9. Migraine and coronary artery disease: An updated open study on the genetic polymorphism

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Summary. Genetic factors that increase susceptibility to oxidative stress, endothelial disfunction and, possibly, stroke include angiotensin-converting enzyme gene deletion polymorphism (ACE-DD) and the methylenetetrahydropholate reductase (MTHFR) C677-TT polymorphism. The relationship of ACE-DD genotype to ischemic stroke and cardiovascular disease is controversial, but it has been independently linked to lacunar infarction, in the absence of carotid atheroma. Lea et al. (2005) reported that the ACE DD genotype acts in combination with the MTHFR T/T genotype to increase migraine susceptibility, with the greatest effect in those with aura. The “TT” polymorphism is also associated with an increased risk of migraine with aura, independent of other cardiovascular risk factors. The aim of our study was to evaluate the incidence of ACE and MTHFR genes polymorphisms in a consecutive series of migrainous patients and of patients affected by myocardial infarction. We studied a series of 200 migrainous patients (1), whose age was between 13 and 77 years (146 suffering

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from migraine without aura, MwA, 21 from migraine with aura, MWA, 33 from mixed forms MwA-MWA, according to ICHD-II 2004 criteria) and of 378 patients (2) suffering from ischaemic cardiopathy (myocardial infarction, MI). The analysis, based on Polymerase Chain Reaction (PCR) and on reverse-hybridization, showed as follows:

**MTHFR (C677T):** 113 patients (56.5%) (1) and 218 (57.6%) (2) were heterozygous; 19 patients (9.5%) (1) and 68 (17.9%) (2) were mutated. The result of 1 patient (2) was unknown.

**MTHFR (A1298C):** 102 patients (51%) [1] and 161 (42.6%) [2] were heterozygous, 15 patients (7.5%) (1) and 48 (12.7%) (2) were mutated. The result of 1 patient (2) was unknown.

**ACE** (evaluated on 198 patients (1) and 287 (2)): 87 patients (44%) (1) and 171 (59.5%) (2) had an ID genotype; 82 (41.4%) (1) and 98 (35.5%) (2) had a DD genotype.

The results of our study confirm the high incidence in the genetic polymorphisms ACE and MTHFR in migraineur. These data are confirmed in the sample of patients affected by myocardial infarction. This gives evidence of a strong relationship between migraine and major vascular diseases and let us hypothesize an important role of ACE and MTHFR system in the pathogenetic model of migraine for its capability to interfere with the endothelial regulation tone. Once an effective role in the genesis of migraine and in the increased risk of migrainous patients to evolve into an ischemic pathology has been obviously assigned to this genetic mutation, future researches must aim through wider and more controlled casistics also to clarify the role that drugs acting on these systems may have on the resolution of these diseases.

**Introduction**

Migraine is a common neurovascular disorder. During the last decade, many clinical studies have given evidence of an association between migraine, particularly migraine with aura, and ischaemic stroke. There are several pathophysiological mechanisms implicated in the genesis of ischaemic events in migrainous patients [1].

Recent studies assess the role of migraine as a risk factor for endothelial dysfunction, responsible not only for a reduced availability of vasodilatators and for an increase of vasoconstrictor agents, but also for a release of procoagulant, proinflammatory and proliferative factors, predisposing migraineurs to atherogenesis [2].

Endothelial dysfunction is due to an increased oxidative stress, promotor of inflammatory processes, proposed as implicated in the pathogenesis of migraine [3].

Migraine is related to an intracranial vasospasm followed by the maximum dilatation of extra and intra-cranial arteries responsible for pain. [2-4]. Considering that platelet activation and plasma coagulability are
increased during migraine attacks [5], it is strongly supported that genes affecting vascular endothelial function could play a significant role in cerebral blood flow changes occurring in migraineurs, so, contributing to the aetiology of the migraine [5,6].

In this respect, factor V Leiden, factor V (H1299R), prothrombin G20210A, factor XIII (V34L), β-fibrinogen, MTHFR (C677T), MTHFR (A1298C), APO E, PAI-1, HPA-1 and ACE I/D genetic polymorphisms seem to play a determinant role in vascular diseases in migraine [2-6]. Therefore, the association between genetic polymorphisms and migraine may induce an increased risk of thrombotic disorders development [2-6].

The present review analyzes both the incidence of the above genetic vascular mutations in migraineurs and the most recent developments related to genetic polymorphisms and migraine.

