HHS Public Access

Author manuscript

Ophthalmol Retina. Author manuscript; available in PMC 2019 September 01.

Published in final edited form as:

Ophthalmol Retina. 2018 September ; 2(9): 949–962. doi:10.1016/j.oret.2018.01.016.

The genetics of retinopathy of prematurity: a model for neovascular retinal disease

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Abstract

TOPIC—Retinopathy of prematurity (ROP) is a proliferative retinal vascular disease in premature infants, and is a major cause of childhood blindness worldwide. In addition to known clinical risk factors such as low birth weight and gestational age, there is a growing body of evidence supporting a genetic basis for ROP.

CLINICAL RELEVANCE—While comorbidities and environmental factors have been identified as contributing to ROP outcomes in premature infants, most notably gestational age and oxygen,

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No authors have financial conflicts of interest. Michael F. Chiang is an unpaid member of the Scientific Advisory Board for Clarity Medical Systems (Pleasanton, CA), and a Consultant for Novartis (Basel, Switzerland). R.V. Paul Chan is a member of the scientific advisory board for Visunex Medical Systems (Fremont, CA) and a consultant for Alcon (Fort Worth, TX), Allergan (Irvine, CA), and Bausch and Lomb (St. Louis, MO).

some infants progress to severe disease despite absence of these clinical risk factors. The contribution of genetic factors may explain these differences and allow better detection and treatment of infants at risk for severe ROP.

METHODS—To comprehensively review genetic factors that potentially contribute to the development and severity of ROP, we conducted a literature search focusing on the genetic basis for ROP. Terms related to other heritable retinal vascular diseases like “familial exudative vitreoretinopathy”, as well as to genes implicated in animal models of ROP, were also used to capture research in diseases with similar pathogenesis to ROP in humans with known genetic components.

RESULTS—Contributions across several genetic domains are described including vascular endothelial growth factor, the Wnt signaling pathway, insulin-like growth factor 1, inflammatory mediators, and brain-derived neurotrophic factor.

CONCLUSIONS—Most candidate gene studies of ROP have limitations such as inability to replicate results, conflicting results from various studies, small sample size, and differences in clinical characterization. Additional difficulty arises in separating the contribution of genetic factors like Wnt signaling to ROP and prematurity. Although studies have implicated involvement of multiple signaling pathways in ROP, the genetics of ROP have not been clearly elucidated. Next-generation sequencing and genome-wide association studies have potential to expand future understanding of underlying genetic risk factors and pathophysiology of ROP.

INTRODUCTION

Retinopathy of prematurity (ROP) is a retinal vascular disorder affecting premature low birth weight infants, and is a major cause of childhood blindness in the United States and internationally. Beyond the clinical impact, infancy-acquired visual loss from ROP represents an enormous social and economic burden.[1–4] Furthermore, as the incidence of premature births worldwide increases and as medical technology becomes better able to treat the complications of premature birth, the number of infants at risk for ROP is increasing rapidly.[5–8]

Oxygen plays a central role in ROP.[9–13] Oxygen environment and a key transcription factor that oxygen regulates (e.g. Hypoxia inducible factor [HIF]) are thought to modulate ROP. In terms of ROP pathogenesis, a two-phase hypothesis has been proposed and has become widely accepted.[14,15] In phase 1, there is delayed physiologic retinal vascular development and vasoattenuation, which is aggravated by hyperoxia and loss of nutrients and growth factors. In phase 2, vasoproliferation occurs at the junction of vascularized and avascular retina. Mouse oxygen-induced retinopathy (OIR) model (exposure to 75% oxygen for 5 days followed by room air), a widely used animal model of ROP, best represents the two-phase hypothesis.[16,17] During the vasoproliferative phase, the avascular retina releases pro-angiogenic growth factors such as vascular endothelial growth factor (VEGF), which are induced by hypoxia and may cause aberrant vessel growth and neovascularization. Oxygen fluctuations with intermittent hypoxia is also implicated in development of ROP in clinical studies[18–20] and OIR animal model studies especially in rats (e.g. cycling between 50 and 10% oxygen).[21,22] Growing neovascular vessels lead to fibrovascular membranes that may

pull on the retina, causing tractional retinal detachment and eventual blindness. The phenotype of ROP is classified based on location, extent, and severity of these pathologic changes.[23] Some infants show a rapidly progressing, severe form of ROP, known as aggressive posterior ROP (AP-ROP).[23–27]

Early investigations into ROP risk factors focused primarily on prematurity itself, as well as environmental factors including oxygen exposure after birth.[10,11] Various studies focusing on oxygen exposure have proven its importance as a primary predictor of ROP outcomes.[9–11] However, some high-risk infants with extremely low birth weight (BW) and gestational age (GA) do not develop ROP, whereas some low-risk infants do develop severe ROP. In these infants at phenotypic extremes, a study showed that known clinical risk factors were not significantly associated with development of ROP.[28] In addition, it is not understood why certain infants are predisposed to AP-ROP with very high likelihood of blindness. This heterogeneity of ROP risk suggests that other factors, such as genetics may be involved in creating a predisposition to ROP. Before specific genetic variations were investigated in ROP, epidemiologic studies suggested racial and ethnic differences in ROP incidence.[29–31] The Cryotherapy for ROP (CRYO-ROP) study of 4,099 premature infants found 7.4% of white infants reached threshold disease, while only 3.2% of black infants achieved a similar level of disease.[31] Also, twin and sibling studies have supported the involvement of a genetic component of disease. Two studies of monozygotic and dizygotic twins found that the heritability of ROP was 0.70 and 0.73, respectively.[32,33] Evidence of genetic effects is also supported by data from the oxygen-induced retinopathy (OIR) phenotype in rodent models, in which studies of different rat strains have found differences in the retinal avascular area and VEGF expression between strains.[34–36] Investigations into this genetic component in humans and animal models have implicated the involvement of multiple genes, but have not discovered a genetic component of large effect. It is likely that knowledge of such a genetic component could be used to identify possible targets to improve outcomes of screening and treatment.

