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Endothelial nitric oxide synthase gene polymorphisms in non-arteritic anterior ischemic optic neuropathy

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Abstract *Background:* To examine the association between the polymorphisms of the endothelial nitric oxide synthase (eNOS) gene and the occurrence of non-arteritic anterior ischemic optic neuropathy (NAION). *Methods:* We studied 15 patients with NAION (mean age, 62 years; 60% male). We investigated two polymorphisms of the eNOS gene, Glu298Asp polymorphism of exon 7 and T(–786)C polymorphism of the promoter region. The genotype distribution in NAION was compared with the control (mean age, 63 years; 63% male) distribution. *Results:* There was no significant difference in the genotype distribution of the Glu298Asp polymorphism between the NAION and control groups ($P=1.000$), whereas the genotype dis-

tribution of the T(–786)C polymorphism varied significantly between the patients with NAION and control subjects ($P=0.002$). After adjusting on covariates, individuals with the CC genotype of the T(–786)C polymorphism were more likely to develop NAION compared with those with TT genotype (odds ratio=0.09; 95% CI 0.01–0.86). *Conclusions:* We found an increased prevalence of T(–786)C polymorphism of the eNOS gene in patients with NAION. Our data suggest that the T(–786)C polymorphism of the eNOS gene may be an important risk factor in the development of NAION in Japanese subjects.

Keywords Nitric oxide synthase · Ischemic optic neuropathy · Polymorphism

Introduction

Non-arteritic anterior ischemic optic neuropathy (NAION), caused by vascular insufficiency leading to optic nerve head ischemia, is a disease characterized by acute visual loss [6]. Although the pathogenesis of NAION is poorly understood, Hayreh et al. [5] have postulated that defective vascular autoregulation in the optic nerve head is a factor. Furthermore, Potarazu [13] indicated that endothelial defects in the ocular circulation could impair the efficacy of endothelial-derived relaxation factors in NAION. Thus, impaired autoregulation and vasoconstriction of the nutrient vessels supplying the optic nerve head may be involved in the pathogenesis of NAION.

Endothelium-derived nitric oxide (NO), which is catalyzed by nitric oxide synthase (NOS), plays an important role in the regulation of vascular tone and suppressing platelet aggregation and leukocyte adhesion [3, 9, 12, 14, 15]. Impaired NO levels on vascular system easily lead to vasospasm and vascular thrombosis. Recent studies have shown that the Glu298Asp and T(–786)C polymorphisms of the endothelial nitric oxide synthase (eNOS) gene, which reportedly affects NO levels, are associated with coronary heart disease and myocardial infarction [4, 16, 18, 22]. In particular, T(–786)C polymorphism may be a risk factor for coronary spastic angina in the Japanese population [20].

In the present study, we examined the association between the Glu298Asp and T(–786)C polymorphisms of the

eNOS gene and the occurrence of NAION in a Japanese population. We found an increased prevalence of T(-786)C polymorphism of the eNOS gene in patients with NAION.

Materials and methods

Written informed consent was obtained from all subjects, and the protocol was consistent with the guidelines of the ethics committee at our institution. The study was performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki. Our study included 15 patients with NAION (nine men and six women; mean age, 62 years; age range, 49–84 years). NAION was diagnosed by neuro-ophthalmologists following the criteria of the Ischemic Optic Neuropathy Decompression Trial Research Group [7]. The criteria included 1) acute unilateral painless visual loss, 2) optic disc-related visual field defects, 3) the presence of an afferent papillary defect, 4) optic disc edema, and 5) no other neurological symptoms. The control group consisted of 121 consecutive patients (76 men and 45 women; mean age, 63 years; age range 45–88 years) who were referred to our institution for reasons other than NAION. All of the subjects were Japanese.

Diabetes mellitus was defined as being treated for insulin- or non-insulin-dependent diabetes mellitus. Hypertension was defined to have a history of hypertension treated with antihypertensive drugs. Three patients (Patient 1, 4, and 9) had taken the drugs at bed time. Subjects were classified as having hyperlipidemia when being treated for that condition. Smoking included past and current smokers. Ischemic heart disease included coronary artery disease and myocardial infarction.