Genetic aspects underlying common forms of migraine are not clear, but the wide clinical spectrum of migraine suggests that several polymorphisms may interact to determine its manifestation and gravity, while the effect of a single mutation is thought to be minimal.

Recently, several angiotensin I-converting enzyme (ACE) inhibitors and an angiotensin II receptor blocker were demonstrated to have a clinically important prophylactic effect in migraine. ACE is one of the key enzymes in the rennin-angiotensin-aldosterone system, which modulates vascular tension and blood pressure [7]. In humans, serum ACE levels are strongly genetically determined. Individuals who were homozygous for the deletion (D) allele showed increased ACE activity levels. To investigate the role of ACE polymorphism in headache, we analyzed the ACE insertion (I)/deletion (D) genotypes of 54 patients suffering from migraine with aura (MwA), 122 from migraine without aura, 78 from tension-type headache (TH), and 248 non-headache healthy controls [7]. The ACE D allele were significantly more frequent in the MwA than controls. The incidence of the D/D genotype in MwA (25.9%) was significantly higher than that in controls (12.5%). No differences in the remaining groups were found. These findings indicate that the D allele and the D/D genotype in the ACE gene is a genetic risk factor for MwA thus suggesting a possible relationship between ACE activity and the pathogenesis of migraine [7].

Paterna et al. [8] evaluate if the DD genotype could also be associated with the frequency and duration of migraine without aura. In 302 patients suffering from migraine without aura, the genotypes of the ACE gene, plasma ACE activity, and the frequency (weekly) and duration of migraine attacks were evaluated. No drugs were given before (4 weeks) and during the study. The same evaluations were performed in 201 subjects without migraine. Patients with migraine without aura showed higher incidence of the ACE-DD
gene (48.34%) than control subjects (37.32%). The frequency of migraine (average attacks per week) was higher in patients with DD (2.11 +/- 1.9) than in patients with ID (1.54 +/- 1.44). No difference in duration of migraine attacks (hours per week) was observed. Plasma ACE activity was increased in patients with the ACE-DD gene. These data suggest that ACE-DD gene polymorphism could have an important role in determining migraine attacks and the frequency of these attacks [8].

Also, angiotensin converting enzyme (ACE) gene has been implicated as a genetic factor associated with migraine [9]. A case-control study was performed to investigate the association between ACE and migraine in 240 migraine patients and 200 healthy controls [9]. There was no significant difference in allelic frequency (I and D) and genotype polymorphism (DD, DI and II) of the ACE gene in migraine patients and controls. Analysis of the difference in ACE polymorphism stratified by gender revealed that male migraine patients with the homozygote DD genotype (ACE-DD) were significantly fewer than that of male controls. There was no existence of a difference among the frequency and duration of headache in each subgroup of migraine patients stratified by ACE genotype. These findings indicate that ACE-DD may have a slight protective effect against migraine in male patients [9].

Another study was conducted to determine whether the ACE I/D gene variant is involved in migraine risk and whether this variant might act in combination with the previously implicated MTHFR C677T genetic variant in 270 migraine cases and 270 matched controls [10]. Statistical analysis of the ACE I/D variant indicated no significant difference in allele or genotype frequencies. However, grouping of genotypes showed a modest, yet significant, over-representation of the DD/ID genotype in the migraine group (88%) compared to controls (81%). Multivariate analysis, including genotype data for the MTHFR C677T, provided evidence that the MTHFR (TT) and ACE (ID/DD) genotypes act in combination to increase migraine susceptibility [10]. This effect was greatest for the MA subtype where the genotype combination corresponded to an OR of 2.89 (95% CI: 1.47-5.72, P = 0.002). In Caucasians, the ACE D allele confers a weak independent risk to migraine susceptibility and also appears to act in combination with the C677T variant in the MTHFR gene to confer a stronger influence on the disease [10].

Given the above evidences, the aim of our study was to evaluate the incidence of ACE and MTHFR genes polymorphisms in a consecutive series of migrainous patients and of patients affected by myocardial infarction.
Materials and methods

The sample studied was represented by 200 patients suffering from migraine and 378 from ischaemic cardiopathy. Migrainous patients, 138 females and 62 males, aged 11-76 years, were observed at the Headache Center of S. Luca Hospital, Vallo della Lucania (Sa) between 2004 and 2011. In the same period 378 patients, 248 males and 130 females, suffering from ischaemic cardiopathy (myocardial infarction) at the Coronary Unity of S. Luca Hospital were studied too. Headache patients suffered from migraine with and without aura and from mixed forms of migraine, diagnosed on the base of the International Headache Society criteria (IHS-1988, and revisited with the new ICHD-II 2004 criteria.

