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Identification of genetic factors underlying severe retinopathy of prematurity in preterm infants

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Objective: Retinopathy of prematurity (ROP) is a pathological condition characterized by abnormal proliferation of retinal vessels and it represents the primary cause of visual impairment in preterm infants. There is increasing backing for the involvement of genetic factors in the onset of ROP.

Methods: A prospective cohort study assessed the allele frequency and genotype distribution of gene polymorphisms in angiogenesis, inflammation and oxygen-sensing pathways in preterm infants with severe ROP. The role of genetic polymorphism in ROP development was investigated using next-generation sequencing (NGS) combined with candidate genes and data mining methods.

Results: A total of 47 confirmed severe ROP cases and gestational age, birthweight and days of oxygen therapy plus 35 similar control infants were enrolled in this study. In the initial hypothesis-generating survey, we selected a p value of 0.01 to minimize false positives while retaining true positives. Using this criterion, we identified 19 single-nucleotide polymorphisms across 11 genes that were associated with the occurrence of ROP (*ZNF717*, *IHH*, *SEC22B*, *IGSF3*, *HYDIN)*, *GGT1*, *FRG1*, *CDC27*, *LRRC37A3*, *CTAGE4* and *ADAMTS7*; all $p<0.001$). Compared with the control group, 62 single-nucleotide polymorphisms in 19 candidate genes (*VEGF*, *EPO, EPAS-1*, *HIF1A*, *RUNX1*, *ESR1*, *CFH*, *PDGFB*, *JAK*, *STAT*, *IGF-1*, *IGFBP2*, *GPX4*, *TLR4*, *ROS1*, *CYP*, *TP53BP1*, *NOS3*, *TNF*) representing angiogenic, inflammation, oxygen-sensing pathways and proliferative retinopathic diseases were found to be associated with the development of severe ROP (all $p<0.01$).

Conclusions: Using NGS gene analysis suggests that genetic risk factors may play an important role in susceptibility to the development of ROP.

Retinopathy of prematurity (ROP) is a disease that is associated with abnormal angiogenesis of the retina, characterized by pathological retinal neovascularization leading to visual impairment [1]. The underlying mechanism involves excessive pathological retina neovascularization, triggered by retina hypoxia as a result of vascular regression due to hyperoxia in ROP [2]. Consistent therapeutic targeting of angiogenesis by inhibition of hypoxia-induced vascular endothelial growth factor (VEGF) reduces complications such as intraocular bleeding, retinal detachment and macular edema [3]. Retinal angiogenesis typically occurs in the fetal environment and is characterized by relatively low oxygen tension. However, after birth premature infants are exposed to higher oxygen levels, which is essential for their survival [4]. In developed countries, survival of infants at the extremes of birthweight and gestational age present new problems associated with inadequate postnatal growth and conditions that increase the generation of reactive oxygen and nitrogen species in premature infants who have reduced oxidative reserve [5].

There is also evidence that suggests a strong heritable component to ROP or that genetic variants may predispose premature infants of certain risk strata to ROP [6]. Studies have reported that ROP occurs in white individuals more than in African Americans or Asians [7,8]. A twin study investigating the prevalence of ROP in monozygotic and dizygotic twins revealed that, after controlling for environmental factors, there was a significant genetic contribution to the development of ROP [9]. Studies that assessed the association of genetic variants with any level of ROP severity reported variants in the WNT signaling pathway [10], which is important in development, in EPAS1 [4] or VEGF [11], which are regulated by hypoxia and involved in angiogenesis, and in superoxide dismutase [12,13], which transcribes an antioxidant enzyme.

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On the basis of these considerations, hypothesis-generating (i.e., putting forward possible explanations based on observations, existing knowledge and so on) and hypothesis-testing (i.e, verifying whether the hypothesis is reasonable by means of experiments and data collection) approaches were used to examine the genetic polymorphisms that may be associated with the development of severe ROP. Haplotype reconstruction analysis is mainly based on linkage disequilibrium, family genetic information and population genetics theory. To evaluate this hypothesis, we aimed to identify specific genetic polymorphisms in candidate genes related to angiogenesis, inflammation and oxygen-sensing pathways, as well as those previously associated with other forms of proliferative retinopathy. These polymorphisms were specifically selected for the analysis of severe ROP in preterm infants in this study.