Many signaling molecules and related pathways have been suspected in the pathogenesis of ROP due to known biochemical and clinical associations: VEGF, insulin-like growth factor-1 (IGF-1), erythropoietin (EPO), and inflammatory mediators. In addition to ROP, the growth of abnormal, leaky blood vessels is a common pathologic component of other blinding neovascular eye diseases, such as diabetic retinopathy (DR) and neovascular age-related macular degeneration (AMD), both of which have strong evidence of a genetic predisposition to disease.[37–39] Moreover, because ROP progresses more rapidly and presents with relatively homogeneous clinical characteristics, the correlation of genotype and phenotype is easier than with a chronic disease such as DR or AMD.[15] Thus, the study of ROP genetics may give us important insights into the pathophysiology of other more prevalent adult and pediatric neovascular retinal diseases.

This review summarizes current research into genetic factors contributing to ROP risk in both human and animal models and recommends future directions for research into the underlying genetics of pathways that contribute to disease.

METHODS

Pubmed was queried from January 1980 to June 2017. The following search terms were used: retinopathy of prematurity AND genetics, retinopathy of prematurity AND gene, retinopathy of prematurity AND single nucleotide polymorphism (SNP), retinopathy of prematurity AND variant, and retinopathy of prematurity AND polymorphism. Criteria for inclusion included the relevance, clinical importance, level of statistical evidence provided, and scientific importance of articles to the subject of this paper. Articles cited in the reference lists of other articles were reviewed and included when considered appropriate. All articles with English abstracts were reviewed.

CANDIDATE GENES IN ROP

VEGF and associated receptors

VEGF plays a crucial role in ROP. Increased VEGF in avascular retina stimulates pathological retinal neovascularization, which may result in blinding complications like tractional retinal detachment. Moreover, VEGF is a proven therapeutic target, as intravitreal anti-VEGF therapy has shown efficacy in promoting regression of severe ROP.[40] There have been many genetic studies on associations between the VEGF gene and incidence or severity of ROP.

Table 1 summarizes results of SNP studies in human VEGF gene (*VEGFA*). rs2010963 (also known as −634G>C and +405 G>C) is the most extensively studied SNP. In a British study of 188 preterm infants on rs2010963 in 2004, the G allele was found to have higher frequency among infants with ROP.[41] This result was supported by a 2015 study in 102 preterm infants from Egyptian hospitals showing that G allele was significantly higher in infants with ROP.[42] However, one study in Hungary reported the opposite results – higher frequency of C allele in severe ROP – and 5 other studies found no significant association between rs2010963 and ROP.

In addition, rs833061 (−460C>T) and *VEGFA* +13553C>T have been reported to be associated with ROP. However, replication has not been attempted for +13553C>T and the association of rs833061 and ROP has not been replicated in 3 other studies. *VEGFA* haplotypes have also been reported to be associated with ROP. A study performed in an Italian population of 342 infants focused on the distribution of polymorphisms in a handful of genes implicated in ROP showed evidence that *VEGFA* haplotype (TCCT) decreases risk of ROP.[43]

VEGF promotes angiogenesis and hyper-permeability by binding to the VEGF receptor 2 (VEGFR-2) on vascular endothelium, whereas VEGFR-1 acts as a decoy receptor.[44] However, studies on VEGFR-1 (*FLT1*) and -2 (*KDR*) genes found no associations with ROP (Table 2).

FEVR, Norrie disease and the Wnt pathway

Familial Exudative Vitreoretinopathy (FEVR) and Norrie disease are developmental diseases of the retina with known genetic causes with similar pathology to ROP. Both are hereditary

disorders occurring primarily in full-term infants, characterized by abnormal retinal vascularization leading to retinal detachment.[45,46] While patients with Norrie disease are blind from or shortly after birth, and often have systemic pathologies such as deafness and mental retardation, the clinical manifestations of FEVR are variable but restricted to abnormalities in ocular development.[47] FEVR is known to be caused by mutations in *FZD4, LRP5, TSPAN12, NDP*, etc.,[48–51] and Norrie disease is caused by mutations in the *NDP* gene.[46] These genes encode proteins which are components of the Wnt/beta-catenin signaling pathway – a group of signal transduction pathways with roles in cell survival, proliferation, and migration throughout the body.

The canonical (beta-catenin dependent) Wnt pathway has known roles in a variety of diseases with angiogenic properties including DR and AMD.[52,53] Frizzled-4 and low-density-lipoprotein receptor related protein 5 (LRP-5) are receptors for Wnt ligands, and tetraspanin-12 is an auxillary membrane protein. Norrin, a product of *NDP* gene, binds to the Frizzled-4, LRP-5, and tetraspanin-12 receptor complex and activates signals on endothelial cells. Mutations of these genes have been investigated in ROP (Table 2).

