



Polymorphisms C677T and A1298C of *MTHFR* Gene: Homocysteine Levels and Prothrombotic Biomarkers in Coronary and Pulmonary Thromboembolic Disease

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Abstract

The activity of the enzyme methylenetetrahydrofolate reductase (*MTHFR*) determines homocysteine (Hcy) levels, and polymorphisms in its gene affect the activity of the enzyme. Changes in the enzyme's activity may lead to a higher susceptibility to develop arterial and venous thromboembolic disease. The aim was to analyze the relationship between the C677T and A1298C polymorphisms of *MTHFR*, Hcy levels, and prothrombotic biomarkers in pulmonary embolism (PE) and acute myocardial ischemia (AMI). Clinical files of patients with thromboembolic diseases having complete data and whose doctor had requested an assay to determine the polymorphisms of the *MTHFR* gene, Hcy levels, and prothrombotic biomarkers were studied to search for the correlation between mutations of the *MTHFR* gene and Hcy levels in the different diseases. We included 334 files: 158 were from women and 176 from men (51 [19 SD] years). Sixty-three percent have had thrombosis, 8% AMI, and 31% PE. Patients with thrombosis had elevated frequency of the C677T polymorphism. The CC genotype was higher than the TT genotype ($P = .003$) and CT versus the TT ($P = .009$). In patients with PE, the CC genotype was higher than the TT genotype ($P = .038$). Pulmonary embolism with massive and submassive events had predominant genotypes 677 TT ($P = .003$) and the AA 1298 ($P = .017$). Elevated Hcy levels in the presence of the T allele in the C677T gene and of the A allele in the A1298C gene are associated with AMI and massive and submassive PE.

Keywords

MTHFR, coronary disease, pulmonary thromboembolism, polymorphisms C677T and A1298C

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Introduction

Venous thromboembolic disease (VTD) includes deep vein thrombosis (DVT) and pulmonary embolism (PE). It is the third most common cause of cardiovascular disease having an incidence of 100 to 200 per 100 000 inhabitants. Although VTD and PE may be fatal in their acute or chronic phases, they can be prevented.¹ Smoking² and other endovascular and coagulation risk factors are associated with these diseases.³ Polymorphisms of the gene encoding for the methylenetetrahydrofolate reductase (*MTHFR*) have also been related to an increased susceptibility to develop VTD.

Methylenetetrahydrofolate reductase regulates homocysteine (Hcy) levels.⁴ Homocysteine is an intermediate amino

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acid containing a sulfhydryl group that comes from methionine methylation.⁵ A transference of a methyl group of the 5-methyltetrahydrofolate to Hcy by MTHFR is required during Hcy metabolism.⁶ For this reason, it has been proposed that metabolic alterations in the enzyme's activity could lead to total homocysteine (tHcy) accumulation and lead to the appearance of some pathologies such as rheumatoid arthritis, cancer,^{7,8} or vascular occlusive diseases.^{9,10}

Polymorphisms of the *MTHFR* gene could affect the enzyme's activity. The *C677T* polymorphism has been associated with an increase in Hcy.¹¹ Homozygote individuals with the *C677T* polymorphism have increased plasma tHcy concentrations and this mutation is prevalent in coronary artery disease.¹¹⁻¹⁸ A meta-analysis in an English population concluded that *C677T* and *A1298C* polymorphisms were associated with elevated plasma Hcy in acute myocardial ischemia (AMI), cerebral vascular events, DVT, and PE.¹⁹ Other studies showed that homozygous *677TT* patients have a higher risk of heart disease than homozygous *677CC* individuals.²⁰ There are few studies analyzing the frequency of *C677T* and/or *A1298C* polymorphisms in coronary disease, PE, and their association with DVT.

The analysis of the risk factors associated with these polymorphisms through retrospective studies could lead to a better knowledge of factors prevalent in affected patients. Based on this fact, the aim of the present study was to evaluate the relationship between the *C677T* and *A1298C* polymorphisms of MTHFR enzyme, different prothrombotic biomarkers, and Hcy levels in patients who have had PE and AMI.

Patients and Material and Methods

This was a retrospective study done between March 2011 and December 2016. Six hundred fifty-five studies of the *C677T* and *A1298C* gene polymorphisms of the *MTHFR* gene on patients having had coronary, pulmonary, or other location arterial thrombosis and on whom Hcy, anti-B2 glycoprotein 1, anticardiolipin, and C and S protein had been measured were analyzed. Only 334 files from patients having complete data were included. Patients with missing data were excluded. The determinations of the *C677T* and *A1298C* gene polymorphisms of *MTHFR* were done by PCR with analysis of melting–fusion curves. For the determination of the *C677T* and *A1298C* polymorphism, the “LightMix Kit MTHFR” from Roche (Barcelona, Spain) was employed. The antithrombin activity was determined using the Berichrom kit that determines its plas-matic activity through automatic analyzers. The in vitro coagulometric Siemens kit (España) was used to determine factors II and VIII. FVIII antigen levels were measured at screening by enzyme immunoassay using the Asserachrom VIII:Ag kit (Stago, Asnières sur Seine, France) in all analyzed patients. Factor V: The activity of FV was determined to each of the samples. The assay was processed by coagulometric method in an STArt semiautomatic coagulometer (Diagnostica Stago SAS, Asnières sur Seine, France), which was used for the mechanical method detection. The reagents used for the measurement of FV were STA-Unicalibrator, Owren buffer pH

7.35, STA-PreciClot Plus I, STA-Factor V, and STA-Neoplastin (Diagnostica Stago SAS).

