Investigation of the association between 677C>T and 1298A>C 5,10-methylenetetrahydrofolate reductase gene polymorphisms and normal-tension glaucoma

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Abstract

Purpose Homozygous polymorphism of the 5,10-methylenetetrahydrofolate reductase (*MTHFR*) gene and resultant hyperhomocysteinaemia have been established as an independent risk factor for vascular diseases. There are evidences that vascular abnormalities are involved in the pathogenesis and progression of normal-tension glaucoma (NTG). In the present study, we were to find out the associations between 677C>T and 1298A>C polymorphisms of the *MTHFR* gene and NTG.

Methods This was a retrospective, case-controlled study enrolling 78 NTG patients and 100 controls. DNA from peripheral blood lymphocytes was extracted and the genotypes of polymorphisms (677C>T and 1298A>C) in the *MTHFR* gene were determined using PCR followed by restriction enzyme digestion. The frequencies of the polymorphic genotypes in the patients with NTG and controls were compared.

Results The frequencies of the polymorphisms of the *MTHFR* gene (677C>T and 1298A>C) in the NTG patients were not significantly different from those of controls. But the younger NTG patients (age at diagnosis ≤ 45 years) showed significantly higher prevalence of 677C>T polymorphism than the older NTG patients (age at diagnosis >45 years) (TT genotype, 38.9 vs 11.9%,

$P = 0.006$, OR = 4.71, 95% CI = 1.49–14.9) and than the younger control subgroup (TT genotype, 38.9 vs 6.1%, $P = 0.001$, OR = 9.86, 95% CI = 2.23–42.4).

Conclusions The 677C>T polymorphism was significantly associated with NTG in the younger patients, while 1298A>C polymorphism was not. This suggests that 677C>T polymorphism of the *MTHFR* gene can be a genetic risk factor of NTG in Korean population.

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Introduction

Glaucoma, the second most common cause of blindness worldwide is characterized by the progressive loss of optic nerve axons and visual field damage.¹ A high intraocular pressure (IOP) is a risk factor commonly associated with this type of neuropathy. However, among patients with glaucomatous optic neuropathy there exists a subpopulation with IOPs that do not exceed those of the normal population (≤ 21 mm Hg). This group of patients is classified as having normal-tension glaucoma

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(NTG). Although the exact pathogenesis of NTG has not been elucidated, diseased vessel walls may play a direct role in the pathogenesis of optic disc cupping in glaucoma.² Disc haemorrhages often found in NTG patients is thought to be a cause of a microinfarction and optic nerve head damage.² Patients with arteritic ischaemia develop optic nerve head excavation resembling glaucomatous cupping.³ There are many evidences that convince vascular abnormality is related to the occurrence and progression of NTG.⁴

Hyperhomocysteinaemia has been identified as a risk factor of cerebrovascular, peripheral vascular, and coronary artery diseases.⁵⁻⁸ The deleterious effects of homocysteine include endothelial dysfunction, arterial intimal-medial thickening, arterial stiffening, and procoagulant activity.⁹ Abnormal homocysteine metabolism may be caused by inherited defects in the enzymes controlling homocysteine metabolism, one of which is known to be 5,10-methylenetetrahydrofolate reductase (MTHFR).¹⁰ MTHFR catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, the predominant circulatory form of carbon donor in the re-methylation of homocysteine to methionine. Among nine mutations of *MTHFR* gene associated with loss of function,^{11,12} a point mutation of 677C>T has been investigated regarding the vascular diseases. Homozygous 677C>T mutation (TT genotype) results in a less active thermolabile form of the MTHFR enzyme and significantly elevated plasma homocysteine levels. TT genotype has been proven to be associated with vascular diseases such as cardiovascular and cerebrovascular diseases.^{10,13-16} Another *MTHFR* gene polymorphism of 1298A>C was reported to be associated with lower blood folate levels and higher blood homocysteine levels in some studies.¹⁷

MTHFR gene polymorphism and resultant hyperhomocysteinaemia as a risk factor of vascular diseases prompted us to investigate the association between *MTHFR* gene polymorphism and NTG.

