Association between methylenetetrahydrofolate reductase (*MTHFR*) polymorphisms and susceptibility to Graves' ophthalmopathy

JAE YEUN LEE[1], NAM KEUN KIM[2], YONG WOOK CHO[3] and HELEN LEW[1]

[1]Department of Ophthalmology; [2]Institute for Clinical Research; [3]Department of Endocrinology, Bundang CHA General Hospital, College of Medicine, CHA University, Seongnam, Keonggi-do 463-712, Republic of Korea

Received July 21, 2015; Accepted May 31, 2016

DOI: 10.3892/mmr.2016.5458

Abstract. The pathogenesis of Graves' ophthalmopathy (GO) remains to be entirely elucidated. The present study aimed to determine the association between phenotypic expression of the *MTHFR* gene and susceptibility to GO in patients with Graves' disease (GD). A prospective case-controlled study was conducted with 122 patients with GD and GO (n=72) or without GO (n=50) and 100 healthy controls in South Korea. Patient history, including smoking, nutritional status, thyroid function and antithyroid antibodies were investigated and clinical activity score, VISA classification (which includes vision, inflammation, strabismus and appearance/exposure) and orbit computed tomography were evaluated. Fasting plasma total homocysteine (tHcy) concentration was measured, and genotype analysis of the MTHFR gene was conducted. The TT homozygous genotype was associated with a two-fold increased risk of GO [adjusted odds ratio (AOR), 2.19; 95% confidence interval (CI), 0.78-6.14]. However, this result was not significant. The TT genotype significantly increased the risk of GO compared with that in healthy controls (AOR, 2.92; 95% CI, 1.11-7.65). The *MTHFR* 677CT/1298AA genotype decreased the risk of GO in patients with GD (AOR, 0.26; 95% CI, 0.08-0.91). tHcy levels in patients with GD without GO were significantly higher than in patients with GO, however, they were within the normal limit. The current study identified an association between *MTHFR* polymorphisms and GO. These results will aid understanding of the pathogenesis of GO and facilitate development of genetic therapeutic strategies.

Correspondence to: Dr Helen Lew, Department of Ophthalmology, Bundang CHA General Hospital, College of Medicine, CHA University, 59 Yatap-ro, Bundang-gu, Seongnam, Keonggi-do 463-712, Republic of Korea
E-mail: eye@cha.ac.kr

Key words: Graves' ophthalmopathy, Graves' disease, MTHFR

Introduction

Graves' disease (GD) is the most common organ-specific autoimmune disorder with 0.5% of the population affected. Patients are predominantly 40-60 years old, with a female to male ratio of 5:1 to 10:1 (1). Although the etiology remains to be elucidated, GD is hypothesized to be the result of a complex interaction between genetic and environmental factors (2).

A high prevalence of 5, 10-methylenetetrahydrofolate reductase (*MTHFR*) mutations and increased homocysteine (Hcy) levels have been reported in populations with autoimmune diseases, including multiple sclerosis, vasculitic lesions, and systemic lupus erythematosus, indicating a potential genetic association between *MTHFR* and autoimmune disorders (3-6).

Mao *et al* (7) conducted a case-control study of 199 GD cases and 235 healthy controls to examine the associations between three common *MTHFR* polymorphisms (677C>T, 1298A>C and 1793G>A) and GD in the Chinese population. Notably, a logistic regression analysis demonstrated that the *MTHFR* 677CT + TT genotypes are associated with ~42% reduced risk of GD in females [adjusted odds ratio (AOR), 0.58; 95% confidence interval (CI), 0.3-0.9], compared with the CC genotype, indicating a marked protective effect of the 677CT + TT genotypes.

Graves' ophthalmopathy (GO) is an autoimmune inflammatory process of the orbit, which is associated with GD (8). The underlying etiology of GO remains to be elucidated, however, previous studies have indicated genetic and environmental factors are likely to be important in the development of GO (9,10).

Thus, the present study hypothesized there was an association between genetic polymorphisms in the *MTHFR* gene and the risk of GO. MTHFR is a key enzyme in folate metabolism that catalyzes the reduction of 5, 10-methylenetetrahydrofolate, a thymidylate synthetase substrate, to 5-methyltetrahydrofolate, a Hcy substrate, for conversion to methionine by methionine synthase. Methionine is converted to S-adenosylmethionine, the universal methyl donor for methylation of a number of biological substrates. MTHFR is a central regulator of DNA synthesis, the methylation cycle, and blood Hcy level. Three

common non-synonymous single nucleotide polymorphisms (SNPs; 677C>T, 1298A>C, and 1793G>A) of the *MTHFR* gene have been demonstrated to result in altered enzymatic activity (7).

The present study aimed to investigate the importance of the *MTHFR* polymorphisms in susceptibility to GO in Korean patients.

