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Association analysis of the functional monoamine oxidase A gene promotor polymorphism in migraine

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Summary. Migraine affects about 15% of the adult population. Serotonergic and dopaminergic systems are believed to be involved in its pathophysiology. One of the key enzymes in the degradation of serotonin and to a lesser extent of dopamine is monoamine oxidase A (MAO-A). In this study we investigated a functionally relevant gene-linked polymorphic repetitive sequence (LPR) located approximately 1.2 kb upstream of the ATG codon in the MAO-A-promotor gene. 119 patients with migraine and 229 controls were tested. The allelic distribution of the controls and the migraine patients did not show significant differences with respect to the low- and high-activity alleles. Moreover, effectiveness of the potent serotonergic antimigraine agents, triptans, which are metabolized by MAO-A, was clinically not affected by the MAO-A-LPR in our patients. These findings thus indicate that there is no association between the functional MAO-A-LPR and susceptibility to migraine.

Keywords: Functional MAO-A promotor polymorphism, migraine.

Introduction

Migraine is a common disorder which affects between 14 to 18% of the general population (Breslau and Rasmussen, 2001). The two most frequent varieties are migraine without aura (MO) and migraine with aura (MA). Population based studies and twin studies indicate a significant familial risk of migraine (Montagna, 2000) and the genetic trait appears to differ between MA and MO (Russell et al., 2002). Migraine is nowadays regarded as a polygenic disease, and serotonergic and as well dopaminergic systems seem to play an important

role in its pathophysiology; for a detailed review on the role of serotonin (5-HT) in migraine see (Johnson et al., 1998). Triptans, highly selective 5-HT1B/D agonists, are the most effective drugs in acute migraine (Silberstein, 2000). An association study of the distribution of allelic polymorphisms of the serotonin transporter (5-HTT) gene on chromosome 17q11.2 revealed a higher frequency of the short and less active form in patients with migraine with aura (Marziniak et al., 2001). Dopaminergic hypersensitivity has been proposed to play an important role in migraine by several authors, for review see (Del Zompo, 2000). Nausea, frequently accompanied by vomiting, often precedes the headache and can be suppressed by dopamine D2 receptor antagonists (Dahlof and Hargreaves, 1998). Flunarizine, a calcium channel blocker, is an effective prophylactic agent in migraine and shows significant dopamine antagonist properties and a moderately high affinity for the DRD2 receptor (Ambrosio and Stefanini, 1991).

An ideal candidate linking both neurotransmitters would be the monoamine oxidase (MAO). The two isoforms MAO-A and MAO-B are mitochondrial enzymes which are involved in the degradation of several different biological amines. MAO-A has a high affinity to endogenous neurotransmitters (e.g. serotonin and norepinephrine), and a lesser affinity to dopamine. A complete MAO-A deficiency due to a nonsense mutation in the coding region of the gene on chromosome Xp11.23–p11.4 (Ozelius et al., 1988) is associated with disturbed amine metabolism, borderline mental retardation and impulsive aggressive behavior in affected males (Brunner et al., 1993). In the treatment of migraine, moclobemide, a MAO-A inhibitor, that is frequently used as an antidepressant, showed a positive effect as a prophylactic agent in an open trial (Meienberg and Amsler, 1996). Furthermore MAO-A is one of the most important enzymes for the elimination of the triptans (Bigal et al., 2003).

Recently, a novel functional repeat polymorphism of the MAO-A gene promotor VNTR (variable number tandem repeat) has been described, which consists of a 30base pair repeated sequence with 2, 3, 3.5, 4 or 5 copies (Sabol et al., 1998). The longer alleles 3.5, 4, and 5 were functionally more active than the short allele 3 in luciferase assays (Deckert et al., 1999).

Thus, the MAO-A gene-linked polymorphic region (LPR) might be involved in disorders that are associated with abnormalities in monoaminergic neurotransmission, like migraine. The present association study was designed to test the hypothesis that length variation of the regulatory MAO-A-LPR is associated with susceptibility to migraine.

