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GENETICS

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Functional serotonin 5-HTTLPR polymorphism is a risk factor for migraine with aura

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Abstract In the present work, we report that the functional serotonin transporter gene promoter (5-HTTLPR) polymorphism is involved in migraine pathogenesis. The distribution of 5-HTTLPR genotypes was significantly different in MA patients (S/S *vs.* S/L *vs.* L/L=32.7 *vs.* 42.3 *vs.* 25.0%), MO patients (18.5 *vs.* 39.1 *vs.* 42.4%) and CON (18.0 *vs.* 51.3 *vs.* 30.7%; chi-square test, $p<0.05$). In 5-HTTLPR S/S carriers, the odds ratio for MA risk was 2.60 (95% confidence interval [95%CI]=1.75–3.85) compared to CON, and it was 2.14 (95%CI=1.42–3.21) compared to MO. These data provide a further insight on the complex genotype-phenotype relationship involved in MA pathogenesis, and might eventually result in new and individualised prognostic and therapeutic measures.

Key words 5-HTTLPR • Serotonin • Polymorphism • Migraine • Aura

Introduction

It has been reported that genetic background linked to serotonin (5-HT) metabolism is involved in migraine pathogenesis. Indeed, the most effective drugs in acute migraine are the triptans, highly selective 5-HT1B/1D agonists.

There is evidence that 5-HT activity is regulated by a functional polymorphism within the promoter region of the 5-HT transporter gene (5-HTT gene-linked promoter region, 5-HTTLPR) [1]. The 5-HTTLPR provides the primary mechanism for reuptake of 5-HT after its release into the synaptic cleft and is thus critical to the maintenance of brain 5-HT homeostasis. *In vitro* studies evidenced that the basal activity of *5-HTTLPR* allele with a 44-base pair insertion (long variant, L) leads to nearly twice as much 5-HTT transcription compared to the other allele (short variant, S) [2].

Different studies investigated the role of genetic variations of 5-HT receptors as risk factors for migraine, their role being still not completely understood [3–5]. A recent work suggested a link between 5-HTTLPR polymorphism and migraine with aura [6].

In the current study, we further evaluate the role of functional 5-HTTLPR polymorphism as a risk factor for migraine.

Methods

Subjects

One hundred and forty-four consecutive migraine patients and 105 nonheadache unrelated healthy volunteers were enrolled at Headache Centres of University of Brescia and "Città di Brescia" Hospital. All migraine patients and healthy controls were interviewed by an experienced neurologist. A standardised record of all demographic characteristics, family history for migraine, cerebrovascular disease, cardiovascular disease and neurological disorders was obtained. The presence of other comorbidities was also evaluated.

All subjects performed a clinical and neurological work-up, and a blood drawing for 5-HTTLPR genotyping.

The migraine patients were diagnosed as having migraine without aura (MO) or migraine with aura (MA) according to the International Headache Society (IHS) criteria [7].

The study was conducted in accordance with local clinical research regulations and informed consent was required from all the subjects.

Polymorphism analyses were performed blinded to diagnosis and genotype.

5-HTTLPR genotyping

Genomic DNA was prepared from 10 ml of blood using the salting out method. Primers 5'-GGCGTTGCCGCTCTGAATGC-3' and 5'-GAGGGACTGAGCTGG ACAACC-3' were used to assess GC-rich regions composed of 20–23 base pair (bp) repeating units in the 5-HTTPR gene.

The 50-µl reactions contained 50 nmol genomic DNA, 0.17 mmol/l each of dATP, dCTP and dTTP; 0.083 mmol/l of dGTP; 1.5 mmol/l $MgCl_2$, 0.1 µg of each primer, and 1 unit Taq polymerase. Following an initial denaturation step at 95°C for 3 min, DNA was amplified in 35 PCR cycles (95°C for 45 s; 66°C for 1 min; 72°C for 1 min); the final extension step was 72°C for 7 min.

A 15-µl aliquot of PCR product was resolved on 2.5% agarose gel, and genotype was determined by fragments' size of 484 bp (short allele, S) or 528 bp (long allele, L).

