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Original Article

Correlation of interactions between *NOS3* polymorphisms and oxygen therapy with retinopathy of prematurity susceptibility

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Abstract: Aim: This study was aimed to detect the correlation of nitric oxide synthase 3 (NOS3) gene polymorphisms (T-786C and G894T) and retinopathy of prematurity (ROP) susceptibility. Interaction between NOS3 gene polymorphisms and the duration of oxygen therapy was also explored in ROP babies. Methods: Genotypes of NOS3 gene polymorphisms were genotyped by MassArray method. Hardy-Weinberg equilibrium (HWE) was used to calculate the representativeness of the cases and controls. Crossover analysis was utilized to explore the gene environment interactions. Relative risk of ROP was presented by odds ratios (ORs) with corresponding 95% confidence intervals (95% CIs). Results: Among the subject features, oxygen therapy had obvious difference between case and control groups (P<0.05). There existed significant association between-786C allele and ROP susceptibility (P=0.049, OR=0.669, 95% CI=0.447-0.999). Genotypes of T-786C polymorphism and genotypes and alleles of G894T polymorphism did not related to the susceptibility of ROP. Interactions were existed between NOS3 gene polymorphisms and oxygen therapy duration. When the duration of oxygen therapy was less than 17 days, both -786CC genotype and 894GT genotype were correlated with ROP susceptibility (P=0.020, OR=0.115, 95% CI=0.014-0.960; P=0.011, OR=0.294, 95% CI=0.100-0.784). Conclusion: -786C allele might have a protective effect for ROP. Interactions of -786CC and 894GT genotype with oxygen therapy duration (less than 17 days) were both protection factors of ROP.

Keywords: *NOS3* gene, polymorphisms, oxygen therapy, interaction, ROP

Introduction

Retinopathy of prematurity (ROP), the most common cause of blindness in children in middle-income developing countries [1-3], is a disorder of the retina among prematurely born babies. ROP generally occurs in preterm neonates who born at ≤ 32 week of gestation [4]. It is characterized by: retinal ischemia, new blood vessels form, fibrosis, seriously affecting the development of children's visual acuity and eyeball. ROP is first reported in 1942 [5], recent years along with the development of neonatal intensive care unit (NICU), the survival rate of premature infant is increased. Then the high risk group is extending. Meanwhile the incidence of ROP has an increased trend.

Pathogenesis of ROP is still unclear by now, multiple factors may be involved in the process of it [6-8]. Development of retina blood vessel is beginning in 16th week of gestation and finishing at 4 weeks after born. With small gestational age, the retinal vascular development is not mature, wide non-vascular area will become in premature infants [9]. The difference of partial pressure of oxygen inside and outside of uterus may be lead to the injury of retinal vascular endothelial cells. A high blood oxygen saturation and long time of oxygen therapy might aggravate the damage [10]. Individual susceptibility could lead to the different effect of oxygen therapy. Genetic factor play an important role in the individual susceptibility. Nitric oxide synthase 3 (NOS3), also known as eNOS, is an enzyme in endothelial cell taking a role in angiogenesis. *NOS3* gene is located at chromosome 7q36, and contains 29 exons. Polymorphisms in this gene are related to susceptibility of many diseases [11-14].

Table 1. Features of participants

Features	Case n=118	Control n=134	*P* value
Gender			0.962
Male	62	70	
Female	56	64	
Gestational age (week)			0.704
≤28	53	57	
>28	65	77	
Birth weight (g)			0.681
≤1500	55	59	
>1500	63	75	
Duration of oxygen therapy (day)			0.008
≤17	49	78	
>17	69	56	

In this case-control study, we explored the association of *NOS3* gene polymprphisms (T-786C and G894T) and the susceptibility of ROP. The interaction between the single nucleotide polymorphisms (SNPs) of *NOS3* gene and the duration of oxygen therapy was analyzed, the role of the interaction in ROP was detected too.

Materials and methods

Participant features

This case-control study was approved by institutional review boards of The First Affiliated Hospital of Nanchang University. From January 2011 to January 2015 in The First Affiliated Hospital of Nanchang University, 118 ROP patients were identified during routine ROP examinations among 314 preterm infants (gestational age ≤32 weeks, birth weight ≤2000 g). Preterm infants who had any cardiovascular diseases and eye diseases were excluded, and then 134 preterm infants were recruited as controls. Features of neonates such as gender, gestational age, birth weight, the duration of oxygen therapy were listed in **Table 1**. All participators were unrelated Chinese Han population. Written informed consent was signed by all families.

