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SHORT REPORT

A second mutation in the methylenetetrahydrofolate reductase gene and the risk of venous thrombotic disease

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Summary. We assessed the effect of a recently described mutation in the MTHFR gene (1298 A→C) on the risk of deep venous thrombosis (DVT) by determining its prevalence in 190 patients with verified DVT and in age-, race- and gender-matched controls. MTHFR 1298 A→C was found in 42·1% of patients and in 41·1% of controls. The OR for venous thrombosis was 1·07 (95% CI 0·70–1·65) for heterozygotes and 0·83 (95% CI 0·33–2·08) for homozygotes. The OR for the factor V Leiden (FVL) mutation was 3·40 (95% CI 1·22–9·48), for FII 20210 G→A was 5·22 (95% CI 1·12–24·2) and for MTHFR 677 C→T, 1·24 (95% CI 0·82–1·87). No significant increased risk for venous thrombosis was found when MTHFR 1298 A→C was coinherited with FVL (OR 2·85, 95% CI 0·88–9·23), FII 20210 G→A (OR 7·19, 95% CI 0·87–59·4) or MTHFR 677 C→T (OR 1·44, 95% CI 0·71–2·92). These data do not support a critical role of MTHFR 1298 A→C in the predisposition to DVT.

Keywords: MTHFR 1298 A→C, MTHFR 677 C→T, thrombosis, risk factor, mutation.

Methylenetetrahydrofolate reductase (MTHFR) is a critical enzyme in the remethylation pathway of the homocysteine metabolism (Rozen, 1997). Molecular defects in the MTHFR gene may result in enzyme deficiency and hyperhomocysteinaemia, a risk factor for venous thrombosis, atherosclerosis and neural tube defects (NTD) (Boers, 1997; Rozen, 1997). To date, several different MTHFR mutations have been identified (Rozen, 1997). The relationship between venous thrombophilia and a frequent mutation in the MTHFR gene (677 C→T), which is associated to a thermolabile phenotype, decreased enzyme activity and mild hyperhomocysteinaemia, is still debatable (Seligsohn & Zivelin, 1997; Brown *et al*, 1998). Recently, a novel MTHFR mutation (1298 A→C) was reported to occur with a high carrier frequency in the general population (van der Put *et al*, 1998; Weisberg *et al*, 1998). Isolated, this frequent mutation does not result in severe enzyme deficiency or hyperhomocysteinaemia (van der Put *et al*, 1998; Weisberg *et al*, 1998). However, combined heterozygosity for both MTHFR 677 C→T and 1298 A→C mutations was found to be associated with diminished enzyme activity, hyperhomocysteinaemia, decreased plasma folate levels and (apparently) increased risk for NTD (van der Put *et al*, 1998; Weisberg *et al*, 1998). The role of the MTHFR 1298 A→C mutation (isolated or in combination with MTHFR 677 C→T) in venous thrombophilia is unknown. We therefore sought to assess the relationship between this novel MTHFR variant and venous thrombotic disease.

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SUBJECTS AND METHODS

Subjects. The patient group included 190 consecutive and unrelated individuals younger than 65 years (male/female ratio 1·0/1·6; mean age 41 years, range 1–65) admitted to a University Hospital with an episode of deep venous thrombosis between 15 October 1996 and 28 August 1998. The patients included were consecutively admitted to three University Hospitals in the same geographical area (State of São Paulo, Brazil): School of Medicine of Ribeirão Preto (University of São Paulo, USP), School of Medicine of Botucatu (State University of São Paulo, UNESP) and Federal University of São Paulo (UNIFESP). For all the patients ultrasonography and/or phlebography objectively confirmed the clinical diagnosis of venous thrombosis. None of the

patients had evidence of malignant disease. 156 patients were Caucasian, 19 were Blacks and 15 were Mulattos. 190 unrelated individuals were recruited as controls (male/female ratio 1·0/1·6; mean age 41 years, range 2–64). Specifically, for each patient entering the study, a sex-, age- (±3 years) and race-matched control subject was identified among donors in the local Blood Centre and invited to participate in the study as a volunteer. We selected as controls asymptomatic and apparently healthy subjects who denied a personal and family history of venous thromboembolism. In some cases a matched control was not found even after this procedure, and in this context controls were selected according to the same criteria (unrelated, asymptomatic, apparently healthy individuals free from a personal and family history of thrombosis) among employees of the University Hospital. 169/190 (89%) controls were blood donors and 21/190 (11%) were hospital employees. All individuals enrolled came from the same geographical area, i.e. the State of São Paulo, south-eastern Brazil. Informed consent was obtained from all participants.

