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Original Paper

Comparison of the incidence of MTHFR C677T homozygosity and hyperhomocysteinemia in patients with and without thromboembolism.

Abstract

Background: The association between methylene tetrahydrofolate reductase (MTHFR) polymorphisms, high homocysteine (HCY) and the risk of thrombosis is still ambiguous. **Aims**: The aim of our study was to analyze retrospectively the incidence of homozygosity for MTHFR, HCY levels and thrombosis. **Materials and methods**: We retrospectively analyzed the clinical data of 407 subjects followed up by our centre: 270 with homozygosis for MTHFR C677T mutations and normal or elevated HCY levels compared to 137 subjects without thrombophilia. **Results**: In both groups with MTHFR C677T homozygosity, the incidence of thrombosis was lower than in the group of subjects without MTHFR mutation and normal HCY levels, but this difference was statistically significant only when we compared subjects without thrombophilia to those with homozygosis for MTHFR C677T and normal HCY. **Conclusions**: Differently from other studies, we did not observe a correlation between thrombotic risk and MTHFR C677T homozygosity. We confirmed the importance of homocysteine in the etiopathogenesis of thrombosis, although it is probably still not clear which level of hyperhomocysteine should be considered as a real risk factor for thrombosis.

Key-words

Genetics, Homocysteine, MTHFR, Thrombophilia

Introduction

Homocysteine (HCY) is an intermediary aminoacid formed by the conversion of methionine to cysteine. Homocysteine is metabolized by one of two divergent pathways: trans-sulfuration to cystathionine, which requires vitamin B6, and re-methylation to methionine, which requires folate and vitamin B12 (1). Methylene tetrahydrofolate reductase (MTHFR) is a key enzyme that catalyzes the conversion of 5,10- methylenetetrahydrofolate to 5-methyltetrahdydrofolate, a co-substrate for the re-methylation of homocysteine to methionine (2). Previous studies have demonstrated that homozygosis for the MTHFR C677T mutation is associated with an increased risk of thrombosis, even in the absence of hyper-homocysteinemia (hHCY) (3,4), unlike other authors who observed that homozygosity for the 677T>C MTHFR polymorphism (MTHFR C677T) is linked to an increase in homocysteine level (5,6), but it is not clearly linked to an increase in thrombophilic events (7,8). Other researches showed that MTHFR polymorphisms do not increase the risk of thromboembolic disease, but only when found in a heterozygous state (9). For these reasons, the measurement of homocysteine, or MTHFR C677T genetic variant, is still a part of routine thrombosis or thrombophilia work-up in many thrombosis centres all over the world, even though the association between hyper-homocysteinaemia (hHCY) and the risk of recurrent venous thrombosis is still ambiguous (10). The aim of this study was to determine

the incidence of thrombosis in patients homozygous for C677T MTHFR mutation, with or without hHCY, compared to healthy individuals with the same characteristics and to healthy subjects without any thrombophilia.

Material and Methods:

We retrospectively analysed the clinical data of 407 subjects followed up by our centre in the last 10 years: 106 males, 301 females; average age: 40.3 (7-84). We divided them into three groups: the first consisted of 135 patients (15 m, 120 f; average age: 40.7; range 12-83) with homozygosis for MTHFR C677T mutations and normal HCY levels, the second group consisted of 135 patients (70 m, 65 f; average age: 39.4; range 15-84) with homozygosis for MTHFR C677T mutations and hHCY, and the third one was composed of 137 patients (21 m, 116 f; average age: 40.7; range 7-77) without any congenital or acquired thrombophilia. Demographic and clinical characteristics of patients and controls are shown in table 1. The incidence of thrombosis was analysed in all groups and the data obtained were analysed using chi-square test: a $p < 0.05$ was considered significant. All arterial and venous thrombotic events were included in the data collection. All subjects with other congenital or acquired thrombophilia coagulation defects (antithrombin deficiency, protein C or protein S deficiency, activated protein C resistance, factor V Leiden, prothrombin G20210A polymorphism, presence of lupus anticoagulant or phospholipid-binding antibodies) were excluded from the study. HHCY has been defined if greater than 15 mM. The significance of those differences observed between the groups was tested using chi-square analysis. Statistical significance was considered a P value less than 0.05.

