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The association between headache and Val158Met polymorphism in the catechol-O-methyltransferase gene: the HUNT Study

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Abstract The catechol-*O*-methyltransferase (*COMT*) gene contains a functional polymorphism, Val158Met, that has been found to influence human pain perception, and one study has found that migraine was less likely among those with the Val/Val polymorphism. In the 1995–97 Nord-Trøndelag Health (HUNT) Study, the association between the Val158Met polymorphism and headache was evaluated in a random sample of 2451 individuals. No association between Val158Met polymorphism and migraine was found. Among women, a lower prevalence of non-migrainous headache was found among individuals with the Val/Val genotype than among those with other genotypes (26.2% *vs.* 33.6%, p=0.04). That non-migrainous headache was less likely among women with the Val/Val genotype may be an incidental finding, but should be investigated in further studies.

Keywords Headache • Migraine • Population-based • COMT gene • Norway

Introduction

Genetic factors are involved in migraine [1], and may also play a role e.g., in chronic tension-type headache [2]. The catechol-*O*-methyltransferase (*COMT*) gene is one of several potential headache genetic determinants. *COMT* is an enzyme that inactivates catecholamines and catechol-containing drugs, and a substitution of valine (Val) by methionine (Met) at codon 158 affects the activity of

the *COMT* enzyme. Individuals with the Val/Val genotype have a three- to fourfold higher activity of the *COMT* enzyme than those with Met/Met genotype [3]. Individuals with the Val/Val genotype have been found to be less susceptible to pain and to have an enhanced opioid system response to pain compared with those with other genotypes [4]. In accordance with this result, two case-control studies have found that migraine and fibromyalgia were less frequent among those with the Val/Val genotype [5, 6].

Based on these findings, our hypothesis was that headache and migraine were less likely among individuals with the Val/Val genotype compared with those with other genotypes. In this population-based study performed among unselected adults we evaluated the relationship between Val158Met polymorphism and headache, migraine included.

Material and methods

Study population

Between August 1995 and June 1997, all inhabitants aged 20 years or older in Nord-Trøndelag county in Norway were invited to participate in the Nord-Trøndelag Health Survey ("Helseundersøkelsen i Nord-Trøndelag"=HUNT). In brief, of 92 566 eligible individuals, 64 560 (70%) participated. Two questionnaires including >200 health-related questions were administrated to the participants. The first questionnaire (Q_1) was enclosed with the invitation letter and delivered during attendance at the health examination. The second questionnaire (Q_2) was filled in after the examination and returned by mail. The population in Nord-Trøndelag County is ethnically homogeneous (less than 3% non-Caucasian), making it suitable for epidemiological genetic research [7, 8].

Headache diagnosis

The headache questions in Q_2 and the prevalence of headache are published elsewhere [9, 10]. The headache questions were designed mainly to determine whether or not the person had headache, determine frequency of headache and diagnose migraine according to a modified version of the migraine criteria of the Headache Classification Committee of the IHS [11]. Subjects who answered 'yes' to the question "Have you suffered from headache during the last 12 months?" were classified as headache sufferers. Based on data from the subsequent 12 headache questions, they were classified into two groups of either migraine or non-migrainous headache. The diagnoses were mutually exclusive. Persons were classified as migraineurs if they reported having migraine or fulfilled the following three criteria: (i) headache attacks lasting 4–72 hours (<4 hours was accepted for those who reported visual disturbances *often* before headache); (ii) headache with at least one of the following three characteristics: (a) pulsating quality, (b) unilateral location, (c) aggravation by physical activity; (iii) during headache, at least one of the following symptoms: (a) nausea and (b) photophobia and/or phonophobia. A headache that did not fulfil the criteria for migraine was classified as a non-migrainous headache. Based on a question about headache frequency during the last year, this variable was divided in three categories; less than 7 days/month, 7–14 days/month and more than 14 days/month.

The classification of the subjects has been described in detail previously, and has been validated by interview diagnoses [9]. In short, for migraine, the positive predictive value (PPV) was 84% and the negative predictive value (NPV) was 78%; for non-migrainous headache, the PPV was 68% and the NPV was 76% [9].

Genotyping of the COMT locus

Blood sampling was done whenever subjects attended, and details for the procedure and the HUNT biobank are described elsewhere [12].