METHODS

Study design: Following approval from a local medical ethics committee, we conducted a prospective cohort study to evaluate the allele frequency and genotype distribution of gene polymorphisms associated with angiogenesis, inflammation and oxygen-sensing pathways in a preterm population. Using haplotype reconstruction analysis, we assessed whether the combination of selected polymorphisms was linked to the occurrence of ROP.

Patient population: We studied preterm infants born at less than 32 weeks of gestational age who were continuously hospitalized at the Children's Hospital Affiliated with Zhengzhou University from September 2017 to January 2021. Infants with major congenital malformations, inherited metabolic disorders or any other congenital syndromes were excluded from the study. DNA analysis was performed on dry blood spots collected 48 h after birth for use in local screening programs. The Life Science Ethics Committee of the Children's Hospital affiliated to Zhengzhou University approved this study and signed consent forms were obtained from all participating families.

All enrolled infants were screened for ROP by an ophthalmologist according to the ROP screening schedule [14], and a case with severe ROP was confirmed by an ophthalmologist using The International Classification of Retinopathy of Prematurity revisited, 2005 (Figure 1) [15]. Control infants were defined as a preterm infant without any stage of ROP. Controls were matched with ROP cases for similar gestational age, birthweight and days of oxygen therapy. Severe ROP, meeting the criteria for treatment of ROP, was defined as prethreshold disease type 1, threshold disease or aggressive posterior ROP [16,17]. Type 1 ROP was any-stage ROP with "plus" disease in zone I, or stage 3 ROP without "plus" disease in zone I, or stage 2 or 3 ROP with "plus" disease in zone II. Threshold disease ROP was defined as five contiguous or eight cumulative clock hours of stage 3 ROP in zone 1, or stage 3 ROP with "plus" disease in zone 2 [15,18]. Aggressive posterior ROP is characterized as a rapidly progressing and uncommon severe form of ROP. If left untreated, ROP usually progresses to stage 5 [15].

Sequencing and variant annotation using next-generation sequencing: Exome sequencing was performed by the Institute of Pediatrics of Children's Hospital, affiliated to Zhengzhou University, Zhengzhou, China. Sequencing and genotyping services were conducted at Henan Provincial Key Laboratory of Children's Genetic Metabolic Diseases, Zhengzhou, China. Peripheral blood samples were collected from each individual and DNA was extracted using the QIAamp DNA Blood Mini Kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA fragments were enriched for clinical exome sequencing, which used the Agilent (Santa Clara, CA) ClearSeq Inherited Disease panel kit (including 2,742 genes) or trio-WES using the Agilent SureSelect XT Human All Exon V5 kit. Quality was filtered according to GATK 3.0-recommended filters; variants with "QUAL ≤50.0, ABHet >0.75, HRun >4.0, QD <5.0, SB ≥0.10, number of ALT ≥1, MQ <40.0 or FS >60.0" were removed. QUAL represents the Phred-scaled probability that supports the existence of ALT, ABHet represents the allele balance measure for heterozygosity, HRun represents the largest contiguous homopolymer run, QD represents coverage-normalized quality score, SB is strand bias, MQ is RMS mapping quality and FS represents Fisher's strand bias. We used ANNOVAR (2015 Mar22 version) to annotate all identified variants and functionally classified the exonic variants into categories including synonymous, nonsynonymous, stop gain/loss, splice site gain/loss, and so on [19].

The harmful effects of the variants observed among these candidate genes were predicted using the software SIFT [20], PolyPhen-2 [21], Mutation Taster [22], REVEL [23], GERP [24], MaxEntScan [25], SpliceAI [26] and AlphaMissense [27]. Only variants with a very low frequency (MAF <0.05) in both the overall and East Asian populations (1KGP [28] and gnomad-v2.1.1 [29]) and that were absent from infants without ROP in our in-house database were further considered to be pathogenic [30].

Gene analysis: In the initial phase of this study, we employed hypothesis-generation (data mining) methods to investigate potential genetic risk factors for severe ROP. Using a subset of samples from infants born at less than 32 weeks' gestation, we analyzed the genotypic data for 388 single-nucleotide polymorphisms (SNPs) across 145 genes to identify risk factors

associated with ROP. In the subsequent phase, the selection of candidate genes was informed by a thorough review of the current literature and considerations of biologic plausibility. Specifically, candidate genes involved in angiogenesis, inflammation and oxygen-sensing pathways, as well as those previously linked to other forms of proliferative retinopathy, were chosen for analysis in the context of ROP in pre-term infants. Following the identification of relevant genes, SNPs within these genes were selected for analysis based on haplotype blocks, ensuring comprehensive coverage of the genes with a minimal number of SNPs. Currently, a total of 59 SNPs across 17 genes have been identified for genotyping.

