalence of ROP
ROP remains a significant and prevalent cause of morbidity among preterm infants worldwide. Most reports on ROP incidence show that there are about 60% babies less than 1500g in nurseries in high income countries.5-8 In middle income countries this figure is quite variable depending on the birth and survival rates of premature infants and the fact that ROP occurs in much older and bigger babies than in high income countries because of varying standards of neonatal care. Globally, there are
at least 50000 children blind from ROP, which remains an important cause of childhood blindness in developed countries, such as the United States and is also emerging as a major cause of childhood blindness in developing countries such as Latin America, Eastern Europe, India and China. In India, Charan et al. prospectively analyzed data from patients with birth weight (BW) ≤1700g and found the incidence of 47% of ROP, including all stages and drew attention to the high prevalence of ROP among patients with BW ≤1700g, with 12.8% requiring treatment. In a retrospective study of patients with BWs more than 1250g referred for ROP examination, Vinekar et al. reported that 45% had threshold ROP, demonstrating that severe ROP occurs in bigger babies in India. A recent multicenter trial from North America of newborns with a gestational age (GA) of 22 to 28 weeks and birth weight of 401 to 1500g gives an overall ROP (any stage) incidence of 59% with a 16% incidence of severe ROP. The worldwide incidence of ROP is shown in (Table 1).

### Risk Factors of ROP

Preterm deliveries, the leading identifiable causes of ROP have been shown to be associated with systemic infections and maternal risk factors. Three factors have shown consistent and significant association with ROP: low gestational age, low birth weight and prolonged exposure to supplementary oxygen following delivery. Evidence suggests that almost all infants with ROP have a gestational age ≤32 weeks and/or birth weight ≤1250g. These indications are generally used to decide whether a baby should be screened for ROP but some centers may extend birth weight screening criteria to 1500g. Retinal assessment with scleral depression is generally recommended for patients born before 30-32 weeks gestation or 4-6 weeks of life, whichever is earlier. It is then repeated every 1-2 weeks until vascularization is complete. Other putative risk factors include mechanical ventilation, sepsis, intraventricular hemorrhage, surfactant therapy, anemia, frequent blood transfusions and apnea.

### Identifying the Genes of Retinopathy of Prematurity

Identifying the genes that contribute to the pathogenesis of ROP has been challenging despite being a major focus of research over the past few decades. A large number of putative genes and genetic variants have been reported in the literature but few of these have been consistently replicated. Identifying genes for ROP is more challenging due to the greater complexity of the disease which may have more multifactorial, polygenic and environmental influences. Potential candidate genes that have been evaluated known pathophysiologic mediators involved in progression of ROP. These have included primarily mediators of angiogenesis in the developing retina, with particular focus on vascular endothelial growth factor (VEGF) and the Norrie disease gene (NDP) and angiotensin converting enzyme. Recent genome wide association studies have reported a number of genetic variants that are consistently associated with risk of ROP. Using the candidate gene approach, the EPAS1 gene