Mutations in the *FZD4* gene were found in up to 7.5% of patients with severe ROP (Table 2).[54–57] A 2015 study of 421 patients displaying various vitreoretinopathies found a significant association between the *FZD4* double missense mutation [P33S(;)P168S] and both ROP and FEVR.[57] A study of 53 Japanese patients with advanced ROP was performed using direct sequencing of *FZD4, TSPAN12, NDP*, and *LRP5*. Investigators identified six nonsynonymous DNA variants in the coding regions of *FZD4* and *LRP5*, but detected no changes in *NDP* or *TSPAN12*, demonstrating involvement of Wnt with ROP.[56]

Mutations in the *NDP* gene have also been found in ROP patients with variable frequencies (Table 2).[58–60] SNP studies in Kuwaiti populations have supported evidence of a link between *NDP* and ROP,[60] while other studies have implied that mutations in the regulatory region of *NDP* are also a contributor to the development of ROP.[61] The relationship between SNPs residing in the UTR of *NDP* and progression of ROP to advanced disease has also been investigated. The Kuwaiti study by Haider found that 83% of patients with severe disease possessed *NDP* 597C>A polymorphisms in their UTR, while none of those whose disease resolved spontaneously possessed this polymorphism.[60]

Taken together, these findings intriguingly suggest involvement for the Wnt pathway and associated genes in ROP development, and serve as strong candidates for further sequencing research. It should be noted that it may be difficult or nearly impossible to differentiate ROP from FEVR in premature infants. This has recently been proposed as a new classification, ROPER (ROP vs. FEVR) due to the clinical similarity of the two conditions.[62] In future studies, in-depth analysis of clinical features, retinal imaging with fluorescein angiography, genetic and phenotypic analysis of relatives, and functional analysis of genetic variants may be helpful for better understanding of genetics in ROP as well as FEVR.

IGF-1

Insulin-like growth factor 1 (IGF-1), a growth hormone promoting somatic growth and maturation, has also been proposed as a contributing factor to ROP progression.[63] IGF-1-

deficient mice showed a decrease in vascular development[61] and lower birth weight[64] than those of controls. In human babies, low IGF-1 levels were also associated with low birth weight,[65] and persistent low serum IGF-1 levels were associated with severity of ROP.[63,66] Based on these findings, IGF-1 replacement therapy has recently been investigated.[67] A phase 2 trial of administering a complex of recombinant human IGF-1 and IGFBP-3 to prevent ROP was undertaken, but the study did not meet its primary endpoint of reducing severity of ROP.[68]

Investigations of specific polymorphisms of *IGF-1* gene have been unsuccessful finding a significant association. A study linked a c.3174G>A polymorphism in the IGF-1 receptor gene (*IGF1R*) to low levels of plasma IGF-1.[69] A 2006 study of 392 infants in Hungary was unable to detect a difference in the prevalence of the *IGF1R* c.3174G>A among severe ROP, mild ROP and full-term groups (Table 2).[70] A 2007 study in an American population was also unable to find a link between advanced ROP and *IGF1R* c.3174G>A polymorphism (Table 2).[71]

eNOS

Endothelial nitric oxide synthase (eNOS) is one of the constitutive enzymes that synthesize NO, which is known to play a regulatory role in retinal and choroidal blood flow.[72,73] In an eNOS-deficient mouse OIR model, neovascularization and vaso-obliteration were both reduced.[74] Moreover, eNOS gene polymorphisms have shown reduced NO levels.[75] Thus, the association between ROP and eNOS gene (*NOS3*) polymorphisms have been investigated. A literature search showed that 3 SNPs (rs2070744, rs1799983 and rs61722009) and one variable number tandem repeat (VNTR), 27-bp VNTR in intron 4, had been observed in ROP patients (Table 2). Although some studies reported positive associations between rs2070744, rs1799983, or the 27-bp VNTR and ROP, others found contradictory results (Table 2).

Inflammatory Mediators

Growing evidence suggests that perinatal inflammation and infection may increase the risk for ROP by direct proangiogenic effects and/or modifying known risk factors.[76] Studies have reported higher plasma levels of inflammatory cytokines including IL-6, Il-8, and TNF[77] and higher vitreous levels of inflammatory cytokines including IL-6, IL-7, IL-10, IL-15, etc. in eyes with advanced ROP.[78]

Dammann et al investigated 4 SNPs of inflammation-associated genes (IL1B, TNF, IL10, TLR4) in preterm patients, but none showed significant association, although there were trends towards higher stage of ROP with the presence of *TNF* and *IL1B* SNPs (Table 2).[76] *TNF* −308G>A polymorphism also showed no significant associations with ROP (Table 2).

A recent study has also shown an angiogenic role for mast cells and associated factors including mast cell tryptase and monocyte chemotactic protein-1, making them a potential target for ROP research.[79]

Brain-derived neurotrophic factor

Brain-derived neurotrophic factor (BDNF), a neuronal trophic factor in brain and retina, may promote survival of several types of retinal neurons.[80–83] Although the exact role of BDNF in retinal angiogenesis is unknown, reduced BDNF levels have been demonstrated in patients with severe ROP, suggesting a possible role of BDNF in development of severe ROP.[84–86] In an animal model study, the retinal level of BDNF was lower in the OIR mouse model compared to that in normal controls.[86]

In a large-scale candidate gene study, which analyzed 1614 Tag SNPs of the 145 candidate genes in 817 infants in the discovery cohort and 543 in the US replication cohort, it was found that two SNPs (rs7934165 and rs2049046) in the intronic region of *BDNF* were associated with severe ROP. Although these results were not independently confirmed in the replication cohort, the association with rs7934165 did increase in significance with severe ROP in their meta-analysis of the combined data. Interestingly, reduced serum BDNF in the severe ROP group was also found in the same discovery cohort.[87] Further studies on the functional effects of intronic variants of *BDNF* and replication studies in different populations are warranted.