Genotyping method

Genomic DNA was extracted from 5 ml peripheral blood samples of all participators using the TIANamp Blood DNA Kit (TIANGEN BIOTECH, China). Spectro-Designer software (SEQUENOM, USA) was used to design the polymerase chain reaction (PCR) primers and extension primers. Genotypes of *NOS3* SNPs (T-786C and G894T) were done with MassArray system (SEQUENOM, USA).

Statistic analysis

PASW 18.0 was used to execute the statistic analysis. Hardy-Weinberg equilibrium (HWE) was calculated to inspect the representative of cases and controls. Genotype and allele distribution differences of *NOS3* T-786C and G894T polymorphisms and participant features differences between case and control groups were compared using the Chi-square test. Interactions of *NOS3* polymorphisms and duration of oxygen therapy were assessed by crossover analysis. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were used to present the relative risk of ROP.

Results

Participant features

As shown in **Table 1**, only the duration of oxygen therapy had significant difference between case and control groups ($P<0.05$). Other features, such as gender, gestational age and birth weight had no obvious differences between the two groups ($P>0.05$).

Correlation of NOS3 SNPs (T-786C and G894T) and ROP susceptibility

Genotype distributions of T-786C and G894T polymorphisms were all in accordance with HWE ($P>0.05$), indicating the subjects were had a good representativeness. All of the genotypes of T-786C SNP had no significant association with the susceptibility of ROP (**Table 2**, $P>0.05$). -786C allele was significantly correlated with ROP susceptibility ($P=0.049$, OR=0.669, 95% CI=0.447-0.999). Meanwhile, no obvious association was found between genotypes and alleles of *NOS3* G894T SNP with the susceptibility of ROP (**Table 2**).

Interaction between NOS3 gene polymorphisms (T-786C and G894T) and oxygen therapy duration in ROP

Interaction analysis results were shown in **Tables 3** and **4**. For the interaction of T-786C

Table 2. Genotype and allele distributions of *NOS3* gene polymorphisms (T-786C and G894T) in ROP

SNP	Case n=118 (%)	Control n=134 (%)	χ^2	*P* value	OR (95% CI)
T-786C					
TT	72 (61.02)	68 (50.75)			
CT	39 (33.05)	51 (38.06)	1.438	0.231	0.722 (0.424-1.230)
CC	7 (5.93)	15 (11.19)	2.926	0.087	0.441 (0.169-1.147)
T	183 (77.54)	187 (69.78)	-	-	-
C	53 (22.46)	81 (30.22)	3.878	0.049	0.669 (0.447-0.999)
G894T					
GG	87 (73.73)	89 (66.42)	-	-	-
GT	28 (23.73)	43 (32.09)	2.031	0.154	0.666 (0.380-1.186)
TT	3 (2.54)	2 (1.49)	0.217	0.641	1.534 (0.250-9.409)
G	202 (85.59)	221 (82.46)	-	-	-
T	34 (14.01)	47 (17.54)	0.912	0.340	0.791 (0.489-1.280)

polymorphism and the duration of oxygen therapy, when the duration of oxygen therapy was less than 17 days, the -786CC genotype might relate to the susceptibility of ROP (*P*=0.020, OR=0.115, 95% CI=0.014-0.960). When oxygen therapy duration was more than 17 days, all of the genotypes of *NOS3* T-786C polymorphism had no obvious correlation with ROP susceptibility (*P*>0.05). Interaction was also existed in oxygen therapy duration and *NOS3* G894T polymorphism (**Table 4**). 894GT genotype maybe relate to the susceptibility of ROP when the oxygen therapy duration was less than 17 days (*P*=0.011, OR=0.294, 95% CI= 0.100-0.784).