Patients and methods

Subjects

119 patients (94 women, 25 men) with migraine were diagnosed according to the International Headache Society (IHS) criteria and were recruited from our Headache Clinic after informed consent and approval by the local ethics committee. Patients completed a standardized headache questionnaire and were subject to a full neurological examination. The 119 migraine patients received a second standardized questionnaire by mail and were asked to indicate the medication

they took for acute migraine treatment and how successful the treatment with triptans was (completely pain free or partial relief, time to onset of relief) and whether there was headache recurrence.

The control group (134 men, 95 women) was recruited from the blood donor center at the University of Würzburg. All subjects were of German Caucasian descent and from the area around Würzburg.

Genotyping

Venous blood samples of all patients and control subjects were obtained. Genomic DNA was extracted by salting out with saturated NaCl solution. PCR fragments were amplified from genomic DNA using primers MAOAFor2 (5′-CCCAGGCTGCTCCAGAAAC) and MAOARev2 (5′-GGACCTGGGAGTTGTGC) (Deckert et al., 1999). Briefly, PCR (40 sec at 94°C, 40 sec at 59°C, 60 sec at 72°C for 35 cycles) was performed in a final volume of 25 µl containing 60 µg of genomic DNA, 10 pmol of each primer, 200 µM of each dNTP, 1.5 $MgCl_2$, 75 mM Tris-HCl (pH 9.0 at 25°C), 20 mM $(NH_4)_2SO_4$, 0.01% Tween 20 and 0.5 U of Taq DNA polymerase (Eurogentec). The PCR products were separated on a 2% SeaKem LE agarose gel and visualized by ethidium bromide staining.

Statistical analysis

For statistical analysis, alleles were divided into two groups with low-activity (3-repeat units, group 1) and high-activity (4 and 5 repeat units, group 2). Chi-square (χ^2) test was performed for comparison of the distribution of the genotype and allele frequency between the patient and the control groups. Symptoms and history data were compared for a correlation between MA and MO and for the three genotypes. The Mann-Whitney-U-test was used for non-parametric variables and the t-test for the parametric variables of the clinical symptoms (Table 1) for the comparison between patient groups of MA and MO.

Table 1. Clinical symptoms of the patient groups with MA and MO

Characteristics	Total	MA	MO
No. of subjects	119	62	57
Men/women	25/94	17/45	8/49
Age, y	43.3 ± 12.4	42.4 ± 12	44.4 ± 12.8
Mean age at onset, y	19.7 ± 11.4	20.3 ± 13.2	18.9 ± 9
Mean duration of one attack, h	23 ± 20.4	24.7 ± 20.9	21.3 ± 19.8
Mean no. of attacks per month	2.47 ± 2.34	2.51 ± 2.47	2.43 ± 2.23
Mean no. of 1st grade relatives with migraine	0.89	0.76	1.04
Mean intensity of pain (VAS 1–10)	7.01 ± 1.48	6.95 ± 1.25	7.07 ± 1.67
Incidence of symptoms			
Unilateral headache	81%	79%	82%
Throbbing pain	71%	69%	72%
Nausea	82%	84%	80%
Vomiting	53%	56%	51%
Photophobia	81%	82%	81%
Phonophobia	42%	39%	46%

No statistical significance of the clinical variables (except of the gender distribution) between the groups with migraine with and without aura. The Mann-Whitney-U-test was used for non-parametric variables and the t-test for the parametric variables of the clinical symptoms for the comparison between patient groups of MA and MO. *MA* migraine with aura; *MO* migraine without aura

Results

Demographic and clinical data of the 119 patients with MA and MO are presented in Table 1. Sixty-two patients had migraine with aura and fifty-seven had migraine without aura. Our patient group comprised mainly females, but no sex differences of the MAO-A-LPR allele distribution were observed in our control population. The female and the male control group both showed an allele frequency of 35% for the low-activity polymorphism and 65% for the high-activity polymorphism.