Compared with the Caucasian population, the Korean population could be a better candidate ethnic group for the genetic study of NTG because of a single ethnic origin of Korean and the higher prevalence of NTG in adult Korean (2.04%) than in Caucasian (0.6%).^{18,19}

Materials and methods

Subjects

The study population comprised 78 patients with NTG and 100 healthy volunteers. All subjects were of Korean origin and were not related. NTG patients were all diagnosed at the Glaucoma Clinic of Seoul National University Hospital. Healthy volunteers were recruited

at the Health Promotion Clinic of Seoul National University Hospital. Human subject participation and receipt of informed consent from each subject were approved by the Institutional Review Board of Seoul National University Hospital Clinical Research Institute. The diagnostic criteria for NTG were normal IOP, glaucomatous optic disc cupping, glaucomatous visual field defects, open anterior chamber angle, and the absence of any contributing ocular or systemic disorders. IOP was measured using a Goldmann applanation tonometer. Normal IOP was defined as a diurnal IOP persistently below 21 mm Hg without any medication. Visual fields of NTG patients were evaluated using the 30-2 program of the Humphrey Visual Field Analyzer Model 750 (Zeiss Inc., San Leandro, CA, USA) or Model 630 (Allergan Inc., San Leandro, CA, USA) and mean deviation (MD) of visual fields were obtained. The MD index signifies overall severity of visual field loss.

One hundred volunteers had completed medical and ophthalmic examinations at the Health Promotion Clinic and served as controls. They had normal visual acuity and normal IOP measured with pneumatic tonometer (Model TX10, Canon Inc., Tokyo, Japan). Subjects with any of the suspicious findings of glaucoma in the disc and fundus (cup-to-disc ratio of more than 0.6, notch in the neural rim, vertically oval cup, zone β -peripapillary atrophy, retinal nerve fibre layer defect, choroidal sclerosis and abnormalities of the disc vessels, that is, nasalization, baring, and bayoneting)²⁰ were excluded. Those having vascular diseases (hypertension, ischaemic heart disease, or cerebrovascular diseases) were not included in controls. Subjects and controls were compared with respect to age, gender ratio, IOP, and family history of glaucoma.

Genetic analysis

Peripheral blood samples of NTG patients and controls were collected from the antecubital vein and contained in an EDTA tube for less than 1 day. Genomic DNA extraction was performed using either the salting-out technique²¹ or a Gentra PureGene DNA isolation kit (Gentra System Inc., Minneapolis, MN, USA). PCR was performed on a final volume of 20 μ l, which contained 50 ng genomic DNA, 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM MgCl₂, 0.01% gelatin, 200 μ M of each dNTP, 10 pmol of each primer (677C>T: the forward primer, 5'-TGAAGGAGAAGGTGTCTGCGGA-3' and the reverse primer, 5'-AGGACGGTGC GG T GAGAGTG-3'; 1298A>C: the forward primer, 5'-CAAGGAGGAGCTGCTGAAGA-3' and the reverse primer, 5'-CCACTCCAGCATCACTCACT-3')¹⁰ and 0.5 U of Taq polymerase (AmpliTaQ Gold™, Applied

Biosystems, Foster City, CA, USA). The PCR conditions were initial denaturation at 95°C for 10 min, and 35 cycles of 95°C for 30 s, 57°C (1298A>C) or 62°C (677C>T) for 30 s and 72°C for 1 min, and a final extension at 72°C for 7 min.

Genotyping the 677C>T polymorphism was performed by using the restriction enzyme *HinfI* (New England BioLabs, Beverly, MA, USA). The T allele sequence was cut into two fragments (175 and 23 bp) by *HinfI*, whereas the C allele sequence remained intact (198 bp). Genotyping the 1298A>C polymorphism was performed by using the restriction enzyme *MboII* (New England BioLabs). The C allele sequence was cut into two fragments (100 and 28 bp) by *MboII* and the A allele sequence was cut into three fragments (72, 28, and 28 bp).

To confirm the accuracy of genotype with the use of the restriction analysis, we randomly selected nine DNA samples and subjected them to PCR and restriction enzyme assay and direct DNA sequencing of the PCR products using the antisense PCR primer and a BigDye terminator cycle sequencing ready reaction kit v2.0 (Applied Biosystems). In each instance, the genotype determined by restriction assay was identical to that determined by the sequencing.

Genotype and phenotype analysis

The alleles with or without 677C>T polymorphism are represented as T or C; the normal homozygote as CC, the heterozygote of the polymorphism as CT and the homozygote of the polymorphism as TT. For 1298A>C polymorphism, A or C, AA, AC, and CC were used to represent the alleles and genotypes.

The frequencies of genotypes and alleles of the *MTHFR* gene were compared between NTG patients and controls using the χ^2 test.

Clinical features of NTG patients with homozygous polymorphisms were compared with the other NTG patients using the Mann–Whitney test and the χ^2 test. Further genetic comparisons were performed between groups divided by the clinical phenotype, which had shown significant association with *MTHFR* gene polymorphism.