Plasma D-dimer levels were evaluated using an automated latex-enhanced quantitative immunoturbidimetric assay, performed with a Sysmex CA-1500 instrument (Sysmex Corporation, Kobe, Japan). The Innovance D-Dimer kit and its quality control materials and calibrators were produced by Siemens AG (Munich, Germany). According to the manufacturer's instructions, a value of 0.5 mg/L fibrinogen equivalent units was selected as the threshold for excluding thromboembolic diseases. The patients were segregated on the basis of their serum.

Detection of binding to cardiolipin by modified (serum-free) ELISA: In order to eliminate β 2GPI from a standard CL ELISA, antibody combinations were first expressed in a serum-free postelectroporation medium containing Ultrosor G (Gibco, Paisley, United Kingdom) in place of fetal calf serum (FCS). A modified CL ELISA was performed by replacing 10% FCS with 10% Bovine Serum Albumin (BSA) (Sigma, Poole, United Kingdom) in each relevant step. The method was otherwise identical to the standard CL ELISA.

Free protein S levels were determined using the Asserachrom Free Protein S immunoassay (Diagnostica Stago), which utilizes a monoclonal antibody sandwich technique. High-sensitivity C-reactive protein (hs-CRP) concentration was determined by immunonephelometry (Cardiophase hs-CRP; Siemens Healthcare Diagnostics Products GmbH, Marburg, Germany) on a BN ProSpec nephelometer according to the manufacturer's procedures. Inter and intra-assay coefficients of variation were less than 6%.

Statistical Analysis

Excel software and SPSS version 19 were used. Nominal and dichotomic variables were reported as percentages and quantitative variables having a normal distribution were reported as mean and standard deviation. Correlation analysis was done with the Spearman correlation test. A significant statistical difference was considered when a $P > .05$ value was reached.

Results

Files from 655 individuals were obtained; however, only 334 had the complete set of data. One hundred fifty-eight (47%) files were from women and 176 (53%) were from men. The average age was of 51 (19) years. Determination of the other prothrombotic biomarkers and thrombosis was present in 63% of these reports. Of the 334 individuals with complete data, 212 patients have had thrombosis, and out of these, 16 patients (8%) have had AMI and 65 patients (31%) have had PE.

The clinical characteristics of the patients divided by gender are shown in Table 1. There were significant differences in age ($P = .02$), total cholesterol ($P = .01$), high-density lipoprotein cholesterol ($P = .0001$), and smoking habits ($P = .08$). The distribution of thrombophilic biomarkers in patients with and without thrombosis, AMI, and PE is shown in Table 2. In

Table 1. Demographic Characteristics of Patients.^a

	Males	Females	P
Age (years)	54 (18)	49 (18)	.02
Platelets (mm ³ /mL)	280 000 (36-511 000)	346 500 (28 000-684 000)	.052
Triglycerides (mg/dL)	129 (27-4320)	123 (41-1224)	NS
Homocysteine	11 (2-198)	10 (3-144)	NS
D-dimer (ng/mL)	1450 (36-10 000)	2044 (73-12 000)	NS
Total cholesterol (mg/dL)	183 (54)	191 (58)	.01
HDL-C (mg/dL)	38 (16)	50 (22)	.0001
C protein (%)	87 (30)	86 (30)	NS
S protein (%)	83 (25)	83 (21)	NS
Antithrombin III	79 (28)	78 (27)	NS
Factor VIII (%)	118 (30)	120 (33)	NS
Dyslipidemia (%)	56 (17%)	66 (20%)	NS
Smoking habit (%)	88 (26%)	64 (19%)	.08

Abbreviations: HDL-C, high-density lipoprotein cholesterol; NS, Non significant; SD, standard deviation.

^aValues are expressed as mean (SD) and in median (min-max) accordingly.

Table 2. Distribution of Thrombophilia Biomarkers in Patients With and Without General Thrombosis, Acute Myocardial Ischemia, and Pulmonary Thromboembolism.