Statistical analysis

The Hardy-Weinberg equilibrium was verified for all tested populations. The differences in genotype frequencies and other risk factors were analysed by the χ^2 test. Demographic characteristics in the groups were compared by Student's *t*-test or one-way ANOVA and Bonferroni post-hoc analysis. Odds ratio (OR) and 95% confidence intervals (95% CI) were also calculated. Results were expressed as mean±standard deviation (SD). The level of significance was taken at $p<0.05$.

Results

One hundred and forty-four migraine patients and 105 non-headache migraine subjects were enrolled. Migraine patients were classified into two subgroups according to the presence (MA, $n=52$) or the absence (MO, $n=92$) of

Table 1 Demographic and clinical characteristics of migraine patients according to migraine subtypes and of nonheadache controls

Variable	CON	MO	MA	*p**
n	105	92	52	–
Age, years	37.3±7.7	35.1±11.8	33.3±9.9	n.s.
Gender, F%	79.4	80.2	78.8	–
Age at onset, years	–	20.0±9.8	21.0±9.5	–
FH migraine, %	8.7	75	77.8	0.0001
FH cerebrovascular disease, %	15.0	39.3	22.5	0.001
FH cardiovascular disease, %	22.3	40.4	22.5	0.02
FH neurological disease, %	9.5	8.3	2.5	n.s.
Smoking, %	18.8	25.8	19.6	n.s.
Hypertension, %	2.3	8.4	6.2	n.s.
Cardiomyopathy, %	1.6	1.2	12.5	0.001
Hypercholesterolaemia, %	0.0	21.4	19.2	0.0001
Dismetabolism, %	1.6	3.6	2.0	n.s.
Asthma, %	3.9	6.0	6.2	n.s.
Allergy, %	9.4	26.5	23.0	0.01
Head trauma, %	5.5	8.4	18.8	0.05
Epilepsy, %	1.5	1.2	4.1	n.s.
Gastritis/gastric disease, %	3.9	27.1	14.6	0.0001

CON, nonheadache unrelated healthy volunteers; *MO*, migraine patients without aura; *MA*, migraine patients with aura; *F*, female; *FH*, family history

*Controls *vs.* MO *vs.* MA

aura. MA and MO subgroups did not differ for demographic characteristics, family history of migraine or other associated comorbidities.

Patients with migraine showed an increased incidence of allergies, previous head trauma, hypercholesterolaemia, gastritis or gastric disease, and cardiomyopathy compared to the control sample. Family history of migraine was more common in migraine patients than in nonheadache volunteers.

The distribution of 5-HTTLPR genotypes was significantly different in MA patients (S/S *vs.* S/L *vs.* L/L=32.7 *vs.* 42.3 *vs.* 25.0%), MO patients (18.5 *vs.* 39.1 *vs.* 42.4%) and CON (18.0 *vs.* 51.3 *vs.* 30.7%; chi-square test, $p<0.05$).

5-HTTLPR S/S was found to be associated with MA, its incidence being higher in this group (32.7%) compared to CON (18.0%, chi-square test, $p<0.05$) and to MO patients (18.5%, $p<0.05$). No difference in demographic characteristics, i.e., gender or age at onset, family history of migraine and other associated comorbidities between 5-HTTLPR S/S and 5-HTTLPR non-S/S carriers was observed.

In 5-HTTLPR S/S carriers, the OR for MA risk was 2.60 (95%CI=1.75–3.85) compared to CON, and it was 2.14 (95%CI=1.42–3.21) compared to MO.

Discussion

The relationship between the 5-HT pathway and migraine is well established. Thus, different studies have investigated a possible link between genetic background linked to 5-HT metabolism and migraine pathogenesis. It has been suggested that T102C polymorphism of *5-HT2A* gene is a risk factor for migraine [3], and a recent work has supported the view that 5-HTTLPR genetic variation is related to migraine with aura [6].

Our results confirm and extend previous studies, and may have several implications for clinical practice. These data suggest that MA and MO have distinct genetic predisposing factors. Moreover, the well known role of this polymorphism on 5-HT transcription [2] may reflect a different response to 5-HT agonist drugs, such as triptans, whose migraine symptomatic effect is still unpredictable.

Further studies are required to elucidate the role of 5-HTTLPR polymorphism in migraine. Notwithstanding, these data provide a further insight on the complex genotype-phenotype relationship involved in migraine pathogenesis, and might eventually result in new and individualised prognostic and therapeutic measures.

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