Parametric and nonparametric tests of significance were performed when appropriate. Continuous data were presented as mean $\pm$ SD. The results were taken to be statistically significant when the *P*-value was <0.05. Conformance with the Hardy–Weinberg equilibrium for the genotype distributions was determined using the χ^2 test for the goodness of fit. The statistical analyses were performed using SPSS for windows version 10.0 program (Statistical Package for Social Sciences Ver. 10.0, SPSS Inc., Chicago, IL, USA).

Results

Clinical features of NTG patients and controls are depicted in Table 1. No significant differences were found between the two groups in terms of age, gender ratio, and IOP. The frequency of family history of glaucoma was significantly higher in the NTG patients (15.4%) than controls (0%) (*P*<0.001).

The distributions of the genotypes of the *MTHFR* gene in NTG patients and controls were compared and shown in Table 2. For the 677C>T polymorphism in 78 NTG patients, the CC genotype was found in 25 patients (32.1%), the CT genotype in 34 (43.6%), and the TT genotype in 19 (24.4%). In 100 controls, the CC genotype was found in 31 (31.0%), the CT genotype in 50 (50.0%), and the TT genotype in 19 (19.0%). For the 1298A>C polymorphism, in NTG patients, the AA genotype was found in 57 patients (73.1%), the AC genotype in 19 (24.4%), and the CC genotype in 2 (2.6%). In controls, the AA genotype was found in 75 (75.0%), the AC genotype in 22 (22.0%), and the CC genotype in 3 (3.0%). Neither CC/TT genotype of 677C>T polymorphism nor AA/CC genotype of 1298A>C polymorphism was significantly associated with NTG. Comparison of the allele distributions also showed no significant difference between NTG patients and controls. The combination of 677C>T and 1298A>C polymorphism showed no significant association with NTG. In both groups, the genotype distributions were in accordance with the Hardy–Weinberg equilibrium.

The clinical features of NTG patients with homozygous polymorphisms were compared with the

Table 1 Clinical features of NTG patients and controls

	NTG patients (n = 78)	Controls (n = 100)	P-value
Age (years)	46.2 $\pm$ 11.7	49.3 $\pm$ 9.2	NS ^a
Gender ratio (male : female)	32 : 46	47 : 53	NS ^b
IOP (mm Hg)	15.7 $\pm$ 2.5 ^c	14.9 $\pm$ 2.6	NS ^a
Maximum IOP (mm Hg)	17.1 $\pm$ 2.6		
Minimum IOP (mm Hg)	14.3 $\pm$ 3.0		
Bilaterality	45 (58%)		
Family history of glaucoma ^d (%)	12 (15.4%)	0 (0.0%)	<0.001 ^e
MD (dB)	−6.53 $\pm$ 5.26 (−25.25 to 0.04)		

IOP, intraocular pressure; MD, mean deviation of program 30–2 of Humphrey Visual Field Analyzer, which signifies overall severity of visual field loss; NTG, normal-tension glaucoma; NS, not significant; .

^a*P*-values by independent *t*-test.

^b*P*-value by χ^2 test.

^cMean of diurnal IOPs.

^dGlaucoma comprises all types of glaucoma including NTG.

^e*P*-value by Fisher's exact test.

Table 2 Comparison of the frequencies of genotypes and alleles of the 677C>T and 1298A>C polymorphism in the *MTHFR* gene between patients with NTG and controls

Genotypes/alleles	NTG patients (n = 78/156)	Controls (n = 100/200)	P-value*	Remark
C677T				
CC	25 (32.1%)	31 (31.0%)	0.88	CC vs CT + TT
CT	34 (43.6%)	50 (50.0%)		
TT	19 (24.4%)	19 (19.0%)	0.39	CC + CT vs TT
C	84 (53.8%)	112 (56.0%)	0.69	C vs T
T	72 (46.2%)	88 (44.0%)		
A1298C				
AA	57 (73.1%)	75 (75.0%)	0.77	AA vs AC + CC
AC	19 (24.4%)	22 (22.0%)		
CC	2 (2.6%)	3 (3.0%)	1.00	AA + AC vs CC
A	133 (85.3%)	172 (86.0%)	0.84	A vs C
C	23 (14.7%)	28 (14.0%)		

MTHFR, 5,10-methylenetetrahydrofolate reductase; NTG, normal-tension glaucoma.

*P value by χ^2 test.