Renin-Angiotensin System

The Renin Angiotensin system (RAS) has been linked to retinal vascular development and pathological angiogenesis. Blockade of RAS with inhibitors of angiotensin-converting enzyme (ACE) and angiotensin receptor blockers ameliorated OIR, suggesting that inhibiting RAS may be beneficial in ROP.[88] A SNP study of *ACE* gene showed association with DR.[87]

However, results from genetic studies on RAS component genes in ROP are inconclusive (Table 2). A study in Italy showed no associations between ROP and SNPs of ACE gene (*ACE*), angiotensinogen gene (*AGT*) and angiotensinogen type 1 receptor gene (*AGTR1*). In a study of 181 premature Kuwaiti infants on 287-bp insertion(I)/deletion(D) in intron 16, the frequency of II genotype was higher in ROP patients compared to normal controls, but the frequency of DD genotype was higher in advanced ROP patients compared to regressed ROP.[90] A candidate gene study of 228 infants with ROP and 102 controls found a SNP in the AGTR1 gene to be associated with ROP, though this association was not significant after Bonferroni correction.[91]

Angiopoietins

Angiopoietin(Ang)-1 and -2 are growth factors that are essential for retinal vascular development. Ang-1 binds tyrosine kinase receptor Tie2 and promotes vascular maturation and stabilization.[92] In an OIR model, intravitreal Ang-1 promoted normal vascular regeneration while inhibiting pathological angiogenesis and vascular leakage.[93] In contrast, Ang-2, a competitive antagonist of Ang-1/Tie-2, promotes neovascularization in animal models.[94,95] Vitreous levels of Ang-1 and Ang-2 in eyes of stage 4 ROP were higher than those of control eyes.[96] However, in two studies of Ang-2 gene promoter polymorphism (*ANGPT2* −35G>C), no association was found with ROP (Table 2).

Erythropoietin

Erythropoietin (EPO), a hormone known to stimulate red blood cell formation in bone marrow, and EPO receptors are expressed in retina, and their expression is regulated by oxygen status.[97,98] Mouse models of ROP have shown that vascular stability is affected by EPO levels, with exogenous restoration of EPO leading to a reduction in blood vessel dropout during the first phase of ROP.[96] Conversely, elevated levels of EPO during the second stage of ROP exacerbated vasoproliferation, and the vitreous level of EPO is elevated in eyes with stage 4 ROP. Increased erythropoietin receptor signaling has also been shown to influence severe OIR models of disease through VEGFR2-mediated angiogenesis, making it an important target for clinical research in human patients.[99,100] While a variant of EPO was investigated in a candidate-gene study by Mohamed et. al., significance for this variant was not reported in the study results.[91]

Hypoxia inducible factor

HIF-1 plays a central role in oxygen homeostasis.[101] According to the oxygen environment, HIF-1 regulates transcription of genes such as VEGF, VEGFR1, PDGF, SDF-1 and Ang2, which have been suggested to play important roles in retinal angiogenesis.[94] In a study of Hif1α knockout mice in an OIR model of disease, disruption of HIF-1 was shown to lead to decreased VEGF abundance, indicating a possible role in neovascularization.[102] Additionally, organ system pharmacology studies in mouse models have indicated that stabilization of HIF-1 may be important for protection against oxygen toxicity in premature infants.[103]

Likewise, homologous recombination models in mice studying HIF-1a-like factor (HLG) and HIF2α found decreasing expression of these genes led to decreased EPO expression and resistance to hyperoxia treatments meant to induce ROP.[104] HIF1α was also shown to upregulate annexin A2 expression in OIR mice during hypoxia, supporting a role in OIR models.[105]

HIF2α's closest human analogue, known as Endothelial PAS Domain Protein 1 (EPAS1), serves as the main regulator of EPO induction and has also been shown to have a connection to ROP.[106] A candidate gene study of 153 genes in 347 infants under 32 weeks gestational age found an association between EPAS1 with development of severe ROP.[91]

Heme oxygenase-1

Heme oxygenase-1 plays important roles in inflammatory responses, oxidative stress, iron-metabolism, and vascular physiology. However, in a candidate gene study, rs3074372 in *HMOX1* showed no significant association with ROP (Table 2).

Other candidate factors—In addition to the above described factors and pathways, a number of other potential targets and mechanisms have been identified that lack genetic studies in patients with ROP. The 'a' disintegrin and metalloproteinase (ADAM) family of proteases are involved in the degradation of extracellular matrix components as well as interactions mediated by integrin.[107] Several subtypes of ADAM family are implicated in the pathogenesis of ROP. ADAM17 knockout mice showed less neovascularization in OIR

models without affecting normal vascular development.[108] Moreover, ADAM 8, 9, and 10 was found to play a role in development of plus disease in OIR mouse models. Adam8−/− and Adam9−/− mice and mice lacking ADAM10 in endothelial cells showed less severe tortuosity and dilation mimicking less plus disease in ROP.[109] Further evaluations in humans including genetic analysis are warranted.