Materials and methods

Patients. The study population comprised 122 patients with GO (n=72) or GD without GO (n=50), in addition to 100 control subjects. The patients were enrolled and recruited between December 2010 and February 2012 at CHA General Hospital (Seongnam, Korea).

All the subjects were unrelated ethnic Koreans from Seongnam and the surrounding regions. Patients were a consecutive series of cases at the Department of Endocrinology, CHA General Hospital, CHA University. The history included smoking, nutritional status, and thyroid function. The antithyroid antibody and clinical activity scores were measured, and the VISA classification (which includes vision, inflammation, strabismus and appearance/exposure), and orbit computed tomography (2-mm slice) were evaluated. GD was diagnosed by the presence of hyperthyroidism and serum antithyroid stimulating hormone receptor antibody and/or an increased radioactive ^{131}I uptake ratio with diffuse uptake. GO was defined as class 3 or higher using the American Thyroid Association mnemonic NO SPECS scheme (11). Patients for the GD without GO group were selected from those who did not have any features of ophthalmopathy, including features of class 1-2. Control samples were from healthy individuals selected randomly from the same regions as the patients. All subject information was collected with informed consent from the participants and the approval of the appropriate institutional review board of the CHA Medical Center (Seongnam, Korea).

Genotype analysis. Blood samples were collected from anticoagulant tubes (EDTA-tube) and stored at 4˚C. Centrifugation at 1,500 x g for 15 min at room temperature was then used to separate leukocytes. Genomic DNA was extracted from leukocytes using a CpGenome™ DNA Modification kit (EMD Millipore, Billerica, MA, USA) according to the manufacturer's protocol. The *MTHFR* 677C>T and 1298A>C genotypes were identified. Regions containing the two polymorphisms were amplified separately. The primers used were as follows: Forward, 5'-GCACTTGAAGAGAAG GTGTC-3' and reverse, 5'-AGGACGGTGCGGTGAGAG TG-3' for the nucleotide 677C>T polymorphism; and forward, 5'-CTTTGGGGAGCTGAAGGACTACTAC-3' and reverse, 5'-CACTTTGTGACCATTCCGGTTTG-3' were used for the nucleotide 1298A>C polymorphism. Human genomic DNA (200 ng) was amplified with 100 pmol of each forward and reverse primer, 1.5 mM $MgCl_2$, 0.2 M of each deoxynucleotide triphosphate and 1 unit of Taq polymerase (Takara Bio, Inc., Otsu, Japan) in a total reaction volume of 100 μl. The polymerase chain reaction (PCR) conditions were as follows: Denaturation at 94˚C for 5 min; followed by 35 cycles at 94˚C for 30 sec, 51˚C for 30 sec, 72˚C for 30 sec; and a final terminal elongation at 72˚C for 5 min. PCR products were digested with *Hin*fI (for nucleotide 677) or *Fnu*4HI (for nucleotide 1,298) for 2 h at 37˚C.

Agarose (3%) gel electrophoresis was used to determine the success of the amplification. For the nucleotide 677C>T polymorphism, an undigested PCR product (203 bp) indicated a homozygous wild-type, three bands at 203, 173, and 30 bp indicated the heterozygous genotype, and two bands of 170 and 30 bp indicated the homozygous genotype. For the nucleotide 1298A>C polymorphism, a single band of 138 bp indicated a wild type, and two fragments of 119 and 19 bp indicated the homozygous genotype.

All the blood samples were processed using a standard protocol. Overnight fasting (12 h) blood samples for plasma total Hcy (tHcy) and folate levels were collected in tubes containing trisodium EDTA, promptly centrifuged at 1,500 x g for 15 min at room temperature, and stored at -20˚C until used in analysis. Plasma tHcy levels were determined using a fluorescence polarization immunoassay (IMx; Abbott Laboratories, Chicago, IL, USA).

Statistical analysis. Patients with GD with or without GO and the controls were compared according to plasma tHcy level and *MTHFR* 677C>T genotype and the MTHFR 1298A>C genotype. For analysis of baseline characteristics, the χ^2 test was used for categorical data and the two-sample t-test was used for continuous data. Genotype and allele frequencies were calculated by direct counting. Conformity to the Hardy-Weinberg equilibrium among each population was performed. AORs and 95% CIs were used to estimate relative risks of the plasma tHcy level and *MTHFR* genotype. All statistical analyses were performed using the SPSS 20.0 package (IBM SPSS, Armonk, NY, USA). $P<0.05$ and false discovery rate (FDR)<0.05 was considered to indicate a statistically significant difference.

