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Frequency of the C677T variant of the methylenetetrahydrofolate reductase (*MTHFR*) gene in patients with migraine with or without aura – a preliminary report

Częstość występowania wariantu C677T genu MTHFR *u pacjentów z migreną z aurą i bez aury – doniesienie wstępne*

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Abstract

Background and purpose: The aim of our study was to evaluate the frequency of the C677T variant in the methylenetetrahydrofolate reductase (*MTHFR*) gene in patients with migraine with or without aura and to find an association between this variant and vascular lesions in magnetic resonance imaging of the head, presence of patent foramen ovale (PFO) and increased level of homocysteine.
Material and methods: Ninety-one patients with migraine, aged 19-57, were investigated in this study. The *MTHFR* C677T variant was genotyped in this group and levels of homocysteine, folic acid and vitamin B_{12} were measured. Transcranial Doppler sonography with test for PFO detection by injection of air contrast during the Valsalva manoeuvre was performed in each patient.
Results: Frequency of the C677T variant in the *MTHFR* gene was similar in patients and controls. Hyperhomocysteinaemia was significantly more frequent in migraine patients with the C677T variant. The prevalence of PFO was significantly higher in migraine patients with aura and the homozygous variant of the *MTHFR* gene.
Conclusions: Frequency of the C677T variant in the *MTHFR* gene was similar in patients and controls. Significantly more

Streszczenie

Wstęp i cel pracy: Celem pracy była ocena częstości występowania wariantu C677T genu *MTHFR* u chorych na migrenę oraz określenie, czy istnieje związek pomiędzy wariantem genu a stężeniem homocysteiny w surowicy, obecnością ogniskowych zmian naczyniopochodnych w badaniu za pomocą rezonansu magnetycznego (RM) głowy oraz występowaniem drożnego otworu owalnego.
Materiał i metody: Badaniem objęto 91 chorych. U wszystkich wykonano rutynowe badanie neurologiczne i internistyczne, oznaczenia stężenia witaminy B_{12}, kwasu foliowego i homocysteiny w surowicy, badanie na obecność wariantu C677T genu *MTHFR*, badanie dopplerowskie tętnic mózgowych z podaniem kontrastu celem diagnostyki drożnego otworu owalnego, a także RM głowy.
Wyniki: Częstość występowania wariantu C677T genu *MTHFR* była podobna u osób z migreną i w grupie kontrolnej. Zwiększone stężenie homocysteiny w surowicy obserwowano znamiennie częściej u chorych na migrenę, u których stwierdzono obecność wariantu C677T genu *MTHFR*. Drożny otwór owalny występował znamiennie częściej u chorych na migrenę z aurą, u których stwierdzono obecność homozygoty wariantu C677T genu *MTHFR*.

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frequent prevalence of PFO in migraine patients with aura (with homozygous recessive genotype of *MTHFR*) probably suggests their common genetic basis. Hyperhomocysteinaemia was significantly more frequent in migraine patients with the C677T variant, which could be an additional risk factor of this disease.

Key words: migraine, *MTHFR* gene, patent foramen ovale.

Wnioski: Częstość występowania wariantu C677T genu *MTHFR* u pacjentów z migreną jest podobna jak u osób bez tej choroby. Znamiennie częstsze występowanie drożnego otworu owalnego u osób z migreną z aurą, u których stwierdzono obecność homozygoty wariantu C677T genu *MTHFR*, mogłoby sugerować związek tego typu migreny z anomalią rozwojową serca i ich ewentualne wspólne podłoże genetyczne. Znamiennie częstsze występowanie zwiększonego stężenia homocysteiny w surowicy u pacjentów z migreną, u których stwierdzono obecność wariantu C677T genu *MTHFR* mogłoby być dodatkowym czynnikiem ryzyka wystąpienia migreny.

Słowa kluczowe: migrena, gen *MTHFR*, drożny otwór owalny.

Introduction

Diagnostic and therapeutic difficulties in some cases of migraine inspire the continued search for new causes and novel treatments for this disease. The significance of genetic factors has been highlighted in recent years, leading to the consideration of migraine as a polygenic disease [1]. Numerous reports show an association between the C677T variant of the *MTHFR* gene (coding methylenetetrahydrofolate reductase) and the prevalence of migraine with aura [2-4]. The impact of the mutations within that gene (especially homozygous form) in hyperhomocysteinaemia, recognized as an independent vascular risk factor, is also stressed [3,4].

