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Research report

Significance of the catechol-O-methyltransferase gene polymorphism in migraine

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Abstract

The objective was to assess the significance of the catechol-o-methyltransferase (COMT) enzyme polymorphism in migraine. For this reason, 62 migraineurs and 64 healthy volunteers were included in the study. The analysis of COMT polymorphism was performed using PCR. The H/H genotype was more frequent in the control group than in the patients group ($P=0.032$). The homozygous or heterozygous L allele was over represented in the migraineurs compared with the controls ($P=0.013$). The L/L genotype was over represented in the migraineurs who also had a family history of migraine ($P=0.003$). There was no relationship between aura and COMT genotypes. In conclusion, the COMT polymorphism may be of potential pharmacological importance regarding the individual differences in the metabolism of catechol drugs in migraineurs. Although altered catechoamine activity due to polymorphism of COMT gene may be one of the mechanisms involved in the pathogenesis of migraine, these mechanisms are not related to presence or absence of aura.

Keywords: Catechol-O-methyltransferase; Migraine; Polymorphism

1. Introduction

Migraine is a common neurovascular disorder. Diagnosis is made by the combination of history, physical examination and exclusion of other diseases. The characteristics of the disorder are severe episodes of headache, and autonomic and neurological symptoms. Although migraine is believed to be the manifestation of a hereditary abnormal sensitivity of neurovascular reactions to sudden changes in the internal or external environment or to cyclic changes in the central nervous system, the real underlying mechanism is unclear. It is likely that catecholamines are involved in migraine related symptoms.

Catechol-O-methyltransferase (COMT) is an enzyme which inactivates catecholamines or catechol containing drugs. The gene encoding for COMT is mapped to chromosome 22q11 [4]. This gene has been implicated to be involved in the pathogenesis of neuropsychiatric disorders, schizophrenia, bipolar affective disorder and Parkinson's disease [2,5,11,13].

COMT is genetically polymorphic in human red blood cells and liver. This genetic polymorphism results in a 3–4-fold difference in COMT activity, and due to a G→A substitution at codon 158 of the membrane-bound forms of COMT, which corresponds to codon 108 of the soluble or cytoplasmic form, leading to a valine to methionine substitution. A valine at codon 108/158 results in the heat-stable, high-activity COMT variant (H), whereas a methionine at this position results in the heat-labile, low-activity variant (L) [12]. The two alleles (Val 108/158 or H, and Met 108/158 or L), and the three genotypes (Val 158/Val 158 or H/H, Val 158/Met 158 or H/L and Met 158/Met 158 or L/L) can be identified with a polymerase chain reaction (PCR)-based restriction fragment length polymorphism (RFLP) analysis using the restriction enzyme Nla III [3].

For the purpose of this study, the significance of the COMT polymorphism was assessed in migraine. This

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study will also be addressing the polymorphic patterns of the COMT both in migraineurs and in the healthy population in this country.

2. Materials and methods

A total of 62 migraine patients and 64 healthy volunteers were included in the study. There were 11 (17.7%) male and 51 (82.3%) female migraineurs with ages ranging from 21 to 52 (mean, 32±5.2) years, and 14 (21.8%) male and 50 (78.2%) female healthy controls with ages ranging from 19 to 58 (mean, 30.7±6.3) years. The migraineurs and controls were unrelated. The migraineurs and healthy controls were nearly age and sex matched. The diagnosis of migraine was made on the basis of the criteria of International Headache Society, 1988.

Those who had mental retardation, drug dependence, and metabolic or psychiatric or neurological illness were excluded. Those whose first-degree relatives had endogenous psychoses or alcoholism were also excluded. An informed consent was obtained from the subjects who participated in the study. The migraineurs and healthy controls were from the same geographic region and of the same ethnic origin.

Physical and psychiatric examinations were carried out, and blood samples were taken for a complete blood count and blood chemistry (electrolytes, glucose, renal and liver function tests, and thyroid hormone level) assessments as well as for molecular analysis of COMT polymorphism.

2.1. Molecular analysis

DNA was extracted from peripheral blood leukocytes, and a PCR based restriction fragment length polymorphism assay was performed to detect the presence of the G→A transition at position 1947 in COMT (accession no. Z26491). PCR was used to amplify a 185-bp fragment of genomic DNA containing the polymorphism. Briefly, the primer sequences were 5′-GGAGCTGGGGGCCTACTGTG-3′ (forward) and 5′-GGCCCTTTTTCCAGGTCTGACA-3′ (reversed). PCR was performed in a 50-μl volume with 20–100 ng DNA, 100 μM dNTPs, 20 pmol of each primer, 1 mM $MgCl_2$, 20 μM Tris–HCl, pH 8.6, 50 μM KCl, 0.2% (w/v) bovine serum albumin and 1 U Taq polymerase (MBI Fermentas). Amplification was performed on an automated thermal cycler (Techne Genius). PCR conditions were 3 min for initial denaturation at 94°C; 35 cycles at 94°C for 1 min for denaturation, 1 min at 60°C for annealing and 1 min at 72°C for extension, followed by 7 min at 72°C for final extension. The resulting PCR products were subjected to restriction digestion for 3 h at 37°C using 5 U Nla III (BioLabs). The digested products were resolved at 100 V for 20–30 min on a 4% NuSieve 3:1 Agarose (FMC BioProducts) containing 0.5 μg/ml ethidium bromide. A 100-bp marker (100-bp DNA Ladder, MBI Fermentas) was used as a size standard for each gel lane. The gel was visualized under UV light using a gel electrophoresis visualizing system (Vilber Lourmat). The COMT-LL genotype was represented by 114-, 36- and 35-bp fragments; COMT-HH by 96-, 35-, 36- and 18-bp fragments: COMT-HL by 114-, 96-, 36-, 35- and 18-bp fragments. The 18-bp fragment was difficult to visualize because of both its small size and co-migration with the similarly size primer residue; however, detection of this fragment was not critical in determining genotypes. Genotyping was based upon independent scoring of the results by two reviewers who were unaware of case/control status.