	Total (N = 334), n (%)	Without Thrombosis (n = 122), n (%)	With Thrombosis (n = 212), n (%)	P
Increased homocysteine >12.4	123 (31)	38 (32)	85 (41)	.07
Low C protein <70% activity	65 (23)	27 (26)	38 (21)	NS
Low S protein <70% activity	54 (20)	23 (23)	31 (18)	NS
Positive anticardiolipin	13 (5)	1 (1)	12 (7)	.03
Antithrombin III <70% activity	95 (30)	27 (24)	68 (34)	.056
Increased D-dimer	255 (79)	84 (71)	171 (83)	.01
Factor VIII <70% activity	9 (4)	8 (4)	1 (3)	NS
Factor V Leiden	32 (12)	5 (6)	27 (14)	.02
		Without AMI (n = 298)	With AMI (n = 16)	P
Increased homocysteine >12.4	123 (38)	113 (37)	10 (63)	.03
Low C protein <70% activity	65 (23)	63 (23)	2 (15)	NS
Low S protein <70% activity	54 (20)	50 (19)	4 (33)	.18
Positive anticardiolipin >15 U	13 (5)	12 (5)	1 (11)	NS
Antithrombin III <70% activity	95	91 (30)	4 (27)	NS
Increased D-dimer >500 ng/mL	255	245 (80)	10 (63)	.11
Factor VIII <70% activity	9 (4)	9 (4)	0	NS
Factor V Leiden	32 (12)	31 (12)	1 (9)	
		Without PTE (n = 253)	With PTE (n = 65)	P
Increased homocysteine >12.4	123 (38)	94 (36)	29 (44)	.15
Low C protein <70% activity	65 (23)	51 (22)	14 (25)	NS
Low S protein <70% activity	54 (20)	45 (20)	9 (16)	NS
Positive anticardiolipin	13 (5)	7 (3)	6 (11)	.03
Antithrombin III <70% activity	95 (30)	77 (30)	18 (30)	NS
Increased D-dimer	255 (79)	191 (74)	64 (99)	.0001
Factor VIII <70% activity	9 (4)	8 (4)	1 (3)	NS
Factor V Leiden	32 (12)	20 (9)	12 (19)	.02
		Without DVT	With DVT	P
Increased homocysteine >12.4	132 (40)	86 (36)	46 (48)	.03
Low C protein <70% activity	65 (23)	47 (24)	18 (21)	NS
Low S protein <70% activity	54 (20)	41 (21)	13 (16)	NS
Positive anticardiolipin	13 (5)	9 (5)	4 (5)	NS
Antithrombin III <70% activity	95 (30)	60 (27)	35 (38)	.06
Increased D-dimer	255 (79)	168 (73)	87 (93)	.0001
Factor VIII <70% activity	9 (4)	5 (3)	4 (5)	NS
Factor V Leiden	32 (12)	13 (7)	19(21)	.001

Abbreviations: AMI, acute myocardial ischemia; DVT, deep venous thrombosis; PTE, pulmonary thromboembolic; NS, Non significant.

Table 3. Genotypic and Allelic Frequencies of the *C677T* and *A1298C* Gene According to the Type of Disease.

<i>C677T</i>	CC, n (%)	CT, n (%)	TT, n (%)	C, n (%)	T, n (%)
With thrombosis	77 (36.3)	106 (50)	29 (13.7)	260 (61.3)	164 (38.6) ^a
Without thrombosis	33 (27)	54 (44.3)	35 (28.7)	120 (49.1)	124 (50.8)
With DVT	25 (26.3)	55 (57.9) ^b	15 (15.8)	105 (55.2)	85 (44.7)
Without DVT	85 (35.6)	105 (43.9)	49 (20.5)	275 (57.5)	203 (42.5)
With PTE	19 (27.9)	42 (61.7) ^b	7 (10.3) ^b	80 (58.8)	56 (41.2)
Without PTE	92 (34.3)	119 (44.4)	57 (21.3)	303 (56.5)	233 (43.4)
With AMI	3 (18.7)	9 (56.2)	4 (25)	15 (46.9)	17 (53.1)
Without AMI	107 (33.6)	151 (47.5)	60 (18.9)	365 (57.3)	271 (42.6)
<i>A1298C</i>	AA, n (%)	CC, n (%)	CC, n (%)	A, n (%)	C, n (%)
With thrombosis	125 (59)	75 (35)	12 (6)	352 (76.6)	99 (23.3)
Without thrombosis	81 (66)	29 (24)	12 (10)	191 (78.3)	53 (21.7)
With DVT	60 (63)	32 (34)	3 (3)	152 (80.0)	40 (20.0)
Without DVT	146 (61)	72 (30)	21 (9)	364 (76.1)	114 (23.9)
With PTE	42 (64)	21 (32)	3 (5)	105 (79.5)	27 (20.4)
Without PTE	164 (61)	83 (31)	21 (8)	411 (76.6)	125 (23.3)
With AMI	11 (69)	3 (19)	2 (13)	25 (78.1)	7 (21.9)
Without AMI	195 (61)	101 (32)	22 (7)	491 (77.2)	145 (22.8)

Abbreviations: AMI, acute myocardial ischemia; DVT, deep venous thrombosis; PTE, pulmonary thromboembolism.

^a0.05 compared between groups allele vs C.

