Methylenetetrahydrofolate reductase (MTHFR-677 and MTHFR-1298) genotypes and haplotypes and plasma homocysteine levels in patients with occlusive artery disease and deep venous thrombosis

Igor Spiroski¹, Sashko Kedev¹, Slobodan Antov¹, Todor Arsov², Marija Krstevska³, Sloboda Dzhekova-Stojkova³, Gordana Bosilkova³, Stojanka Kostovska⁴, Dejan Trajkov², Aleksandar Petlichkovski², Ana Strezova², Olivija Efinska-Mladenovska² and Mirko Spiroski²

¹Clinic for Cardiology, Faculty of Medicine, ²Institute of Immunobiology and Human Genetics, ³Institute of Medical and Experimental Biochemistry, Faculty of Medicine, University “Ss. Kiril and Metodij”, Skopje, Republic of Macedonia; ⁴Institute of Transfusion, Skopje, Republic of Macedonia

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The aim was to investigate different genotypes and haplotypes of methylenetetrahydrofolate reductase (MTHFR-677, -1298) and plasma concentration of total homocysteine (tHcy) in Macedonian patients with occlusive artery disease (OAD) and deep venous thrombosis (DVT). Investigated groups consists of 80 healthy, 74 patients with OAD, and 63 patients with DVT. Plasma tHcy was measured with Microplate Enzyme Immunoassay. Identification of MTHFR genotypes and haplotypes was done with CVD StripAssay. The probability level (P-value) was evaluated by the Student’s t-test. Plasma concentration of tHcy in CC and CT genotypes of MTHFR C677T was significantly increased in patients with OAD and in patients with DVT. Plasma concentration of tHcy in AC genotype of MTHFR A1298C was increased in patients with OAD and in patients with DVT. Plasma concentration of tHcy was significantly increased in AA genotype of patients with OAD, but not in patients with DVT. We found a significant increase of plasma tHcy in patients with OAD in comparison with healthy respondents for normal:heterozygote (CC:AC), heterozygote:normal (CT:AA), and heterozygote:heterozygote (CT:AC) haplotypes. Plasma concentration of tHcy in patients with DVT in comparison with healthy respondents was significantly increased for normal:normal (CC:AA), normal heterozygote (CC:AC), and heterozygote:heterozygote (CT:AC) haplotypes. We conclude that MTHFR C677T and MTHFR A1289C genotypes and haplotypes are connected with tHcy plasma levels in Macedonian patients with OAD and DVT.

Keywords: MTHFR-677, MTHFR-1298, plasma total homocysteine, occlusive artery disease, deep venous thrombosis, Macedonians

INTRODUCTION

Total homocysteine (tHcy) plasma level is an independent risk marker for venous thrombosis, myocardial infarction, stroke, congestive heart failure, osteoporotic fractures, and Alzheimer disease. tHcy levels are determined by the interaction of genetic and environmental factors. The 677C-T polymor-
phism in the gene encoding 5,10-methylenetetrahydrofolate reductase (MTHFR; 607093.0003) has consistently been associated with plasma tHcy levels. Methylenetetrahydrofolate reductase (EC 1.5.1.20) catalyzes the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, a co-substrate for homocysteine remethylation to methionine.

The human MTHFR gene (MIM *607093) has been localized to chromosome 1p36.3 (Goyette et al., 1994) and is composed of 11 exons (Goyette et al., 1998). MTHFR thermolabile polymorphisms [MTHFR, C677T, A1298C, GLU429ALA] were investigated in several diseases. The mutation in the heterozygous or homozygous state correlated with reduced enzyme activity and increased thermolability in lymphocyte extracts. Individuals homozygous for the mutation had significantly elevated plasma tHcy levels. There have been indications that even a slight excess of homocysteine in blood can result in increased risk of cardiovascular diseases (Refsum & Ueland, 1998), therefore a successful method of plasma homocysteine measurement must be characterised by high accuracy and precision. An improved chromatographic method of total plasma homocysteine measurements was developed (Sawula et al., 2008) in order to obtain higher sensitivity, reliability and reproducibility. But according to others, there is no rationale for measuring the MTHFR C677T variant for clinical purposes (Bezemer et al., 2007). There are a lot of published papers connecting the MTHFR mutations, mostly MTHFR C677T, with plasma tHcy levels. Several meta-analyses showed positive association with vascular diseases (Klerk et al., 2002; Cronin et al., 2005), however, in other meta-analyses associations were not found (Lewis et al., 2005; Ariyaratnam et al., 2007; Keijzer et al., 2007).