Results

Patient characteristics. A summary of selected characteristics, including age and gender of the patients with GO (n=72), those with GD without GO (n=50), and the 100 controls are presented in Table I. The frequency matching variables were compared between the cases and controls. All individuals belonged to the Korean population. The mean age of the GO group (39.11±13.23 years, mean ± standard deviation) was similar of that of the GD without GO group (38.84±13.99 years). However, the mean age of the GO group was slightly lower than that of the controls (53.54±9.48 years; $P<0.001$).

Matching of gender was imperfect, the cases had a markedly lower percentage of males (30.1%) than the controls (32.0%), due to GD predominantly affecting females. This variable was further adjusted by the AOR to balance the difference of gender. No significant differences in tHcy or folate levels between the controls and GO groups, or the controls and the GD without GO group were observed.

Serum tHcy concentration was significantly higher in patients with GO compared with those with GD but without GO (8.72±3.60 vs. 10.92±4.44 μmol/l; $P=0.005$). However, the two values were within normal limits. No significant difference in folate concentrations was observed between the GO and GD without GO groups.

Table I. Characterization of the GO, GD without ophthalmopathy patients, and control groups.

Parameter	Control (n=100)	GO (n=72)	P-value	GD (n=50)	P-value
Male (%)	32	22 (30.1)	>0.05	18 (36.0)	>0.05
Age (years, means ± SD)	53.54±9.48	39.11±13.23	<0.0001	38.84±13.99	<0.001
Smokers (n (%))	29	17 (23.9)	>0.05	13 (26.0)	>0.05
TFT status [hyper/euthyroidism (%)]	0/100	69.70/31.30	>0.05	61.0/39.0	>0.05
TSHR Ab (U/l) [0-9][a]	0	16.94±50.0	0.005	18.17±34.36	0.005
TSAb (%) [0-140][a]	0	314.14±211.91	<0.001	176.32±222.36	<0.001
tHcy (μmol/l) [5-15][a]	8.36±2.68	8.72±3.60	>0.05	10.92±4.44	>0.005
Folate (nmol/l) [3.45-13.77][a]	8.30±4.27	8.70±5.52	>0.05	6.83±4.01	>0.05

[a]Normal value range. Values are expressed as the mean ± SD. tHcy concentration was significantly higher in patients with GO compared to those with GD but without GO (8.72±3.60 vs. 10.92±4.44 μmol/l, P=0.005). GD, Grave's disease; GO, Grave's ophthamopathy; TSHR Ab, thyroid-stimulating hormone receptor antibody; TSAb, thyroid-stimulating antibody; SD, standard deviation; tHcy, total homocysteine.

Table II. tHcy and folate levels in Graves' ophthalmopathy patients with the *MTHFR* 677C>T and 1298A>C genotypes.

Parameter	677CC	677CT	677TT	P-value	1298 AA	1298 AC	P-value
tHcy (μmol/l) [5-15][a]	8.66±2.32	7.80±2.32	11.16±5.50	0.008	8.88±4.00	8.33±2.93	0.607
Folate (nmol/l) [3.45-13.77][a]	8.12±4.21	9.85±7.71	7.63±3.24	0.537	8.50±5.27	9.15±6.00	0.701

tHcy concentrations were significantly higher in the 677TT group than those in the CC or CT groups (P=0.008, Kruskal-Wallis test). [a]Normal value. tHcy, total homocysteine.

Table III. *MTHFR* 677C>T and 1298A>C genotype frequencies in GO, GD without ophthalmopathy patients, and controls.

Genotype	Control (n=100)	GO (n=72)	AOR[a] (95% CI)[a]	GD (n=50)	AOR[a] (95% CI)[a]
MTHFR 677C>T					
CC	35 (35.0)	25 (34.2)	1.00 (reference)	10 (20.0)	1.00 (reference)
CT	53 (53.0)	30 (42.5)	0.67 (0.29-1.54)	25 (50.0)	1.96 (0.72-5.34)
TT	12 (12.0)	17 (23.3)	**2.19 (0.78-6.14)**	15 (30.0)	**4.43 (1.23-15.95)**
Dominant (CC vs. CT + TT)			0.97 (0.45-2.06)		2.45 (0.93-6.48)
Recessive (CC + CT vs. TT)			**2.92 (1.11-7.65)**		**2.59 (0.94-7.13)**
MTHFR 1298A>C					
AA	72 (72.0)	49 (68.1)	1.00 (reference)	39 (78.0)	1.00 (reference)
AC	26 (26.0)	23 (31.9)	1.02 (0.46-2.29)	11 (22.0)	0.77 (0.30-1.94)
CC	2 (2.0)	0 (0.0)	NA	0 (0.0)	NA
Dominant (AA vs. AC + CC)			0.97 (0.43-2.15)		0.73 (0.29-1.83)
Recessive (AA + AC vs. CC)			NA		NA

