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Association of -634 C/G Polymorphism at Vascular Endothelial Growth Factor Gene with Risk Retinopathy of Prematurity

Mohammadamin Ghadyani[1], Mahmood Noorishadkam[2], Seyed Hamed Hosseini-Jangjou[3*], Reza Bahrami[4], Elahe Akbarian[5], Maryam Saeida-Ardekani[2], Hossein Neamatzadeh[2,6], Zahra Zare[2]

[1] Department of Advanced Medical Sciences and Technologies, Islamic Azad University, Science and Research Branch, Tehran, Iran
[2] Mother and Newborn Health Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[3] Department of Pediatrics, Iranshahr University of Medical Sciences, Iranshahr, Iran
[4] Neonatal Research Center, Shiraz University of Medical Sciences, Shiraz, Iran
[5] Children Growth Disorder Research Center, Shahid Sadoughi University of Medical Sciences, Yazd, Iran
[6] Department of Medical Genetics, Shahid Sadoughi University of Medical Sciences, Yazd, Iran

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Corresponding author:
Seyed Hamed Hosseini-Jangjou

Email:
shhosseinij@gmail.com

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ABSTRACT

Background: Retinopathy of prematurity (ROP) is a major cause of blindness in newborn infants worldwide. It is well known that neovascularization of the retina is prominent in the proliferative stages of ROP. It is suggested that vascular endothelial growth factor (VEGF) may play a role in the development of ROP. The aim of this study was to evaluate the association of the VEGF -634C/G polymorphism at VEGF with risk of ROP.

Methods: In the study 54 neonates diagnosed with ROP and 55 healthy neonates served as controls. The VEGF -634 C/G polymorphism was genotyped by restriction fragment length polymorphism (PCR-RFLP) technique.

Results: The CC, CG, and GG genotypes of VEGF -634C/G polymorphism were found in 33.3%, 38.9%, and 27.8% of neonates with ROP, respectively. In controls, CC, CG, and GG genotypes were seen in 43.6%, 45.4%, and 10.9%, respectively. Frequency of mutant allele (C) was 52.8% in neonates with ROP and 66.4% in healthy neonates. There was a significant difference in the distribution of VEGF -634C/G polymorphisms between cases and controls. Moreover, there was a significant association between VEGF -634C/G polymorphisms and ROP risk (OR = 3.141, 95% CI 1.115-8.851, P = 0.030).

Conclusion: This study results revealed that VEGF -634C/G polymorphism might serve as a risk factor for development of ROP. Thus, clinicians should be aware of the ROP risk in infant with the VEGF -634C/G polymorphism and ROP risk in infants. However, large sample size and well-designed studies are necessary to validate our findings.

Introduction

Retinopathy of prematurity (ROP), also known as retrolental fibroplasia (RLF) and Terry syndrome, was first reported in 1942 by Terry.[1,2] ROP is an important cause of preventable blindness in children worldwide.[3,4] It is a sight-threatening vasoproliferative disorder of the retina primarily affecting severely premature infants.[5,6] ROP is a multifactorial disease which several risk factor such as neonatal immaturity hyperoxia, low nutrient supply, intrauterine fetal demise of one twin, and apnea have been described in development of this disease.[7,8] It is estimated that ROP causes about 10% of childhood blindness worldwide and 6-18% of childhood blindness in developed countries.[9] Early screening and treatment of ROP prevent blindness and associated with child better overall growth.[1-3,10]

The etiology of ROP is multifactorial which genetic and environmental factors.[11-13] Several epidemiological studies have showed that genetic variants in specific candidate genes, such as vascular endothelial growth factor (VEGF) have a relationship with development and severity of ROP.[14] It is showed that increased VEGF in avascular retina stimulates pathological retinal neovascularization, resulting in pathology of ROP.[15] Thus, VEGF could be used as a potential therapeutic target for prevention regression of severe ROP.[16,17]