^b $P < .05$. Compared between groups with and without lesion between the same genotypes or alleles.

patients with AMI, the frequency of high Hcy levels (>12.4) was statistically higher than in patients without it. Additionally, in patients with PE or DVT, the presence of positive anticardiolipin and elevated D-dimer and of mutations on Leiden factor V was higher than in patients without these diseases ($P \leq .05$). The distribution of the genotypes according to the presence of thrombosis, to its type, and to the presence of AMI, in both the *C677T* and the *A1298C* polymorphisms, is shown in Table 3. In the *C677T* polymorphism, there was a statistically significant difference in the *CC* genotype against the *TT* genotype ($P = .003$) and in the *CT* genotype versus the *TT* genotype ($P = .009$), as well as in the allele *T* ($P = .003$) in patients with thrombosis. In patients having pulmonary thromboembolism (PTE), there was a statistical difference between the *CC* genotype and the *TT* genotype ($P = .038$). Regarding the *A1298C* polymorphism, the highest frequency was found in the *AA* genotype in comparison to the *AC* and *CC* genotypes; however, there were no statistically significant allelic or genotypic differences between groups.

The results from the evaluation of the association between seric Hcy concentration, the MTHFR polymorphisms studied, and the different types of thrombosis are shown in Table 4. There were significant differences in patients having MTHFR *C677T* against the total population in the *CC* genotype against the *TT* genotype ($P = .004$) and the C versus T allele ($P = .044$). In patients having thrombosis versus the MTHFR genotypes, a difference between the *CC* genotype and the *CT* and *TT* genotypes was present ($P = .009$ and $.003$, respectively) in the *T* allele ($P = .047$). Furthermore, in PTE, there was a difference in *CT* genotype and the *TT* genotype ($P = .038$). Regarding MTHFR *A1298C*, there were no statistically significant differences in any of the lesions studied.

When the analysis between the genotypic frequencies according to the type of thromboembolism and the levels of Hcy was done, there was an increase in Hcy and massive and submassive thromboembolism when compared to segmentary and subsegmentary thromboembolism in *C677T TT* ($P = .003$) and in *AA 1298* ($P = .017$; Table 5).

Gestational losses happened in 8 (2%) women and the Hcy levels were 7 (4). Only in 2 women, there were recurrent losses and the Hcy levels were 12 and 10.

In this study, 20 patients had autoimmune diseases and 9 had neoplastic diseases. Out of the patients with cancer, there was thrombosis at different locations in 5. There was AMI and PTE in only 1 case. From the patients having cancer, 1 had endometrial cancer and all of them had elevated Hcy levels. All cases having thrombosis in other places than the myocardium or lungs were homozygous for the *AA* allele of the *A1298C* polymorphism and heterozygous for the *CT* in the *C677T* polymorphism. The cases having AMI or PE were homozygous for the *AA* of the *A1298C* gene and homozygous for the *CC* allele of the *C677T* (Table 6).

There were brain vascular ischemic events or brain hemorrhagic events in 36 cases out of the 115 cases studied: 3 were homozygous in the *A1298C* gene, 1 in the *C677T*, and 6 and 12 were heterozygous, respectively. Thrombosis recurrence in the same or another location happened in 40 (12%) patients.

Discussion

Methylenetetrahydrofolate reductase plays an important role in the regulation of the Hcy levels. An increase in Hcy has been associated with ischemic pathology, Alzheimer, dementia, osteoporosis, and psychiatric disorders.²¹⁻²³

Table 4. Total Seric Homocysteine Concentration According to the Genotype MTHFR C677T and MTHFR A1298C.

C677T	CC	CT	TT	C	T
In the total population	11.51 (6.4)	13.18 (12.43)	16.46 (12.71) ^a	12.52 (10.4)	14.10 (12.57) ^a
With thrombosis	11.83 (6.1)	14.75 (14.6) ^a	14.82 (8.4)	13.59 (11.9)	14.77 (13.5) ^a
Without thrombosis	10.6 (7.1)	10.04 (4.5)	17.81 (15.3)	10.25 (5.6)	13.07 (10.8)
With DVT	11.84 (5.2)	13.3 (5.3)	17.07 (10.2)	12.84 (5.3)	14.07 (6.7)
Without DVT	11.44 (6.7)	13.2 (14.8)	16.28 (13.1)	12.38 (12)	14.12 (14)
With PTE	11.83 (5.6)	13.54 (6.0)	12.71 (5.4)	13.02 (5.4)	13.42 (5.9)
Without PTE	11.44 (6.8)	13.06 (14)	16.94 (13.3)	12.38 (11.5)	14.29 (13.8)
With AMI	16.33 (8.5)	14.78 (4.5)	17.25 (7.6)	15.17 (5.3)	15.54 (5.4)
Without AMI	11.37 (6.3)	13.08 (12.7)	16.41 (13)	12.39 (10.6)	14.01 (12.08)
A1298C	AA	AC	CC	A	C
In the total population	13.68 (11.3)	12.14 (10.9)	14.59 (7.6)	13.18 (11.2)	12.59 (10.4)
With thrombosis	14.01 (11.5)	13.24 (12.3)	14.8 (6.4)	13.73 (11.8)	13.36 (11.6)
Without thrombosis	13.16 (11.1)	9.22 (5.1)	15.0 (9.2)	12.16 (10.1)	10.89 (6.9)
With DVT	13.69 (7.0)	12.91 (5.2)	15.33 (5.0)	13.41 (6.4)	13.11 (5.2)
Without DVT	13.67 (12.7)	11.78 (12.8)	14.47 (8.1)	13.07 (12.7)	12.37 (11.9)
With PTE	13.3 (12.4)	12.57 (4.6)	11.0 (4.5)	13.8 (5.4)	12.38 (4.5)
Without PTE	13.77 (12.4)	12.03 (12.1)	15.16 (7.9)	13.20 (12.3)	12.60 (11.4)
With AMI	16.36 (5.5)	15.67 (8.1)	12.0 (5.6)	16.21 (5.8)	14.2 (6.7)
Without AMI	13.52 (11.6)	12.03 (11.0)	14.85 (7.9)	13.03 (11.4)	12.52 (10.6)