Results:

We found a slightly higher thrombosis rate in homozygous subjects with hHCY, 24/135 (17.8%), compared to those with normal homocysteine, 21/135 (15.5%), even if no statistical significance emerged (p=0.24).

The incidence of thrombosis in both groups was lower than in the group of subjects without MTHFR mutation and normal HCY levels: 38/137 (27.7%); this difference was statistically significant only when we compared subjects with no thrombophilia to homozygous MHTFR C677T and normal HCY (p=0.014) but not in comparison to homozygous MHTFR C677T and hHCY (p=0.06). The comparison between the three groups with statistical significance is shown in Table 2.

Table 1: Demographic and clinical characteristics of patients and controls

	Average age (range)	Sex	Thrombosis	No Thrombosis
Homozygosis MTHFR C677T and nHCY (n=135)	40.7 (12-83)	15 M, 120 F	21 (15.5%)	114 (84.5%)
Homozygosis MTHFR C677T and hHCY (n=135)	39.4 (15-84)	70 M, 65 F	24 (17.8%)	135 (82.2%)
Subjects with no congenital or acquired thrombophilia) (n=137)	40.7 (7-77)	21 M, 116 F	38 (27.7%)	99 (72.3%)
Total (n= 407)	40.3 (7-84)	106 M, 301 F	83 (20.4%)	324 (79.6%)

C677T MTHFR: 677T>C Methylene tetrahydrofolate reductase polymorphism; nHCY: normal homocysteine; hHCY: hyperhomocysteine;

Table 2: Comparison and statistical significance of the different groups of patients

Comparison of homozygous C677T MTHFR subjects with hHCY, to those with nHCY		
	Thrombosis	No thrombosis
Homozygous C677T MTHFR and nHCY (≤15 µmol/L)	21 (15.5%)	114
Homozygous C677T MTHFR and hHCY (>15 µmol/L)	24 (17.8%)	111
	p= 0.24 (NS)	
Comparison of homozygous C677T MTHFR subjects with nHCY, to those without thrombophilia		
Homozygous C677T MTHFR and nHCY (≤15 µmol/L)	21 (15.5%)	114
No thrombophilia	38 (27.7%)	99
	P=0.014	
Comparison of homozygous C677T MTHFR subjects with hHCY, to those without thrombophilia		
Homozygous C677T MTHFR and hHCY (>15 µmol/L)	24 (17.8%)	111
No thrombophilia	38 (27.7%)	99
	P=0.06 (NS)	

C677T MTHFR: 677T>C Methylene tetrahydrofolate reductase polymorphism; nHCY: normal homocysteine; hHCY: hyperhomocysteine; NS: no statistical significance

Discussion:

Contrary to what many studies report in the scientific literature, we found a higher percentage of thrombosis in the population without homozygous MHTFR C677T and without hHCY compared to the population with homozygous MHTFR C677T, both with or without hHCY, but statistical significance was achieved only when we excluded hHCY. The increase in homocysteine was in fact decisive in the comparison only between patients homozygous for MTHFR, even if without statistical significance. This confirms the importance of homocysteine as an independent risk factor in the etiopathogenesis of thrombosis, although it is probably still not clear which level of hHCY should be considered as a real risk factor for thrombosis. The lack of direct correlation between thrombotic risk and MTHFR mutation we have noted, could be indirectly confirmed if we observe the results about the high prevalence of C677T MTHFR in the general population, as was reported by other studies that showed that the homozygous C677T MTHFR is particularly common in Mexico (32%), in Southern Italy (26%) and in Northern China (20%) (11), or that the homozygous C677T MTHFR ranges from less than 1% among African Americans to 20% plus among some Caucasian populations and Hispanics, while Asian populations have a prevalence of around 11% (12). In our

opinion, prospective multicentre studies comparing thrombotic patients to healthy subjects are necessary to confirm definitively the absence of independent thrombotic risk from the MTHFR mutation in both heterozygosity and homozygosity, and to define better the exact degree of danger of the hHCY in the aetiopathogenesis of thrombosis

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