Statistical analysis: Descriptive methods were employed to summarize the genetic outcomes and demographic data of the enrolled infants. The Chi-square test was used for categorical variables, while the Student *t* test was applied for continuous variables. The crude relative risk was estimated along with 95% confidence intervals. A p value of less than 0.05 was considered statistically significant. Statistical analysis and data management were conducted using SPSS version 21.0 (SPSS Inc., Chicago, IL).

RESULTS

Demographic characteristics: A total of 81 patients were diagnosed with severe ROP, among whom 34 patients declined to participate because of congenital anomalies; informed consent was not obtained. Thus, 47 patients with severe ROP were enrolled in this study, as well as 35 infants with similar gestational ages, birthweights and days of oxygen therapy without ROP as the control group. Most of the maternal characteristics and infant clinical characteristics were similar between the two groups, although some infant characteristics varied (Table 1).

Initially, a data mining investigation was conducted using genetic information related to preterm births from prior studies. We analyzed 388 SNPs across 145 genes to assess their association with ROP. As indicated in Table 2, 11 SNPs exhibited p values less than 0.01; formally, a p value of less than 0.0001 necessitates the application of stringent statistical significance criteria. A Bonferroni correction was thus applied for the nearly 500 SNPs evaluated. Currently, there is an ongoing debate regarding the applicability of this conservative correction in the context of genetic data mining research [31]. A critical question arises as to whether these studies test a single hypothesis multiple times or whether they assess multiple hypotheses independently. Regardless, the overarching aim of any statistical correction in genetic studies examining multiple loci is to minimize false positives while ensuring that true positives are not overlooked.

We selected a p value of 0.01 to minimize false positives without losing true positives in this initial hypothesis-generating investigation. Using these criteria, 11 genes were found to be associated with the development of ROP: zinc finger protein 717 (*ZNF717*), Indian hedgehog (*IHH*), vesicle trafficking protein (*SEC22B*), immunoglobulin superfamily member 3 (*IGSF3*), hydrocephalus-inducing protein (*HYDIN*), gamma-glutamyltransferase 1 (*GGT1*), facioscapulohumeral muscular dystrophy region gene 1 (*FRG1*), cell division cycle 27 (*CDC27*), leucine rich repeat containing 37 member A3 (*LRRC37A3*), CTAGE family member 4 (*CTAGE4*) and ADAM metallopeptidase with thrombospondin type 1 motif 7 (*ADAMTS7*; Table 2).

A true candidate gene approach was next used by using family-based and non–family-based statistical models to study the genetic risk factors for ROP. Compared with the control group, we found 19 candidate genes associated with

Table 1. Demographic data of enrolled infants.

Characteristics	Non-ROP n=35	Severe ROP n=47	P value
Males n (%)	18(51)	27(57)	0.657
Mean gestational age (weeks)	29.8±1.7	29.1±1.8	0.078
Mean birthweight (g)	1294.3±313.4	1286.8±309.2	0.914
Antenatal steroids, n (%)	1(3)	4(8)	0.387
Hypertension or pre-eclampsia, n (%)	4(11)	7(15)	0.751
Clinical chorioamnionitis, n (%)	2(6)	5(11)	0.693
Caesarean section, n (%)	17(49)	26(55)	0.656
Mean Apgar Scores at Five minutes	7.8±1.6	7.7±1.4	0.764
Days on oxygen therapy (mean)	32.7±5.5	34.2±9.5	0.406
Sepsis, n (%)	7(20)	19(40)	0.058
Bronchopulmonary dysplasia, n (%)	5(14)	12(26)	0.276

Table 2. Signicant data mining genes and genetic mutational analysis.