Discussion

ROP is an eye disease affecting preterm babies who generally receive neonatal care. It is thought to be caused by aberrant growth of retinal blood vessels which may lead to scarring and retinal detachment [15, 16]. ROP may be resolved spontaneously, but it also leads to blindness in serious cases. Although the pathogenesis of ROP is unknown, it is believed that low birth weight, oxygen toxicity and hypoxia could contribute to the development of it [17-20]. However, not all the prematurely born babies undergo the occurrence of ROP. There exists individual difference, and genetic factors might decide the difference. NOS3, as a nitric oxide synthase in endothelial cell, is involved in the regulating of vessels. Previous studies indicated that *NOS3* gene relate to the incidence of retinopathy [21, 22]. T-786C and G894T is the two widely studied SNPs in *NOS3* gene. T-786C polymorphism is in the promoter region of *NOS3* gene, and G894T (Glu298Asp) SNP is a missense mutation which locates in exon 7 of the gene. Therefore we analyzed the association between *NOS3* gene polymorphisms (T-786C and G894T) and the susceptibility of ROP in this study.

Analysis results showed that -786CC and -786CT genotype frequencies were higher in controls than that in cases, but the differences were not significant. -786C allele of *NOS3* gene was significantly high in cases, and might play a protection role for ROP. This result was agreed with previous study which focused on diabetic retinopathy [23], and another study found that -786C allele could increase the ROP risk [22]. However a previous research performed by Rusai et al. indicated that T-786C SNP had no association with ROP [24]. For *NOS3* gene G894T polymorphism, genotypes and alleles of it had no significant association with the susceptibility of ROP, denominating G894T polymorphism was not related to ROP susceptibility. That was different from previous study which indicated 894T allele is a significant risk factor for the occurrence of ROP [22]. However Azmy et al. suggested GG genotype of G894T SNP could increase the risk of diabetic retinopathy [25].

Because features analysis indicated that oxygen therapy duration was associated with the risk of ROP, we carried out the interaction analysis to explore whether the interactions were existed between the duration of oxygen therapy and the two *NOS3* gene polymorphisms. As for T-786C SNP, if not considered the effects of oxygen therapy, -786CC genotype was not related to ROP risk. If take into the interaction, there was a significant change. When oxygen therapy duration was less than 17 days, -786CC genotype was correlated with 0.115 fold decreased risk of ROP. When the duration of oxygen therapy was more than 17 days -786CC genotype has no significant association with the occur-

Table 3. Interaction between *NOS3* gene T-786C polymorphism and oxygen therapy in ROP

Duration of oxygen therapy (day)	T-786C	Case n=118	Control n=134	χ^2	*P* value	OR (95% CI)
≤17	TT	31	32	-	-	-
	CT	17	37	3.776	0.052	0.474 (0.222-1.012)
	CC	1	9	5.388	0.020	0.115 (0.014-0.960)
>17	TT	41	36	0.266	0.634	1.176 (0.604-2.290)
	CT	22	14	1.305	0.253	1.622 (0.076-3.729)
	CC	6	6	0.003	0.960	1.032 (0.300-3.548)

Table 4. Interaction between *NOS3* G894T polymorphism and oxygen therapy in ROP

Duration of oxygen therapy (day)	G894T	Case n=118	Control n=134	χ^2	*P* value	OR (95% CI)
≤17	GG	40	51	-	-	-
	GT	6	26	6.425	0.011	0.294 (0.100-0.784)
	TT	3	1	1.490	0.222	3.825 (0.383-38.179)
>17	GG	47	38	2.260	0.133	1.577 (0.870-2.859)
	GT	22	17	1.697	0.193	1.650 (0.775-3.515)
	TT	0	1	0.778	0.378	1.020 (0.982-1.059)

rence of ROP. Meanwhile 894GT genotype becomes the protective factor for the development of ROP, when the duration of oxygen therapy was less than 17 days. Before interacted with the oxygen therapy 894GT genotype had no obvious association with the risk of ROP. All of the results suggested that there existed interactions between *NOS3* SNPs and the duration of oxygen therapy.

Our study is the first study focused on the interaction between *NOS3* gene polymorphisms and the duration of oxygen therapy in the development of ROP. The interaction could affect the susceptibility of ROP, and alter the action of *NOS3* gene polymorphisms in ROP susceptibility. Under certain conditions, *NOS3* gene T-786C and G894T SNPs might play crucial role in the occurrence of ROP. We suggested that ROP is a complex disease which influenced by gene and environment factors. Although we obtained a meaningful result, the etiology of ROP was still unclear. In order to get a credible evidence to certify the pathogenesis of it, further study with a big sample size and more loci was necessary.

Disclosure of conflict of interest

None.

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