The metabolism of homocysteine requires an adequate supply of vitamin B_{12} and folic acid for normal remethylation reaction. Presence of the C677T variant of *MTHFR* decreases the activity of methylenetetrahydrofolate reductase, leading therefore to an increased level of homocysteine [5]. In recent years attention has been drawn to the common mechanism of migraine and persistent foramen ovale (PFO). The coincidence of these two entities may be found in as many as 40% of patients [6].

The aim of the present study was to evaluate the frequency of the C677T variant of the *MTHFR* gene in patients with migraine and to find an association between this variant and increased level of homocysteine, presence of vascular lesions in magnetic resonance imaging (MRI) of the head, or presence of PFO.

Material and methods

The study comprised 91 patients, including 71 women and 20 men, aged between 19 and 57 years (mean 38 ± 10.3 years), diagnosed with migraine according to the criteria of the International Headache Society [7]. All patients were hospitalized in the Department of Neurology within the Seventh Independent Public Teaching Hospital (Medical University of Silesia) in Katowice-Ochojec and were followed up in the outpatient neurological clinic. Fifty-one patients, aged between 22 and 54 years (mean 37.9 ± 10.2 years), had migraine with aura, and the other 40 patients, aged between 19 and 57 years (mean 39.2 ± 10.4 years), had migraine without aura.

The control group consisted of 48 subjects, including 36 women and 12 men, aged between 24 and 57 (mean 37 ± 8.6 years).

All patients with migraine underwent the physical examination (both general and neurological one), along with measurements of serum concentration of vitamin B_{12}, folic acid, and homocysteine. MRI of the head was performed in each patient and the contrast transcranial Doppler study (c-TCD) was used to assess the presence of PFO.

The C677T variant of the *MTHFR* gene was tested with the polymerase chain reaction (PCR) using restriction enzyme digestion, strictly according to the previously described method [2].

DNA was isolated from whole blood, either fresh or frozen, with the DNA isolation kit Blood Mini (A&A Biotechnology, Poland). The quantity and quality of the isolated DNA were checked spectrophotometrically (Biomate3 spectrophotometer) at the wavelength of 260 nm and 280 nm. Purified DNA fragments containing the site of the studied *MTHFR* variant were amplified by PCR using the PCR primer sequences published by Kowa *et al*. [8]. The sense primer sequence was

5'-TGA AGG AGA AGG TGT CTG CGG GA-3', and the antisense primer sequence was 5'-AGG ACG GTG CGG TGA GAG TG-3'.

PCR reaction was performed in a 25-μL sample containing 150 ng of genomic DNA, 1 U of Fast Start polymerase (Roche, Germany), 1 × standard PCR buffer with $MgCl_2$, nucleotides mixture at the baseline concentration of 200 mM and distilled water mixed to the final volume of 25 μL. The following PCR parameters were used: one cycle at 95°C for 5 min for an initial denaturation, followed by 35 cycles of denaturation for 30 s at 95°C, primer annealing for 30 s at 65°C, primer extension for 30 s at 72°C and a final extension for 5 min at 72°C. The mixture was then cooled to 4°C. This amplification reaction led to the synthesis of a 198-bp fragment. Those fragments were separated using a 1% agarose gel (Top Vision LE GQ Agarose, Fermentas, USA) with 1 × TAE buffer (50X TAE Electrophoresis Buffer, Fermentas, USA). The reaction mixture (5 μL) and length marker (5 μL) (O'GeneRuler Low Range DNA Ladder, Fermentas, USA) were added to the agarose gel with ethidium bromide. Electrophoretic separation of DNA fragments was visualized under ultraviolet light and the images were captured with a digital camera.

The PCR product mixture (about 0.2 μg) was incubated with 1 IU of *Hin*fI endonuclease (FastDigest – Fermentas, USA). FastDigest buffer (2 μL) was added to the reaction mixture with distilled water to a final volume of 30 μL. The reaction mixture was incubated for 5 min at 37°C and then inactivated for 20 min at 65°C.

The restriction reaction products were separated using a 2.5% agarose gel (Top Vision LE GQ Agarose, Fermentas, USA) with 1 × TAE buffer (50X TAE Electrophoresis Buffer, Fermentas, USA). The length of the restriction fragments was identified with a length marker (2 μL) (O'GeneRuler Low Range DNA Ladder, Fermentas, USA). The C to T substitution at nucleotide 677 in the *MTHFR* gene results in a restriction site for the *Hin*fI enzyme, cleaving this fragment into 175-bp and 23-bp fragments.

TCD was performed in a strictly standardized manner, using the Pioneer TC 2020 (EME) apparatus with 2-MHz and 4-MHz probes [9]. The middle cerebral artery was assessed at the depth of 50-60 mm with the access through the temporal bony window. TCD technique in search of the PFO was in accordance with the international consensus [10] with the use of Pioneer TC 2020 (EME) apparatus with dedicated software, enabling simultaneous registration at several depths.