Gene	Exon	Location in the gene	cDNA change	SNP ID number	CytoBand	Highest-MAF	P value	Previously reported	Prediction (SIFT/ polyphen2/Mutaton Taster)
ZNF717	**Exon5**	**chr3–75787486**	**c.1288G>A**	**rs77649281**	**3p12.3**	**0.015**	**<0.001**	**/**	**T/B/N**
	Exon5	chr3–75786252	c.2522C>A	rs140641854	3p12.3	0.003	<0.001	/	D/P/D
IHH	UTR3	chr2–219919754	c.*175C>G	rs3099	2q35	0.421	<0.001	Yes	–/–/-
SEC22B	intron	chr1–145112288 145,112,288	N-N-G-GCA	-	1q21.1	0.000	<0.001	/	–/–/-
IGSF3	Exon3	chr1–117156585	c.634C>T	rs139013364	1p13.1	0.001	<0.001	/	–/–/D
	Exon3	chr1–117156459	c.760G>A	rs61786651	1p13.1	0.000	<0.001	/	D/P/D
HYDIN	Exon15	chr16–71101200	c.2068A>G	rs10744982	16q22.2	0.002	<0.001	/	T/D/D
	Exon22	chr16–71054178	c.3229A>G	rs6416709	16q22.2	0.004	<0.001	/	T/B/N
	Exon40	chr16–70989335	c.6259C>T	rs1774541	16q22.2	0.004	<0.001	/	D/P/D
	Exon44	chr16–70972620	c.6892C>G	rs1774360	16q22.2	0.000	<0.001	/	D/P/D
GGT1	Exon14	chr22–25024072	c.1361G>T	rs199681469	22q11.23	0.000	<0.001	/	D/B/D
	Exon12	chr22–25023441	c.1063C>T	rs200419006	22q11.23	0.001	<0.001	/	D/P/D
FRG1	Exon7	chr4–190881969	c.604G>A	rs6846627	4q35.2	0.000	<0.001	/	T/B/D
CDC27	Exon12	chr17–45214699	c.1441A>G	rs62075623	17q21.32	0.000	<0.001	/	D/P/D
LRRC37A3	UTR5	chr17–62893457	c.-82A>T	rs11650755	17q24.1	0.376	<0.001	/	–/–/-
CTAGE4	exon1	chr7–143882864	c.2268T>C	rs200691220	7q35	0.048	<0.001	/	–/–/-
	exon1	chr7–143882889	c.2293C>T	rs201820489	7q35	0.043	<0.001	/	T/B/N
	exon1	chr7–143882922	c.2326A>G	rs200196623	7q35	0.085	<0.001	/	T/B/N
ADAMTS7	Exon19	chr15–79058307	c.3946T>A	rs200436984	15q25.1	0.025	<0.001	/	T/B/N

SIFT-D Deleterious, SIFT-T Tolerated, polyphen2-B Benign, polyphen2-D Probably damaging, polyphen2-P Possibly damaging, MutationTaster-D Disease_causing: probably deleterious, MutationTaster-N Polymorphism: probably harmless,

the development of severe ROP (p<0.01): *VEGF*, erythropoietin (*EPO*), endothelial PAD domain protein 1 (*EPAS-1*), hypoxia-inducible factor 1 alpha (*HIF1A*), RUNX family transcription factor 1 (*RUNX1*), estrogen receptor 1 (*ESR1*), complement factor H (*CFH*), platelet-derived growth factor subunit B (*PDGFB*), Janus kinase (*JAK*), signal transducer and activator of transcription (*STAT*), insulin-like growth factor 1 (*IGF-1*), insulin like growth factor binding protein 2 (*IGFBP2*), glutathione peroxidase 4 (*GPX4*), Toll-like receptor 4 (*TLR4*), receptor tyrosine kinase (*ROS1*), cytochrome P450 (*CYP*), tumor suppressor p53-binding protein 1 (*TP53BP1*), nitric oxide synthase 3 (*NOS3*) and tumor necrosis factor (*TNF*). The 19 candidate genes were chosen and are shown in Table 3 representing angiogenic, inflammation, oxygen-sensing pathways and proliferative retinopathic diseases

In addition to the initial hypothesis-generating investigation of 11 genes and the candidate gene approach involving 19 genes, consistent with previous reports, we identified genes associated with angiogenic, inflammatory, antioxidant and proliferative processes in retinopathic disorders. The genes implicated in previous ROP studies are listed in Table 4.