VEGF is a multifunctional cytokine produced by cells that stimulates the formation of blood vessel on the endothelium. Human VEGFA (OMIM: 192240) was mapped on chromosome 6p31.3, consists of 8 exons and 7 introns.[1,18] VEGF belongs to a supergene family group of dimeric glycoproteins characterized by 8 conserved cysteines and functions as a homodimer structure, which includes the placental growth factor (PlGF), VEGF-A to F.[19,20] To date, several variants have identified in the untranslated regions (3′-and 5′-UTRs) and coding region of VEGF gene which modulate VEGF protein expression.[18] To date, several studies were evaluated the association between ROP risk and VEGF -634 C/G polymorphism. However, their results were inconclusive and inconsistent. Here, we performed a study to examine the association of VEGF -634 C/G polymorphism with risk of ROP.

Materials and Methods

Study Population: A total of 54 infants with ROP as case and 55 healthy infants as control groups admitted to the neonatal intensive care unit (NICU) from April 2017 to May 2018 were recruited. All infants were examined by the ophthalmologist and neonatologist between 1 and 2 weeks intervals from the 4th postnatal week onwards. Maximum ROP stage was evaluated and therapy was decided after consultation with a neonatal ophthalmologists. All procedures used in this study are consistence with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki.

Genotyping: Genomic DNA was isolated from whole blood samples as the manufacturer's protocol using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). Then, we evaluated the extracted DNA concentration and purity by the NanoDrop ND1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA). We followed the PCR-restriction fragment length polymorphism (PCR-RFLP) technique for genotyping VEGF -634 C/G polymorphism as previously described.[21] The DNA obtained from the PCR was cut with restriction enzymes.

Statistical Analysis: All statistical analyses were performed by the Statistical Package for Social Science (SPSS) version 20 (IBM Corp., Armonk, N.Y., USA). The association of VEGF -634 C/G polymorphism with ROP and healthy neonates were tested using Chi-square or Fisher Exact test. Odds ratio (ORs) and their corresponding 95% confidence

intervals were calculated for estimating ROP risk corresponding to genotypes VEGF -634 C/G polymorphism. The Court software was applied to assess Hardy-Weinberg equilibrium of VEGF -634 C/G polymorphism. *P*-value of < 0.05 was considered as statistically significant.

Results

The main characteristics of the neonates with ROP and healthy neonates (control group) are presented in Table 1. The study included 54 neonates with ROP (gestational age: 27 ± 4.3 weeks) and 55 healthy neonates (gestational age: 29.51 ± 4.23 weeks). The genotype frequencies of the VEGF -634 C/G polymorphism was presented in Table 2. The genotype frequencies of VEGF -634 C/G polymorphism in control group was in accordance to Hardy-Weinberg equilibrium (P = 0.336). Moreover, minor allele frequency (MAF) in the control group was 33.6%.

As shown in Table 2, the VEGF CC, CG, and GG genotypes were found in 33.3%, 38.9%, and 27.8% of neonates with ROP, respectively. In controls, CC, CG, and GG genotypes were seen in 43.6%, 45.4%, and 10.9%, respectively. Frequency of mutant allele (C) was 52.8% in neonates with ROP and 66.4% in healthy neonates. There was a significant difference in the distribution of VEGF -634C/G polymorphism mutant allele between cases and controls (P = 0.040). Moreover, there was a significant association between VEGF -634C/G polymorphisms and ROP risk (OR = 3.141, 95% CI 1.115-8.851, P = 0.030).