### Table 1: Worldwide incidence of ROP

| Country | Period of study | No. of babies | BW and/or GA | Incidence of ROP (%) | Reference |
|---------|----------------|---------------|--------------|----------------------|-----------|
| Australia | 1992-2009 | 373 | GA 23.0–25.6 wk | 15.0 | Gunn et al., 2012 |
| Brazil | 2004-2006 | 3437 | BW ≤2000g or/ GA <37 wk | 3.4 | Zin et al., 2010 |
| China | 2009-2010 | 2185 | BW ≤2000g or GA ≤34 wk | 13.1 | Li et al., 2012 |
| Colombia | 2001-2005 | 1138 | BW ≤1500g and GA <32 wk | 8.0 | Zuluaga et al., 2006 |
| Germany | 1978-1992 (P1) 1993-2007 (P2) | 1473 | BW ≤1000g | 19.5 (P1) 14.8 (P2) | Schwarz et al., 2011 |
| India | 1999-2002 | 1083 | BW ≤2000g GA ≤36 wk | 11.0 | Jalali et al., 2006 |
| Kuwait | 2001-2003 | 599 | BW ≤1501g or GA <36 wk | 7.8 | Wani et al., 2010 |
| Romania | 2002-2007 | 1783 | BW ≤2000g or GA ≤34 wk | 15.2 | Vatavu et al., 2010 |
| Saudi Arabia | 2009-2011 | 386 | BW ≤1500g or/ GA ≤32 wk | 6.4 | Amer et al., 2012 |
| Singapore | 1988-2001 | 564 | BW ≤1500g | 8.0 | Shah et al., 2005 |
| United Kingdom | 1987-1990 (P1) 1990-1998 (P2) | 484 | BW ≤1500g (P1) BW ≤1500g or/ GA ≤32 wk (P2) | 5.2 | Brennan et al., 2003 |
| United States | 2000-2009 | 355806 | BW 501–1500g | 6.8 | Horbar et al., 2012 |

GA-Gestational age; BW-Birth weight; P1-Period 1; P2-Period 2
was found to be associated with the development of ROP. When a multiple logistic regression analysis was performed evaluating the genes and associated risk factors, EPAS1 and CFH were found to be associated with the development of ROP.30

Candidate Gene Studies
Most research to date has focused on identifying genetic susceptibility to ROP through the candidate gene approach. This study design typically selects participants with and without the disease of interest (i.e. cases and controls) and compares the frequency of genetic variants between the groups. Most studies also attempt to adjust for some factors such as birth weight, gestational stage, sex etc. Although relatively simple to perform, such studies have the important drawback of lacking study power. Studies evaluating polymorphisms in specific candidate genes, such as VEGF have also demonstrated an association between genetic factors and susceptibility to ROP.25,26 VEGF protein increases in the ocular fluid of patients with intravitreous neovascularization including ROP31 and VEGF mRNA was detected in the avascular retina of a human infant with stage 3 ROP. A variety of endothelial cell growth factors have been identified including fibroblast growth factor, vascular endothelial growth factor, hepatocyte growth factor, placental growth factor, insulin like growth factor 1, and platelet derived endothelial cell growth factor.

Vascular Endothelial Growth Factor
VEGF plays as a ‘master switch’ in the neovascularization formation in retinopathy of prematurity and in disorganized growth of retinal blood vessels which may result in scarring and retinal detachment. Normally during angiogenesis, VEGF levels should rise due to hypoxia condition. Its low levels decrease angiogenic signaling and allow the retraction of blood vessels by apoptosis of endothelial cells. However in phase 1 of ROP, these levels decrease within 6 hours under normoxic or hyperoxic conditions.32 VEGF is strictly under the control of oxygen; between 17% and 45% oxygen, the extent of vasculogenic cell division is inversely proportional to the level of oxygen. The promoter region of VEGF has several polymorphisms, some of which have been associated with retinopathy of prematurity. Some of them are in the promoter and 5′ untranslated region (5′-UTR) but some of them are in the 3′ untranslated region (3′-UTR). Several polymorphisms within the VEGF gene are correlated with variation in VEGF protein production.33 Previously it was reported that the CC genotype of +936 C/T polymorphism in the 3′-UTR of VEGF gene was associated with an increase in the VEGF level in the peripheral blood as compared with CT and TT genotypes.34 In support of the VEGF gene involvement in ROP some studies have shown an association of VEGF gene polymorphism and ROP risk,25,26 but in a meta-analysis study some other studies did not show any association.35 Results of this meta analysis indicate that advanced ROP is associated with VEGF gene -460T/C polymorphism but -634G/C, -2578C/A and 936C/T polymorphisms did not show any association. The majority of published studies appear to replicate associations of VEGF gene polymorphisms with retinopathy of prematurity, highlighting the promise that this gene may hold. Several clinical trials are currently investigating the efficacy of anti-VEGF agents in treatment of retinopathy of prematurity.