Abbreviations: AMI, acute myocardial ischemia; DVT, deep venous thrombosis; PTE, pulmonary thromboembolism; Seric Hcy, Seric homocysteine.

^aP ≤ .05.

Table 5. Relation Between the Genotype and the Type of Pulmonary Thromboembolism According to the Levels of Homocysteine.

C677T	Massive and Submassive	Segmentary and Subsegmentary	P
CC	n = 5; 10.80 (3.70)	n = 14; 11.93 (3.75)	.578
CT	n = 17; 13.59 (4.38) ^a	n = 21; 11.67 (5.50)	.029
TT	n = 2; 19.50 (0.07) ^a	n = 5; 10.00 (3.53)	.003
A1298C	Massive and Submassive	Segmentary and Subsegmentary	P
AA	n = 20; 14.10 (4.44)	n = 19; 10.47 (4.62)	.017
AC	n = 3; 8.67 (2.51)	n = 19; 12.95 (4.63)	.068
CC	n = 1; 16.00	n = 2; 8.50 (2.12)	.212

^aStatistical difference Compared between groups Massive and Submassive vs Segmentary and subsegmentary.

In the present study, the increases in Hcy levels were associated with the presence of the genotype *677TT* and with the T allele in the general population and there was a significant difference in patients having thrombosis. This association had been previously reported and the Hcy elevation seemed to have a marked influence upon every disease.²⁴

Information regarding the prevalence of polymorphisms of the thermolabile variant of MTHFR differs greatly from one paper to another. The substitution of *677 C>T* results in an amino acid change from alanine to valine in the 225 codon. This substitution diminishes the enzyme's activity in 35% of patients having the *677CT* variant and in 50% to 70% of patients having the *677TT* variant.²⁵ This increase is related to elevated Hcy levels and to a higher thrombotic and cardiovascular risk in the Mexican population and in other ethnic groups.²⁶⁻³⁰

Patients with *677TT* and *1298CC* polymorphisms have elevated Hcy levels, independently of whether they have

thrombosis or not.³¹ These findings are in agreement with our results on the T allele in patients with high seric Hcy levels. In these patients, there was a tendency to elevate Hcy levels in the presence of the *677T* polymorphism independently of their thrombotic condition. Recent studies in patients with DVT or PE have shown that there is an increased risk of arterial disease and an important risk of PE in heterozygous patients with the genic polymorphism *C677T* of MTHFR where the cytosine is substituted by thymidine in the 677 nucleotide of the gene (*C677T*). This is also true in other thrombotic diseases such as congenital heart disease.³² Furthermore, it is in accordance with the data found in the present study. A high frequency of the *C677T* polymorphism has been found in Mexico varying from 44% to 58%, but the implication on cardiovascular risk had not been previously reported.²⁶⁻³⁰

An association of elevated Hcy in the presence of thrombosis was confirmed by this study. The *A1298C* and *C677T*

Table 6. Neoplastic and Autoimmunity Diseases, Genotype of MTHFR Gene and Thrombosis Site.

