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M. Mochi • S. Cevoli • P. Cortelli • G. Pierangeli • S. Soriani • C. Scapoli • P. Montagna

A genetic association study of migraine with dopamine receptor 4, dopamine transporter and dopamine-beta-hydroxylase genes

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Abstract We assessed the role of some dopamine metabolism genes in the genetic susceptibility to migraine. We performed an association study using three functional polymorphisms: a 48-base-pair (bp) tandem repeat in the D4 dopamine receptor gene (*DRD4*), a 40-bp tandem repeat in the dopamine transporter gene (*DAT*) and a dinucleotide repeat in the dopamine beta-hydroxylase (*DBH*) gene. Allelic and genotypic frequencies for each polymorphism were assayed in two migraine populations (93 individuals with migraine with aura (MA) and 101 with migraine without aura (MO)) and were compared with those in a control group (117 individuals). No significant differences were found between control and migraine groups for *DAT* and *DBH* polymorphisms. Instead, the distribution of alleles for the *DRD4* gene in the MO group was significantly different from those in both MA and control groups, with the shortest and longest alleles being less frequent in MO. Our data indicate that MO, but not MA, shows significant genetic association with *DRD4*.

Key words Migraine • Genetic association • Dopamine • DRD4 • Dopamine-beta-hydroxylase • Dopamine transporter

M. Mochi (✉) • S. Cevoli • G. Pierangeli • P. Montagna
Department of Neurological Sciences
University of Bologna
Via Ugo Foscolo 7, I-40123 Bologna, Italy

P. Cortelli
Institute of Clinical Neurology
University of Modena and Reggio-Emilia, Modena, Italy

S. Soriani
Institute of Pediatrics, University of Ferrara, Ferrara, Italy

C. Scapoli
Department of Evolutive Biology, University of Ferrara, Ferrara, Italy

Introduction

Migraine is a common neurological disease affecting 10%–20% of the Caucasian population [1, 2]. The disease has a substantial genetic component, even though the mode of transmission is not simple [3]. Different pathogenetic models have been proposed, in particular the involvement of ionic channels following the discovery of the role of *CACNA1A*, a gene located on 19p13 and coding for a P/Q type calcium channel responsible for familial hemiplegic migraine (FHM) [4]. Other pathogenic models have also been suggested. Based on pharmacological evidence [5], several genetic association studies have addressed the possible pathogenic role of the dopaminergic system in migraine. Studies of several dopamine receptor genes, performed in different populations, gave proof of a positive association of the D2 dopamine receptor gene (*DRD2*) with migraine without [6] and with [7] aura. Polymorphisms in the dopamine-beta-hydroxylase (*DBH*) gene have been linked to typical migraine [8]. Some of these results were subsequently contradicted [9].

Following our previous negative association studies of migraine with the *DRD3*, *COMT* and *MAO-A* genes [10], we went on to investigate other genes involved in dopamine metabolism, in particular the dopamine D4 receptor (*DRD4*), dopamine transporter (*DAT*) and dopamine-beta-hydroxylase (*DBH*) genes. We selected the *DRD4* gene on chromosome 11p15.5 because several studies implicated this locus in normal [11, 12] and pathological [13] personality traits, and because an increased density of dopamine D4 receptors on lymphocytes of migraine patients had been shown [14].

The *DBH* gene is located on chromosome 9q34, and encodes an enzyme that catalyzes the conversion of dopamine to norepinephrine. Elevated serum levels of DBH are found in migrainous patients during headache attacks [15], and an allelic association between *DBH* polymorphism and migraine has already been reported [8]. The dopamine transporter is involved in the presynaptic uptake of dopamine by dopaminergic neurons of the substantia nigra. The *DAT* gene (named *SLC6A3*) is located on chromosome 5p15.3 and has been associated with neurological [16, 17] and psychiatric [18, 19] disorders. We performed a genetic association study using three functional polymorphisms within these genes to understand their involvement in the pathogenesis of migraine.