Table 3 Comparison of the clinical features of NTG patients between with and without homozygous polymorphisms in *MTHFR* gene (TT and CC genotype)

Polymorphism	Clinical features	Genotype		P-value*
		CC + CT (n = 59)	TT (n = 19)	
677C>T	Age at diagnosis (years)	47.7 ± 11.9	41.5 ± 10.0	0.030
	Gender ratio (male : female)	25/34	7/12	0.670
	IOP (mm Hg)	15.8 ± 2.7	15.5 ± 2.1	0.686
	MD (dB)	-6.57 ± 4.82	-6.37 ± 6.47	0.489
1298A>C		AA + AC (n = 76)	CC (n = 2)	
	Age at diagnosis (years)	46.3 ± 11.8	43.5 ± 2.1	0.703
	Gender ratio (male : female)	31/45	1/1	0.990
	IOP (mm Hg)	15.6 ± 2.5	17.5 ± 0.7	0.342
	MD (dB)	-6.58 ± 5.31	-4.29 ± 1.34	0.755

IOP, intraocular pressure; MTHFR, 5,10-methylenetetrahydrofolate reductase; MD, mean deviation of program 30-2 of Humphrey Visual Field Analyzer, which signifies overall severity of visual field loss; NTG, normal-tension glaucoma.

*P-value by Mann-Whitney test and χ^2 test.

other NTG patients and are shown in Table 3. The age at diagnosis of NTG was significantly different among patients having different genotypes of 677C>T polymorphism. There was a tendency of decreasing mean age at diagnosis as the number of T allele increased (CC, 49.9 years; CT, 46.2 years; and TT 41.5 years). TT genotype showed significantly earlier age at diagnosis (41.5 ± 10.0 years) than CC + CT genotypes (47.7 ± 11.9 years) ($P = 0.030$ by Mann-Whitney test). Other clinical features of gender ratio, IOP, and MD values were not significantly different between patients with TT genotype and CC + CT genotypes. All the clinical features of patients did not show any significant association with genotypes of 1298A>C polymorphism.

Genotypes and alleles of 677C>T polymorphism in the younger NTG patients (age at diagnosis ≤ 45 years) were compared with those of the older ones (age at

diagnosis > 45 years) and are shown in Table 4.

Reference age was set according to the mean age of the NTG patients (46.2 years). The younger NTG patients showed significantly different distribution of genotypes and alleles compared with the older ones. The frequencies of TT genotype and T allele were significantly higher in the younger NTG patients than the older ones (TT genotype, 38.9 vs 11.9%, $P = 0.006$, OR = 4.71, 95% CI = 1.49–14.9; T allele, 58.3 vs 35.7%, $P = 0.005$, OR = 2.52, 95% CI = 1.32–4.81). MD of visual field was not significantly different between two groups (-5.34 ± 5.04 dB in the younger patients and -7.61 ± 5.28 dB in the older patients, $P = 0.064$ by independent *t*-test).

The younger NTG patients showed significantly higher frequencies of TT genotype and T allele in comparison with the younger control subgroup (age ≤ 45 years)

Table 4 Comparison of the genotypes and alleles of 677C>T polymorphism in the *MTHFR* gene between younger NTG patients (age at diagnosis ≤ 45 years) and older ones (age at diagnosis ≥ 45 years)

	Younger NTG patients (n = 36/72)	Older NTG patients (n = 42/84)	P-value*	Remarks
CC	8 (22.2%)	17 (40.5%)	0.09	CC vs CT + TT
CT	14 (38.9%)	20 (47.6%)		
TT	14 (38.9%)	5 (11.9%)	0.006, OR = 4.71 (1.49–14.9)	TT vs CC + CT
C	30 (41.7%)	54 (64.3%)		
T	42 (58.3%)	30 (35.7%)	0.005, OR = 2.52 (1.32–4.81)	T vs C

MTHFR, 5,10-methylenetetrahydrofolate reductase; NTG, normal-tension glaucoma; OR, odds ratio with 95% confidence interval.

*P-value by χ^2 test.

Table 5 Comparison of the genotypes and alleles of 677C>T polymorphism in the *MTHFR* gene between younger NTG patients (age at diagnosis ≤ 45 years) and younger control subgroup (age ≤ 45 years)

	Younger NTG patients (n = 36/72)	Younger control (n = 33/66)	P-value*	Remarks
CC	8 (22.2%)	10 (30.3%)	0.45	CC vs CT + TT
CT	14 (38.9%)	21 (63.6%)		
TT	14 (38.9%)	2 (6.1%)	0.001, OR = 9.86 (2.23–42.4)	TT vs CC + CT
C	30 (41.7%)	41 (62.1%)		
T	42 (58.3%)	25 (37.9%)	0.016, OR = 2.30 (1.16–4.55)	T vs C

MTHFR, 5,10-methylenetetrahydrofolate reductase; NTG, normal-tension glaucoma; OR, odds ratio with 95% confidence interval.

