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Association between *FVL* G1691A, *MTHFR* C677T and A1298C Polymorphisms with Risk for Retinopathy of Prematurity

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ABSTRACT

Background: Retinopathy of prematurity (ROP) is an important cause of preventable blindness in children. The aim of this study was to examine the association of the polymorphisms at Factor V Leiden (*FVL*) and methylene tetrahydrofolate reductase (*MTHFR*) gene with risk of ROP.

Methods: A total of 106 neonates with ROP and 110 healthy neonates were enrolled. The *FVL* G1691A and *MTHFR* C677T and A1298C polymorphisms were genotyped by PCR-RFLP assay.

Results: There was a significant association between *FVL* G1691A polymorphism and an increased risk of ROP. However, the *MTHFR* C677T and A1298C polymorphisms were not associated with risk of ROP.

Conclusion: *FVL* G1691A polymorphism may be risk factor for development of ROP in neonates. However, there was no significant association between *MTHFR* C677T and A1298C polymorphisms and risk of ROP. However, it is critical that larger and well-designed studies in different ethnicities are needed to confirm our conclusions.

Introduction

Abnormal development of retinal blood vessels in premature infants leads to a potentially blinding disease named retinopathy of prematurity (ROP).[1-3] When Terry described ROP first in 1942, it was termed retrolental fibroplasia.[4] Consequently, 6-18% of all childhood blindness is linked to preterm birth in developed countries.[5] Newborn eye screening provides an opportunity to identify retinal damage, prevent blindness, and have effective treatment.[6,7] The degree of the immaturity of the neonates, race, and ethnicity are major risk factors for ROP.[8,9] Moreover, the availability, access to, and quality of neonatal care are the other factors associated with the frequency and risk of ROP.[10,11] Data from 1354 infants screened for ROP described that Asian and Hispanic neonates were more likely to develop ROP compared with White non-Hispanic neonates, while Black non-Hispanic infants were less likely to develop this disease.[12] In the American guideline, screening is suggested for all infants weighing ≤1500 g or those with gestational ages ≤30 weeks, as well as those between 1500 and 2000 g or gestational age of >30 weeks with an unstable clinical course, including those requiring cardiorespiratory support or those who are at high risk for ROP.[13,14] ROP has been extensively studied worldwide because of its enormous impact of lifetime vision impairment or blindness on the quality of affected infants' life.[11,15] It is evident that environmental factors (such as length of stay on a neonatal intensive care unit (NICU), duration of artificial ventilation (AV), administration of postnatal glucocorticoids, duration of oxygen supplementation, and fluctuations in oxygen saturation) contribute to the development of ROP in premature neonates.[16,17]

The exact mechanism of ROP is not fully known, but many interacting factors including genetic susceptibility have been identified.[1,18,19] It is recommended that ROP cases are tested for both inherited and acquired thrombophilia.[20,21] However, the role of thrombophilic risk factors has become increasingly controversial and there is no sufficient data to understand the role of *FVL* and *MTHFR* genes in the development of ROP.[20] Three common genetic variants including the substitution of arginine by glutamine at amino-acid residue 506 in the *FVL* gene, C to T transition at nt. 677 polymorphism and a G to A transition at position 20210 *MTHFR* may be associated with ROP possibly due to increased plasma homocysteine levels. To date, only two studies have evaluated the association between thrombophilia and ROP risk their results are inconclusive and inconsistent.[20,21] Here, we performed a study to examine the association of *FVL* G1691A, *MTHFR* C677T, and A1298C polymorphisms with susceptibility to ROP in Iranian neonates.

Materials and Methods

Study Population: All infants admitted to the neonatal intensive care unit (NICU) from April 2018 to May 2019 for ROP screening were included in the study. A neonatologist and ophthalmologist examine the neonates every 1 to 2 weeks beginning at the 4th postnatal week. The maximum ROP stage was evaluated and therapy began after performing an ophthalmology consultation. The study population included 109 neonates with ROP, with a gestational age of 32 weeks or less at birth and birth weight of ≥ 1500 grams or less. The control group enrolled 110 healthy infants without ROP. All procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Declaration of Helsinki.

DNA Analysis: We collected and extracted whole blood samples according to the manufacturer's protocol using the QIAamp DNA blood mini kit (Qiagen, Hilden, Germany). We also determined DNA concentration and purity using the NanoDrop ND1000 spectrophotometer (Thermo Fisher

Scientific, Waltham, MA, USA). In order to genotyping *FVL* G1691A (rs6025), *MTHFR* C677T (rs1801133), and *MTHFR* A1298C (rs1801131) polymorphisms, we followed the PCR-restriction fragment length polymorphism (PCR-RFLP) technique that has been used previously.[22,23] The DNA obtained from the PCR was cut with restriction enzymes. MnI1 restriction enzyme was used to detect *FVL* rs6025, MspI restriction enzyme for *MTHFR* C677T, and MboII restriction enzyme for *MTHFR* A1298C.