Venous blood was collected from all subjects and genomic DNA was isolated from peripheral leukocytes according to a kit protocol (Qiagen, Hilden, Germany). We

examined two polymorphisms of the eNOS gene. Genotypes of the two polymorphisms were determined with a fluorescence- or colorimetry-based allele specific DNA primer assay system as described previously (Toyobo Gene Analysis, Tsuruga, Japan) [21]. The sequences of the primers used in this assay of the Glu298Asp polymorphism were as follows: sense primer, 5'-ACGGTCGCTTC GACGTGCT-3'; antisense primer, 5'-GCACCTCAAGG ACCAGCTC-3'. The sequences of the primers used in this assay of the T(-786)C polymorphism were as follows: sense primer, 5'-ATCAAGCTCTCCCTGGTG-3'; antisense primer, 5'-TCAGCAGAGAGACTAGGGCTGA-3'. Each reaction mixture included 20 ng DNA, 5 pmol of each primer, 0.2 mmol of each deoxynucleotide triphosphate per liter, 3 mmol magnesium chloride per liter, and 1 IU of DNA polymerase buffer. The amplification protocol comprised an initial period of denaturation at 95°C for 5 min, 35–45 cycles of denaturation at 95°C for 30 s, annealing at 55°C for 30 s, extension at 72°C for 30 s, and a final period of extension at 72°C for 2 min.

To confirm the accuracy of genotyping with the use of this method, we subjected DNA samples to PCR, and restriction-fragment-length polymorphism analysis or to direct DNA sequencing of the PCR products. In each instance, the genotype determined by the allele-specific DNA-primer-probe assay system was identical to that determined by the confirmatory methods.

Statistical analysis was performed using a statistical package (STATA 8.0; College Station, Tex., USA). Logistic regression analysis was employed to evaluate the independent effect of eNOS mutations on the occurrence of NAION by adjusting on other covariates such as diabetes mellitus, hypertension, hyperlipidemia and smoking status. The eNOS mutations and other covariates were coded by indication variables. All tests were two-sided. The criterion for statistical significance was $P < 0.05$.

Table 1 Clinical characteristics of patients with non-arteritic anterior ischemic optic neuropathy

Patient No.	Age	Sex	DM	HT	HL	Smoking	IHD	Glu298Asp	T(-786)C
1	64	F	-	+	-	-	-	Glu/Glu	TT
2	77	F	+	+	-	-	-	Glu/Glu	TT
3	67	M	+	-	-	+	-	Glu/Asp	TT
4	54	F	-	+	-	-	-	Glu/Glu	TT
5	67	M	+	-	-	+	-	Glu/Glu	CC
6	56	M	-	-	-	-	+	Glu/Glu	TC
7	71	M	-	-	-	-	-	Glu/Glu	TT
8	84	M	+	-	-	+	-	Glu/Glu	TT
9	56	F	+	+	+	-	-	Glu/Glu	CC
10	53	F	+	-	+	-	-	Glu/Glu	TC
11	67	F	-	-	+	-	-	Glu/Glu	TT
12	67	M	-	-	-	-	+	Glu/Glu	TT
13	53	M	+	+	-	-	-	Glu/Glu	CC
14	51	M	+	-	-	+	-	Glu/Glu	TT
15	49	M	-	+	+	-	-	Glu/Glu	TC

F=female; M=male; +=yes; -=no; DM=Diabetes Mellitus; HT=Hypertension; HL=Hyperlipidemia; IHD=Ischemic Heart Disease

Table 2 Genotype frequencies of eNOS gene polymorphisms in control and NAION

Genotype	Controls no. (%)	NAION no. (%)
Glu298Asp polymorphism		
Glu/Glu	110(91)	14(93)
Glu/Asp	11	1(7)
Asp/Asp	11	1(7)
T(-786)C polymorphism*		
TT	94(78)	9(60)
TC	25(20)	3(20)
CC	2(2)	3(20)

NAION indicates non-arteritic anterior ischemic optic neuropathy subjects

For T(-786)C polymorphism: * $P=0.002$ between control and NAION

Results

Table 1 shows the clinical and demographic characteristics of the 15 patients with NAION. The genotype and allele frequencies of the eNOS Glu298Asp and T(-786)C polymorphisms in the control group and in NAION are shown in Table 2 and Table 3, respectively.

Distribution of Glu298Asp genotypes

In the control group, the frequencies of the G and T alleles were 95% and 5%, and the frequencies of the GG, GT, and TT genotypes were 91%, 9%, and 0%, respectively. In NAION patients, the frequencies of the GG, GT, and TT genotypes were 93%, 7%, and 0%, respectively, while the gene frequencies of G and T alleles were 97% and 3%, respectively. The genotype distribution of the Glu298Asp polymorphism was not significantly different between NAION patients and controls ($P=1.000$). There was no significant difference in the frequency of homozygosity for the Glu298Asp polymorphism of the eNOS gene between the NAION patients and control groups ($P=0.755$).