*P-value by χ^2 test.

(TT genotype, 38.9 vs 6.1%, $P = 0.001$, OR = 9.86, 95% CI = 2.23–42.4; T allele, 58.3 vs 37.9%, $P = 0.016$, OR = 2.30, 95% CI = 1.16–4.55) (Table 5).

The frequency of vascular diseases was 16.7% (6/36) in the younger NTG patients and 28.6% (12/42) in the older NTG patients. To avoid the bias from unequal distribution of vascular diseases between patients and controls, we excluded patients having vascular diseases from the NTG patients and performed genotype comparison between patients and controls. Eighteen NTG patients were excluded because of having vascular diseases. In comparison between 60 NTG patients and 100 controls, there was no significant association of 677C>T or 1298A>C polymorphism with NTG. In comparison of frequencies of 677C>T polymorphism between younger NTG patients and older ones, the younger NTG patients also showed significantly higher frequencies of TT and T allele than the older ones (TT genotype, 43.3 vs 16.7%, $P = 0.024$, OR = 3.82, 95% CI = 1.15–12.7; T allele, 61.7 vs 41.7%, $P = 0.028$, OR = 2.25, 95% CI = 1.08–4.68).

Discussion

The principal finding of this study is the association of 677C>T polymorphism of the *MTHFR* gene with NTG in the younger patients, and our study is the first, to our best knowledge, to report this association. The frequency of TT genotype in younger NTG patients in our study (38.9%) is much higher when it is compared with the

results reported in different ethnic populations (2–17.9%).^{10,13,14,16,22–29} Genetic traits of a disease should be more important in younger patients than in older ones. If one has a genetic predisposition to a disease, it is likely that the disease should develop earlier. One may argue that there is no way to know when the disease has developed in our patients. However, there was statistically no difference in the severity of visual field loss represented with MD values between the younger patients and the older ones. Therefore, it is less likely that the younger patients were detected earlier in the progress of the disease than the older ones. Aung *et al*³⁰ reported the mutation of optineurin gene is associated with the early onset (mean age of 40.8 years), more severe form of NTG. This result also could be interpreted as the early onset NTG has a distinct genetic pathogenesis from the late onset NTG. Our result of higher frequencies of 677C>T *MTHFR* gene polymorphism in the younger NTG patients suggests that the polymorphism may play a role in the pathogenesis of NTG or early-onset NTG subgroup in Korean population.

In our result, *MTHFR* gene polymorphism was not significantly associated with the whole NTG patients, and this is of the same result reported by Mabuchi *et al*²⁹ in Japanese population. NTG is not considered as a disease of a single gene abnormality, but rather the sum of various genetic alterations and various pathomechanisms.^{31–33} This may explain why the whole NTG patients did not show association with TT genotype of 677C>T *MTHFR* gene polymorphism and only a

subgroup of younger patients showed association. We do not think this polymorphism is a major causative factor of NTG. Polymorphism of the *MTHFR* gene may be a contributory factor in the subgroup of NTG and further research is necessary to characterize its role in the development of NTG.

Although the TT genotype is more frequent in the younger NTG patients, the prevalence of vascular diseases was less frequent in the younger NTG patients than the older ones (16.7 *vs.* 28.6%). Considering the increasing prevalence of vascular diseases with age, this is an expected result and could not be a source of bias in the comparison of genotypes.

The lack of association of 1298A>C polymorphism in the *MTHFR* gene with NTG indicates that it has low possibility of being related to the pathogenesis of NTG. Although 1298A>C polymorphism is associated with higher level of blood homocysteine when it is combined with 677C>T polymorphism¹⁷, researchers reported that 1298A>C polymorphism alone was not a major risk factor of vascular diseases and Alzheimer's disease.^{34,35}