DISCUSSION

ROP is a complex disease where evidence suggests it is influenced by both genetic and environmental factors. This prospective cohort study was designed to assess genetic polymorphisms in candidate genes and evaluate genes that were previously genotyped and associated with preterm birth or growth delay. Low birthweight and growth retardation are well-established predictors of worsening ROP [32]. Using this approach, 11 genes (*ZNF717*, *IHH*, *SEC22B*, *IGSF3*, *HYDIN*, *GGT1*, *FRG1*, *CDC27*, *LRRC37A3*, *CTAGE4* and *ADAMTS7*) were found to have an association (p<0.01) with the development of ROP. Using the candidate gene approach, *VEGF*, *EPO*, *EPAS-1*, *HIF1A*, *RUNX1*, *ESR1*, *CFH*, *PDGFB*, *JAK*, *STAT*, *IGF-1*, *IGFBP2*, *GPX4*, *TLR4*, *ROS1*, *CYP*, *TP53BP1*, *NOS3* and *TNF* were found to be positively associated with the development of ROP compared with control group infants. These genes represented angiogenic, inflammation, oxygen-sensing pathways and proliferative retinopathic diseases.

Previous studies found a positive association between small-for-gestational-age status or restricted growth and severe ROP [32,33]. One report suggested that recessive mutations in *ZNF717*, *SEC22B*, *LRRC37A3* and *CTAGE4* were are associated with restricted growth [34]. *ZNF717* may be involved in transcriptional regulation, and *SEC22B* is an endoplasmic reticulum-Golgi intermediate compartment-resident

Table 3. Candidate genes chosen for analysis.

Gene	Gene product	No.SNPs
VEGF	Vascular endothelial growth factor	3
EPO	Erythropoietin	2
EPAS-1	Endothelial PAD domain protein 1	3
HIF1A	Hypoxia inducible factor 1 alpha	2
RUNX1	RUNX family transcription factor 1	3
ESR1	estrogen receptor1	2
CFH	Complement factor H	1
PDGFB	platelet derived growth factor subunit B	8
JAK	Janus kinase	3
STAT	signal transducer and activator of transcription	3
IGF-1	insulin-like growth factor 1	12
IGFBP2	insulin like growth factor binding protein 2	2
GPX4	glutathione peroxidase 4	1
TLR4	toll-like receptor 4	1
ROS1	receptor tyrosine kinase	1
CYP	Cytochrome P450	8
TP53BP1	tumor suppressor p53-binding protein 1	2
NOS3	nitric oxide synthase 3	1
TNF	tumor necrosis factor	4

Table 4. The genes implicated in previous ROP studies.

Gene	Gene product	Gene category	Reference (PMID)
SerpinF1	Pigment epithelium derived factor	angiogenic	18787502
ALDH2	Aldehyde dehydrogenase	angiogenic	18787502
IL1A	Interleukin 1A	inflammation	18787502
ILIB	Interleukin 1B	inflammation	18787502
TRAF2	TNF-receptor-associated factor 2	inflammation	18787502
NGF	Nerve growth factor	angiogenic	30680928
COX2	cyclooxygenase 2	inflammation	12601017
SOD	superoxide dismutase	antioxidant	22574884
BDNF	brain-derivated neurotrophic factor	angiogenic	34346275
NDP	Norrie disease protein	proliferative retinopathic diseases	32884843
FZD4	frizzled-4	proliferative retinopathic diseases	32884843
WIF1	Wnt inhibitor factor 1	proliferative retinopathic diseases	35080047
ATG5	autophagy-related protein 5	angiogenic	31070476
FOXC2	forkhead box protein C2	inflammation	29380370

soluble NSF attachment protein receptor (SNARE) that controls phagosome maturation and function in macrophages and dendritic cells. The secretory pathway controls the release of cytokines and may also impact the secretion of nitric oxide, which is synthesized by Golgi-active inducible nitric oxide synthase [35] and achieves synthesis of nitric oxide, particularly in endothelial cells known to have an important role in angiogenesis, vasculogenesis and ROP [36].

Hedgehog proteins, including Indian hedgehog (IHH) and Sonic hedgehog (SHH), play critical roles in the development of various tissues during embryogenesis, such as the lungs, intestines, limbs and bones [37]. Previous research has also highlighted the involvement of Hedgehog signaling in angiogenesis [38]. In a separate study examining the role of IHH in yolk sac vascular development in mice, it was found that IHH knockout mice were able to initiate angiogenesis; however, they exhibited incomplete vascular remodeling when compared with wild-type or heterozygous mice [39]. *HYDIN* is involved in endothelial cell development and *GGT1* may play a potential pathophysiological role in vascular disease [40–42].