Genomewide linkage scan for genes affecting plasma Hcy levels have shown the strongest linkage signal on 11q23 in the vicinity of the NNMT (nicotinamide N-methyltransferase) gene, which is involved in the metabolism of homocysteine. Haplotype analyses of ten SNPs within this gene identified one haplotype associated with plasma Hcy levels \( p = 0.0003 \). It was concluded that the NNMT gene may be a major genetic determinant of plasma homocysteine levels in Spanish families and that since this gene encodes an enzyme involved in homocysteine synthesis, the finding would be consistent with known biochemical pathways (Souto et al., 2005).

We published a distribution of the total homocysteine values in female Macedonian population and found normal distribution with mean value \( \pm S.D. \) of 7.4±2.8 μmol/L (Krstevska et al., 2001). Plasma tHcy concentrations were not significantly higher in postmenopausal than in premenopausal women (Krstevska, 2001). We analyzed association of methylenetetrahydrofolate reductase (MTHFR-677, -1298) polymorphisms with occlusive artery disease and deep venous thrombosis in Macedonians, and concluded that significant association of MTHFR-677, and -1289 polymorphisms with occlusive artery disease or venous thrombosis in Macedonians was not found, except for the protective association between the MTHFR/CA:CC diplotype and occlusive artery disease (Spiroski et al., 2008). The aim of this study was to investigate total homocysteine plasma concentration in different genotypes and haplotypes of methylenetetrahydrofolate reductase (MTHFR-677, -1298) in Macedonian patients with occlusive artery disease (OAD) and deep venous thrombosis (DVT).

**MATERIALS AND METHODS**

**Investigated groups.** The total studied sample consists of 217 individuals composed of three different groups: healthy individuals, patients with occlusive artery disease, and patients with deep venous thrombosis. a) Healthy individuals \((n=80)\), 40 female and 40 male, aged 40.7±11.3 years, born in different parts of Macedonia attending the Institute for Transfusion for blood donation. Inclusion of healthy individuals was random, if medical doctor declared their health as acceptable (on the basis of medical documentation, completed interview, and physical examination). From the investigation were excluded individuals with family history of vascular diseases. b) Occlusive artery disease \((n=74)\), 28 female and 46 male patients with proved and documented myocardial infarct \((n=2)\), brain infarct \((n=20)\), or peripheral artery thrombosis \((n=2)\), aged 63.3±9.6 years hospitalized at the Institute of Heart Diseases, Faculty of Medicine, and Institute for Transfusion, Skopje for outpatient treatment. c) Deep venous thrombosis \((n=63)\), 43 female and 20 male patients (diagnosed by ultrasonography and/or venography), aged 57.7±11.8 years, attending the Institute of Heart Diseases, Faculty of Medicine, and Institute for Transfusion, Skopje for outpatient treatment.

All individuals are of Macedonian origin, and residents of different geographical areas of the Republic of Macedonia. All patients and healthy individuals included in this study signed a written consent to participate in the study which was approved by the Committee of the Ministry of Education and Science from the Republic of Macedonia (No. 13-1672/4-02).

**Genomic DNA isolation and storage.** Blood samples were collected after written consent; DNA was isolated from peripheral blood leukocytes by the phenol-chlorophorm extraction method or with BioRobot EZ1 workstation (QIAGEN) (Towner,
The quality and quantity of DNA was analyzed by GeneQuant (Pharmacia). Isolated DNA samples were stored in Macedonian Human DNA Bank (hDNAMKD) (Spiroski et al., 2005).

**Typing methods.** Assay for the identification of MTHFR mutations is based on polymerase chain reaction (PCR) and reverse-hybridization with CVD StripAssay (ViennaLab Labordiagnostica GmbH, Austria). The procedure includes three steps: 1) DNA isolation, 2) PCR amplification using biotinylated primers, 3) hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences are detected using streptavidin-alkaline phosphatase and colour substrates. The assay covers two mutations: MTHFR C677T and MTHFR A1298C. The genotype of a sample is determined using the enclosed Collector™ sheet or using the software StripAssay Evaluator, ver. 2.0, ViennaLab Diagnostics GmbH.