[a]Adjusted by age and gender. The TT homozygous genotype was associated with a two-fold increased risk of GO compared with the healthy controls (AOR, 2.19; 95% CI, 0.78-6.14), however, this difference was not significant. The TT genotype significantly increased the risk of GO compared with that in the normal controls (AOR, 2.92; 95% CI, 1.11-7.65) and it was associated with a significant four-fold increased risk of GD without GO compared with the controls (AOR, 4.43; 95% CI, 1.23-15.95). The TT genotype increased the risk of GD without GO compared with the normal controls due to the recessive effect of the T allele (AOR, 2.59; 95% CI, 0.94-7.13). GO, Graves' ophthalmopathy; GD, Graves' disease; NA, not applicable; AOR, adjusted odds ratio; CI, confidence interval.

No significant differences in tHcy and folate levels were demonstrated between smokers and non-smokers.

Serum tHcy levels of patients with GO according to *MTHFR* polymorphism are presented in Table II. tHcy concentrations

Table IV. *MTHFR* 677C>T and 1298A>C genotype frequencies in GO and GD without ophthalmopathy patients.

Genotype	GO (n=72)	GD (n=50)	AOR[a] (95% CI)
MTHFR 677C>T			
CC	25 (34.2)	10 (20.0)	1.00 (reference)
CT	30 (42.5)	25 (50.0)	0.47 (0.19-1.18)
TT	17 (23.3)	15 (30.0)	0.43 (0.15-1.22)
Dominant (CC vs. CT + TT)			0.48 (0.20-1.11)
Recessive (CC + CT vs. TT)			0.73 (0.32-1.65)
MTHFR 1298A>C			
AA	49 (68.1)	39 (78.0)	1.00 (reference)
AC	23 (31.9)	11 (22.0)	1.66 (0.72-3.83)
CC	0 (0.0)	0 (0.0)	NA
Dominant (AA vs. AC + CC)			1.66 (0.72-3.83)
Recessive (AA + AC vs. CC)			NA

[a]Adjusted by age and gender. GO, Graves' ophthalmopathy; GD, Graves' disease; NA, not applicable; AOR, adjusted odds ratio; CI, confidence interval.

were higher in the 677TT group than those in the CC or CT groups (P=0.008, Kruskal-Wallis test). No differences in folate or tHcy concentrations according to *MTHFR* 1298A>C polymorphism were observed.

Distribution of the MTHFR genotype. Table III presents the distributions of the *MTHFR* 677C>T and 1298A>C genotypes and allele frequencies. The genotype frequencies of *MTHFR* 677C>T were 34.2% (CC), 42.5% (CT), and 23.3% (TT) in patients with GO, 20% (CC), 50% (CT) and 30% (TT) in patients with GD without GO, and 35% (CC), 53% (CT), and 12% (TT) in the control subjects.

The AORs for the TT vs. CC genotypes of the *MTHFR* 677C>T SNP and the AA vs. CC genotypes of the *MTHFR* 1298A>C SNP in the case group are presented in Table IV. The AORs of the GO group in comparison with the healthy controls and the AORs of the GD without GO group compared with the healthy controls were calculated. The TT homozygous genotype was associated with a two-fold increased risk of GO when the patients with GO were compared with the healthy controls (AOR, 2.19; 95% CI, 0.78-6.14); however, the difference was not significant.

In addition, the dominant (TT or CT) vs. CC and recessive TT vs. (CT or CC) effects of the allele were also compared. To investigate any dominant or recessive effects of the T allele, two further comparisons were performed.

Considering the dominant effect of the T allele, the CT or TT genotype did not significantly increase the risk of GO (AOR, 0.97; 95% CI, 0.45-2.06), whereas the TT genotype significantly increased the risk of GO compared with that in the healthy controls (AOR, 2.92; 95% CI, 1.11-7.65; P<0.001). The AA and CC genotypes of the *MTHFR* 1298 A>C SNP were compared, but no significant difference was found.

The TT homozygous genotype was associated with a significant four-fold increased risk of GD without GO when the GD without GO group was compared with the controls (AOR, 4.43; 95% CI, 1.23-15.95; P<0.001). The TT genotype increased the risk of GD without GO compared with that in the healthy controls due to the recessive effect of the T allele (AOR, 2.59; 95% CI, 0.94-7.13). No significant differences in the AOR of 1298 A>C were observed when the GO group was compared with the controls or the GD without GO group was compared with the controls.

The AORs for the TT vs. CC genotype of *MTHFR* 677C>T SNP and AA vs. CC genotype of *MTHFR* 1298 A>C SNP in the GO and GD without GO groups are presented in Table IV. No significant differences in AOR was detected between the GO and GD without GO groups.