Table 1. Demographic characteristics of ROP cases and controls

Variables	ROP (n = 106)	Control (n = 55)	*P*-value
Gender			
Males	25 (46.3)	26 (47.3)	0.918
Females	29 (53.7)	29 (52.7)	
Gestational Age (week)			
≤ 28	23 (42.5)	25 (45.4)	0.763
> 28	31 (57.4)	30 (54.6)	
Birth weight (gram)			
≤ 1500	24 (44.4)	27 (49.1)	0.481
> 1500	30 (55.6)	28 (50.9)	

Discussion

To date, several studies evaluated the association of *VEGF* -460T/C, -634G/C, +405G/C and +936C/T polymorphisms with ROP risk in different populations.[22] Moreover, studies showed that VEGF serum concentration on the 20th day of life appears to be a promising approach to screening infants at high risk of the development of advanced ROP.[23] However, previously data described that the association of the VEGF polymorphisms and the risk of ROP is not considerable.[23]

In this study we evaluated the VEGF -634C/G polymorphism at VEGF with risk of ROP. Our results revealed that VEGF -634C/G polymorphism is associated with risk of ROP.

Ali et al., in a case-control study evaluated the association of VEGF 634C/G and VEGF 936C/T polymorphisms in 102 preterm infants with Egyptian neonates with ROP. The results support the hypothesis that the carrier state of VEGF 634 C/G polymorphism has an impact on the risk of ROP in infants.[21]

Table 2. Distribution of VEGF -634C/G polymorphisms in ROP cases and controls

Polymorphism	ROP (n = 54)	Control (n = 55)	Odds Ratio		P
			OR	90% CI	
Genotypes					
CC	18 (33.3)	24 (43.6)	Ref.		
CG	21 (38.9)	25 (45.5)	0.764	0.356-1.637	0.488
GG	15 (27.8)	6 (10.9)	3.141	1.115-8.851	0.030
Alleles					
C	57 (52.8)	73 (66.4)	Ref.		
G	51 (47.2)	37 (33.6)	1.765	1.022-3.050	0.042

OR: Odds Ratio; CI: Confidence Interval.

Moreover, Cooke et al., showed that the G allele was associated with an increased risk of ROP.[13] Kusuda et al., in a prospective study from a tertiary center that enrolled ROP developed in 127 infants with ROP and 77 healthy Japanese infants for evaluation VEGF (g.-634 G > C, g.+13553C > T) and VEGF-receptor (KDR g.+4422 A/C, Flt-1 c.+6724 C/T) gene polymorphisms. Their results showed that the frequency of polymorphisms did not differ between ROP and non-ROP patients. Moreover, they suggested that a genotype of the VEGF pathway weakly affects the severity of ROP compared with other clinical factors.[24]

Our results are not in agreement with previous meta-analyses. Malik et al., in a meta-analysis of six case-control studies including 355 cases with ROP and 471 healthy controls examined the genetic association of VEGF-634G/C polymorphism with susceptibility to ROP. Their combined ORs described that the VEGF-634G/C polymorphism not significant risk factor for development of ROP.[25] Moreover, Luo et al., in a meta-analysis evaluated the association of four polymorphism at VEGF gene including-460 T/C, +936 C/T, -634 G/C, and -2578 C/A with susceptibility to ROP. Their results showed that only VEGF polymorphism -460 T/C was associated with an increased risk ROP.[26] Shukla et al., also in a meta-analysis showed that the VEGF -460T/C and +936C/T polymorphism were associated with ROP risk.[27] Gohari et al., in a meta-analysis of 27 case-control studies with 5,748 ROP cases and 6,146 controls assessed the association between the VEGF-A polymorphisms and susceptibility of ROP. Their results showed that only VEGF-A -460T/C polymorphism may contribute to the susceptibility to ROP.[1] Shastry et al., also in a meta-analysis revealed that the association of the VEGF gene promoter polymorphism did not associate with risk of advanced ROP.[28] Liu et al., in a meta-analysis reported that risk of advanced ROP is associated with VEGF -460T/C polymorphism, but not -634G/C, -2578C/A and 936C/T.[29]

Conclusion

In summary, this study results revealed that VEGF -634C/G polymorphism might serve as a risk factor for development of ROP. Thus, clinicians should be aware the association of VEGF -634C/G polymorphism with ROP risk in infants. However, large sample size and well-designed studies are necessary to validate our findings.

Conflict of Interests

Authors have no conflict of interests.

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