A rare report of thrombophilic derangements related to migraine with aura in monozygotic twins.

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Abstract

Recent evidences indicate that migraine may be involved as a risk factor for cerebral infarction in young women, thus indicating an undisputable connection between migraine and cerebral ischaemia [1-3]. Although the nature of this connection remains essentially unknown, several studies have been devoted to the detection of markers of a prothrombotic tendency in migrainous subjects [1-4]. In the present study, we have evaluated the possible relationship between migraine and prothrombotic genetic risk factors. MN and MM, 52-year old monozygotic male twins, and MA 27-year old daughter of MM are suffering from migraine with aura (International Headache Society, 2004 criteria) since 15 years of age and have a familial history of cardio-cerebral vascular disease. MN is affected with angina pectoris and MM with myocardial infarction and transient ischemic accident. Data of laboratory examinations for screening of the prothrombotic state revealed: MN: Factor XI:c 128.2% (range 70-120), Factor XII:c 150.4% (range 70-120), Activated Protein C-resistance (APT) 0.6 (range >0.7), Plasma Homocysteine 29 μmol/L (range 5-15), MTHFR gene (TT/CC). MM: Factor XII:c 138.6% (range 70-120), APC-resistance 0.6 (range >0.7), Plasma Homocysteine 40.2 μmol/L (range 5-15), MTHFR gene (TT/CC). MA: Factor XII:c 132.2% (range 70-120), APC-resistance 0.5 (range >0.7), Plasma Homocysteine 30.6 μmol/L (range 5-15), MTHFR gene (TT/CC), PAI-1 4G/5G.

Although there are no data in literature on prothrombotic genetic risk factors in patients suffering from migraine, our data seem to demonstrate a strict association between migraine with aura history, cardio-cerebral vascular disease and prothrombotic genetic risk factors, thus suggesting that haemostatic risk factors for arterial thrombosis could play an important role in the clinical study of these patients.

Keywords: Migraine, cerebral ischaemia, prothrombotic genetic risk factors

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Introduction

The possible relationship between migraine and risk of ischaemic stroke is an important public health concern. However, the mechanism underlying this relationship is complex and not fully clear. Migraine and stroke can coexist: stroke may occur with the clinical features of migraine, or it may be induced by migraine [1-3]. There are good epidemiological evidences about the relationship of migraine not only with an increased risk of stroke (which is stronger in young adults, but may persist in the elderly) but also with any vascular ischaemic event, myocardial infarction included [2].

The exact mechanism by which migraine with aura may lead to ischaemic vascular events is still unknown and probably very complex. Prospective data give no evidence that migraine without aura is associated with increased risk of any ischaemic vascular events [2]. Several studies showed an increased risk of stroke in peo-
people suffering from migraine; some others failed in finding this association. The probable mechanism is thought to be partly a platelet hyperaggregability and a cerebral blood flow reduction, usually occurring in migraine with aura [2].

Unlike previous studies, showing no increase in the risk of haemorrhagic stroke in migraineurs, Etminam et al. [3,4], gave evidences about a causal relation between migraine and stroke.

Possible mechanisms for this association include irregularities in blood flow, cardiac abnormalities, and abnormal production of prostaglandins as well as noradrenergic or cholinergic transmitters and receptors [1-4].

The Genetic Epidemiology of Migraine study showed that, compared with controls, migraineurs are more probably smokers, with a familial history of early myocardial infarction. The same study highlighted that migraineurs with aura have an unfavorable cholesterol profile, hypertension, frequently report a history of early onset coronary heart disease or stroke and present a two-fold increased 10-year risk of coronary heart disease, compared with the Framingham score, even after adjusting for age [2].

A reduction of cerebral blood flow in some regions and an increased plateau activity, factors contributing to the risk of thrombosis, were noticed in migraine [4]. The detailed study of migraine pathophysiology announced that a dysfunction of brain cells and arteries is a major component of this disorder. The involvement of cerebral arteries, in fact, together with the high prevalence of migraine in young people with stroke induced Kurth et al. [2] to hypothesize that migraine may be a risk factor for stroke and to find the potential biological mechanisms underlying this connection.

The above study suggested several hypotheses:
1) Migraine might be a direct cause of an ischaemic event (i.e., migraineous infarct),
2) The pathophysiology of migraine might affect the endothelial function and by this alone or in combination with pre-existing factors might increase the risk of stroke outside of a migraine attack,
3) Migraine might be associated to an increased prevalence of risk factors for ischaemic vascular events,
4) The connection might be provoked by migraine-specific drugs,
5) Migraine and ischaemic vascular events might be connected through a genetic component. As regards to ischaemic stroke, some congenital heart defects e.g., patent foramen ovale, were discussed as potential biological mechanisms [2].

The accepted neurovascular theory of migraine integrates the phenomena of headache and aura, the focal neurologica
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mic stroke mechanisms, involving the vasculature not only in headache, but also in migraine-related infarction. It is still unclear whether endothelial dysfunction may be a cause or a consequence of headache, or whether they coexist for other reasons.

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.

Among genetic factors that increase susceptibility to oxidative stress and to endothelial dysfunction, with a consequent increase in the risk of stroke, the Angiotensin-Converting Enzyme (ACE) gene deletion polymorphism and the Methylene tetrahydrofolate reductase (MTHFR) C677T gene polymorphism are involved [6]. In an attempt to individuate genes potentially implicated in the etiopathogenesis of migraine, ACE gene is probably the most studied gene [10,11].