A total of six combinations were possible (Table V). However, no individual with 677CC/1298CC occurred in the GD with or without GO groups. No significant differences in AOR were observed when the GO and control groups were compared (data not shown). The AORs for comparisons of the GO and GD without GO groups were calculated. The 677CT/1298AA compound genotype indicated a 74% decreased risk of GO (AOR, 0.26; 95% CI, 0.08-0.91).

In Table VI, MTHFR 677C>T and 1298A>C allele frequencies in the three groups were described and analyzed. No significant differences in AOR were observed when the GO and control groups were compared. However there were significant differences in AOR when the GD and controls were compared (P<0.05). The T allele increased the risk of GD without ophthalmopathy by ~2 times compared with the controls (AOR, 1.95; 95% CI, 1.20-3.18). The haplotype of MTHFR 677T/1298A also increased the risk of GD without ophthalmopathy by ~2 times compared with the controls (AOR, 1.95; 95% CI, 1.16-3.30).

Discussion

GO is the most common extrathyroid manifestation of GD and it affects 25-50% of patients with GD (12-14). GO is manifested by proptosis, orbital inflammation, edema, strabismus

Table V. Analysis of the *MTHFR* 677C>T and 1298A>C combination in GO and GD without ophthalmopathy patients.

Genotype	GO (n=72)	GD (n=50)	AOR (95% CI)[a]
MTHFR 677/1298			
CC/AA	16 (22.2)	5 (10.0)	1.00 (reference)
CC/AC	9 (12.5)	5 (10.0)	0.52 (0.11-2.42)
CC/CC	0 (0.0)	0 (0.0)	NA
CT/AA	16 (22.2)	19 (38.0)	**0.26 (0.08-0.91)**
CT/AC	14 (19.4)	6 (12.0)	0.68 (0.16-2.95)
TT/AA	17 (23.6)	15 (30.0)	0.35 (0.10-1.20)

[a]Adjusted by age and gender. The 677CT/1298AA compound genotype indicated a 74% decreased risk of GO (AOR, 0.26; 95% CI, 0.08-0.91). GO, Graves' ophthalmopathy; GD, Graves' disease; NA, not applicable; AOR, adjusted odds ratio; CI, confidence interval.

Table VI. *MTHFR* 677C>T and 1298A>C allele frequencies in GO, GD without ophthalmopathy patients, and controls.

Genotype	Controls (2n=200)	GO (2n=144)	AOR (95% CI)[a]	GD (2n=100)	AOR (95% CI)[a]
MTHFR 677C>T					
C allele	123 (61.5)	80 (55.6)	1.00 (reference)	45 (45.0)	1.00 (reference)
T allele	77 (38.5)	64 (44.4)	1.28 (0.83-1.97)	55 (55.0)	**1.95 (1.20-3.18)**
MTHFR 1298A>C					
A allele	170 (85.0)	121 (84.0)	1.00 (reference)	89 (89.0)	1.00 (reference)
C allele	30 (15.0)	23 (16.0)	1.08 (0.60-1.95)	11 (11.0)	0.70 (0.34-1.46)
MTHFR 677/1298 haplotype					
C-A	93 (46.5)	57 (39.6)	1.00 (reference)	34 (34.0)	1.00 (reference)
C-C	30 (15.0)	23 (16.0)	1.25 (0.66-2.36)	11 (11.0)	1.00 (0.45-2.22)
T-A	77 (38.5)	64 (44.4)	1.36 (0.85-2.17)	55 (55.0)	**1.95 (1.16-3.30)**

[a]Adjusted by age and gender. MTHFR 677C>T and 1298A>C allele frequencies in three groups were described and analyzed. No significant differences in AOR were observed when the GO and control groups were compared. However there was significant diffreneces in AOR when the GD and controls were compared. T allele increased the risk of GD without ophthalmopathy by ~2 times the risk, when compared with the controls (AOR, 1.95; 95% CI, 1.20-3.18). The MTHFR 677T/1298A haplotype increased the risk of GD without ophthalmopathy by ~2 times, when compared with the controls (AOR, 1.95; 95% CI, 1.16-3.30). GO, Graves' ophthalmopathy; GD, Graves' disease; AOR, adjusted odds ratio; CI, confidence interval.

and optic neuropathy (15). The etiology of the disease requires further elucidation, however, genetic and environmental factors are likely to be involved (9,16).

