Apolipoprotein E gene polymorphisms and migraine

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Abstract

Nitric oxide plays an important role in the pathogenesis of migraine. Studies suggest that the expression of molecules involved in the pathogenesis of headache (i.e., nitric oxide-interleukin) is influenced by apolipoprotein E (APOE) and is gene specific. Hence, we hypothesized that APOE polymorphism may be associated with migraine.

Our study analysed the incidence of genetic polymorphism Apolipoprotein E in a sample of migraineurs and a control group of the patients with ischemic cardiopathy.

In this study 70 consecutive patients aged 10-66 years (mean age 39.2 years), suffering from migraine [¹] (58 migraine without aura, 12 migraine with aura, ICHD-II criteria) and 70 patients aged 36-71 years (mean age 45.8 years), with ischemic cardiopathy [²] were studied with Polymerase Chain Reaction (PCR) for genetic polymorphism Apolipoprotein E.

ApoE: 51 patients (75%) [¹] and 47 (67%) [²] had an E3/E3 genotype; 11 (16%) [¹] and 11 (16%) [²] had a E3/E4 genotype; 5 (7%) [¹] and 7 (10%) [²] had a E2/E3 genotype; 1 (1%) [¹] and 5 (7%) [²] had a E4/E4 genotype; 2 (1.5%) [¹] and 0 [²] had a E2/E4 genotype; 0 [¹] and 0 [²] had a E2/E2 genotype.

Our results highlighted a more or less equivalent prevalence of apolipoprotein E gene polymorphisms in migraineurs and in subjects suffering from ischemic cardiopathy. Further research is required to confirm the findings of the present study in a larger sample and to elucidate the role of APOE polymorphism in headache.

Key words: Apolipoprotein E, gene polymorphisms, migraine
Introduction

Migraine is a neuro-vascular syndrome characterised by recurrent headache attacks associated with photophobia, phonophobia, nausea and vomiting. Migraine occurs in about 18% of women and 6% of men, regardless of race or geographical location [1]. Currently, migraines are divided into two categories: migraine without aura (previously termed common migraine), and migraine with aura (previously also termed classical migraine) preceded by a 15-20 minute episode of visual or sensory aura. Auras are most commonly visual alterations, such as hemianopsic field defects and scotomas that enlarge and spread peripherally [2]. Visual auras are associated with spreading cortical depression; sensory auras are usually experienced as paraesthesias of the arm and face.

While the exact etiology of migraine headaches is unknown, several theories have been proposed. The vascular theory 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 [3]. 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 [4]. 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 [5].

Moskowitz's theory involves the trigeminovascular complex, which links the aura and the headache of migraine [6]. In this theory the trigeminovascular neurons release substance P and other neurotransmitters in response to various triggers.

Nitric oxide (NO) is thought to play a central role in the pathogenesis of migraine as well as tension-type headache. Up-regulation of the endogenous L-arginine/NO pathway and increased NOS expression (perhaps the constitutive form) have been hypothesized in migraineurs during spontaneous migraine attacks [7]. Whether this up-regulation is expressed only at the endothelial level or also occurs at the neural level, especially in the pain transmission pathways, has been a matter of controversy among headache researchers [8].

There is evidence that trigeminal neurons contain nitric oxide and its activity is regulated by nNOS, as suggested by the increase in c-fos immunoreactivity of trigeminal nucleus caudalis following administration of nitrate donor [9]. Furthermore L-NAME, a nitric oxide synthetase inhibitor, can reduce this activity [10]. Nitric oxide has its role not only in the development of migraine, but also in the pathogenesis of tension-type headache [11]. It is suggested that nitroglycerin increases the pre-existing central sensitization in chronic tension-type subjects, and nitric oxide synthetase inhibitors are helpful in the management of chronic tension-type headache by reducing the central sensitization [11].