Methods. Genomic DNA from the peripheral blood was isolated by the salting-out method (Miller *et al*, 1988). The following primers were used for determination of the MTHFR 1298 A/C genotypes: P1: 5′-CTTTGGGGAGCTGAAGGACTACTAC-3′ and P2: 5′-CAGTTTGTGACCATTCCGGTTTG-3′. PCR products were submitted to restriction digestion with the enzyme *Mbo*I, in order to identify the three possible genotypes, as reported (van der Put *et al*, 1998). The detection of the FVL, MTHFR 677 → T and FII 20210 G → A mutations was performed as previously described (Bertina *et al*, 1994; Frosst *et al*, 1995; Poort *et al*, 1996).

Statistics. Odds ratio (OR) as a measure of the relative risk for venous thrombosis and 95% confidence interval (95% CI) were calculated by standard methods.

RESULTS

The results for the analysis of the MTHFR 1298 A → C mutation in patients and controls are given in Table I.

Table I. MTHFR 1298 A → C mutation in patients with verified venous thrombosis and in healthy controls.

Genotype	Patients ($n = 190$)	Controls ($n = 190$)	OR (95% CI)
AA	110 (57·9%)	112 (58·9%)	1·0*
AC	71 (37·4%)	67 (35·3%)	1·07 (0·70–1·65)
CC	9 (4·7%)	11 (5·8%)	0·83 (0·33–2·08)
AC + CC	80 (42·1%)	78 (41·1%)	1·04 (0·69–1·57)

AA: wild-type genotype; AC: heterozygous; CC: mutant homozygous.

Table II. MTHFR 1298 A → C, MTHFR 677 C → T, factor V Leiden (FVL) and FII 20210 G → A mutations in patients with verified venous thrombosis and in healthy controls.

		Patients ($n = 190$)	Controls ($n = 190$)	OR (95% CI)
MTHFR 1298 A → C	MTHFR 677 C → T			
−	−	30	30	1·0*
+	−	41	51	0·80 (0·41–1·54)
−	+	80	82	0·97 (0·53–1·76)
+	+	39	27	1·44 (0·71–2·92)
MTHFR 1298 A → C	FVL			
−	−	107	111	1·0*
+	−	69	74	0·96 (0·63–1·47)
−	+	3	1	3·11 (0·31–30·3)
+	+	11	4	2·85 (0·88–9·23)
MTHFR 1298 A → C	FII 20210 G → A			
−	−	107	110	1·0*
+	−	73	78	0·96 (0·63–1·45)
−	+	3	1	3·08 (0·31–30·1)
+	+	7	1	7·19 (0·87–59·4)

(−) refers to the wild-type genotypes and (+) refers to both the heterozygous and the homozygous state for each mutation in the case of MTHFR and to the heterozygous state in the case of FVL and FII 20210 G → A mutations. No subject was homozygous for both MTHFR mutations.

* Reference category.

MTHFR 1298 A→C was found in 80/190 patients (carrier frequency 42·1%, allele frequency 0·234) and in 78/190 controls (carrier frequency 41·1%, allele frequency 0·234). The OR for venous thrombosis related to the MTHFR 1298 A→C was 1·07 (95% CI 0·70–1·65) for heterozygotes and 0·83 (95% CI 0·33–2·08) for homozygotes. The overall OR (for both heterozygotes and homozygotes combined) was 1·04 (95% CI 0·69–1·57). In addition, no differences were detected in different age groups: the OR for venous thrombosis in the age group <35 years was 1·13 (95% CI 0·64–2·00), whereas the OR in the age group ⩾35 years was 0·95 (95% CI 0·53–1·71).

In the control group, the MTHFR 1298 A→C mutation was found with a carrier frequency of 41·6% (allele frequency 0·240) in Caucasians, 46·6% (allele frequency 0·266) in Mulattos and 31·6% (allele frequency 0·158) in Blacks. In the patient group the mutation was found with a carrier frequency of 44·9% (allele frequency 0·246) in Caucasians, 33·3% in Mulattos (allele frequency 0·200) and 26·3% (allele frequency 0·158) in Blacks. On the basis of these findings, it can be suggested that the MTHFR 1298 A→C mutation exhibited a heterogenous racial distribution. However, since the number of non-Caucasians analysed in our study was relatively small, a possible ethnic variability linked to this mutation should be further investigated. Of note, the OR for venous thrombosis related to the MTHFR 1298 A→C mutation remained essentially unchanged after we restricted the analysis to Caucasians. Specifically, the following OR and 95% CI were obtained in this context: for heterozygotes: 1·21 (0·76–1·93); for homozygotes: 1·13 (0·72–1·78); overall OR: 0·74 (0·26–2·03).