Exclusion criteria from the study were:

- positive anamnesis for abuse of analgesics
- presence of serious medical diseases, which obliged patients to treatments interfering with the study
- need to take drugs for other disorders

A clinical schedule was compiled for each patient presenting for the first time at the Headache Center. Once reported personal data and information about the general personal and familial physiological anamnesis, in the second part of the questionnaire the semiological characteristics of headache were identified: familiarity, age of onset, course, frequency, recurrence. Later, in the third part, information about the description of headache, such as seat and kind of onset, type of pain (pulsating, gravitative, constrictive, etc.) localization, diffusion, intensity were collected. In order to correctly diagnose headache, the potential presence, localization, kind-order-duration of appearance of visual (flickering lights, spots or lines), sensory, motor, as well as speech symptoms.

Several local and/or general symptoms, associated to migraine, as well as possible trigger factors precipitating headache, i.e. psycho-physical stress (the more frequently reported) were identified. Finally, every patient was asked whether he/she used drugs, particularly analgesics, or what kind of measures he/she adopted during attacks, to reduce pain. This aspect was very important, as most patients, particularly those suffering from MwA, aim to press temples, to reduce pain, while patients who refer photo-phonophobia prefer laying on bed in the dark.

Subsequently, dietary habits were investigated (number of daily meals, quality and quantity of food consumed, liquids daily taken etc.), as well as the existence of clinical signs indicating probable nourishment deficits.
Data collected were integrated with a general clinical and neurological exam, then every patient was given a diary, to report attacks occurring in the period between the first and the second visit (number of attacks, duration and intensity). For fertile female patients, the diary was also useful to verify the probable combination of headache attacks and menstruations.

Patients also received a schedule to search for probable trigger factors and a prescription of tests to complete the diagnosis (supra-aortic trunk doppler, CT and/or brain MR, EEG test, Ocular Fundus, blood tests to determine basal homocysteine (by HPLC), fibrinogen, antitrombina III, folates and vitamin B12 levels).

Finally, a series of test to determine genetic polymorphisms was prescribed. Our study focused on the evaluation of the following polymorphisms:

- MTHFR (C677T)
- MTHFR (A1298)
- ACE I/D

At the end of the first visit, each patient was suggested an attack therapy, based on headache characteristics.

The assay for the identification of genetic mutations associated with cardiovascular diseases was based on polymerase chain reaction (PCR) and reverse-hybridization and included three steps:

1) DNA isolation
2) PCR amplification using biotinylated primer
3) Hybridization of amplification products to a test strip containing allele-specific oligonucleotide probes immobilized as an array of parallel lines. Bound biotinylated sequences were detected using streptavidin-alkaline phosphatase and color substrates.


Amplification mix, Taq dilution buffer, conjugate solution, wash solution B contained 0.05% NaN₃. Conjugate solution contained streptavidin-alkaline phosphatase. Color developer contained nitro blue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl phosphate (BCIP).

Store all reagents at 2-8°C when not in use. Store Taq polymerase at -20°C.
Results

Tables 1 and 2 report the results of our study.

**Table 1.** Comparison of MTHFR polymorphisms.

<table>
<thead>
<tr>
<th>POLYMORPHISM</th>
<th>MIGRAINE GROUP</th>
<th>MYOCARDIAL GROUP</th>
<th>INFARCT GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% WT</td>
<td>% HET</td>
<td>% MUT</td>
</tr>
<tr>
<td>MTHFR (C677T)</td>
<td>34</td>
<td>56,5</td>
<td>9,5</td>
</tr>
<tr>
<td>MTHFR A1298C</td>
<td>41,5</td>
<td>51</td>
<td>7,5</td>
</tr>
</tbody>
</table>

**Table 2.** Comparison of ACE polymorphisms.

<table>
<thead>
<tr>
<th>POLYMORPHISM</th>
<th>MIGRAINE GROUP</th>
<th>MYOCARDIAL INFARCT GROUP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% II</td>
<td>% ID</td>
</tr>
<tr>
<td>ACE ID</td>
<td>14,6</td>
<td>44</td>
</tr>
</tbody>
</table>

Migrainous patients came almost consecutively at our observation. 146 of them suffered from MwA (73%), 21 from MWA (10,5%) and 33 were affected by mixed forms MwA-MWA (16,5%). 80 patients (40%) referred that one relative at least suffered from an unknown form of headache and 15 (7,5%) had a positive familial history for vascular diseases.

