Methylenetetrahydrofolate Reductase Gene Polymorphisms (C677T and A1298C) and Hemorrhagic Stroke in Moroccan Patients

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Background: The number of deaths from hemorrhagic strokes is about twice as high than the number of deaths from ischemic strokes. Genetic risk assessment could play important roles in preventive and therapeutic strategies. The present study was aimed to evaluate whether the *MTHFR* gene polymorphisms could increase the risk of cerebral hemorrhage in Moroccan patients. *Methods:* A total of 113 patients with hemorrhagic stroke and 323 healthy controls were included in this case-control study. The C677T (rs1801133) and A1298C (rs1801131) *MTHFR* gene polymorphisms were genotyped by Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) method in all patients and controls. The genotype and allele frequencies were compared between groups using appropriate statistical analyses. *Results:* Both groups, patients and controls, were in accordance with the Hardy–Weinberg Equilibrium. For the C677T polymorphism, the frequencies of the CC, CT, and TT genotypes were 50.44% versus 46.13%, 39.82% versus 43.03, and 9.73% versus 10.84% in controls versus patients, respectively, whereas for the A1298C polymorphism, the frequencies of the AA, AC, and CC genotypes were 56.64% versus 57.59%, 40.71% versus 37.15, and 2.65% versus 5.26% in controls versus patients, respectively. No statistically significant difference has been proved between patients and controls frequencies ($P > .05$) for all additive, recessive, and dominant models. Additional analyses including genotypes combination, allelic frequencies, and hemorrhagic stroke patient subtypes did not show any statistically significant difference between controls and patients/subgroup patients. *Conclusions:* Our findings suggested no association between *MTHFR* gene polymorphisms and susceptibility to hemorrhagic strokes in Moroccan patients. Further investigations should be conducted to elucidate the roles of other gene variants in the pathogenesis of this condition. **Key Words:** Hemorrhagic stroke—*MTHFR*—C677T and A1298C polymorphisms—risk assessment—Morocco.

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Introduction

Stroke is the second-leading cause of death in the world with 6.5 million stroke deaths in 2013 and about 795,000 people experience a stroke in the United States each year.[1] In Morocco, a lower middle-income country, stroke is a public health concern with prevalence estimated to be 284 per 100,000.[2] It has been estimated that stroke mortality will double worldwide by 2020, owing to an ageing population and an increasing incidence in developing countries.[3] It is not only one of the major causes of death, but is also the leading cause of long-term disability, making it very costly to the economy.[4] About 80% to 85% of stroke cases are ischemic, whereas 15% to 20% are hemorrhagic. However, in younger adults (20-64 years), the number of deaths from hemorrhagic strokes (HS) is about twice as high than the number of deaths from ischemic strokes.[5] In HS, blood seeps from a hole in a blood vessel wall into either the brain itself (intracerebral hemorrhage), or the space around the brain (subarachnoid hemorrhage).[3] Stroke is a multifactorial disease and numerous risk factors including age, gender, ethnicity, family history, genetic predisposition, hypertension, diabetes mellitus, hyperlipidemia, smoking, oral *Streptococcus mutans*, anticoagulant and thrombolytic therapy, obesity, and alcohol consumption have been identified.[6-16] Literature data suggest a strong familial contribution to strokes and the heritability for overall intracerebral hemorrhage (ICH) risk was estimated at 44% suggesting a genetic predisposition.[17-19] Candidate gene, genome-wide, and meta-analysis approaches have been used to identify genetic components associated to HS risk or to one of its subgroups. Therefore, some variants in genes related to the pathogenesis of cerebral amyloid angiopathy hemostasis, lipid metabolism, inflammation, and the central nervous system microenvironment (*ACE*, *APOE*, *COL4A2*, *MTHFR*, *PMF1*, *SLC25A44* and *TRHDE*) has been considered as the most likely candidates for variants that increase the risk of intracerebral hemorrhage (HIC).[20-24]

The *MTHFR* gene, which encodes for the methylenetetrahydrofolate reductase enzyme, is one of the most investigated genes associated with stroke for its important role in vascular function through the homocysteine.[25] Otherwise, the coding enzyme is involved in the transformation of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which acts as a methyl donor for methylation of homocysteine to methionine and plays therefore a critical role in modulating plasma levels of homocysteine. The common *MTHFR* gene variants, C677T (rs1801133) and A1298C (rs1801131), are known to be associated with reduced enzymatic activity resulting in hyperhomocysteinemia.[26] Some studies have reported that hyperhomocysteinemia may induce endothelial dysfunction and therefore associated with hemorrhagic and ischemic strokes by acting on coagulation or through the rupture of microaneurysms, probably increasing vessel fragility, respectively.[27,28] Although, the association of *MTHFR* variants and the risk of hemorrhagic stroke were strongly shown in some ethnic populations, however, a weak association was found in other groups.[20,28-34]

The present study has been undertaken to evaluate the association of the common variants in *MTHFR* gene with the risk of hemorrhagic stroke in Moroccan patients in global goal to understand the genetic baseline of this pathology in our population.

Methods

Subjects

Patients enrolled in this study included 113 hemorrhagic stroke cases who presented to the Neurosurgery Service of CHU of Casablanca, Morocco, between July 2010 and October 2012. They were from diverse geographic regions of the country. Hemorrhagic stroke was confirmed in all participating subjects by neuroimaging (computed tomography scan or magnetic resonance imaging). Only nontraumatic cases were included in the range of selection. Exclusion criteria included trauma, brain tumor, and hemorrhagic transformation of a cerebral infarction. Baseline demographic, clinical features, and laboratory findings of patients were noted. After clinical data collection, whole blood samples were obtained for the genetics analysis. In addition, 323 individuals without any medical history of stroke or any neurological disorders according to the results of an interview and review of medical records were recruited as healthy controls. The informed consent was obtained from all the participants or their families. This work was carried out in accordance with The Declaration of Helsinki.

Genotyping

All DNA samples were isolated from frozen blood, checked for the quality and quantity using Nanovue plus spectrophotometer and normalized to a concentration of 20 ng/ul.

MTHFR C677T and A1298C polymorphisms were genotyped by using PCR-RFLP method. The primers (F: 5′-TGAAGGAGAAGGTGTCTGCGGGA-3′ and R : 5′-AGGACGGTGCGGTGAGAGTG-3′) and (F 5′-CTTTG GGGAGCTGAAGGACTACTAC-3′ and R 5′-CACTTTGTG ACCATTCCGGTTTG-3′) were used to amplify 198 bp and 163 bp sequences including the polymorphic sites of C677T and A1298C variants, respectively. The polymerase chain reaction condition was for both single nucleotide polymorphisms (SNP): an initial denaturation step of 5 minutes at 95°C, 35 cycles of 94°C for 30 seconds, 55°C for 30 seconds, and 72°C for 30 seconds, and a final extension step of 72°C for 10 minutes. The PCR products were digested by *HinfI* and *MboII* restriction enzymes, respectively, and then separated by electrophoresis in 3% Agarose gel. For the C677T variant, digestion of the C allele (normal)