FRG1 was found to be mutated in ROP-group infants, which is crucial for angiogenesis in different diseases [43–45]. A previous study also reported that *CDC27* is a downstream molecule of the transforming growth factor-beta (TGF-β)/Smads pathway, and phosphorylation of *CDC27* participates in TGF-β-induced anaphase-promoting complex (APC)/cyclosome (C) activation, leading to the occurrence of corresponding inhibitory gene transcription and cancer suppression [46]. TGF-β, as an important effector molecule involved in maintaining immunity and tolerance, has been confirmed to play a crucial role in the occurrence and development of proliferative vitreoretinopathy [47] or proliferative diabetic retinopathy [48]. One study demonstrated that the ADAMTS7 Serine214-to-Proline substitution arising from a CAD-associated variant affected angiogenesis [49], which plays a pathophysiological role in ROP and is characterized by pathological retinal neovascularization [50].

For the candidate gene representing angiogenic, inflammation, oxygen-sensing pathways and proliferative retinopathic diseases. A comprehensive analysis of its expression patterns, regulatory mechanisms, and potential therapeutic implications is urgently needed. EPAS1 encodes components of the basic helix–loop–helix/PAS domain transcription factor family and is predominantly expressed in vascular endothelial cells [51]. Similar to hypoxia-inducing factor 1-α (HIF-1α), EPAS1 remains stable under hypoxic conditions, forms heterodimers with aromatic nuclear receptor transporters and activates VEGF promoters. In a recent study investigating ROP development in newborn mice using a hyperoxic/normoxic model, HIF-2α (the murine equivalent of EPAS1) knockout mice exhibited no signs of retinal neovascularization compared with control mice [52]. Furthermore, the expression of EPO mRNA was significantly decreased in the knockout mice relative to wild-type mice [52].