Except for the presence or absence of aura, patients groups did not differ regarding history or clinical criteria. Data of the genotype and allele frequencies of the patients and the control group are shown in Table 2. The frequency of the short allele with 3 copies of the MAO-A-LPR as opposed to the long alleles with 4 or 5 copies showed no differences between the patients with MA or with MO or the controls.

Fifty-two of the 119 patients returned correctly completed questionnaires about the effectiveness of their migraine medication. Of these, 31 had taken triptans, the others had used non steroidals, mostly aspirin. Eight patients complained about the recurrence of migraine headache (2/11 after zolmitriptan, 2/9 after sumatriptan, 4/16 after rizatriptan, and 0/6 after naratriptan). The genotype distribution of the eight patients who complained about headache was not different from the whole patient group (1 male, 7 female patients, genotypes: 1×3/3, 3×3/4, 3×4/4, 1×3 (male patient)).

Table 2a. Genotype frequencies of the MAOA-LPR in female patients and controls

Female patients	Controls (n = 95)	MA (n = 45)		MO (n = 49)
Genotype 3/3	18 (18.9%)	5 (11.2%)		5 (10.2%)
Genotype 3/4, 3/5	30 (31.6%)	20 (44.4%)		24 (49%)
Genotype 4/4, 4/5	47 (49.5%)	20 (44.4%)		20 (40.8%)
Genotype controls vs. MA patients		$\chi^2 = 2.72$	$df = 2$	$p = 0.257$
Genotype controls vs. MO patients		$\chi^2 = 4.68$	$df = 2$	$p = 0.096$

Table 2b. Allele frequencies of the MAOA-LPR in female patients and controls

Female patients	Controls (n = 190)	MA (n = 90)		MO (n = 98)
Allele 3	66 (34.7%)	30 (33.3%)		34 (34.7%)
Allele 4 and 5	124 (65.3%)	60 (66.7%)		64 (65.3%)
Allele controls vs. MA patients		$\chi^2 = 0.05$	$df = 1$	$p = 0.893$
Allele controls vs. MO patients		$\chi^2 = 0.33$	$df = 1$	$p = 0.611$

No significance of the genotype and the allele distribution of female patients with migraine with and without aura and the controls in the Chi-square (χ^2) test. *MA* migraine with aura; *MO* migraine without aura

Table 3. Allele frequencies of the MAOA-LPR in male patients and controls

Male patients	Controls (n = 134)	MA (n = 17)		MO (n = 8)
Allele 3	47 (35.1%)	8 (47.1%)		2 (25%)
Allele 4 or 5	87 (64.9%)	9 (52.9%)		6 (75%)
Male allele controls vs. MA patients		$\chi^2 = 0.94$	$df = 1$	$p = 0.423$
Male allele controls vs. MO patients		$\chi^2 = 0.34$	$df = 1$	$p = 0.72$

No significance of the allele distribution of male patients with migraine with and without aura and the controls in the Chi-square (χ^2) test. The coding region for the MAO-A is on the X-chromosome, therefore in males genotype and allele frequency are identical. *MA* migraine with aura; *MO* migraine without aura

Discussion

This is the first association study between the functional MAO-A polymorphism, MAO-A-LPR, and migraine. We did not find a significant difference in the genotype distribution or the allele frequencies of the MAO-A-LPR between patients with migraine with aura and without aura and the controls. We also did not find a significant difference in the effectiveness and the effective period of triptans (metabolized by MAO-A) between the different genotypes, although these findings are preliminary because of the small number of investigated patients.

The functional relevance of the MAO-A promotor polymorphism has clearly been shown in three independent studies. Thus, the 3-repeat allele was less active than the 4-repeat allele in driving MAO-A gene transcription in studies of neuroblastoma cells (Sabol et al., 1998; Deckert et al., 1999) and choriocarcinoma cells (Sabol et al., 1998); moreover, MAO-A activity in skin fibroblasts was lower in cells harboring the 3-repeat allele than in those cells with the 4-repeat allele (Denney et al., 1999). Thus, there is unequivocal evidence of the functionality of the MAO-A promotor polymorphism.