Table 1
COMT polymorphism in the migraineurs and healthy controls

	COMT genotypes		
	L/L	L/H	H/H
Migraineur *n* (%)	13 (21)	40 (64.5)	9 (14.5)
Control *n* (%)	12 (18.8)	30 (46.9)	22 (34.4)

2.2. Statistics

The analyses of data were performed using SPSS 8.0 for Windows, and Pearson χ^2 or Fisher's exact tests were used.

3. Results

The H/H genotype was over represented in the healthy controls compared with the migraineurs ($\chi^2=6.890$, $P=0.032$). The homozygous or heterozygous L allele was over represented in the migraineurs compared with the controls ($\chi^2=6.695$, $P=0.013$) (Table 1). The L/L genotype was over represented in the migraineurs who also had a family history of migraine (Fisher's exact test, $P=0.003$) (Table 2).

Of 62 migraineurs, 29 had migraine without aura (MOA) and 33 had migraine with aura (MA), (Table 3). The distribution of the COMT variants in MOA and MA were similar ($\chi^2=0.833$, $P=0.659$).

Table 2
Relationship between COMT polymorphism and family history of migraine

Family history	COMT genotypes		
of migraine	L/L	L/H	H/H
Absent *n* (%)	1 (3.6)	20 (71.4)	7 (25)
Present *n* (%)	12 (35.3)	20 (58.8)	2 (5.9)

Table 3
Relationship between COMT polymorphism and aura

Migraine	COMT genotypes		
	L/L	L/H	H/H
Without aura *n* (%)	7 (24.1)	17 (58.6)	5 (17.2)
With aura *n* (%)	6 (18.2)	23 (69.7)	4 (12.1)

4. Discussion

In the majority of migraine diseases, there is a multifactorial inherited character. The level of complexity is further increased by the effect of modifying genes, and by the non coincidental association with other neurological diseases [9]. Genetic linkage and association studies have been performed worldwide in an effort to unveil the genetic basis of migraine [8]. Therefore, genetic determinants are certainly at the basis of some migraine forms, and the role of genetics is now increasing due to the better phenotypical characterization rendered possible by the 1988 criteria.

The increased frequency of the dopamine D2 receptor gene allele NcoI C was found in patients with migraine with aura. The primary role for the dopaminergic system in migraine pathogenesis is unconvincing. Based on well established interactions between central amines, a reduced release of serotonin between attacks could lower dopamine release which would lead to receptor hypersensitivity [7]. An up-regulation of dopamine receptors (D3 and D4) was also found in migraine, and was thought to reflect central and/or peripheral dopamine receptor hypersensitivity due to hypofunction of the dopaminergic system [1]. Clinical and pharmacological evidence supports the hypothesis of a hypersensitivity of dopamine receptors in migraine patients [6]. Degradation of catecholamines like dopamine can be influenced by the level of activity of COMT enzyme which is involved in the catecholamine inactivation.

Although the association of catecholamines with migraine has been researched, the significance of the COMT and its polymorphism, which determines the degree of catecholamine degradation, is unclear. Since the enzyme has high (H/H), intermediate (L/H) and low activity (L/L) alleles depending upon the polymorphism of its gene, degradation rates of the catecholamines or catechol drugs varies depending on the existence of different alleles.

In the presence of H/H genotype, we would expect decreased catecholamines levels in the medium, which in turn could lead to up regulation of the catecholamine receptors. Although this should have been the condition responsible for the receptor up regulation, according to our results, the H/H genotype was over represented in the controls than in the migraineurs. Therefore, dopamine receptor up regulation should be resulting from some other factors that we do not know.

The L/L or L/H genotypes were over represented and H/H genotype less represented in the migraineurs. These genotypic variations of the gene encoding COMT may affect the kinetics of the drugs being used in the migraine treatment. In addition to this, the presence of low COMT activity, which is determined by L/L and L/H variants, is a risk factor for migraine disease while the presence of H/H variant may have a protective role in migraine.

The interactions of dopaminergic system are complex, and the control of dopamine activity may be determined by variation in enzymes involved in dopamine synthesis and catabolism, and by affinity of the various dopamine receptors. Because many of these enzymes and receptors are polymorphic, the interaction of individual genetic variants may be additive, synergistic, or antagonistic-.Therefore, studying a single factor involved in the dopaminergic activity may not end up with clear conclusions. These factors and their interactions should be studied in a selected population in the further studies.

It was proposed that susceptibility to MA and MOA has a genetic component and these disorders are distinct [10]. In this study, there was no relationship between the aura and COMT variants. Therefore, COMT activity, which is determined by the variants of COMT gene, does not play a role in aura in migraine.

In conclusion, the COMT polymorphism may be of potential pharmacological importance regarding the individual differences in the metabolism of catechol drugs in migraineurs. Although altered catechoamine activity due to polymorphism of COMT gene may be one of the mechanisms involved in the pathogenesis of migraine, these mechanisms are not related to presence or absence of aura.

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