**Total plasma homocysteine determination.** Blood samples were taken after 12 h fasting from the antecubital vein. After about 45 min the samples were centrifuged at 3000 × g for 10 min. Plasma fractions were aspirated and transferred to plastic tubes and were stored at −20°C until analyzed. Total plasma homocysteine was measured with a Microplate Enzyme Immunoassay, EIA, method (Bio Rad Laboratories, USA) (Frantzen et al., 1998).

**Statistical methods.** The population genetics analysis package, PyPop, (Lancaster et al., 2003; Single et al., 2007) was used for analysis of the MTHFR data for this report. Plasma concentration of total homocysteine data were analyzed using standard statistical program Statgraphics Plus for Windows ver. 2.1 (Microsoft Corp., Redmond, WA, USA). The probability level (P-value) was evaluated by the Student’s t-test. The results are presented as the arithmetic mean ± standard deviation (S.D.). P values of 0.05 or less were considered significant.

**RESULTS**

**Genotypes of MTHFR and total homocysteine**

Plasma concentration of total homocysteine (mean value ± standard deviation, in μmol/L) in different genotypes of MTHFR C677T in healthy respondents, patients with occlusive artery disease (OAD), and patients with deep venous thrombosis (DVT) is given in Fig. 1.

We can see that the lowest plasma concentration of total homocysteine was found in healthy respondents with CC genotype of MTHFR C677T (10.78 ± 2.59 μmol/L), with small (insignificant) increase in CT genotype (11.44 ± 2.81 μmol/L, P = 0.314), and significant increase in TT genotype of MTHFR C677T (14.83 ± 5.49 μmol/L, P < 0.001). Plasma concentration of total homocysteine in CC genotype of MTHFR C677T was additionally increased in patients with occlusive artery disease (13.47 ± 3.18 μmol/L, P < 0.001), and in patients with deep venous thrombosis (14.36 ± 3.42, P < 0.001). Plasma concentration of total homocysteine was significantly increased in CT genotype of patients with occlusive artery disease (16.13 ± 6.41 μmol/L, P < 0.001), and patients with deep venous thrombosis (13.24 ± 3.24 μmol/L, P < 0.001). Plasma concentration of total homocysteine in TT genotype of MTHFR C677T was not significantly different between healthy respondents and patients with occlusive artery disease, and patients with deep venous thrombosis. There was not significant difference in plasma concentration of total homocysteine between genotypes of MTHFR C677T in patients with occlusive artery disease and deep venous thrombosis.

Plasma concentration of total homocysteine in different genotypes of MTHFR A1298C in healthy respondents, patients with occlusive artery disease (OAD), and patients with deep venous thrombosis (DVT) is given in Table 1.

As shown in Table 1, the lowest plasma concentration of total homocysteine was found in healthy respondents with AC (heterozygote) genotype of MTHFR A1298C (10.79 ± 2.65 μmol/L), with small (insignificant) increase in CC genotype (11.37 ± 2.14 μmol/L, P = 0.714), and significant increase in AA genotype (12.50 ± 4.02 μmol/L, P = 0.030). Plasma concentration of total homocysteine in AC genotype of MTHFR A1298C was...
additionally increased in patients with occlusive artery disease (15.86 ± 5.86 μmol/L, P < 0.001), and in patients with deep venous thrombosis (14.45 ± 3.08, P < 0.001). Plasma concentration of total homocysteine was significantly increased in AA genotype of patients with occlusive artery disease (15.68 ± 5.82 μmol/L, P = 0.038), but not in patients with deep venous thrombosis (13.75 ± 3.36 μmol/L, P = 0.167). Plasma concentration of total homocysteine in CC genotype of MTHFR A1298C was not significantly different between healthy respondents and patients with occlusive artery disease, and patients with deep venous thrombosis. There was not significant difference in plasma concentration of total homocysteine between genotypes of MTHFR A1298C in patients with occlusive artery disease. Patients with deep venous thrombosis with CC genotype of MTHFR A1298C had lowest plasma concentration of total homocysteine (8.40 ± 1.70 μmol/L), while it was significantly increased in AA (13.75 ± 3.36 μmol/L, P = 0.034) and AC genotype (14.45 ± 3.08 μmol/L, P = 0.011).