Patients and methods

We collected blood from an overall sample of 194 unrelated migraine patients, 101 with migraine without aura (MO) and 93 with migraine with aura (MA), aged 12–60 years. All patients gave informed consent to participate in the study. All patients were diagnosed by a neurologist after direct interview; the diagnostic criteria of the International Headache Society (IHS) were applied [20]. We also analysed a control population of 117 unrelated individuals, which included the healthy spouses of patients, healthy laboratory staff and neurological patients without migraine, aged at least 40 years to reduce bias related to age at onset of migraine. Patients and controls originated from the same Italian region (Emilia-Romagna).

DNA was extracted from blood by standard methods [21] and the following polymorphisms were analysed:

1. The *DRD4* polymorphism was a 48-base-pair (bp) tandem repeat in exon III of the gene. Primers and polymerase chain reaction (PCR) methods used were those described by Ebstein et al. [11].
2. The *DBH* (GT)n polymorphism was detected by means of PCR and enzymatic digestion by HaeIII restriction endonuclease, according to Wei et al. [22].
3. The *DAT* polymorphism was a 40-base-pair tandem repeat in the 3' untranslated region of the gene, which regulates the translation of the DAT protein [23]. Primers and PCR methods used were those described by Mercier et al. [17].

DNA fragments were separated on MetaPhor agarose (FMC) gel, at a 2% concentration for *DRD4* and *DAT*, and 5% for *DBH*. Due to technical difficulties (DNA exhaustion, PCR failure), the number of individuals examined in the different samples varied in the three association studies.

The allelic genotypic and frequencies were compared for statistical evaluation by means of the exact test for population differentiation for r x k contingency table (as implemented on Arlequin Software, Ver2.0) [24] based on Markov chain (MC) procedure (number of steps in MC, 1 000 000; number of dememorization steps=100 000) and by Fisher's exact test together with odds ratios and 95% confidence intervals.

Results

We measured the allelic frequencies of *DRD4*, *DBH* and *DAT* polymorphisms in 194 migraine patients and in 117 healthy controls. The allelic distributions of the *DAT* and *DBH* genes were similar in all groups (Tables 1, 2).

Table 3 shows the allelic frequencies in the migraine and control groups for the 48-bp *DRD4* polymorphism. According to the exact test, there was no significant difference between controls and MA patients (p=0.3144±0.0040). There was, how-

Table 1 Dopamine transporter (*DAT*) gene allelic frequencies in healthy controls and migraine patients. Values are number (percentage) of chromosomes tested

Allele number	Controls (n=194)[a]	Migraine without aura (n=202)	Migraine with aura (n=186)
6	1 (0.5)	0 (0)	0 (0)
9	55 (28.4)	57 (28.2)	58 (31.2)
10	135 (69.6)	145 (71.8)	123 (66.1)
11	3 (1.5)	0 (0)	5 (2.7)

[a] Data are missing for 20 subjects due to technical problems during the analysis

Table 2 Dopamine-beta-hydroxylase (*DBH*) gene allelic frequencies in healthy controls and migraine patients. Values are number (percentage) of chromosomes tested

Allele number	Controls (n=234)[a]	Migraine without aura (n=202)	Migraine with aura (n=186)
3	133 (56.8)	127 (62.9)	117 (62.9)
4	77 (32.9)	63 (31.9)	58 (31.2)
5	22 (9.4)	12 (5.9)	11 (5.9)
6	2 (0.9)	0 (0)	0 (0)

Table 3 Allelic frequencies of the 48-bp tandem repeat polymorphism at the D4 dopamine receptor gene (*DRD4*) in healthy controls and migraine patients. Values are number (percentage) of chromosomes tested