DNA for genotyping was extracted from peripheral blood leukocytes from whole blood or blood clots stored in the HUNT biobank, using the Puregene kit (Gentra Systems Inc.) manually or with an Autopure LS (Gentra Systems Inc.). Laboratory technicians were blinded to the headache status of the samples. *COMT* genotypes were determined using the LightCycler (Roche Diagnostics Scandinavia AB, Bromma, Sweden) fluorescence resonance energy transfer method [10]. Polymerase chain reaction (PCR) amplifications were performed in 20-µl reactions on a LightCycler System, using 2 µl genomic DNA and the LightCycler-FastStart DNA Master Hybridization Probes kit (Roche Diagnostics Scandinavia AB, Bromma, Sweden). PCR primers (Eurogentec, Seraing, Belgium) and fluorescence labelled probes (PROLIGO, Paris, France) are shown in Table 1. Based on melting curve profiles, participants were classified as having Val/Val, Val/Met or Met/Met genotypes. Details on PCR and melting curve conditions are available on request.

Table 1 Primers and hybridisation probes used for *COMT* Val158Met genotyping

	Sequence
Primers	
Forward	5'-ACGCCGTGATTCAGGAGCA-3'
Reverse	5'-GTCTTTCCTCAGCCCCAG-3'
Probes	
Sensor	5'-TCA<u>C</u>GCCAGCGAAATCCA-Fl-3'
Anchor	5' LC Red 640-ATCCGCTGGGTGATGGCG-3'

Fl, fluorescein; *LC Red 640,* LightCycler Red 640
Underlined C indicates polymorphic site

Participation

Out of the 92 566 invited individuals, a total of 51 383 subjects (56%) completed the headache questionnaire in Q_2. Details of the non-participants are described elsewhere [7, 10].

In the HUNT biobank a total of 62 664 DNA samples are stored. At the time of the 1995–1997 HUNT study, participants were not sufficiently informed about possible genetic DNA-based research. Therefore, an extensive information campaign about functional genomic research was performed in 2002, and each surviving adult HUNT participant (n=61426) received an information folder and a personal letter asking for re-consent to include genetic research. In total, 1185 (1.9%) persons withdrew their consent [7, 8]. Out of the remaining group of 60 241 participants, *COMT* gene polymorphism analyses were performed on 3048 individuals. Out of the 3048 individuals, a total of 2451 (80%) subjects also had responded to the headache questions and were subjects for the present study.

Out of the 3048 individuals with known *COMT* genotype, approximately 70% of these were selected completely at random. The remaining 30% had been randomly selected among an older group of individuals. This latter group was generated in connection with a planned genetic study on diabetes that needed age-matched controls to a diabetic population. Because diabetes patients as a group are older than the general population, somewhat older individuals were needed as controls. As a consequence, the total group of 3048 individuals was somewhat older than the HUNT population as a whole.

Ethics

The study was approved by the Regional Committee for Ethics in Medical Research, and by the Norwegian Data Inspectorate.

Statistical analysis

Differences between continuous variables were tested with analyses of variance (one-way ANOVA) and between dichotomous variables with the χ^2 test. Analyses used two-tailed estimation of significance, and p<0.05 was considered to be statistically significant. Overall, our sample of 982 headache sufferers and 1469 controls had the power to detect a 5% difference in headache prevalence between genotypes with 95% certainty and 80% power. The sample of 305 migraine patients and 1469 controls had the power to detect a 3% difference in migraine prevalence between genotypes with 95% certainty and 80% power.

Statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS), version 13.0 (SPSS Inc, Chicago).

Results

The distribution of genotypes among the 2451 individuals was in Hardy-Weinberg equilibrium. Out of these, a total of 982 persons (346 men and 636 women) with a mean age of 46.8 years suffered from headache, and the remaining 1468 individuals with a mean age of 55.5 years were headache-free controls. Among the 982 headache sufferers, 305 (93 men and 212 women) had migraine and 677 (253 men and 424 women) non-migrainous headache.

The demographic data according to *COMT* genotypes are shown in Table 2. No difference in distribution of gender, mean age or education level was found between the genotype groups. However, the individuals with known *COMT* genotype were significantly older than those without *COMT* data available (p<0.01) (Table 2).

Comparison of allele distribution of *COMT* Val158Met polymorphism by gender failed to detect significant differences between headache sufferers and controls (Table 3). Similarly, the distribution of the *COMT* Val158Met genotypes was similar between controls and headache (all types) and migraine. When the Met/Met and Val/Met genotypes were pooled, headache was slightly less likely among women with the Val/Val genotype (44.0%) than in women with other genotypes (49.3%) (p=0.13). This tendency was explained by a lower prevalence of non-migrainous headache among women with the Val/Val genotype than among those with Val/Met or Met/Met genotypes (26.2% *vs*. 33.6%, p=0.04).