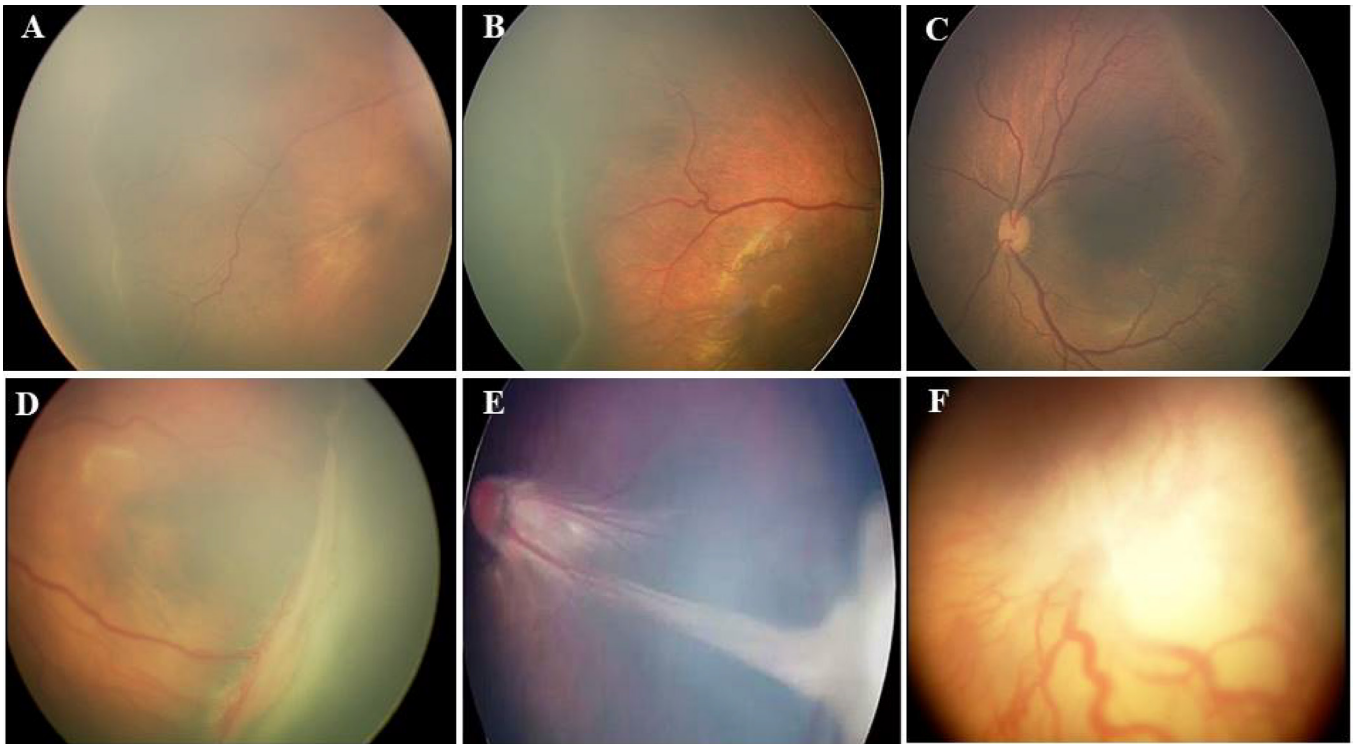

Figure 1. Stages of retinopathy of prematurity. A. Stage 1 of retinopathy of prematurity (ROP); B. Stage 2 of ROP; C. Stage 3 of ROP; D. Stage 4A of ROP; E. Stage 4B of ROP; F. Stage 5 of ROP.

RUNX1, also known as RUNt-associated transcription factor 1, serves as a mediator of abnormal retinal angiogenesis and is linked to the progression of ROP. This association suggests that the downregulation of RUNX1, in conjunction with anti-VEGF therapies, may play a crucial role in the future management of pathologic ocular angiogenesis [53]. Previous studies have shown that ESR1 is involved in the process of proliferative diabetic retinopathy [54,55], which has molecular mechanisms related to vascular dysfunction, similar to ROP. CFH serves as an inhibitor of the complement pathway and binds to the complement component C3b, thereby preventing the production of C3 invertase and inhibiting progression of the cascade. Two SNPs in CFH have been identified as being associated with the development of ROP and are included within these haplotypes [4]. Pericyte–endothelial interactions are indispensable for the integrity and functionality of retinal neurovascular units, including vascular cells, retinal neurons and glial cells. The essential autocrine and paracrine signaling pathways, such as VEGF, PDGFB, Notch, angiopoietin, Norrin and TGF-β, have been well characterized in the regulation of pericyte–endothelial interactions in neovessel formation processes (vasculogenesis and angiogenesis) during embryonic development [56].

IGFBP2 production is promoted by HMGA2 and contributes to vascular development and sprouting angiogenesis [57]. GPX4 and reactive oxygen species (ROS) deficiency elevate the risk of ferroptosis in retinal pigment epithelium induced by ROS. ROS contributes to retinal pigment epithelium cell death through iron-mediated apoptosis and inhibits glutathione peroxidase 4, thereby enhancing the pathogenicity of LAMP2-knockdown cells [58]. Furthermore, TLR4 plays a significant pathophysiological role in oxygen-induced retinopathy. A recent study demonstrated that intravitreal injection of the TLR4 antagonist TAK-242 reduced the area of nonperfusion, inhibited abnormal angiogenesis and increased retinal vascular density in mice with oxygen-induced retinopathy [59]. CYPs are cytochrome P450 epoxygenases involved in the process of retinal angiogenesis that reduce VEGF production and VEGF-induced angiogenic responses in endothelial cells, which may provide a rational therapy against retinal neovascularization by inhibiting CYP [60,61]. TP53BP1, TNF, JAK and STAT pay an important role in inflammation, which increases the risk of the development of ROP [62]. TP53BP1 deficiency promotes pathological neovascularization in proliferative retinopathy [63] and TNF-α acts as a predictor of central nervous system damage and retinopathy in preterm infants [64].

In summary, through next-generation sequencing gene analysis and data mining of previous genotyping data, we identified 19 SNPs in 11 genes associated with the occurrence of ROP, as well as 62 SNPs in 19 candidate genes related to angiogenesis, inflammation, oxygen-sensing pathways and the promotion of retinopathy. Replication of these SNPs in larger, independent populations is necessary to confirm the association between these common genetic variants and the development of ROP. Ultimately, the identification of allelic associations may inform modifications to environmental factors contributing to ROP, such as the timing and volume of oxygen supplementation.

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