An insertion/deletion (I/D) polymorphism localized within intron 16 of the ACE gene, is due to the presence (allele I-Insertion) or absence (allele D-Deletion) of a 287-bp alu repeat sequence and it can produce three different genotypes:

- II insertion homozygosis
- ID insertion/deletion heterozygosis
- DD deletion homozygosis [12].

Several studies reported an association between DD homozygous genotype and the intermediate phenotype (circulating concentrations of the enzyme). DD homozygosis provokes a 56% increase in ACE activity compared with I allele homozygous [13].

Several clinical studies [14-25] demonstrated that ACE-DD genotype acts in combination with MTHFR-TT genotype, to increase susceptibility to migraine, particularly migraine with aura. MTHFR-TT genotype is also associated with an increased risk of migraine with aura, independently from other cardiovascular risk factors [14-27].

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 in patients affected by myocardial infarction.

Materials and Methods

MN and MM, 52-year old monozygous male twins and MA, 27-year old daughter of MM, represented the samples studied. They are suffering from migraine with aura (International Headache Society, 2004 criteria) since 15 years of age, with a familial history of cardio-cerebral vascular disease. MN is affected with angina pectoris and MM with myocardial infarction and transient ischemic accident. They were observed at the Headache Center of S. Luca Hospital, Vallo della Lucania (Sa), between the periods 2004 - 2009.

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

Several local and/or general symptoms, associated with migraine, as well as possible trigger factors precipitating headache, e.g., 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 experience photo-phono-phobia 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 Data collected were integrated with general clinical and neurological examinations. 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 examination, 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 (A1298C)
ACE I/D

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

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).

All reagents were stored at 2-8°C when not in use. Taq polymerase was stored at -20°C.

Results

Table 1 reports the results of our study. Our data indicate:

<table>
<thead>
<tr>
<th>Test</th>
<th>M.N.</th>
<th>M.M.</th>
<th>M.A.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Factor XI (n.v. 70-120)</td>
<td>128,20%</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Factor XII (n.v. 70-120)</td>
<td>150,40%</td>
<td>138,60%</td>
<td>132,20%</td>
</tr>
<tr>
<td>Activated Protein C-resistance (n.v. &gt;0,7)</td>
<td>0,6</td>
<td>0,6</td>
<td>0,5</td>
</tr>
<tr>
<td>Homocysteine (n.v. 5-15 micromol/l)</td>
<td>29</td>
<td>40,2</td>
<td>30,6</td>
</tr>
<tr>
<td>MTHFR (gene C677T)</td>
<td>TT/CC</td>
<td>TT/CC</td>
<td>TT/CC</td>
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**Figure 1.** Potential mechanisms of stroke in migraine

**Figure 2.** Integrated pathogenetic model of migraine
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 [6].

According to Moskowitz theory, trigeminovascular neurons release substance P and other neurotransmitters in response to various triggers. Substance P is associated with vasodilatation, mast cell degranulation, increase in vascular permeability and edema of the meninges, events that configure the so-called “neurogenic inflammation”. Excessive trigeminal discharge and neurovascular inflammation of the meninges ensue in migraine headache [27].

Recent studies [1-3] assess the role of migraine as a risk factor for endothelial dysfunction, responsible not only for a reduced availability of vasodilators and for an increase of vasoconstrictor agents, but also for a release of procoagulant, proinflammatory and proliferative factors, predisposing migraineurs to atherogenesis.

Endothelial dysfunction is due to an increased oxidative stress, promoter of inflammatory processes, proposed as implicated in the pathogenesis of migraine [6].

High homocysteic acid (Hcy) levels, provoked by hyperhomocysteinemia, have marked excitatory effects on neurons. By acting as an endogenous agonist of NMDA receptors homocysteic acid contributes to the excitability of CNS and it has a predominant role in the initiation, propagation and duration of cortical spreading depression involved in migraine pathogenesis. It can furthermore, sensitize dura mater and cerebral arteries, and/or promote trigeminovascular system activation, predisposing subjects to migraine attacks or increasing their gravity [27].

Among the potential mechanisms of hyperhomocysteinemia-induced vascular damage, very relevant is the hypothesis that reactive oxygen species are generated during this amino acid metabolism, and they are implicated in the development of atherosclerotic processes.

So there is a strict proportionality between plasma Hcy levels and its oxidant power. In addition to the correlation between homocysteinemia and nitric oxide production Hcy is also a crucial risk factor for vasculopathies and at the same time implicated in migraine genesis [28].

Several studies showed that Ang-II exerts important proinflammatory effects on vessel wall, inducing Reactive Oxygen species (ROS), inflammatory cytokines and adhesion molecule production [28,29].

Ang-II increases blood monocytes migration and differentiation into macrophages, in atherosclerotic plaque; by interacting with AT1 receptors, it stimulates NADPH-oxidase system and promotes ROS production in vascular cells and in macrophages, particularly activators of cytoplasmic signaling cascades, such as NFkB and of other mechanisms increasing oxidative stress in vessel wall and leading to an activation of redox-sensitive genes, i.e. those encoding for proinflammatory cytokines (IL-6). Other mechanism by which ROS may induce the development of atherosclerosis include PAI-1 mediated thrombotic mechanisms and proinflammatory cytokines stimulation [29].

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


16. Prothrombotic genetic risk factors in migraine


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