	# of Cases	A1298C			C677T			Hcy Level	Thrombosis Site
		AA	AC	CC	CC	CT	TT		
Neoplasia									
Metastatic Ca	1	-	+	-	-	+	-	9	No
Endometrial	1	+	-	-	-	+	-	14	CVD
CLL	1	+	-	-	-	+	-	12	No
NHL	1	-	+	-	-	+	-	14	No
Myelodysplastic	1	+	-	-	+	-	-	14	No
Multiple myeloma	1	+	-	-	-	+	-	8	No
Pancreas (head) Ca	1	-	+	-	-	+	-	12	No
Cranial sarcoma	1	+	-	-	-	+	-	2	No
Germinal tumor	1	+	-	-	-	-	+	5	No
Total	9	6	3	0	1	7	1		
Autoimmunity									
Addison + AFS	1	-	+	-	-	+	-	22	T in hemorrhoids
RA	1	+	-	-	-	+	-	12	AMI
Factor V deficiency	3	3+	-	-	-	3+	-	13	CVD(1) DVT(1) PTE (1)
S protein deficiency	1	+	-	-	-	+	-	9	No
SLE	2	2+	-	-	-	2+	-	18	DVT (2)
SLE + secondary AFS	2	2+	-	-	-	2+	-	9	No
Primary AFS	7	4+	+	2+	3+	2+	2+	13	DVT (4)
Obliterating thromboangiitis	2	+	+	-	+	+	-	19	MT (1) DVT (1)
Wegener	1	+	-	-	+	-	-	5	No
Total	20	15	3	2	5	13	2		

Abbreviations: AFS, antiphospholipid syndrome; AMI, acute myocardial ischemia; Ca, cancer; CLL, chronic lymphocytic leukemia; CVD, cerebral vascular disease; DVT, deep venous thrombosis; Hcy, homocysteine; MT, mesenteric thrombosis; NHL, non-Hodgkin Lymphoma; RA, rheumatoid arthritis; PTE, pulmonary thromboembolism; SLE, systemic lupus erythematosus; T, thrombosis.

polymorphisms of the *MTHFR* gene are associated with a higher risk of venous thromboembolism.³³ In our series, this combination was present in patients having non-Hodgkin lymphoma and this finding would deserve to be further investigated.³⁴

In this series of patients, those having autoimmune diseases or neoplasms had elevated Hcy levels. The frequency of autoimmunity was 6%, and in these patients, there was thrombosis in different locations. However, one patient had AMI and another PE and both were homozygous for *A* in the *A1298C* gene and homozygous for the allele *C* of *C677T*. Out of the patients having cancer, only 1 patient developed thrombosis manifesting as VDT.

Another clinical aspect associated with thrombosis and increased Hcy levels is recurrent pregnancy losses and infertility.³⁵⁻³⁸ In this series, 8 women experienced fetal losses, out of whom 5 (62%) had a mutation in *C677T* and 2 had recurrent abortions. In the remaining 3, there was a mutation in *A1298C* and elevated Hcy levels. This result is similar to the one reported in the Hindu population, although in that study alterations in the plasminogen activator factor plasminogen activator inhibitor-1 PAI-1 were also found.³⁹ In this series, infertility was present in only 3 women who also had miomatosis and elevated Hcy levels. Elevated Hcy levels in infertility and gestational losses had already been previously found.⁴⁰

In patients with primary antiphospholipid syndrome who have moderately high Hcy levels, the heterozygous point

mutations in *C677T* and *A1298C* of the *MTHFR* gene have been proposed as the possible causes for the thrombotic disease and also as the cause of the presence of generalized brain lesions and idiopathic hypoparathyroidism.⁴¹ This situation was not found in our cases and could be due to ethnicity and/or to a folate deficiency in the diet.

Recurrence in thrombosis was present in 11% of the cases. Out of these patients, the majority (7%) corresponded to DVT and the remaining 4% corresponded to brain vascular disease, alterations in the retina, and mesenteric artery.

Limitations of the Study

This retrospective revision did not allow for the obtainment of laboratory data on other complementary prothrombotic biomarkers in the cases where the genomic determination had been done. Nevertheless, the number of cases included was enough to report significant data. Another limitation of the present study was that we could not determine the ingestion of folates and B₁₂ vitamin. It is a retrospective study that of course assumes selection bias and therefore the risks cannot be calculated according to each type of thrombosis. Because the lifestyle cannot be planned in the selected individuals we do not know if there is coparticipation of a diet deficit in the food that an individual consumes. We could not analyze the influence of these cofactors that may have an association with the results. Therefore, other controlled assays are required.

Conclusion

Venous thromboembolic disease is associated with the presence of polymorphisms in the gene encoding for the MTHFR enzyme and with elevated Hcy levels. The increase in the Hcy levels in presence of the *T* allele in the *C677T* gene and of the *A* allele in the *A1298C* gene is associated with AMI and massive and submassive PTE. Multicentric, prospective studies considering ethnicity, cardiovascular risk factors, nutritional habits, lifestyle, and a specific search for the participation of dietary folates are required and should be proposed to determine the relevance of these findings.

Authors' Note

Eulo Lupi-Herrera and María Elena Soto contributed to the conception and design of the study, drafting the article or revising it critically for important intellectual content, acquisition of data, analysis and interpretation of data, and final approval of the version to be submitted. María Elena Soto, Antonio de Jesús Lugo-Dimas, and Marcela Elizabeth Núñez-Martínez contributed to acquisition of data, drafting the article, translation and revision of the language in English, and revising it critically for important intellectual content.