In human postmortem frontal cortex MAO-A enzyme activity was also lower in individuals with the 3-repeat allele, as opposed to those individuals with the 4-repeat allele, although the difference was not significant (Balciuniene et al., 2002). This is due to two effects: first, counter regulation in the setting of complex neuronal networks and, of special importance, the effect on neurodevelopment. Thus, polymorphisms such as the MAO-A promotor polymorphism exert a major effect during neurodevelopment. This is best demonstrated by mice lacking the MAO-A gene due to transgenic technology. Thus, MAO-A deficient mice develop persistent behavioral abnormalities during a critical period of neurodevelopment. These behavioral as well as neuroanatomical abnormalities persist the whole life, although monoamine levels in brain attain normal levels within several months after reaching adulthood (Cases et al., 1995; Seif et al., 1999). Finally, even minor neurochemical changes often have important consequences, as demonstrated in a landmark study on the behavioral effects of the MAO-A promotor polymorphism in humans (Caspi et al., 2002).

Positive association studies imply a clinical relevance for the examined polymorphism. The short promotor region is positively associated to antisocial

alcoholics in comparison to controls and alcoholics without antisocial behavior (Samochowiec et al., 1999) and to patients with panic disorders in comparison to healthy controls (Deckert et al., 1999). Taking into consideration that the MAO-A-LPR influences not only the transcriptional activity but also the mean cellular activity of MAO-A (Denney et al., 1999) and the well known influence of MAO-A on the serotonin homeostasis, it has been speculated that an excess of high activity MAO-A gene promotor alleles should result in elevated MAO-A activity and elevated serotonin degradation. This pathophysiological mechanism might lead to the increased susceptibility to depression (Schulze et al., 2000). Immunolabeling experiments in MAO-A-deficient mice emphasize the physiological importance since in these mice 5-HT accumulates transiently within the midbrain, the diencephalon and the brain stem (Cases et al., 1998), anatomic regions that are very important in the generation of migraine. In view of the negative result of the present study and in accordance with recent investigations (Balciuniene et al., 2002) it might be that a further, yet unknown functional polymorphism, in linkage disequilibrium with the VNTR promotor, might effect enzyme levels in the brain.

Secondly, the altered MAO-A expression might be compensated by other regulatory mechanisms in the serotonin homeostasis.

Our results indicate that the functional MAO-A polymorphism is unlikely to be involved in the biological susceptibility to migraine either with or without aura. Furthermore the genotype of the MAO-A-LPR appears to have no important clinical relevance on the effectiveness and the effective period of triptans, although the pharmacological findings must be seen as preliminary because of the small number of patients investigated.

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References

Ambrosio C, Stefanini E (1991) Interaction of flunarizine with dopamine D2 and D1 receptors. Eur J Pharmacol 197: 221–223

Balciuniene J, Emilsson L, Oreland L, Pettersson U, Jazin E (2002) Investigation of the functional effect of monoamine oxidase polymorphisms in human brain. Hum Genet 110: 1–7

Bigal ME, Bordini CA, Antoniazzi AL, Speciali JG (2003) The triptan formulations: a critical evaluation. Arq Neuropsiquiatr 61(2-A): 313–320

Breslau N, Rasmussen BK (2001) The impact of migraine: epidemiology, risk factors, and comorbidities. Neurology 56 [Suppl 1]: S4–S12

Brunner HG, Nelen M, Breakefield XO, Ropers HH, van Oost BA (1993) Abnormal behavior associated with a point mutation in the structural gene for monoamine oxidase A. Science 262(5133): 578–580

Cases O, Seif I, Grimsby J, Gaspar P, Chen K, Pournin S, Muller U, Aguet M, Babinet C, Shih JC, et al. (1995) Aggressive behavior and altered amounts of brain serotonin and norepinephrine in mice lacking MAOA. Science 268(5218): 1763–1766