MTHFR C677T mutation was found in 19 patients (9,5%), while the heterozygous genotype was found in 113 patients (56,5%), and the normal one in 78 patients (34%).

15 patients (7,5%) were mutated for the A1298C variant of MTHFR, 102 (51%) were heterozygous and 83 (41,5%) were normal (Table 1).

Finally, ACE polymorphism, evaluated on 198 patients (99%), was in its I/I form in 29 patients (14,6%), I/D in 87 patients (44%) and D/D in 82 patients (41,4%) (Table 2).

The control sample was composed by 378 patients suffering from Acute Myocardial Infarction (IMA), admitted, in the same period, at the Intensive Coronary Care Unit of S.Luca Hospital, in Vallo della Lucania.
They underwent the same series of screening for genetic polymorphisms, and results were the following:

The evaluation of C677T variant of MTHFR identified 68 mutated (17.9%), 218 (57.6%) heterozygous and 92 (24.5%) normal patients (Table 1). The result of 1 patient was unknown.

The normal genotype of MTHFR (A1298C) was present in 169 patients (44.7%); 161 (42.6%) were heterozygous and 48 (12.7%) were mutated (Table 2). The result of 1 patient was unknown.

ACE polymorphism, evaluated on 287 patients, was in its I/I form in 18 patients (5%), I/D in 171 patients (59.5%) and D/D in 98 patients (35.5%) (Table 2).

**Discussion**

Migraine is a common neurovascular disorder. During the last decade, many clinical studies have given evidence of an association between migraine, particularly migraine with aura, and ischaemic stroke. There are several pathophysiological mechanisms implicated in the genesis of ischaemic events in migrainous patients [11-14].

Although several studies were conflicting, they suggested a higher frequency of some prothrombotic genetic abnormalities in migrainous patients [11-14].

While the exact etiology of migraine headaches is unknown, several theories have been proposed. The vascular theory, as proposed in 1938 by Graham and Wolff [15], attributes migraines to an initial intra-cranial arterial vasoconstriction, resulting in reduced blood flow to the visual cortex, followed by a period of extra-cranial vasodilation [16]. Modern imaging techniques have shown that during a common migraine attack there are in fact only minor changes in cerebral blood flow, and the proposed initial vasoconstrictive phase may actually last much longer than the aura [17]. It has also been hypothesised that migraine sufferers have an inherent vasomotor instability and are more susceptible to the vasodilatory effects of certain physical and chemical agents. This point of view has been reinforced by the observation that organic nitrates, which are capable of delivering nitric oxide, trigger migraine attacks in migraineurs, at low doses, ineffective in normal subjects [18].

Moskowitz’s theory involves the trigeminovascular complex, which links the aura and the headache of migraine [19-20]. In this theory the trigeminovascular neurons release substance P and other neuro-transmitters in response to various triggers (Fig.1).
Figure 1. Potential mechanisms of stroke in migraine.

Figure 2. Integrated pathogenetic model of migraine.
Among genetic factors that increase susceptibility to oxidative stress and endothelial dysfunction, polymorphisms of ACE and MTHFR genes may, through their influence on plasma Ang-II and Homocysteine levels, respectively, play a key role both in migraine and in cardiovascular diseases pathogenesis.

Starting from these hypotheses, our study focused on the identification of ACE and MTHFR (C677T and A1298C) genotypes in a group of migraineurs and in a control group suffering from vascular diseases.

Our results showed essentially comparable frequencies of the three polymorphisms, thus confirming a common etiopathogenesis.

On the basis of our data, the pathogenetic model of migraine was integrated with genetic polymorphism, for their capability to interfere with endothelial function.

References


11. Iniesta, JA; Corral, J; Gonzà-Conejero, R.; Ortuno, AD; Navarro, MLM; Vicente, V; Role of factor XIII (Val 34 Leu) polymorphism in patients with migraine; *Cephalalgia* 2001, 8: 837-841.