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References

- Konstantinides SV, Torbicki A, Agnelli G, et al. Task Force for the Diagnosis and Management of Acute Pulmonary Embolism of the European Society of Cardiology (ESC). 2014 ESC guidelines on the diagnosis and management of acute pulmonary embolism. *Eur Heart J*. 2014;35(43):3033-3069. doi:10.1093/eurheartj/ehu283.
- Heit JA, Silverstein MD, Mohr DN, Petterson TM, O'Fallon WM, Melton LJ III. Predictors of survival after deep vein thrombosis and pulmonary embolism: a population-based, cohort study. *Arch Intern Med*. 1999;159(5):445-453.
- Kahn SR, Kearon C, Julian JA, et al. Extended Low-intensity Anticoagulation for Thrombo-embolism (ELATE) Investigators. Predictors of the post-thrombotic syndrome during long-term treatment of proximal deep vein thrombosis. *J Thromb Haemost*. 2005;3(4):718-723.
- Kluijtmans LA, Young IS, Boreham CA, et al. Genetic and nutritional factors contributing to hyperhomocysteinemia in young adults. *Blood*. 2003;101(7):2483-2488.
- Chai AU, Abrams J. Homocysteine: a new cardiac risk factor? *J Clin Cardiol*. 2001;24(1):80-84.
- Dudman NP, Guo XW, Gordon RB, Dawson PA, Wilcken DE. Human homocysteine catabolism: three major pathways and their relevance to development of arterial occlusive disease. *J Nutr*. 1996;126(4 suppl):1295S-1300S.
- Roubenoff R, Dellaripa P, Nadeau MR, et al. Abnormal homocysteine metabolism in rheumatoid arthritis. *Arthritis Rheum J*. 1997;40(4):718-722.
- Peytremann R, Thorndike J, Beck WS. Studies on N5-methyltetrahydrofolate-homocystein methyltransferase in normal and leukemia leukocytes. *J Clin Invest*. 1975;56(5):1293-1301.
- Boers GH, Smals AG, Trijbels FJ, et al. Heterozygosity for homocystinuria in premature peripheral and cerebral occlusive arterial disease. *N Engl J Med*. 1985;313(12):709-715.
- Clarke R, Daly L, Robinson K, et al. Hyperhomocysteinemia: an independent risk factor for vascular disease. *New Engl J Med*. 1991;324(17):1149-1155.
- Kang SS, Wong P, Susmano A, Sora J, Norusis M, Ruggie N. Thermolabile methylenetetrahydrofolate reductase: an inherited risk factor for coronary artery disease. *Am J Hum Genet*. 1991;48(3):536-545.
- Jacques PF, Bostom AG, Williams RR, et al. Relation between folate status, a common mutation in methylenetetrahydrofolate reductase, and plasma homocysteine concentrations. *Circulation*. 1996;93(1):7-9.
- Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet*. 1995;10(1):111-113.
- Kluijtmans LA, van den Heuvel LP, Boers GH, et al. Molecular genetic analysis in mild hyperhomocysteinemia: a common mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for cardiovascular disease. *Am J Hum Genet*. 1996;58(1):35-41.
- Ma J, Stampfer MJ, Hennekens CH, et al. Methylenetetrahydrofolate reductase polymorphism, plasma folate, homocysteine, and risk of myocardial infarction in the US physicians. *Circulation*. 1996;94(10):2410-2416.
- Van Bockxmeer FM, Mamotte CD, Vasikaran SD, Taylor RR. Methylenetetrahydrofolate reductase gene and coronary artery disease. *Circulation*. 1997;95(1):21-23.
- Christensen B, Frosst P, Lussier-Cacan S, et al. Correlation of a common mutation in the methylenetetrahydrofolate reductase gene with plasma homocysteine in patients with premature coronary artery disease. *Arterioscler Thromb Vasc Biol*. 1997;17(3):569-573.
- Verhoef P, Kok FJ, Kluijtmans LA, et al. The 677 C3T mutation in the methylenetetrahydrofolate reductase gene: associations with plasma total homocysteine levels and risk of coronary atherosclerotic disease. *Atherosclerosis*. 1997;132(1):105-113.
- Lewis SJ, Shah E, Smith G. Meta-analysis of MTHFR 677 C→T polymorphism and coronary heart disease: does totality of evidence support casual role for homocysteine and preventive potential of folate. *BMJ*. 2005;331(7524):1053-1070.
- Clarke R, Bennett DA, Parish S, et al; MTHFR Studies Collaborative Group. Homocysteine and coronary heart disease: meta-analysis of MTHFR case-control studies, avoiding publication bias. *PLoS Med*. 2012;9(2):e1001177.