### Haplotypes of MTHFR and total homocysteine

Plasma concentration of total homocysteine in different haplotypes of MTHFR C677T:A1298C is lowest in normal: normal (CC:AA) haplotype in healthy respondents (10.94 ± 2.06 μmol/L) and in patients with occlusive artery disease (12.24 ± 2.91 μmol/L), and is increased in the haplotypes with combinations of heterozygotes and homozygotes. Statistically significant increase was found only for the haplotype heterozygote:heterozygote (CT:AC) in patients with occlusive artery disease (P = 0.049). The lowest plasma concentration of total homocysteine in patients with deep venous thrombosis was found in the normal:homozygote (CC:CC) haplotype (8.40 ± 1.70, P = 0.021), but the number of samples is too small to be accepted.

### Table 1. Different genotypes of MTHFR A1298C and plasma concentration of total homocysteine in healthy respondents, patients with occlusive artery disease, and patients with deep venous thrombosis

<table>
<thead>
<tr>
<th>MTHFR A1298C genotype</th>
<th>Healthy (n = 80)</th>
<th>OAD (n = 74)</th>
<th>DVT (n = 63)</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>AA (wild)</td>
<td>12.50 ± 4.02 (38)</td>
<td>15.68 ± 5.82 (45)</td>
<td>13.75 ± 3.36 (32)</td>
<td>0.038</td>
<td>0.167</td>
</tr>
<tr>
<td>AC (heterozygote)</td>
<td>10.79 ± 2.65 (39)</td>
<td>15.86 ± 5.86 (25)</td>
<td>14.45 ± 3.08 (29)</td>
<td>0.0001</td>
<td>0.0001</td>
</tr>
<tr>
<td>CC (homozygote)</td>
<td>11.37 ± 2.14 (3)</td>
<td>14.50 ± 2.24 (4)</td>
<td>8.40 ± 1.70 (2)</td>
<td>0.121</td>
<td>0.203</td>
</tr>
<tr>
<td>P (AA/AC)</td>
<td>0.030</td>
<td>0.925</td>
<td>0.401</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (AA/CC)</td>
<td>0.635</td>
<td>0.785</td>
<td>0.034</td>
<td></td>
<td></td>
</tr>
<tr>
<td>P (AC/CC)</td>
<td>0.714</td>
<td>0.654</td>
<td>0.011</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations: P1, significant difference between healthy respondents and patients with occlusive artery disease; P2, significant difference between healthy respondents and patients with deep venous thrombosis.

### Table 2. Different haplotypes of MTHFR C677T:A1298C and plasma concentration of total homocysteine in healthy respondents, patients with occlusive artery disease, and patients with deep venous thrombosis

<table>
<thead>
<tr>
<th>MTHFR haplotype</th>
<th>Healthy (n = 80)</th>
<th>OAD (n = 74)</th>
<th>DVT (n = 63)</th>
<th>P1</th>
<th>P2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal:Normal (CC:AA)</td>
<td>10.94 ± 2.06 (7)</td>
<td>12.24 ± 2.91 (9)</td>
<td>14.85 ± 2.68 (6)</td>
<td>0.334</td>
<td>0.013</td>
</tr>
<tr>
<td>Normal:Heterozygote (CC:AC)</td>
<td>10.66 ± 2.84 (24)</td>
<td>14.05 ± 3.54 (12)</td>
<td>15.11 ± 3.09 (12)</td>
<td>0.863</td>
<td>0.004 &lt;0.001</td>
</tr>
<tr>
<td>Normal:Homozygote (CC:CC)</td>
<td>11.37 ± 2.14 (3)</td>
<td>14.50 ± 2.24 (4)</td>
<td>8.40 ± 1.70 (2)</td>
<td>0.021</td>
<td>0.122 0.203</td>
</tr>
<tr>
<td>Heterozygote:Normal (CT:AA)</td>
<td>11.77 ± 3.10 (20)</td>
<td>15.43 ± 6.05 (26)</td>
<td>12.44 ± 3.30 (16)</td>
<td>0.126</td>
<td>0.018 0.535</td>
</tr>
<tr>
<td>Heterozygote:Heterozygote (CT:AC)</td>
<td>10.99 ± 2.39 (15)</td>
<td>17.53 ± 7.13 (13)</td>
<td>13.99 ± 3.08 (17)</td>
<td>0.507</td>
<td>0.002 0.005</td>
</tr>
<tr>
<td>Heterozygote:Homozygote (TT:AA)</td>
<td>14.83 ± 5.49 (11)</td>
<td>19.42 ± 14.78 (10)</td>
<td>15.19 ± 3.26 (10)</td>
<td>0.833</td>
<td>0.348 0.859</td>
</tr>
</tbody>
</table>