Previous studies have also aimed to identify gene polymorphisms in patients with GO. Thyroid stimulating hormone receptor gene polymorphisms, for example, rs2268458 in intron 1, do not exhibit notable associations with GO susceptibility (17,18). Similarly, polymorphisms of the protein tyrosine phosphatase non-receptor 22 gene, which encodes an important negative regulator of T-cell activation, are not associated with GO (19). Previous studies into interleukin (IL)-12B, IL-13, and IL-18 have produced similar results (20-22). However, the results of previous studies into the association between cytotoxic T-lymphocyte-associated antigen-4 gene polymorphisms are conflicting, as positive results have been reported for the -318C/T polymorphism (23,24), however, other polymorphisms (for example, -1722A>G, -1661A>G, +49G>A, 60C>T) do not appear to be associated with the development of GO (25). Khalilzadeh *et al* (26) investigated the association between GO with SNPs in the IL-1 family [IL-1a, IL-1b, IL-1 receptor (IL-1R) and IL-1R antagonist (IL-1RA)] and demonstrated that polymorphisms in the IL-1a and IL-1RA genes are significantly associated with GO.

The present study investigated two common *MTHFR* gene polymorphisms to determine their association with susceptibility to GO in patients with GD. The results suggest that the *MTHFR* 677 TT genotype is associated with a 292% increase in the risk of GO (AOR, 2.92; 95% CI, 1.11-7.65), compared with that of the CC + CT genotypes and healthy controls. The *MTHFR* 677CT/1298AA combination exerted a protective effect against GO (AOR, 0.26; 95% CI, 0.08-0.91). Furthermore, from the comparison of MTHFR 677C>T and 1298A>C allele frequencies in three groups, T allele increased the risk of GD without ophthalmopathy by ~2 times compared with the controls (AOR, 1.95; 95% CI, 1.20-3.18). The haplotype of MTHFR 677T/1298A increased the risk of GD without ophthalmopathy

by ~2 times compared with the controls (AOR, 1.95; 95% CI, 1.16-3.30).

tHcy levels in patients without GO were significantly higher than those in patients with GO. However, this result was not significant as the number of cases was too small and the two values were within normal limits.

No difference in tHcy levels between patients with GO and healthy controls was observed. Furthermore, no difference in folate levels was observed among the three groups, and no significant differences in genotype, and tHcy and folate levels between smokers and non-smokers were observed.

As smoking is a known important acquired factor associated with GO, the present study suggests that smoking is associated with genotype, tHcy, or folate. However, no significant results, suggesting that the small number of smokers in the current study and the background of the participants prevented identification of a significant difference. The majority of smokers in Korea are traditionally male, but the majority of participants in the present study were female due to the high prevalence of GD in females.

The majority of previous studies have focused on the *MTHFR* 677C>T polymorphisms associated with stroke, cancer, birth defects, recurrent abortion and cardiovascular disorders (27-32). However, fewer previous studies have assessed the association of a second common polymorphism, 1298A>C, with ischemic stroke, cancer, and heart disease (33,34).

To the best of our knowledge, the present study is the first case-control study to provide evidence for an association between *MTHFR* polymorphisms and GO. As genetic polymorphisms often vary among ethnic groups or geographic areas, further studies are required to further elucidate the association between *MTHFR* polymorphisms and GO in diverse ethnicities.

The present study had a number of limitations as it was conducted in a hospital-based population, and the patient and control groups were small. Other possible limitations are due to the difficulty of diagnosis of GO due to its continuous course. Indeed, patients without GO during the study may develop the condition at a later time. Thus, a large, community-based random population and long-term follow up is required to resolve these limitations in future studies. Furthermore, certain results were determined to be significant using the P-values, however, following application of false discovery rate, the P-values were no longer considered to indicate a significant result, thus, careful interpretation of the results of the present study is required.

Despite these limitations, the current study was the first to focus on associations between the *MTHFR* 677C>T and 1298A>C polymorphisms and GO in a Korean population. It demonstrates that 677TT is an independent risk factor for GO.

Thus, genotype screening of GD patients and special management of those with *MTHFR* 677TT is recommended. Physicians should explain the course of GO to patients, and close follow-up is required. These actions may facilitate early intervention in GO and make patients more comfortable.

In addition, combinations of the *MTHFR* 677/1298 polymorphisms should be assessed in terms of their association with the risk of GO, as the risk is decreased by 677CT/1298AA.

In conclusion, the present study observed an association between GO and *MTHFR* polymorphisms in Koreans. As this is the first study of the association between *MTHFR* and GO, additional investigations that include different and larger populations are required. The results of the current study provide novel insight into the genetic factors in GO, and may facilitate elucidation of the factors associated with its pathogenesis, and development of genetic therapeutic strategies.

Acknowledgements

This study was supported by the Basic Science Research Program through National Research Foundation of Korea Grants funded by the Korean Government (grant no. NRF-2015R1D1A1A09057432), Republic of Korea.