21. Paramonov AD, Moiseev SV, Fomin VV, et al. Hyperhomocysteinemia and acute phase proteins in various forms of ischemic heart disease [in Russian]. *Ter Arkh.* 2004;76(6):67-70.
22. McCully KS. Front hyperhomocysteinemia, suppressed immunity, and altered oxidative metabolism caused by pathogenic microbes in atherosclerosis and dementia. *Front Aging Neurosci.* 2017;9:324. doi:10.3389/fnagi.2017.00324.
23. Kim JI, Moon JH, Chung HW, Kong MH, Kim HJ. Association between homocysteine and bone mineral density according to age and sex in healthy adults. *J Bone Metab.* 2016;23(3):129-134. doi:10.11005/jbm.2016.23.3.129.
24. Moiz B. Homocysteine as a biomarker for thrombosis: what is the learning curve? *J Coll Physicians Surg Pak.* 2017;27(3):121-122.
25. Zetterberg H, Regland B, Palmér M, et al. Increased frequency of combined methylenetetrahydrofolate reductase C677T and A1298C mutated alleles in spontaneously aborted embryos. *Eur J Hum Genet.* 2002;10(2):113-118.
26. Mutchinick OM, López MA, Luna L, Waxman J, Babinsky VE. High prevalence of the thermolabile methylenetetrahydrofolate reductase variant in Mexico: a country with a very high prevalence of neural tube defects. *Mol Genet Metab.* 1999;68(4):461-467.
27. Ni J, Liu Y, Zhou T, Wu X, Wang X. Single nucleotide polymorphisms in key one-carbon metabolism genes and their association with blood folate and homocysteine levels in a Chinese population in Yunnan. *Genet Test Mol Biomarkers.* 2018;22(3):193-198. doi:10.1089/gtmb.2017.019.
28. Lazzarini PE, Capocchi PL, Selvi E, et al. Hyperhomocysteinemia, inflammation and autoimmunity. *Autoimmun Rev.* 2007;6(7):503-509.
29. Kumar J, Das SK, Sharma P, Karthikeyan G, Ramakrishnan L, Sengupta S. Homocysteine levels are associated with MTHFR A1298C polymorphism in Indian population. *J Hum Gen.* 2005;50(12):655-663.
30. Onrat ST, Akci O, Söylemez Z, Onrat E, Avşar A. Prevalence of myocardial infarction polymorphisms in Afyonkarahisar, Western Turkey. *Mol Biol Rep.* 2012;39(9):9257-9264. doi:10.1007/s11033-012-1799-1.
31. Wayne TF Jr. Methylenetetrahydrofolate reductase C677T polymorphism, venous thrombosis, cardiovascular risk, and other effects. *Angiology.* 2015;66(5):401-404. doi:10.1177/0003319714548871.
32. Sánchez R, Galaviz C, Sierra J. Polimorfismo 677CT del gen de la metilentradihidrofolato reductasa y cardiopatías congénitas aisladas en población Mexicana. *Rev Esp Cardiol.* 2012;65(2):158-163.
33. Liu F, Silva D, Malone MV, Seetharaman K. MTHFR A1298C and C677T polymorphisms are associated with increased risk of venous thromboembolism: a retrospective chart review study. *Acta Haematol.* 2017;138(4):208-215. doi:10.1159/000480447.
34. Mashhadi MA, Miri-Moghaddam E, Arbabi N, et al. C677T and A1298C polymorphisms of methylene tetrahydrofolate reductase in non-Hodgkin lymphoma: southeast Iran [published online ahead of print April 1, 2017]. *Tumori.* 2017. doi:10.5301/tj.5000634.
35. Blumenfeld Z, Weiner Z, Lorber M, Sujov P, Thaler I. Anticardiolipin antibodies in patients with recurrent pregnancy wastage: treatment and uterine blood flow. *Obstet Gynecol.* 1991;78(4):584-589.
36. Brown HL. Antiphospholipid antibodies and recurrent pregnancy loss. *Clin Obstet Gynecol.* 1991;34(1):17-26.
37. Infante-Rivard C, David M, Gauthier R, Rivard GE. Lupus anticoagulants, anticardiolipin antibodies, and fetal loss: a case-control study. *N Eng J Med.* 1991;325(15):1063-1066.
38. Dossenbach-Glaninger A, von Trotsenburg M, Krugluger W, et al. Elevated coagulation factor VIII and the risk for maternal early pregnancy loss. *Thromb Haemost.* 2004;91(4):694-699.
39. Parveen F, Tuteja M, Agrawal S. Polymorphisms in MTHFR, MTHFD, and PAI-1 and recurrent miscarriage among North Indian women. *Arch Gynecol Obstet.* 2013;288(5):1171-1177. doi:10.1007/s00404-013-2877-x.
40. D'Uva M, Di Micco P, Strina I, et al. Hyperhomocysteinemia in women with unexplained sterility or recurrent early pregnancy loss from Southern Italy: a preliminary report. *Thromb J.* 2007;5:10.
41. Nucera C, Vaccaro M, Moleti M, et al. Antiphospholipid antibodies syndrome associated with hyperhomocysteinemia related to MTHFR Gene C677T and A1298C heterozygous mutations in a young man with idiopathic hypoparathyroidism (DiGeorge syndrome). *J Clin Endocrinol Metab.* 2006;91(6):2021-2026.