Allele number	Controls (n=212)[a]	Migraine without aura (n=202)	Migraine with aura (n=186)
2	22 (10.4)	13 (6.4)	27 (14.5)
3	7 (3.3)	6 (3.0)	9 (4.8)
4	146 (68.9)	171 (84.7)	121 (65.1)
5	6 (2.8)	0 (0)	1 (0.5)
6	2 (0.9)	0 (0)	0 (0)
7	28 (13.2)	12 (5.9)	27 (14.5)
8	1 (0.5)	0 (0)	1 (0.5)

[a] Data are missing for 11 subjects due to technical problems during the analysis

ever, a significant difference between MO patients and controls (p=0.0009±0.0001) and between MO and MA patients (p=0.0002±0.0001), due to an overrepresentation of allele 4 in MO group with respect to both controls and MA patients. Extreme alleles, i.e. those having less than 4 tandem repeats, e.g. alleles 2 and 3, and those with more than 4 repeats, e.g. alleles 5, 6, 7 and 8 (particularly alleles 2 and 7, since the others are very rare) showed a decreased frequency in MO.

Considering the frequency of allele 4 against the frequencies of all the other alleles (Table 4), MO group again resulted statistically different from both MA and control groups, the latter two samples presenting comparable allelic frequencies.

Table 5 shows the genotypic distributions for the *DRD4* polymorphism in the analysed samples. The distribution in

Table 4 Comparison between allele 4 and all other *DRD4* allele frequencies. Differences between control and migraine with aura groups were not significant (p=0.45; OR=1.19; 95% CI=0.78–1.81)

	Controls (n=212)[a]	Migraine without aura (n=202)	Migraine with aura (n=186)
Allele 4, n (%)	146 (68.9)	171 (84.7)	121 (65.1)
Other alleles, n (%)[a]	66 (31.1)	31 (15.3)	65 (34.9)
Fisher's exact p	0.0002[b]	–	<0.0001[b]
OR (95% CI)	2.49 (1.54–4.04)[b]	–	2.96 (1.82–4.83)[b]

[a] Alleles 2, 3, 5, 6, 7 and 8

[b] Versus migraine without aura group

Table 5 Genotypic frequencies of the *DRD4* polymorphism in healthy controls and migraine patients. Values are number (percentage) of subjects tested

Genotype	Controls (n=106)[a]	Migraine without aura (n=101)	Migraine with aura (n=93)
44	54 (50.9)	73 (72.3)	40 (43.0)
47	13 (12.3)	8 (7.9)	14 (15.1)
34	4 (3.8)	6 (5.9)	7 (7.5)
24	16 (15.1)	11 (10.9)	20 (21.5)
77	4 (3.8)	1 (1.0)	5 (5.4)
27	4 (3.8)	2 (2.0)	1 (1.1)
22	1 (0.9)	0 (0)	3 (3.2)
37	2 (1.9)	0 (0)	1 (1.1)
38	0 (0)	0 (0)	1 (1.1)
45	4 (3.8)	0 (0)	0 (0)
78	1 (0.9)	0 (0)	0 (0)
35	1 (0.9)	0 (0)	0 (0)
46	1 (0.9)	0 (0)	0 (0)
56	1 (0.9)	0 (0)	0 (0)
57	0 (0)	0 (0)	1 (1.1)

[a] Data are missing for 11 subjects due to technical problems in the analysis

the control sample was wider, since it included some rare genotypes not present in the migraine samples. We observed a borderline, not-significant difference between control and migraine without aura groups (p=0.053) and a significant difference (p=0.002) between MO and MA groups. The difference between control and migraine with aura groups was not significant (p=0.29).