In conjunction with ADAM17, studies have also considered the family of tissue inhibitor of metalloproteinases (TIMP) family of proteins. The TIMP-3 protein specifically is a known physiological ADAM17 inhibitor.[110] Mouse model investigations into the application of this protein as a potential treatment showed that TIMP-3 application was linked to decreased neovascular tuft formation.[109]

In addition to these studies, large candidate gene studies of ROP have been successful identifying targets with undiscovered connections to ROP. The previously mentioned study by Mohamed et al. implicated genes with function in embryonic development (*IHH*), transcription (*TBX5*), and protein localization (*GP1BA*, *CETP*) (Table 2).[91] The same study also found an association between ROP and complement factor H (*CFH*), known to be associated with development of AMD.[38]

DISCUSSION

Summary of previous studies

Most genetic studies in ROP have used the candidate gene approach and focused on genes related to angiogenesis, inflammation, and retinal (neuro)development. Among them, *VEGFA* polymorphisms and FEVR-related genes have been most extensively studied in different populations. However, no *VEGFA* polymorphisms have been proven to be associated with ROP, because most positive studies have not been replicated in other populations (Table 1). Variants of Wnt pathway genes, which are known to cause FEVR or Norrie disease, have been also found in ROP patients, suggesting possible associations of these variants in at least a small proportion of severe ROP patients (Table 2). However, these results also have limitations in that we may not confidently distinguish between premature infants with severe ROP and FEVR-related genetic variants and prematurely-born infants with FEVR, as Hartnett et al. pointed out.[27] In addition the polygenic nature of many diseases makes identification of causative variants difficult in small sample sizes focused on a small number of variants.[111] Recently, results of a large-scale candidate gene study using Tag SNPs of the 145 candidate genes in a multiracial cohort were reported.[87] Although no SNPs were significantly associated with the presence versus absence of ROP in this study, one SNP of *BDNF* gene was significantly associated with severe ROP in their meta-analysis combining the discovery and replication cohorts, which warrants further genetic and biological studies.

Limitations of previous studies

It is difficult to draw meaningful conclusions from most of the candidate gene studies reviewed here due to the following limitations: (1) the sample sizes of most individual studies were small; (2) no replication study has been performed for many variants; (3) there

are conflicting results among studies of the same variants; (4) most studies were conducted using only one or a few clinical sites; (5) ocular phenotype was not standardized; (6) confounding variables were not reported or standardized; (7) meta-analysis is not possible for most variants due to different study protocols between studies; (8) there are variabilities in neonatal care such as oxygen treatment protocol[9], incidence of (severe) ROP, and diagnosis and management of ROP between physicians, study hospitals, study countries and study periods.[8,112–115] Differences in neonatal care may affect survival rate, systemic morbidities of prematurity, incidence of ROP and severity of ROP, making it difficult to find exact roles of genetic variants. Moreover, there are unexplained differences in outcome of premature birth such as mortality. Also, differences in diagnosis and management of ROP may cause bias in phenotypic categorization of subjects, which is a huge problem in genetic studies. It should be noted that genetic risk factors for stage 1-3 ROP and stage 4 or 5 ROP could be different, as different biochemical processes may be involved and management protocols and treatment outcomes of study centers are also important factors for stage 4 or 5 ROP.

Most importantly, candidate gene studies have inherent limitations of not being able to find novel genetic factors. Other approaches to detect novel variants or genes associated with ROP are necessary.

Future Directions of studying ROP genetics

It is very challenging to study the genetics of multifactorial diseases such as ROP. To overcome the current limitations mentioned above and to study the contribution of genetics efficiently, it is necessary to improve the methodology for studying the genetics of ROP. It is essential that investigators leverage new methods that interrogate genetic factors agnostically and at high sample sizes, in order to maximize study power and facilitate simultaneous investigation of many, rather than single, genetic elements. Genome-wide Association Studies (GWAS) test for association across hundreds of thousands of SNPs simultaneously using array-based technology. GWAS can be helpful to find genes or pathways associated with ROP. In other ophthalmological diseases such as AMD[38,116–118], DR[119,120], glaucoma[121–123] and myopia[124–126], GWAS has been successful in finding susceptibility loci. However, a large-scale GWAS has not been conducted in ROP. Massively parallel sequencing, also called next-generation sequencing (NGS), enables sequencing of specific regions, whole exome, or whole genome in a short period of time at high depth and affordable cost. Whole exome sequencing or targeted exome sequencing can be helpful for finding novel variants with possible functional consequences. Exome genotyping arrays may also provide a method of interrogating for SNPs involved in ROP.

In addition to these genetic evaluations, integration of sequence data with data regarding post-transcriptional and post-translational modification, including transcriptomics, metabolomics, and proteomics, will be important to identify biomarkers that may be useful for early detection, diagnosis, and prediction of treatment response. Studies of epigenetics in DR have also shown promise, with epigenetic changes associated with processes of microvasculature complications[127], mitochondrial dysfunction[128], microRNA expression[129],

and capillary cell apoptosis.[130,131] These findings suggest that interrogation of epigenetic factors may be an important method of discovering new treatments in ROP.

Second, large-scale multi-center collaboration of the type offered by consortium studies can help provide structure to such studies. Consortium approaches facilitate recruitment of larger cohorts and make available more sophisticated computational approaches allowing investigators to control for more complicated confounding effects. Previous large international consortium attempts at examining the role of genetics in multifactorial disease have met with success[38,121,132–134], and two consortium studies investigating the genetic causes of ROP are currently ongoing at centers in North America.[135,136]

Third, standardization of ocular phenotypes and confounding factors is crucial. For this, ocular and systemic factors should be acquired systematically, and known risk factors including GA and BW should be assessed in a standardized fashion and strictly controlled for. Additionally, the importance of environmental effects should be noted, as differences between study populations and sites has the ability to have a profound effect on phenotype. Heterogeneity of study subjects in race, ethnicity, and physical covariates, as well as differences between treatment sites and attending clinicians can affect study outcomes. This is especially important to distinguish genetic variants associated with ROP from those associated with prematurity itself. Also, objective phenotyping such as image-based diagnosis should be considered. Compared to clinical ophthalmoscopic diagnosis, consensus image-based diagnosis may enable reduction of intra- and inter-grader discrepancy in ROP diagnosis.