Table 3 Allele frequencies of eNOS gene polymorphisms in control and NAION

Allele	Controls no. (%)	NAION no. (%)
Glu298Asp polymorphism		
Glu	231(95)	29(97)
Asp	11(5)	1(3)
T(-786)C polymorphism*		
T	213(88)	21(70)
C	29(12)	9(30)

NAION indicates nonarteritic anterior ischemic optic neuropathy subjects

For T(-786)C polymorphism: * $P=0.021$ between control and NAION

Table 4 Logistic regression analysis

Risk factors	Odds ratio	95% CI	P-value
<i>Logistic regression model for each risk factor separately</i>			
Diabetes melitus (yes/no)	2.48	0.69–8.97	0.17
Hypertension (yes/no)	0.45	0.13–1.64	0.23
Hyperlipidemia (yes/no)	1.25	0.33–4.7	0.74
Smoking (yes/no)	0.82	0.21–3.12	0.77
Ischemic heart disease (yes/no)	3.31	0.59–18.71	0.18
eNOS Glu298Asp TT(TT/GG)	1.17	0.12–11	0.89
eNOS T(-786)C CC(CC/TT)	0.09	0.01–0.86	0.01

Distribution of T(-786)C genotypes

The T and C alleles were present in 88% and 12%, respectively, of the control group, and the TT, TC, and CC genotypes were found in 94 (78%), 25 (20%) and 2 (2%) control subjects, respectively, out of 121. In contrast, the T and C alleles were present in 70.0% and 30.0%, respectively, of the NAION group, while the TT, TC, and CC genotypes were found in 9 (60.0%), 3 (20.0%) and 3 (20.0%) NAION patients, respectively, out of 15. The genotype distribution of the T(-786)C polymorphism was significantly different between the NAION patients and controls ($P=0.002$). In addition, there was a significant difference in the frequency of homozygosity for the T(-786)C variant of the eNOS gene between the NAION patients and control groups ($P=0.021$).

Logistic regression analysis

The result of logistic regression analysis is given in Table 4. The CC genotype of the T(-786)C polymorphism was a statistically significant risk factor.

Discussion

In the present study, we demonstrated that there was a significant association between the T(-786)C polymorphism of the eNOS gene and the occurrence of NAION in Japanese subjects. This is the first report of a positive association between an eNOS gene polymorphism and NAION.

Recently, the mechanisms leading to vasospasm associated with the T(-786)C variant of the eNOS gene have been found [11]. Nakayama et al. showed that the T(-786)C variant suppressed eNOS gene transcription, and concluded that this suppression results in reduced endothelial NO production in patients carrying the T(-786)C variant. Since NO is known to suppress production of the potent vasoconstrictors, endothelin and angiotensin II [2,

19], deficient NO production in patients with this polymorphism could result in increased synthesis of these vasoconstrictors. Furthermore, other studies suggest that vasospasms may be responsible for NAION [1, 10]. Hayreh speculated that acute blood loss, a condition associated with NAION, can increase the release of endogenous vasoconstrictor agents, leading to vasoconstriction and NAION [5]. Taken together, we suggest that the homozygous T(-786)C variant in the eNOS gene may be a risk factor for the development of NAION.

In contrast, we found no positive association between the Glu298Asp polymorphism and the development of NAION in the present study. Interestingly, there are reports that the Glu298Asp variant is significantly associated with Behçet disease and giant cell arteritis [8, 17], suggesting that eNOS activity is affected differently by the T(-786)C and Glu298Asp variants. The Glu298Asp variant may affect a mechanism for promoting vessel wall damage in vasculitis.

An important limitation of our study is the small number of cases. However, the distribution of the T(-786)C genotype in the Japanese patients of our study is very similar to the distribution from a study Yoshimura et al. [22], which looked at a large number of Japanese. For this reason, we are confident that the frequencies of the TT, TC, and CC genotypes were accurately determined. However, it will be necessary to examine a large number of cases to substantiate the connection between the eNOS gene polymorphisms and NAION in future studies. Another limitation of our study is that we have not considered nocturnal hypotension which is the most important risk factor for NAION. Future studies will need to investigate association between the eNOS gene polymorphisms and nocturnal hypotension.

In conclusion, we found a high prevalence of T(-786)C polymorphism of the eNOS gene in patients with NAION. Our data suggest that the T(-786)C polymorphism of the eNOS gene may be an important risk factor in the development of NAION in a Japanese population.

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