*Abbreviations: P, significant difference with Normal:Normal (CC:AA) haplotype; P1, significant difference between healthy respondents and patients with occlusive artery disease; P2, significant difference between healthy respondents and patients with deep venous thrombosis.
We found a significant increase of plasma total homocysteine in patients with occlusive artery disease in comparison with healthy respondents for normal:heterozygote (CC:AC) \((P1=0.004)\), heterozygote:normal (CT:AA) \((P1=0.018)\), and heterozygote: homozygote (CT:AC) \((P1=0.002)\) haplotypes. Plasma concentration of total homocysteine in patients with deep venous thrombosis in comparison with healthy respondents was significantly increased for normal:normal (CC:AA) \((P2=0.013)\), normal heterozygote (CC:AC) \((P2<0.001)\), and heterozygote:homozygote (CT:AC) \((P2=0.005)\) haplotypes (Table 2).

Examining the effect of the combination of both mutations (Table 2), the 677TT genotype was always associated with the normal 1298AA genotype in all individuals, and the 1298CC genotype was always associated with the normal 677CC genotype. Double mutant homozygous individuals were not observed.

**DISCUSSION**

In this manuscript we report plasma concentration of total homocysteine (tHcy) in different genotypes and haplotypes of methylenetetrahydrofolate reductase gene (MTHFR-677, -1298) in Macedonian patients with occlusive artery disease and deep venous thrombosis.

We found that plasma concentration of tHcy in CC and CT genotypes of MTHFR C677T was significantly increased in patients with OAD and in patients with DVT. Plasma concentration of tHcy in AC genotype of MTHFR A1298C was also increased in patients with OAD and DVT. Plasma concentration of tHcy was significantly increased in AA genotype of patients with OAD, but not in patients with DVT. Statistically significant increase was found only for the haplotype CT:AC in patients with OAD. We found a significant increase of plasma concentration of tHcy in patients with OAD for CC:AC, CT:AA, and CT:AC haplotypes, as well as in patients with DVT for CC:AA, CC:AC, and CT:AC haplotypes.

Our results in patients with occlusive artery disease are in agreement with those of others where the most important genetic determinant of tHcy in the general population is the common C677T variant of methylenetetrahydrofolate reductase gene (MTHFR) that results in higher tHcy. Individuals with the homozygous mutant (TT) genotype have a significantly higher (14–21%) risk of heart disease. Plasma tHcy is very responsive to intervention with the B vitamins required for its metabolism, in particular folic acid, and to a lesser extent vitamins B\(_{12}\) and B\(_6\). Thus, although primarily aimed at reducing neural-tube defects (van der Linden et al., 2006), folic acid fortification may have an important role in the primary prevention of CVD via tHcy lowering (Sazci et al., 2006; Kothekar, 2007; Freitas et al., 2008; Ghazouani et al., 2008; McNulty et al., 2008; Poduri et al., 2008).

Increased tHcy levels in our patients with deep venous thrombosis are similar with several published results (den Heijer et al., 1998; Wald et al., 2002; Hotoleanu et al., 2007). Meta-analysis of prospective and retrospective studies demonstrates a modest association of homocysteine with venous thrombosis (den Heijer et al., 2005). In a single large study, MTHFR C677T was not associated with the risk of venous thrombosis, and the narrow confidence interval excludes even a small effect. Therefore, mildly elevated homocysteine levels as a result of MTHFR 677TT do not seem to cause venous thrombosis.

In several papers the polymorphisms C677T and A1298C of MTHFR and fasting plasma homocysteine levels do not seem to be significant risk factors for venous thromboembolic disease (Salmon et al., 2001; Domagala et al., 2002). At present, the status of homocysteine as a target for intervention in the prevention of atherothrombotic arterial and venous disease is uncertain. Current evidence does not support the use of B vitamin supplements to reduce vascular risk (den Heijer et al., 2005).