It is also important to note that additional basic research studies using representative animal models such as mouse or rat OIR models are required to test hypotheses. While animal models face many limitations including differences in biology, most notably their use of full-term rather than premature animals, these models' ability to control for phenotypic, environmental, and genetic stratification factors distinguishes them as a valuable method of testing hypotheses and adding insight to human observational studies.

Expected benefits of genetic studies of ROP

Finding genetic variants affecting ROP will be useful in at least three ways. First, genetic risk factors may be incorporated into risk modelling to predict development and progression of ROP. A refined risk analysis system with clinical and genetic risk factors may help clinicians to identify both high- and low-risk patients. Second, identifying specific genes or biological pathways that contribute the pathogenesis of ROP may be helpful for development of new therapeutics. In AMD, genetic studies have revealed the importance of complement pathway in the pathogenesis of AMD, which has led to development of new investigational agents under clinical trials such as lampalizumab, an inhibitor of complement factor D. Third, studying ROP genetics can also contribute to the understanding of pathophysiologies of other ocular vascular diseases such as AMD or DR and other angiogenesis-related diseases like cancer.[15] Fourth, a better understanding of the genetics of retinopathy of prematurity may lead to better understanding of the pathophysiologic mechanisms of common neonatal diseases of prematurity such as chronic lung disease.

Conclusion

Evidence suggests a genetic contribution to ROP, including epidemiologic studies, twin studies and risk analysis studies. To date, a number of candidate gene studies have been performed. However, it is still unclear which genes or variants are significantly and strongly associated with development and progression of ROP. Large-scale studies using NGS and GWAS with standardized phenotyping have potential to expand understanding of genetic contributions and pathophysiology of ROP.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

Supported by grants R01EY19474, P30EY10572, and T32EY023211 from the National Eye Institute (Bethesda, MD); by the National Center for Advancing Translational Sciences; by CTSI grant UL1TR001881; by the National Institute of Diabetes and Digestive and Kidney Disease Diabetes Research Center (DRC) grant DK063491; by grant SCH-1622679 from the National Science Foundation (Arlington, VA); and by unrestricted departmental funding from Research to Prevent Blindness (New York, NY).

ABBREVIATIONS

ROP	Retinopathy of Prematurity
AP-ROP	aggressive posterior retinopathy of prematurity
BW	birth weight
GA	gestational age
CRYO-ROP	cryotherapy for ROP
OIR	oxygen induced retinopathy
DR	diabetic retinopathy
AMD	age-related macular degeneration
FEVR	familial exudative vitreoretinopathy
UTR	untranslated region

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Table 1

Studies Investigating the Association Between VEGFA Genes and Retinopathy of Prematurity (ROP)

Polymorphism	Study country	Subjects	Results	Reference
rs2010963 (−634G>C, +405G>C)	United Kingdom	91 treatment-requiring ROP (BW 779g [440–1185g], GA 25 wk [23–32 wk]), 97 non-treatment-requiring preterm infants (BW 920 g [448–2302g], GA 26 wk ± 2.9 wk)	Higher frequency G allele among infants with threshold ROP	41
	Hungary	115 treatment-requiring ROP (BW 1160g ± 270g, GA 28.5 wk ± 2.0 wk), 86 mild or no ROP (BW 1200g ±270g, GA 29.2 wk ± 2.9 wk)	Higher frequency C allele among treated infants	137
	Turkey	42 treatment-requiring ROP (BW 1097.5g ± 264.3g, GA 28.2 wk ± 2.4 wk), 50 regressed ROP (BW 1253.0g ± 212.2g, GA 29.7 wk ± 2.0 wk), 31 no ROP (BW 1345.6g ± 225.9g)	No significant association	138
	United States	61 stage 4/5 ROP (BW 882g [600–1300g], GA 26 wk [23–30 wk]), 61 normal controls (BW 2430–3960g, GA 34–40 wk)	No significant association	139
	Japan	127 ROP (944g [3778–2168g], GA 27 wk [22–33 wk]), 77 no ROP (BW 1596g [692–2400g], GA 32 wk [22–33 wk])	No significant association	140
	Egypt	62 ROP (BW 1400g [1000–2110g], GA 32 wk [28–34 wk]), 40 no ROP (BW 1640g [1009–2800g], GA 33 wk [29–35 wk])	High frequency of G allele in ROP	42
	Poland	60 treatment-requiring ROP (BW 900g ± 225g, GA 26.7 wk ± 2.3 wk), 20 regressed ROP (BW 1029g ±231g, GA 27.5 wk ± 1.6 wk), 101 no ROP (BW 1153g ±225g, GA 29.2 wk ± 2.05 wk)	No significant association	141
	Iran	15 treatment-requiring ROP (BW 879g ± 81g, GA 27 wk ± 13 wk), 30 regressed ROP (BW 884g ± 63g, GA 27 wk ± 12 wk), 66 no ROP (BW 980g ± 81 g, GA 27 wk ± 10 wk)	No significant association	142
rs1547651	Caucasian	43 ROP, 299 no ROP (all subjects GA ≤ 28 weeks)	No significant association	43
rs3025039 (+936C>T)	Caucasian	43 ROP, 299 no ROP (all subjects GA ≤ 28 weeks)	No significant association	43
	Iran	15 treatment-requiring ROP (BW 879g ± 81g, GA 27 wk ± 13 wk), 30 regressed ROP (BW 884g ± 63g, GA 27 wk ± 12 wk), 66 no ROP (BW 980g ± 81 g, GA 27 wk ± 10 wk)	No significant association	142
	United States	33 stage 4/5 ROP, 49 normal controls	No significant association	143
	Egypt	62 ROP (BW 1400g [1000–2110g], GA 32 wk [28–34 wk]), 40 no ROP (BW 1640g [1009–2800g], GA 33 wk [29–35 wk])	No significant association	42
rs833058	Italy	43 ROP, 299 no ROP (all subjects GA ≤ 28 weeks)	No significant association	43
rs833061 (−460C>T)	Italy	43 ROP, 299 no ROP (all subjects GA ≤ 28 weeks)	No significant association	43
	Hungary	115 treatment-requiring ROP (BW 1160g ± 270g, GA 28.5 wk ± 2.0 wk), 86 mild or no ROP (BW 1200g ± 270g, GA 29.2 wk ± 2.9 wk)	High frequency of 460TT/405CC haplotype in treatment-requiring ROP	137
	Turkey	42 treatment-requiring ROP (BW 1097.5g ± 270g, GA 28.2 wk ± 2.4 wk), 50 regressed ROP (BW 1253.0g ± 212.2g, GA 29.7 wk ± 2.0 wk), 31 no ROP (BW 1345.6g ± 225.9g)	No significant association	138