Besides nitric oxide synthetase, nitric oxide production is also dependent on apolipoprotein E (APOE) polymorphism and this production is gene specific [12]. Available literature suggests that APOE E4 increases the uptake of arginine in microglia as compared to APOE E3 and thus may regulate production of nitric oxide [6]. Relatively increased nitric oxide production has been reported by APOE E4 containing monocytes as compared to the monocytes harbouring APOE E3 gene [13]. This production is independent of the expression of iNOS gene and may be largely dependent on the arginine uptake [12].

The increased production of NO is consistent with the increased nitrative/oxidative stress observed with APOE E4 and may underlie the greater neuronal damage seen after closed head injury and stroke [13]. The production of nitric oxide may be gender dependent, as monocytes from APOE E4/E4 male transgenic mice have been shown to produce more nitric oxide as compared to APOE E3/E3 mice. However, this difference was not observed in...
female APOE transgenic mice [14]. Not only the gender, but also the amount and isoform of APOE might influence the development of inflammation. Higher inflammation activity was associated with the APOE E4 gene as compared to the APOE E3 gene [15]. Similarly, APOE polymorphism also influences the expression of the cytokines that are commonly involved in migraine and tension-type headache [16, 17]. APOE E3 gene containing cells have lower expression of cytokines TNF-a and IL-6 as compared to cells expressing APOE E2 and APOE E4 genes [17]. Moreover, circulating IL-10 levels are also dependent on the presence of APOE E4 gene [18].

Based on these evidences, we hypothesized that APOE gene polymorphism should be associated with migraine and the present study was planned to assess the role of APOE gene polymorphism in migraine patients.

**Patients and Methods**

The study was performed and approved by Neurophysiopatology Service, Headache Centre, S. Luca Hospital, Vallo della Lucania (SA), Italy.

Our study analysed the incidence of genetic polymorphism Apoliporotein E in a sample of migraineurs and a control group of the patients with ischemic cardiopathy.

In this study 70 consecutive patients aged 10-66 years (mean age 39.2 years), suffering from migraine (58 migraine without aura, 12 migraine with aura, ICHD-II criteria ) and 70 patients aged 36-71 years (mean age 45.8 years), with ischemic cardiopathy were studied with Polymerase Chain Reaction (PCR) for genetic polymorphism Apoliporotein E.

**Genotyping**

In a sterile EDTA vacutainer, 3 ml venous blood was collected, at least 3 days after the attack of headache and immediately stored at -20_C. Genomic DNA was extracted with the help of Himedia Pura(R) kit, based on the spincolumn technique to provide 4~9620 lg of genomic DNA from 200 IL of human blood. DNA samples were subjected to polymerase chain reaction according to the method described by Pantelidis et al. [19] with slight modifications. This method involved use of four primers, which were combined to form three sets, each set containing two primers to detect the presence of a given allele of the APOE gene. Because this genotyping system is based on the presence or absence of PCR amplification by allele-specific primers, it is imperative to ensure PCR amplification for those reactions that do not produce allele-specific amplicons.

For this reason, each APOE-specific primer mix also contained a pair of "control primers" (primers 8 and 9), which amplified two regions of chromosome 6 in the HLA-DR locus, to verify PCR amplification in the absence of haplotype-specific amplification in each PCR reaction. Amplification was performed in a thermal cycler (Appendorf) using a high stringency PCR protocol with high annealing temperature to ensure specificity of amplification. The conditions were as follows: initial denaturation for 1 min at 96_C, followed by 5 cycles of 20 s at 96_C, 45 s at 70_C and 25 s at 72_C; 21 cycles of 25 s at 96_C, 50 s at 65_C and 30 s at 72_C; 4 cycles of 30 s at 96_C, 60 s at 55_C and 120 s at 72_C. The PCR products were analyzed by electrophoresis on a 1.5% Tris~borate-DTA/editium bromide agarose gel with 1 IL of loading dye at 6~968 V/cm. For all PCR reactions (APOE e2, APOE e3, and APOE e4), the presence of a 173-bp band indicated the presence of the specific APOE gene and a band at 785 bp depicted the product of the control gene.