Statistical Analysis: All statistical analysis and procedures were performed using the Statistical Package for Social Science (SPSS) version 21 (IBM Corp., Armonk, NY., USA). We computed Hardy-Weinberg equilibrium using Excel-based software for the polymorphisms of *FVL* G1691A, *MTHFR* C677T, and A1298C. The association of *FVL* and *MTHFR* polymorphisms with ROP and healthy neonates was tested using Chi-square or Fisher Exact test. We calculated odds ratios (ORs) and their associated 95% confidence intervals to estimate ROP risk for genotypes FVL G1691A, *MTHFR* C677T, and A1298C polymorphisms. P-value of < 0.05 was considered statistically significant.

Results

The main characteristics of the neonates with ROP and healthy controls are presented in Table 1. The study included 106 neonates with ROP (gestational age: 28 ± 3.8 weeks) and 110 healthy neonates (gestational age: 30.81 ± 3.12 weeks). The genotype frequencies of the *FVL* G1691A, *MTHFR* C677T, and A1298C polymorphisms are shown in Table 2. The genotype frequencies of *MTHFR* polymorphisms for the control group were in accordance to Hardy-Weinberg equilibrium (P = 0.365; and P = 0.750, respectively), except for *FVL* (P = 0.013).

The *MTHFR* C677T genotypes in neonates with ROP were 42.5% CC, 43.3% CT and 13.2% TT, while in healthy neonates consisted of 39.1% CC, 50.0% CT and 10.9% TT. Frequency of mutant allele (T) was 35.4% in neonates with ROP and 35.9% in healthy neonates.

Table 1. Demographic and Clinical Characteristics of Enrolled Infants

Variables	ROP (n = 106)	Control (n = 110)	P
Gender			
Males	50 (47.2)	63 (57.3)	0.137
Females	56 (52.8)	47 (42.7)	
Gestational Age (week)			
≤ 28	49 (46.2)	51 (46.4)	0.983
> 28	57 (53.7)	59 (53.6)	
Birth weight (gram)			
≤ 1500	51 (48.1)	56 (50.9)	0.681
> 1500	55 (51.9)	54 (49.1)	

There was no significant association between TC (OR = 0.797, 95% CI = 0.466-1.361, P = 0.405) and TT (OR = 1.243, 95% CI = 0.543-2.827, P = 0.604) polymorphisms at C677T polymorphism and an increased risk of ROP (Table 2). Moreover, the *MTHFR* A1298C genotypes in neonates with ROP were 36.8% AA, 49.1% AC and 14.1% CC, while in healthy neonates consisted of 32.2% AA, 46.4% AC and 16.4% CC. Frequency of mutant allele (C) was 38.7% in neonates with ROP and 39.6% in healthy neonates. There were no significant differences in the distribution of AC (OR = 1.114, 95% CI = 0.653-1.901, P = 0.692) and CC (OR = 0.842, 95% CI = 0.400-1.773, P = 0.652) A1298C polymorphisms at *MTHFR* gene and an increased risk of ROP (Table 2).

For *FVL* G1691A polymorphism, GG, GA, and AA genotypes were found in 37.7%, 44.36%, and 17.9% in neonates with ROP, respectively. GG, GA, and AA genotypes were found in 56.4%, 30.9%, and 12.7% of healthy neonates, respectively. In neonates with ROP, mutant allele frequency (A) was 40.1%, compared to 44.6% in healthy neonates. There were significant differences in the distribution of heterozygote genotype (GA) of *FVL* G1691A polymorphism (OR = 1.781, 95% CI = 1.020-3.108, P = 0.042) between neonates with ROP and healthy controls (Table 2).

Table 2. Distribution of *MTHFR* and *FVL* Polymorphisms in ROP Cases and Healthy Neonates