Polymorphism	Study country	Subjects	Results	Reference
	United States	61 stage 4/5 ROP (BW 882g [600–1300g], GA 26 wk [23–30 wk]), 61 normal controls (BW 2430–3960g, GA 34–40 wk)	No significant association	139
+13553C>T	Japanese	127 ROP (BW 944g [378–2168g], GA 27 wk [22–33 wk]), 77 no ROP (BW 1596g [692–2400g], GA 32 wk [22–33 wk])	A significant association between the TT genotype and non-severe ROP for gestational age	140
+702C>T	United States	33 stage 4/5 ROP, 49 normal controls	No significant association	143
+1612G>A	United States	33 stage 4/5 ROP, 49 normal controls	No significant association	143
−2578C>A	United States	ROP (BW 2430–3960g, GA 34–40 wk), no ROP (BW 600–1300g, GA 23–30 wk) (number of patients not reported)	No significant association	144
	Hungary	90 treatment-requiring ROP (BW 1160g ± 300g, GA 28.5 wk ± 2.4 wk), 110 mild (stage 1 or 2) or no ROP (BW 1200g ± 280g, GA 28.5 wk ± 2.4 wk)	No significant association	145

Table lists investigated polymorphism and presence of statistical significance. Where noted in the original study, information is provided in parentheses regarding the birth weight (BW) and gestational age (GA) of patients. Brackets denote range of patient values and ± denotes one standard deviation of range of each variable.

Table 2

Summary of candidate gene studies of retinopathy of prematurity other than *VEGFA*

Gene	Variant	Study country	Subjects	Results	Reference
ACE	rs1799752	Italy	299 ROP, 43 no ROP	No significant association	43
	rs4291				
	287-bp insertion in intron 16	Kuwait	74 ROP (53 regressed, 21 stage 4/5 ROP), 107 no ROP	The incidence of the II genotype was higher in ROP cases, while the incidence of the DD genotype was significantly higher in advanced stage ROP cases compared to spontaneously regressing ROP cases. (I, insertion, D, deletion)	90
AGT	rs699	Italy	43 ROP, 299 no ROP	No significant association	43
AGTR1	rs5186	Italy	43 ROP, 299 no ROP	No significant association	43
	rs427832	United States	102 ROP, 228 no ROP	Significant association with ROP at $p < 0.01$ level of significance	91
ANGPT2	−35G>C	United States	Not specified	No significant association	144
		Hungary	90 treatment-requiring ROP, 110 no or mild (stage 1 or 2) ROP	No significant association	145
BDNF	rs7934165 rs2049046	United States	126 treatment-requiring ROP, 467 stage 1/2 ROP	Two intronic SNPs found to be associated with difference between mild and threshold ROP	146
	rs7934165	United States	140 treatment-requiring ROP, 1257 no or mild (stage 1 or 2) ROP	Meta-analysis of two studies provided evidence of association of variant with severe ROP	146
CETP	rs289747	United States	102 ROP, 228 no ROP	Significant association with ROP at $p < 0.01$ level	91
CFH	rs52985	United States	102 ROP, 228 no ROP	Increased protection against ROP as number of T alleles increased ($p = 0.01$)	91
	rs800292	United States	102 ROP, 228 no ROP	Increased protection against ROP as number of T alleles increased ($p = 0.01$)	91
EPAS1	rs1867785	United States	102 ROP, 228 no ROP	Significantly higher incidence of A allele in ROP	91
GP1BA	rs2243093	United States	102 ROP, 228 no ROP	Significant association with ROP at $p < 0.01$ level	91
LRP5	rs143924910 (c.3656G>A), c.4148A>C, rs141407040 (c.4619C>T)	Japan	53 advanced ROP	Direct sequencing of coding regions of LRP5 revealed 3 nonsynonymous DNA variants in 3 patients.	56
	3-bp insertion in exon 1	Japan	17 advanced ROP, 51 no ROP	Single patient with advanced ROP shown to have 3 bp insertion in exon 1 CTG repeat area not observed in 28 unaffected patients.	147