Discussion

The existence of dopaminergic hypersensitivity in migraine is documented on pharmacological grounds [5, 14] and some studies claimed a genetic association between migraine and molecular variations in dopamine metabolism genes *DRD2* [6, 7] and *DBH* [8]. We analysed polymorphisms within three genes involved in dopamine metabolism: *DRD4, DAT* and *DBH*, and compared the allelic frequencies in two migraine samples, MO and MA, with a control sample. All three polymorphisms investigated are functional; in particular, the *DRD4* polymorphism results in physiological differences in ligand binding between the commonest short (4 repeats) and the commonest long (7 repeats) alleles [25]. For the *DAT* polymorphism, Heinz et al. [23] reported different enzymatic activities in the three commonest genotypes (homozygotes 33 and 44 and heterozygotes 34) found in normal individuals. For *DBH*, genotype 9/9 has low enzymatic activity, while genotype 10/10 has high and genotype 9/10 has intermediate enzymatic activity [22]. Our study found no differences in *DAT* and *DBH* allelic frequencies, whereas *DRD4* allelic frequencies differed significantly between MO and MA and between MO and controls. Our data demonstrated an increased frequency of allele 4 and a reduction of extreme alleles 2 and 7 in MO, although allele 4 remained the most frequent in all groups, normal and migrainous. On this basis (although of course *DRD4* cannot be the sole genetic determinant), we propose that MO, but not MA, is genetically associated with the *DRD4* gene.

The 48-base-pair tandem repeat in exon III of *DRD4* presents alleles with pharmacological characteristics differing between short (<4 repeats) and long (>4 repeats) alleles, with the longest alleles causing greatest affinity for anti-psychotic drugs such as clozapine [25, 26]. Some studies performed in psychiatric patients reported an association of long allele 7 with personality traits such as novelty seeking [11, 12], or with attention deficit hyperactivity disorder (ADHD) [13]. Co-morbidity of migraine with psychiatric disorders is well known [27]. On the other hand, dopamine intervenes in the regulation of the cerebral vasculature, especially in intraparenchymal microvessels [28]. Vascular abnormalities are well known to occur during the migraine attack.

In light of these observations, there may be a rationale for envisaging the direct involvement of *DRD4* in the genetic determination of MO. We must, however, acknowledge some difficulties in attributing a direct pathogenic role to *DRD4* on the basis of a positive genetic association study. In particular, it seems unlikely that predisposition for a disease (even one common worldwide like migraine) may be related to the commonest allele at a particular polymorphism. Further confirmatory data on independent populations are needed, and alternative explanations are possible: (a) there may be another functional polymorphism in *DRD4* in linkage dysequilibrium with the 48-bp repeat polymorphism and involved in susceptibility to MO; or (b) the extreme alleles can exert a protective action towards MO but not MA, through an unknown biological activity.

As a corollary, our work shows that MA and MO differ in regard to susceptibility factors, a notion supported by other studies in the literature [6, 7]. Indeed, some authors consider MA and MO distinct nosological entities [29].

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Sommario *Per individuare il ruolo di geni coinvolti nel metabolismo della dopamina nella predisposizione genetica all'emicrania, abbiamo effettuato uno studio di associazione utilizzando tre polimorfismi funzionali: una sequenza ripetuta di 48 basi nel recettore D4 della dopamina (*DRD4*), una sequenza ripetuta di 40 basi nel gene del trasportatore della dopamina (*DAT*) e un dinucleotide ripetuto nel gene della dopamina-beta-idrossilasi (*DBH*). Le frequenze alleliche e genotipiche per ciascun polimorfismo sono state stimate su due campioni di emicranici (93 individui con emicrania con aura, MA; 101 con emicrania senza aura, MO) e confrontate con le frequenze ottenute su un campione di controllo di 117 individui. Non ci sono differenze significative fra il campione di controllo e i due campioni di emicranici per i polimorfismi nei geni* DAT *e* DBH. *È stata evidenziata una differenza significativa fra MO e MA e fra MO e i controlli rispetto al polimorfismo nel gene DRD4, dovuta ad una diminuzione della frequenza degli alleli estremi nella distribuzione delle lunghezze, nel campione di MO. DRD4 sembra mostrare una associazione genetica con MO ma non con MA.*

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