Table 2 Distribution of sex, age and education related to *COMT* genotypes

Characteristics	No COMT genotyping (n=48 932)	Met/Met (n=775)	Met/Val (n=1226)	Val/Val (n=450)
Sex, female (%)	54.0	51.7	54.5	55.1
Age, mean (SD)	48.7 (16.9)	51.9 (18.0)	52.2 (17.7)	51.5 (17.7)
Years of education				
≤9 (%)	35	36	39	35
10–12 (%)	44	43	42	44
>12 (%)	21	21	19	21

Table 3 Headache prevalence related to genotype of *COMT* in men and women

	Men						Women					
	Met/Met		Met/Val		Val/Val		Met/Met		Met/Val		Val/Val	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Genotypes												
Controls	269	34.1	381	48.4	138	17.5	212	31.1	330	48.5	139	20.4
Headache	105	30.3	177	51.2	64	18.5	189	29.7	338	53.1	109	17.1
Migraine	27	29.0	51	54.8	15	16.1	62	29.2	166	50.0	44	20.8
Non-migrainous headache	78	30.8	126	49.8	49	19.4	127	30.0	232	54.7	65	15.3
Headache >14 days/month	9	33.3	15	55.6	3	11.1	6	19.4	21	67.7	4	12.9
Alleles	Met		Val			Met		Val				
Controls	919	58.3	657	41.7		754	55.4	608	44.6			
Headache	387	55.2	305	44.1		716	56.3	556	43.7			
Migraine	105	56.5	81	43.5		230	54.2	194	45.8			
Non-migrainous headache	282	55.7	224	44.3		486	54.1	412	45.9			
Headache >14 days/month	33	61.1	21	38.8		33	53.2	29	46.8			

Discussion

In this population-based study among 2451 unselected adults, no significant association between migraine and the Val158Met polymorphism at the *COMT* gene was found.

Previously, individuals with the *COMT* Val/Val genotype have been found to be less susceptible to pain [4], and in one study migraine was less frequent among those with the Val/Val genotype [5]. In contrast, the distribution of the Val/Val genotype and other genotypes were similar between controls and migraine patients in the present study. In accordance with our results, no association was found between migraine with aura and *COMT* gene polymorphism in a case-control study of 97 patients with migraine with aura and 94 healthy controls [13]. Similarly, no relationship to the Val158Met polymorphism at the *COMT* gene was found in another case-control study of 103 patients with chronic daily headache with drug abuse, 101 patients with episodic migraine without aura and without analgesic abuse, and 117 controls without headache [14]. These conflicting results underline the importance of testing positive findings by replication in other populations, because there is a relatively high risk of positive findings due to coincidence [15].

Of course, non-replications raise concerns about power. However, our sample of 305 migraine patients and 1469 controls should have 80% power to detect a difference in migraine prevalence of 3% between genotypes. A limitation of our study is the use of a simplified headache classification, because headache sufferers were classified into two groups of either migraine or non-migrainous headache. Based on our validation study, we have reason to believe that the majority of individuals with non-migrainous headache suffered from tension-type headache [9].

The observation that *COMT* activity is lower in females than in males and that *COMT* activity may be under hormonal control [16, 17] is the reason for performing separate analyses for each gender. The prevalence of non-migrainous headache tended to be lower among women with the Val/Val genotype than among those with the other genotypes. This may be an incidental finding, but should be investigated in further studies including patients fulfilling the diagnosis of tension-type headache. If the findings could be replicated, the gender-specific finding is of interest in light of the well established gender difference in prevalence of headache, and lower pain threshold in women [18].

Our study could not confirm a strong association between headache and the Val158Met polymorphism. To date, no other functional polymorphisms within the *COMT* gene have been linked to headache. However, two other different genetic haplotypes of the *COMT* gene have been found to be involved in pain perception in a recent case-control study [19], and these may be relevant for headache prevalence.

In conclusion, in this population-based study performed among 2451 unselected adults, no significant

association between migraine and the Val158Met polymorphism at the *COMT* gene was found. Among women, non-migrainous headache tended to be less likely among with those with the Val/Val genotype than among those with the other genotypes.

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