Polymorphism	ROP (n = 106)	Control (n = 110)	Odds Ratio		P
			OR	90% CI	
MTHFR C677T					
Genotypes					
CC	45 (42.5)	43 (39.1)	Ref.		
CT	47 (43.3)	55 (50.0)	0.797	0.466-1.361	0.405
TT	14 (13.2)	12 (10.9)	1.243	0.543-2.827	0.604
Alleles					
C	137 (64.6)	141 (64.1)	Ref.		
T	75 (35.4)	79 (35.9)	0.977	0.659-1.449	0.908
MTHFR A1298C					
Genotypes					
AA	39 (36.8)	41 (32.2)	Ref.		
AC	52 (49.1)	51 (46.4)	1.114	0.653-1.901	0.692
CC	15 (14.1)	18 (16.4)	0.842	0.400-1.773	0.652
Alleles					
A	130 (61.3)	133 (60.4)	Ref.		
C	82 (38.7)	87 (39.6)	0.964	0.665-1.419	0.854
FVL G1691A					
GG	40 (37.7)	62 (56.4)	Ref.		
GA	47 (44.3)	34 (30.9)	1.781	1.020-3.108	0.042
AA	19 (17.9)	14 (12.7)	1.498	0.708-3.167	0.291
Alleles					
G	127 (59.9)	122 (55.4)	Ref.		
A	85 (40.1)	98 (44.6)	0.833	0.569-1.221	0.349

OR: Odds Ratio; CI: Confidence Interval

Discussion

ROP is a multifactorial disease characterized by abnormal retinal vessel growth that can lead to blindness in infants.[24] ROP is a multifactorial and complex disorder with the involvement of genetic and environmental factors. There are well-established observations that support a genetic influence on the development of ROP.[25-27] The incidence and severity of this disease are inversely related to gestational age and birth weight. There is no exact incidence for ROP among Iranian neonates because no large, multicenter prospective studies have been performed. However, in a meta-analysis including 18,000 premature infants, the prevalence of ROP was reported to be 23.5% in Iran.[28,29] For the first time, a study is assessing the association between specific *MTHFR* and *FVL* polymorphisms and ROP among Iranian neonates. *MTHFR* is a vital enzyme involved in folate metabolism which catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methylenetetrahydrofolate, the circulatory form of folate.[30-32] Several epidemiological studies revealed that the *MTHFR* polymorphisms may increase the risk of negative pregnancy outcomes and baby birth weights.[22,33-35] Our results showed that the *MTHFR* C677T and A1298C polymorphisms did not significantly associate with an increased risk of ROP in our population. This study evaluated the alteration in the *MTHFR* gene and its association in predisposition to ROP in the Iranian population. To date, only two studies evaluated the association in Israeli and Turkish populations, and our results are consistent with those studies. In 2003, Kenet et al., evaluated the relationship of genetic prothrombotic markers including *FVL*, *MTHFR*, and FIIG20210A genes and their plasma levels with neonatal complications in preterm infants. They have revealed that preterm infants with thrombophilia are not at increased risk for developing neonatal complications. Moreover, their results did not show a

significant association between *MTHFR* 677 polymorphism and an increased risk of ROP.[20] In 2015, Aydin et al., have performed a study to assess the association of *MTHFR* C677T and A1298C polymorphisms with the development of ROP in a Turkish population. Similarly, they have found that both *MTHFR* C677T and A1298C polymorphisms were not significantly associated with the risk of ROP.[21] However, Tiwari et al., revealed that *MTHFR* C677T polymorphism was an evident genetic risk factor associated with the susceptibility of preterm delivery, negative pregnancy outcome, and low birth weight (LBW) in Northeast Indian population.[33]

There are several shreds of evidence that show acquired and inherited *FVL* mutation have been associated with adverse pregnancy outcomes, such as thrombosis, recurrent pregnancy loss, and placenta-mediated outcomes, such as placental abruption, preeclampsia, gestational age, and preterm birth.[35,36] Our data showed a significant association between heterozygote genotype of *FVL* G1691A polymorphism with risk of ROP. Similarly, Aydin et al., found that the prevalence of *FVL* G1691A polymorphism (16.3%) was higher in neonate ROP than healthy neonates in the Turkish population. Thus, they suggested that the *FVL* G1691A polymorphism was significantly associated with ROP risk in the Turkish population.[21] We found that the frequency of *FVL* mutation was 40.1% in patients with ROP neonates and that the incidence was less than healthy (44.6%). Kenet et al.,[20] showed that 2.4% of ROP neonates had heterozygous *FVL* G1691A polymorphism in an Israeli cohort and Kleinberg et al., reported that 4% of advanced ROP (stage 4 and 5) cases were heterozygous for *FVL* G1691A polymorphism in an Indian cohort. Their results described that *FVL* G1691A polymorphism was not a major risk factor for ROP development.[37] However, Kleinberg et al., found that the *FVL* G1691A polymorphism may be associated with other additive factors as might be expected for a complex genetic trait.[37]

Conclusion

In summary, our results showed that *FVL* G1691A polymorphism may serve as predisposing factors of ROP in our population. However, *MTHFR* C677T and A1298C polymorphisms were not significantly associated with an increased risk of ROP. Due to the limited sample size in this study, it is critical that larger and well-designed studies in different ethnicities are needed to confirm our conclusions.

Conflict of Interests

Authors have no conflict of interests.

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