In the previous reports, plasma homocysteine levels were found to be significantly higher in patients with the TT genotype than in patients with the CC or CT genotype.^{10,13,14} Although the differences in the plasma homocysteine levels were small, previous studies suggested that plasma homocysteine levels after dietary methionine loading were significantly affected by the genotype of the *MTHFR* gene.¹⁰ Experimental studies have suggested that high plasma homocysteine levels can cause atherogenic and thrombotic states by modulating vascular cell proliferation and promoting prothrombotic activities in the vascular wall.¹⁴ In addition, although serotonin has no effect on the vasculature in normal monkeys, in atherosclerotic monkeys it produced vasospasm of the central retinal artery and/or of the posterior ciliary artery. It has been postulated that in some atherosclerotic individuals this mechanism may play an important role in the development of ischaemic disorders of the retina and optic nerve head, and possibly of glaucomatous optic neuropathy, particularly in NTG.³⁶ Vasospastic syndrome is an established risk factor for glaucoma in addition to increased IOP.^{2,37} In NTG where glaucomatous damage occurs without elevated IOP, the phenomenon of vasospasm or ischaemia is assumed to prevail in the pathogenesis over barotraumas.³⁸ This is supported by the observation that optic disc haemorrhages are frequently found before the occurrence of retinal nerve fibre layer defects in patients with NTG, which also implies a vascular insult, such as thrombosis or vasospasm, at the optic nerve head.³⁹ Therefore, vascular abnormalities, such as vascular endothelial dysfunction, atherosclerosis, thrombosis, and structural vessel wall

stiffness induced by elevated level of homocysteine may be the cause of development and progression of NTG.^{9,40} Vascular stiffness has been reported to be related to hyperhomocysteinaemia in normal individuals and hypertensive patients. Reduced arterial distensibility may cause vascular dysregulation throughout the body.⁹ Vascular dysregulation, unstable ocular perfusion, and thereby, ischaemia and reperfusion damage have also been proposed as etiologic factors of optic nerve damage in glaucoma.⁴⁰

In recent reports, the mean plasma homocysteine levels of NTG and primary open-angle glaucoma (POAG) patients showed no significant differences from those of controls.^{41,42} Those were expected results because the frequency of TT genotype in the whole NTG patients was not significantly higher than controls in our study and in the study done by Mabuchi *et al.*²⁹

The association between hyperhomocysteinaemia and other types of glaucoma has also been investigated. Recent researches on the association of 677C>T *MTHFR* gene polymorphism and hyperhomocysteinaemia with high-tension POAG and pseudoexfoliation glaucoma revealed conflicting results and the association is not obvious.^{25–28} As the barotrauma from high IOP is the primary pathogenesis of optic nerve damage in high-tension POAG, there appears to be low possibility of the association between hyperhomocysteinaemia and high-tension POAG.

Although plasma homocysteine level is related to the activity of the *MTHFR* enzyme, other environmental factors, such as dietary intake, are related with the activity of the *MTHFR* enzyme and with the plasma homocysteine level. The uptakes of folic acid, vitamin B₁₂, pyridoxine, and choline are associated with a reduced level of homocysteine.⁴³ The clinical benefits of these nutrients in vascular diseases have not yet been determined. Accordingly, whether the uptake of these nutrients and the reduction of the plasma homocysteine level might be beneficial or not for patients with NTG whose genotypes of the *MTHFR* gene are TT also remains to be determined.

Significantly higher frequency of family history in the NTG patients as shown in Table 1 indicates that NTG must have a genetic pathogenesis. There is a possibility that this polymorphism may have an association with the patients having family history. Although family history of NTG is as high as 15.4% in the patients of our study, we could not prove a significant association between this polymorphism and the family history because of the small sample size of the patients.

The limitation of our study is that the healthy controls were not strictly chosen with diurnal IOP measurements and visual field testing. Although optic disc and retinal nerve fibre layer examinations and single IOP

measurement can rule out most of NTG patients in the control group, there is still a little possibility of NTG patients being included in the controls. Girkin *et al*⁴⁴ reported subjective optic disc grading by glaucoma specialist could diagnose glaucoma very accurately without visual field test (sensitivity of 78.4% and specificity of 91.9%). In addition, considering the low prevalence of NTG in general population (2.04% in Koreans),¹⁸ it is unlikely that this would have significantly altered the results. Another limitation is that we had not checked the corneal thickness in NTG patients. The measurement of corneal thickness would have resulted in more accurate diagnosis of NTG.

In conclusion, the present study demonstrated that the 677C>T polymorphism in the *MTHFR* gene was significantly associated with NTG in the younger patients and suggests it can be a genetic risk factor of NTG. No associations were found between 1298A>C polymorphism and NTG. The association between NTG and the 677C>T polymorphism in the *MTHFR* gene will contribute to the better understanding of the pathogenesis of NTG.

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