Endothelial Nitric Oxide Synthase
Endothelial nitric oxide synthase (eNOS) is a major weapon of endothelial cells to fight vascular disease where it generates the key signaling vasoprotective molecule nitric oxide (NO). eNOS is associated with plasma membranes.
surrounding cells and the membranes of Golgi bodies within cells. NO triggers the gene expression and activation of several vasculogenic, angiogenic, cell-migration and proliferation including vascular endothelial growth factor, fibroblast growth factor-2, and matrix metalloproteinase.\textsuperscript{35,36} eNOS is located on chromosome 7q\textsuperscript{35-36}, it shows a single nucleotide polymorphism in exon 7, Glu298Asp (G894T, rs1799983) which results in a substitution of glutamic acid by aspartic acid at amino acid position 298. The Glu298Asp polymorphism was suggested to be associated with altered NOS3 enzyme activity, reduced NO production, and blunted endothelial dependent vasodilation. The association between eNOS T-786C and eNOS 27-bp repeat functional polymorphisms and the development of severe ROP was found in low birth weight infants.\textsuperscript{37}

**Genome Wide Association Studies**

A comprehensive genome wide association study (GWAS) is the ultimate way to identify those genes that would not be suspected based on current understanding of the biology of parturition. The birth of a premature infant is a complex problem with a devastating impact on individuals, families and society. Advances in genotyping technologies and rapid cost reductions have made assaying hundreds of thousands of single nucleotide polymorphisms feasible, and computational algorithms can relate them to clinical disease and measurable traits. The genetic risk could reside either in the mother and her uterus or in the infant/placenta. One major challenge in genetic factors study of prematurity is that the risk case is not truly established. Identification of genetic factors in the mother and/or infant could provide insights into identifying relevant environmental covariates that may be more amenable to rapid interventions but difficult to find using standard epidemiology alone.

**Conclusion**

Retinopathy of prematurity remains an important cause of blindness in preterm infants and treatment options have limitations. Several lines of evidence point to a considerable genetic influence in susceptibility to ROP and there has been intensive research to uncover the genes responsible. In the highly developed countries of the world, with screening programs and interventions aimed at prevention and treatment, ROP still accounts for 3% to 11% of blindness in children.\textsuperscript{38} The onset of ROP may be influenced strongly by the gestational age and birth weight of the newborn, factors that are unrelated to prematurity are likely involved. Identification of polymorphisms or mutations in genes is only the beginning and it may not solve all the problems. Some previous studies show association between genetic factors and susceptibility to ROP:\textsuperscript{25-29} Potential candidate genes that have been evaluated are known pathophysiologic mediators involved in ROP processes. These have included primarily mediators of angiogenesis in the developing retina, with particular focus on vascular endothelial growth factor,\textsuperscript{25,26} the Norrie disease gene.\textsuperscript{27-29} Linkage studies and candidate gene approaches have suggested many potential genetic variants which may underlie the disease but replication of these results has often been inconsistent. Despite limitations, a few fairly consistent associations involving variants in the NDP, VEGF, EPAS-1 and CFH genes have been demonstrated. No GWAS investigating retinopathy of prematurity has been published to date, but given past successes with this approach, it is expected that this type of analysis particularly if conducted within a population based cohort setting will provide novel insights into genetic susceptibility to ROP. Furthermore, despite treatment for aggressive posterior ROP (AP-ROP), a substantial proportion of eyes develop retinal detachments, most probably leading to very poor vision if any. Anti-VEGF treatment may improve structural and visual outcome of AP-ROP, but the systemic and ophthalmic long term effects are still unknown.

**Financial & competing interest disclosure**

The authors do not have any competing interests in any product/ procedure mentioned in this study. The authors do not have any financial interests in any product / procedure mentioned in this study.

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