References

1. Abraham P and Acharya S: Current and emerging treatment options for Graves' hyperthyroidism. Ther Clin Risk Manag 6: 29-40, 2010.
2. Ayadi H, Hadj Kacem H, Rebai A and Farid NR: The genetics of autoimmune thyroid disease. Trends Endocrinol Metab 15: 234-239, 2004.
3. Mavragani CP, Patronas N, Dalakas M and Moutsopoulos HM: Ill-defined neurological syndromes with autoimmune background: A diagnostic challenge. J Rheumatol 34: 341-345, 2007.
4. Afeltra A, Vadacca M, Conti L, Galluzzo S, Mitterhofer AP, Ferri GM, Del Porto F, Caccavo D, Gandolfo GM and Amoroso A: Thrombosis in systemic lupus erythematosus: Congenital and acquired risk factors. Arthritis Rheum 53: 452-459, 2005.
5. Petri M, Roubenoff R, Dallal GE, Nadeau MR, Selhub J and Rosenberg IH: Plasma homocysteine as a risk factor for atherothrombotic events in systemic lupus erythematosus. Lancet 348: 1120-1124, 1996.
6. Foffa I, Festa PL, Ait-Ali L, Mazzone A, Bevilacqua S and Andreassi MG: Ascending aortic aneurysm in a patient with bicuspid aortic valve, positive history of systemic autoimmune diseases and common genetic factors: A case report. Cardiovasc Ultrasound 7: 34, 2009.
7. Mao R, Fan Y, Zuo L, Geng D, Meng F, Zhu J, Li Q, Qiao H, Jin Y, Bai J and Fu S: Association study between methylenetetrahydrofolate reductase gene polymorphisms and Graves' disease. Cell Biochem Funct 28: 585-590, 2010.
8. Weetman AP: Graves' disease. N Engl J Med 343: 1236-1248, 2000.
9. Gianoukakis AG and Smith TJ: Recent insights into the pathogenesis and management of thyroid-associated ophthalmopathy. Curr Opin Endocrinol Diabetes Obes 15: 446-452, 2008.
10. Anvari M, Khalilzadeh O, Esteghamati A, Esfahani SA, Rashidi A, Etemadi A, Mahmoudi M and Amirzargar AA: Genetic susceptibility to Graves' ophthalmopathy: The role of polymorphisms in proinflammatory cytokine genes. Eye (Lond) 24: 1058-1063, 2010.
11. Werner SC: Modification of the classification of the eye changes of Graves' disease: Recommendations of the Ad Hoc Committee of the American Thyroid Association. J Clin Endocrinol Metab 44: 203-204, 1977.
12. Gianoukakis AG, Khadavi N and Smith TJ: Cytokines, Graves' disease, and thyroid-associated ophthalmopathy. Thyroid 18: 953-958, 2008.
13. Kuriyan AE, Phipps RP and Feldon SE: The eye and thyroid disease. Curr Opin Ophthalmol 19: 499-506, 2008.
14. Khoo TK and Bahn RS: Pathogenesis of Graves' ophthalmopathy: The role of autoantibodies. Thyroid 17: 1013-1018, 2007.
15. Fatourechi V, Garrity JA, Bartley GB, Bergstralh EJ, DeSanto LW and Gorman CA: Graves ophthalmopathy. Results of transantral orbital decompression performed primarily for cosmetic indications. Ophthalmology 101: 938-942, 1994.
16. Esteghamati A, Khalilzadeh O, Mobarra Z, Anvari M, Tahvildari M, Amiri HM, Rashidi A, Solgi G, Parivar K, Nikbin B and Amirzargar A: Association of CTLA-4 gene polymorphism with Graves' disease and ophthalmopathy in Iranian patients. Eur J Intern Med 20: 424-428, 2009.