Gene	Variant	Study country	Subjects	Results	Reference
NOS3	rs2070744 (−786T>C)	Italy	43 ROP, 299 no ROP	No significant association	43
		Hungary	105 treatment-requiring ROP, 127 stage 1 or 2 ROP	No significant association	148
		United States	15 ROP, 131 no ROP	significantly higher frequency of C allele in ROP	149
		United States	19 stage 4/5 ROP, 34 normal	significantly higher frequency of C allele in ROP	150
	rs1799983 (894G>T)	United States	14 stage 4/5 ROP, 32 normal	No significant association	150
		United States	15 ROP, 131 no ROP	significantly higher frequency of T allele in ROP	149
		Italy	43 ROP, 299 no ROP	No significant association	43
	27-bp VNTR in intron 4 (b/a)	United States	15 stage 4/5, 32 normal controls	No significant association	150
		Hungary	105 treatment-requiring ROP, 127 stage 1 or 2 ROP	The aa genotype presented an independent risk factor for ROP requiring treatment.	148
	rs61722009	Italy	43 ROP, 299 no ROP	No significant association	43
FLT1	c.+6724(TG) 13–23 dinucleotide repeat	Japan	127 ROP, 77 no ROP	No significant association	140
FZD4	c.97 C>T; c.502 C>T (double missense mutation)	United States	93 ROP, 98 normal controls	Seven of 93 (7.5%) patients with ROP showed c.97 C>T; c.502 C>T double missense mutation.	57
	rs80358282 (c.205C>T), rs184709254 (c.380G>A), c.631T>C	Japan	53 advanced ROP	Direct sequencing of coding regions of *FZD4* revealed 3 nonsynonymous DNA variants in 4 patients.	56
	c.766A>G	Unspecified	10 sporadic FEVR cases 20 advanced ROP cases	PCR amplification of a large DNA fragment revealed one severe ROP case with c.766A>G. Significance not investigated.	54
	c.1109C>G, c.609G>T	Canada	71 severe ROP, 33 mild or no ROP	Direct sequencing of coding regions of *FZD4* revealed 2 nonsynonymous DNA variants in 2 patients.	55
HMOX1	rs3074372	Italy	43 ROP, 299 no ROP	No significant association	43
IGF1R	c.3174G>A	United States	52 stage 4/5 ROP, 33 normal controls	No significant association	71
		Hungary	108 treatment-requiring ROP, 120 stage 1 or 2 ROP, 164 normal controls	No significant association	70
IHH	rs3099	United States	102 ROP, 228 no ROP	Significant association with ROP at $p < 0.01$ level	91
IL10	−1082G>A	Germany	31 stage 1 or 2 ROP, 13 stage 3 ROP, 29 no ROP	No significant association	76
IL1B	+3953C>T	Germany	31 stage 1 or 2 ROP, 13 stage 3 ROP, 29 no ROP	No significant association	76

Ophthalmol Retina. Author manuscript; available in PMC 2019 September 01.

Gene	Variant	Study country	Subjects	Results	Reference
KDR	32G>A	Turkey	42 treatment-requiring ROP, 50 regressed ROP, 31 normal controls	No significant association	138
	g.+4422(AC)11–14 dinucleotide repeat	Japan	127 ROP, 77 no ROP	No significant association	140
NDP	Sequencing of all 3 exons and UTRs	United States	54 severe ROP, 36 mild or no ROP, 22 normal controls, 31 normal parents	Six of 54 (11 %) infants with severe ROP had polymorphisms in the NDP.	151
	Direct sequencing of coding regions and noncoding exon 1	Japan	53 advanced ROP	No meaningful sequence changes	56
	237A>G	Japan	17 advanced ROP, 51 no ROP	Single patient with AP-ROP found to have heterozygous substitution not observed in 51 unaffected cases	147
	14 bp deletion in exon 1	Australia	31 ROP (Stage 2 or greater), 90 no ROP	Two twins with stage 3 regressed ROP and one unrelated patient with regressed stage 2 ROP displayed 14 bp deletion in CT repeat reagion. Also observed in a control patient. No statistical analysis.	152
	5 bp deletion in exon 1 26C>G 71 bp deletion in exon 1	UK	31 ROP stage 3 or more, 16 regressed ROP, 2 no ROP	One patient had 5 bp deletion and C>G transersion at +26, one patient had 71 bp deletion in same exon 1 region. No statistical analysis.	61
	12 bp insertion in exon 1 14 bp deletion in exon 1	Japan	100 advanced ROP (stage 4/5), 6 regressed stage 3 ROP, 130 no ROP	Two advanced ROP patients found to have disruptions in exon 1 of ND gene. No statistical analysis.	59
	597C>A 110C>G	Kuwait	95 ROP, 115 no ROP	Significant association was found between ROP and 597C>A polymorphism. No significance found between 110C>G polymorphism and ROP.	60
	121C>T R121W L108P	United States	16 ROP, 50 normal controls	One patient with a heterozygous base substitution, one pair of twins with novel R121W mutation, and one pair of twins with L108P missense mutation observed. No statistical analysis.	58
TBX5	rs1895602	United States	102 ROP, 228 no ROP	Significant association with ROP at $p < 0.01$ level	91
TGFB1	−509C>T	United Kingdom	91 treatment-requiring ROP, 97 stage 1/2 or no ROP	No significant association	41
TLR4	rs4986790 (c.896A>G)	Germany	31 stage 1 or 2 ROP, 13 stage 3 ROP, 29 no ROP	No significant association	76
TNF	−308G>A	United Kingdom	91 treatment-requiring ROP and 97 stage 1/2 or no ROP	No significant association	41
		Germany	31 stage 1 or 2 ROP, 13 stage 3 ROP, 29 no ROP	No significant association	76
TSPAN12	Direct sequencing of coding regions of *TSPAN12*	Japan	53 advanced ROP	No meaningful sequence changes	56