Cases O, Lebrand C, Giros B, Vitalis T, De Maeyer E, Caraon MG, Price DJ, Gaspar P, Seif I (1998) Plasma membrane transporters of serotonin, dopamine, and norepinephrine mediate serotonin accumulation in atypical locations in the developing brain of monoamine oxidase A knock-outs. J Neurosci 18: 6914–6927

Caspi A, McClay J, Moffitt TE, Mill J, Martin J, Craig IW, Taylor A, Poulton R (2002) Role of genotype in the cycle of violence in maltreated children. Science 297(5582): 851–854

Dahlof CG, Hargreaves RJ (1998) Pathophysiology and pharmacology of migraine. Is there a place for antiemetics in future treatment strategies? Cephalalgia 18: 593–604

Deckert J, Catalano M, Syagailo YV, Bosi M, Okladnova, Di Bella D, Nothen MM, Maffei P, Franke P, Fritze J, Maier W, Proping P, Beckmann H, Bellodi L, Lesch KP (1999) Excess of high activity monoamine oxidase A gene promoter alleles in female patients with panic disorder. Hum Mol Genet 8: 621–624

Del Zompo M (2000) Dopaminergic hypersensitivity in migraine: clinical and genetic evidence. Funct Neurol 15 [Suppl 3]: 163–170

Denney RM, Koch H, Craig IW (1999) Association between monoamine oxidase A activity in human male skin fibroblasts and genotype of the MAOA promotor-associated variable number tandem repeat. Hum Genet 105: 542–551

Johnson KW, Phebus LA, Cohen ML (1998) Serotonin in migraine: theories, animal models and emerging therapies. Prog Drug Res 51: 219–244

Marziniak M, Moessner R, Lesch KP, Sommer C (2001) Altered allelic distribution of a functional polymorphism within the promotor of the serotonin transporter gene in migraine without aura and migraine with aura. J Neurol 248: S2 II/165

Meienberg O, Amsler F (1996) Moclobemide in the prophylactic treatment of migraine. A retrospective analysis of 44 cases. Eur Neurol 36: 109–110

Montagna P (2000) Molecular genetics of migraine headaches: a review. Cephalalgia 20: 3–14

Ozelius L, Hsu YP, Bruns G, Powell JF, Chen S, Weyler W, Utterback M, Zucker D, Haines J, Trofatter JA, et al. (1988) Human monoamine oxidase gene (MAOA): chromosome position (Xp21-p11) and DNA polymorphism. Genomics 3: 53–58

Russell MB, Ulrich V, Gervil M, Olesen J (2002) Migraine without aura and migraine with aura are distinct disorders. A population-based twin survey. Headache 42: 332–336

Sabol SZ, Hu S, Hamer D (1998) A functional polymorphism in the monoamine oxidase A gene promoter. Hum Genet 103: 273–279

Samochowiec J, Lesch KP, Rottmann M, Smolka M, Syagailo YV, Okladnova O, Rommelspacher H, Winterer G, Schmidt LG, Sander T (1999) Association of a regulatory polymorphism in the promoter region of the monoamine oxidase A gene with antisocial alcoholism. Psychiatry Res 86: 67–72

Schulze TG, Muller DJ, Krauss H, Scherk H, Ohlraum S, Syagailo YV, Windemuth C, Neidt H, Grassle M, Papassotiropoulos A, Heun R, Nothen MM, Maier W, Lesch KP, Rietschel M (2000) Association between a functional polymorphism in the monoamine oxidase A gene promoter and major depressive disorder. Am J Med Genet 96: 801–803

Seif I, De Maeyer E (1999) Knockout mice for monoamine oxidase A. Int J Neuropsychopharmcol 2(3): 241–243

Silberstein SD (2000) Practice parameter: evidence-based guidelines for migraine headache (an evidence-based review): report of the Quality Standards Subcommittee of the American Academy of Neurology. Neurology 55: 754–762

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