The FVL mutation was found in 2·6% of the controls and in 8·4% of the patients (OR 3·40, 95% CI 1·22–9·48). The FII 20210 G→A mutation was detected in 1·1% of the controls and in 5·3% of the patients (OR 5·22, 95% CI 1·12–24·2). Homozygosity and heterozygosity for the MTHFR 677 C→T mutation was identified in 19·5% and 43·1% of patients and in 16·3% and 41·1% of controls, respectively (OR for homozygotes 1·36, 95% CI 0·76–2·41; OR for heterozygotes 1·19, 95% CI 0·76–2·41; overall OR 1·24, 95% CI 0·82–1·87).

We also recalculated OR for venous thrombosis when MTHFR 1298 A→C was present in combination with MTHFR 677 C→T, FVL and FII 20210 G→A. The results of these analyses are given in Table II. No subject was homozygous for both MTHFR mutations. A significant increased risk for venous thrombosis could not be identified when MTHFR 1298 A→C and 677 C→T were co-inherited (OR 1·44, 95% CI 0·71–2·92). The OR for venous thrombosis when MTHFR 1298 A→C was present in combination with FVL was 2·85 (95% CI 0·88–9·23). Although the OR for venous thrombosis tended to be somewhat increased when the MTHFR 1298 A→C mutation was co-inherited with FII 20210 G→A (OR 7·19), this difference was not significant (95% CI 0·87–59·4).

DISCUSSION

In the present investigation we assessed the possibility that a frequent mutation in the MTHFR gene (1298 A→C) might be related to increased risk of venous thrombotic disease. This does not seem to be the case, since the observed OR for venous thrombosis (1·07 for heterozygotes and 0·83 for homozygotes) implied a neutral relative risk and did not point to a significant role of this mutation in the risk of venous thrombosis. We also calculated the OR taking into account the concurrent presence of the MTHFR 677 C→T mutation, since the combined heterozygosity for the two MTHFR mutations was recently found to be associated with decreased enzyme activity, hyperhomocysteinaemia and increased risk of NTD (van der Put *et al*, 1998; Weisberg *et al*, 1998). As shown in Table II, no significant interactive effect seemed to occur when both mutations were present in combination. Of note, no subjects were homozygous for both MTHFR mutations. This finding is in agreement with recent data demonstrating that the two mutations occur in *trans* (van der Put *et al*, 1998). It must be noted that we did not measure homocysteine levels in the subjects included in the present study, and therefore our conclusions are based on the absence of an identifiable relationship between the specific abnormalities in the MTHFR gene and the risk of venous thrombosis.

We also did not observe a significant interaction of MTHFR 1298 A→C with FVL or FII 20210 G→A in influencing the risk of venous thrombosis. However, these stratified analyses were performed on the basis of relatively small numbers of carriers of combined defects and the calculated confidence intervals were therefore wide. We believe that a large number of subjects with these concurrent defects should be analysed before the possibility of an interactive effect may be completely excluded.

At present the homozygous state for the MTHFR 677 C→T mutation is considered to be a risk factor for NTD (van der Put *et al*, 1998). In contrast, its clinical significance in venous and arterial thrombosis is still controversial (Seligsohn & Zivelin, 1997; Brown *et al*, 1998; Flechter & Kessling, 1998). Regarding the specific relationship with venous thrombophilia, case–control studies dealing with large number of subjects failed to demonstrate a significant clinical impact of MTHFR 677 C→T on the risk of venous thrombosis (Brown *et al*, 1998; Kluijtmans *et al*, 1998). Our present data are in line with these studies, insofar as they do not support the MTHFR 677 C→T mutation as a determinant of the risk of DVT. In addition, our findings indicate that the MTHFR 1298 A→C mutation is also unlikely to be a major risk factor for venous thrombosis. We suggest that screening for these two genetic variations is probably not recommended in thrombophilic patients.

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