Homocysteine level is not dependent on MTHFR gene mutations. There is a report that nicotine/N-n-methyltransferase (NNMT) gene may be a major genetic determinant of plasma homocysteine levels and that since this gene encodes an enzyme involved in homocysteine synthesis, the finding would be consistent with known biochemical pathways (Souto et al., 2005). Plasma homocysteine was negatively associated with plasma vitamin B\(_{12}\) concentration and plasma folate, with the degree of correlation between plasma vitamin B\(_{12}\) and homocysteine concentrations dependent on MTHFR genotype (Bailey et al., 2002). The results showed that tHcy at baseline was significantly higher for the 677TT genotype group compared to the 677CC genotype group and that this group responded with a significantly larger increase in tHcy upon coffee exposure than the 677CC and 677CT genotype groups (Strandhagen et al., 2004). Catechol-O-methyltransferase (COMT) Val(158) carriers had significantly higher tHcy than Met(158) homozygotes. The effect was limited to individuals homozygous for the MTHFR T(677) allele. In addition, individuals homozygous for the COMT G(-287) allele tended to have lower tHcy levels. High activity variants of COMT interact with the low activity variant of MTHFR to increase tHcy levels (Tunbridge et al., 2008).

We reported results from MTHFR-677, and -1289 polymorphisms on the same cohort (identical healthy participants and patients as in this study) in
which association with occlusive artery disease and deep venous thrombosis was not found, except for the protective MTHFR/CA:CC diplotype with artery occlusive disease (Spiroski et al., 2008). The most frequent MTHFR-677 genotype in healthy participants was CT with observed frequency of 44.6%, lower frequency was found for CC genotype (42.2%), and the lowest frequency was found for TT genotype, 13.2%. The frequencies of MTHFR-677 CT and TT genotypes were slightly increased in patients with occlusive artery disease and deep venous thrombosis, with a consecutive decrease of CC genotype. All genotypes in healthy participants and patients with blood vessel disease showed a good fit with Hardy-Weinberg equilibrium. The most frequent MTHFR-1298 genotype in healthy participants was AA with observed frequency of 49.4%, lower frequency was found for CA genotype, and the lowest frequency was found for CC genotype. The frequencies of MTHFR-1298 genotypes AA and CC were slightly increased in patients with occlusive artery disease and deep venous thrombosis, with a consecutive decrease of CA genotype. All genotypes in healthy participants and patients with blood vessel disease showed a good fit with Hardy-Weinberg equilibrium.

The significant differences of plasma total homocysteine levels in healthy participants and patients with OAD and DVT with different genotypes and haplotypes of MTHFR-677 and -1298, in spite of a lack of association with MTHFR-677 and -1298 polymorphisms, can be explained with additional genetic and/or external interactions. DNA samples from 6793 participants in the third National Health and Nutrition Examination Survey (NHANES III) during 1991–1994 were genotyped for polymorphisms of genes coding for folate pathway enzymes 5,10-methylenetetrahydrofolate reductase (MTHFR) 677C3T and 1298A3C, methionine synthase reductase (MTRR) 66A3G, and cystathionine-β-synthase 844ins68 (Yang et al., 2008). The results suggest that dietary intake of folic acid can attenuate significantly the negative impact on serum folate and homocysteine of selected polymorphisms of folate-related genes, which could be one of the explanations for our data.

We noticed that the 677TT genotype was always associated with the normal 1298AA genotype in all individuals, and the 1298CC genotype was always associated with the normal 677CC genotype. Double mutant homozygous individuals were not observed in this study. Similar data were reported by others (Freitas et al., 2008; Spiroski et al., 2008). In the meta-analysis with reliable data on combined MTHFR genotypes in general populations (n=5389) the combined data comprised the following totals for each genotype at nucleotide positions 677 and 1298: 838 CC/AA (i.e., 677CC/1298AA), 1225 CC/AC, 489 CC/CC, 1120 CT/AA, 1093 CT/AC, 8 CT/CC, 606 TT/AA, 10 TT/AC, and 0 TT/CC. The estimated haplotype frequencies, and the fractional contribution of each, were 677C/1298A, 0.37; 677C/1298C, 0.31; 677T/1298A, 0.32; and 677T/1298C, 0.0023 to 0.0034. Thus, a vast majority of 677T alleles and 1298C alleles are associated with 1298A alleles and 677C alleles, respectively (Ogino & Wilson, 2003). The absence of MTHFR diplotypes in Macedonians could be as a result of selective pressures or of the small frequencies in the investigated groups.

In summary, MTHFR C677T and MTHFR A1289C genotypes and haplotypes are connected with tHcy plasma levels in Macedonian patients with occlusive artery disease and deep venous thrombosis. The results can be part of the complex interaction between candidate genes and external factors responsible for cardiovascular diseases in Macedonians.

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