Statistical analysis was done with the help of SPSS v 11.0 for Windows. For the categorical variables, v2 test was run. Binary logistic regression was applied to calculate the odds. The results were considered to be significant when P value was less than 0.05. The power of the study was calculated using Ca-TS software (http://csg.sph.umich.edu/) and deviation from Hardy-Weinberg equilibrium (HWE) was calculated manually.
Results

In migraineurs patients group were showed Apo genotype E3/E3 in 51 patients (75%); E3/E4 in 11 (16%); E2/E3 in 5 (7%); E4/E4 in 2 (1.5%); E2/E2 in 0. In patients with ischaemic cardiopathy were showed Apo genotype E3/E3 in 47 (67%); E3/E4 in 11 (16%); E2/E3 in 7 (10%); E4/E4 in 5 (7%); E2/E4 in 0; E2/E2 in 0. (See Table I).

The comparison of the results obtained in the two groups showed a slight percentage difference in subgroups E3/E3 (75% vs. 67%) and E2/E4 (1.5% vs 0%) in favor of migraine patients and inversely in cardiac patients in the subgroups E2/E3 (7% vs 10%) and E4/E4 (1% vs 7%), no difference between subgroups allelic E3/E4 (16% vs 16%), E2/E2 (0% VS0%) (See Fig. 1).

Discussion

Our results highlighted an equivalent prevalence of apoliprotein E gene polymorphisms in migraineurs and in subjects suffering from ischemic cardiopathy thus confirming that cytokines may play a role in primary headaches and in major vascular event[16].

Pro-inflammatory and anti-inflammatory cytokines show different relationships with the migraine attack. Perini et al. [17] found that TNF-α levels were increased during migraine attacks and they quantitatively correlated with the time elapsed after the headache onset. Similarly, levels of IL-1β also elevate during migraine, but that of other proinflammatory cytokines IL-2 and IL-6 remain unchanged. Increase in IL-6 in serum was demonstrated in two studies within 1 h of initiation of migraine attack [20, 21]. Furthermore, microglial cytokines are also influenced by APOE polymorphism and, together, these reports deduce that APOE E3 gene could be protective and APOE E4 might predispose to migraine [17, 18, 22-24]. It is known that APOE E2 lessens the amount of proinflammatory cytokine production from microglia, while APOE E3 and APOE E4 increase it linearly [21]. APOE E4 increases the release of IL-1β, PGE2 as well as TNF-α and IL-6 [23, 24]. Hence, APOE E4 predisposes the person to suffer a sustained inflammatory response both in the central nervous system as well as in the rest of the body [24, 25].

Akin to this study, previous studies also failed to find the association linkage of polymorphisms of various genes including neuronal NOS [26], inducible NOS [27], dopamine transporter gene [28] and interleukin-6 gene [29] with the migraine or its subtypes, probably owing to the heterogenicity of the migraine.

Like any other study, this study also had some limitations.

Migraine is frequently co-existents with other illnesses, e.g., depression, anxiety, allergies, etc., and also with each other [30]. Hence, it was difficult to identify and recruit subjects with pure migraine as well as isolated tension-type headache. The rigorous exclusion increases the validity of our findings, but at the cost of limiting the sample size.

Moreover, our data is consistent with the frequencies of APOE genes described in previous studies [31-33].

In conclusion, the present study shows that APOE E gene polymorphisms increases the risk of migraine. This is theoretically unexpected and a new finding. This finding requires confirmation in a population-based study. Also, further research is required to elucidate the pathophysiological role of APOE gene polymorphism and microglia in migraine.

References


Table I: Apo E genotype

<table>
<thead>
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<th>Apo E Genotype</th>
<th>Migraineurs patients</th>
<th>Patients with ischemic cardiopathy</th>
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<tr>
<td>E3/E3</td>
<td>51 (75%)</td>
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Fig. 1: Apo E genotype: patients migraineurs patients vs ischaemic cardiopathy