17. Yin X, Latif R, Bahn R, Tomer Y and Davies TF: Influence of the TSH receptor gene on susceptibility to Graves' disease and Graves' ophthalmopathy. Thyroid 18: 1201-1206, 2008.
18. Villanueva R, Inzerillo AM, Tomer Y, Barbesino G, Meltzer M, Concepcion ES, Greenberg DA, MacLaren N, Sun ZS, Zhang DM, *et al*: Limited genetic susceptibility to severe Graves' ophthalmopathy: No role for CTLA-4 but evidence for an environmental etiology. Thyroid 10: 791-798, 2000.
19. Ichimura M, Kaku H, Fukutani T, Koga H, Mukai T, Miyake I, Yamada K, Koda Y and Hiromatsu Y: Associations of protein tyrosine phosphatase nonreceptor 22 (PTPN22) gene polymorphisms with susceptibility to Graves' disease in a Japanese population. Thyroid 18: 625-630, 2008.
20. Hiromatsu Y, Fukutani T, Ichimura M, Mukai T, Kaku H, Miyake I and Yamada K: Interleukin-12B gene polymorphism does not confer susceptibility to Graves' ophthalmopathy in Japanese population. Endocr J 53: 753-759, 2006.
21. Chong KK, Chiang SW, Wong GW, Tam PO, Ng TK, Hu YJ, Yam GH, Lam DS and Pang CP: Association of CTLA-4 and IL-13 gene polymorphisms with Graves' disease and ophthalmopathy in Chinese children. Invest Ophthalmol Vis Sci 49: 2409-2415, 2008.
22. Mukai T, Hiromatsu Y, Ichimura M, Fukutani T, Kaku H, Miyake I, Shoji S, Koda Y and Bednarczuk T: Lack of association of interleukin-18 gene polymorphisms with susceptibility of Japanese populations to Graves' disease or Graves' ophthalmopathy. Thyroid 16: 243-248, 2006.
23. Han SZ, Zhang SH, Li R, Zhang WY and Li Y: The common -318C/T polymorphism in the promoter region of CTLA4 gene is associated with reduced risk of ophthalmopathy in Chinese Graves' patients. Int J Immunogenet 33: 281-287, 2006.
24. Zhang Q, Yang YM and Lv XY: Association of Graves' disease and Graves' ophthalmopathy with the polymorphisms in promoter and exon 1 of cytotoxic T lymphocyte associated antigen-4 gene. J Zhejiang Univ Sci B 7: 887-891, 2006.
25. Bednarczuk T, Hiromatsu Y, Fukutani T, Jazdzewski K, Miskiewicz P, Osikowska M and Nauman J: Association of cytotoxic T-lymphocyte-associated antigen-4 (CTLA-4) gene polymorphism and non-genetic factors with Graves' ophthalmopathy in European and Japanese populations. Eur J Endocrinol 148: 13-18, 2003.
26. Khalilzadeh O, Anvari M, Esteghamati A, Mahmoudi M, Tahvildari M, Rashidi A, Khosravi F and Amirzargar A: Graves' ophthalmopathy and gene polymorphisms in interleukin-1alpha, interleukin-1beta, interleukin-1 receptor and interleukin-1 receptor antagonist. Clin Experiment Ophthalmol 37: 614-619, 2009.
27. Frosst P, Blom HJ, Milos R, Goyette P, Sheppard CA, Matthews RG, Boers GJ, den Heijer M, Kluijtmans LA, van den Heuvel LP, *et al*: A candidate genetic risk factor for vascular disease: A common mutation in methylenetetrahydrofolate reductase. Nat Genet 10: 111-113, 1995.
28. Girelli D, Martinelli N, Pizzolo F, Friso S, Olivieri O, Stranieri C, Trabetti E, Faccini G, Tinazzi E, Pignatti PF and Corrocher R: The interaction between MTHFR 677 C->T genotype and folate status is a determinant of coronary atherosclerosis risk. J Nutr 133: 1281-1285, 2003.
29. Heijmans BT, Boer JM, Suchiman HE, Cornelisse CJ, Westendorp RG, Kromhout D, Feskens EJ and Slagboom PE: A common variant of the methylenetetrahydrofolate reductase gene (1p36) is associated with an increased risk of cancer. Cancer Res 63: 1249-1253, 2003.
30. Kim NK, Choi YK, Kang MS, Choi DH, Cha SH, An MO, Lee S, Jeung M, Ko JJ and Oh D: Influence of combined methylenetetrahydrofolate reductase (MTHFR) and thymidylate synthase enhancer region (TSER) polymorphisms to plasma homocysteine levels in Korean patients with recurrent spontaneous abortion. Thromb Res 117: 653-658, 2006.
31. Ko KH, Kim NK, Yim DJ, Hong SP, Park PW, Rim KS, Kim S and Hwang SG: Polymorphisms of 5,10-methylenetetrahydrofolate reductase (MTHFR C677T) and thymidylate synthase enhancer region (TSER) as a risk factor of cholangiocarcinoma in a Korean population. Anticancer Res 26: 4229-4233, 2006.
32. Ou CY, Stevenson RE, Brown VK, Schwartz CE, Allen WP, Khoury MJ, Rozen R, Oakley GP Jr and Adams MJ Jr: 5,10 Methylenetetrahydrofolate reductase genetic polymorphism as a risk factor for neural tube defects. Am J Med Genet 63: 610-614, 1996.
33. Sazci A, Ergul E, Tuncer N, Akpinar G and Kara I: Methylenetetrahydrofolate reductase gene polymorphisms are associated with ischemic and hemorrhagic stroke: Dual effect of MTHFR polymorphisms C677T and A1298C. Brain Res Bull 71: 45-50, 2006.
34. Shen H, Xu Y, Zheng Y, Qian Y, Yu R, Qin Y, Wang X, Spitz MR and Wei Q: Polymorphisms of 5,10-methylenetetrahydrofolate reductase and risk of gastric cancer in a Chinese population: A case-control study. Int J Cancer 95: 332-336, 2001.