The study was performed in a supine position, with the 18-gauge needle inserted into the right cubital vein. The first stage of testing included bolus injection of the contrast agent produced by vigorous mixing of the normal saline (9 mL) and air (1 mL). If no microbubbles were detected within 40 s, the testing was repeated using the Valsalva manoeuvre, increasing the pressure within the thoracic cavity [11]. The third stage of testing (expiration) was made 5 seconds after contrast agent injection. If no embolic signal was detected three times, the study was considered negative. Each patient received a detailed instruction on the appropriate performance during the test, and the efficacy of testing was confirmed by the measured decrease of systolic velocity of the registered blood flow.

A four-grade scale of the right-to-left shunt was used: (1) no microbubbles; (2) 1-10 microbubbles; (3) > 10 microbubbles; and (4) massive microbubbles (so-called 'curtain'). If massive microbubbles were detected, the Valsalva manoeuvre was abandoned. None of the patients reported headache directly after the TCD.

The same panel of tests, with the exception of head MRI and TCD testing for PFO, was performed in controls.

The protocol of the study was approved by the Bioethical Committee of the Medical University of Silesia in June 2009 (L.dz.KNM/0022/KB1/55/I/09).

Statistical analysis

The database of the clinical material was prepared in Excel 2003 (Microsoft), and the statistical calculations were performed in Statistica v.7.1 (StatSoft) and MedCalc v.9.03. A p-value of less than 0.05 was considered statistically significant. The hypothetical distribution conformity of the specific variables with the normal one was tested with the Shapiro-Wilk test.

Unpaired Student's t-test was used for comparisons between groups in the case of normally distributed variables. The Mann-Whitney U-test was used for non-normally distributed variables. Qualitative variables were compared using the chi-square test with Yates correction.

Results

The frequency of the C677T variant of the *MTHFR* gene was similar in patients with migraine and in controls (47.3% and 47.9%, respectively; $p = 0.94$). Increas-

ed serum homocysteine concentration was significantly more prevalent among patients with migraine (regardless of its type) and with the C677T variant of the *MTHFR* gene than in other migraineurs (23.8% vs. 8.3%, respectively; $p = 0.04$). Hyperhomocysteinaemia was similarly prevalent in controls with the C677T variant of *MTHFR* and in those without the C677T variant (4.3% vs. 8.0%, respectively; $p = 0.6$).

The prevalence of vascular lesions in MRI in patients with migraine and with the C677T variant of the *MTHFR* gene was similar to the migraineurs without the C677T variant of the *MTHFR* gene (36.7% vs. 29.4%, respectively; $p = 0.54$).

PFO was found more often in patients with migraine with aura in whom the homozygous C677T variant of the *MTHFR* gene was found (Fig. 1).

PFO was found in similar percentages of patients with migraine without aura, in whom the homozygous or heterozygous C677T variant of the *MTHFR* gene was noted (Fig. 2).

Discussion

Migraine is a common disease, affecting 10-20% of the general population, but its pathophysiology is not completely understood. Besides vascular mechanisms, genetic factors probably also play a role. It is believed that the C677T variant of the *MTHFR* gene, as well as polymorphism of the angiotensin-converting enzyme (ACE) gene, may play an important role in the response to vascular oxidative stress [13]. However, the results of studies on the association between migraine and the C677T variant of the *MTHFR* gene are equivocal [2,8,14-23]. Oterino *et al*. assessed the frequency of the T677 homozygous *MTHFR* gene in the Spanish population and did not find significant differences between 230 patients with migraine and 204 controls. It was noted only that the T allele of the *MTHFR* gene was significantly more frequent in patients with migraine with aura [3]. Finnish studies involving 898 patients with migraine with aura and 900 healthy subjects did not reveal differences in prevalence of *MTHFR* gene variants between those groups. The authors concluded that the significance of that gene for susceptibility to migraine was unlikely [20]. There was no difference in prevalence of the C677T variant of the *MTHFR* gene between migraineurs and controls in the Portuguese population either [17]. Australian authors studied 267 migraineurs with the C677T variant of the *MTHFR* gene and found that this genotype was associated with specific clinical features of migraine [24].

In 2010, Schürks *et al*. reviewed papers reporting genetic studies in migraine published before March 2009. Twelve out of the 21 papers on the C677T variant of the *MTHFR* gene or *ACE* D/I polymorphism in various populations were devoted to the C677T variant of the *MTHFR* gene. The combined results of those studies showed that presence of the T allele was asso-

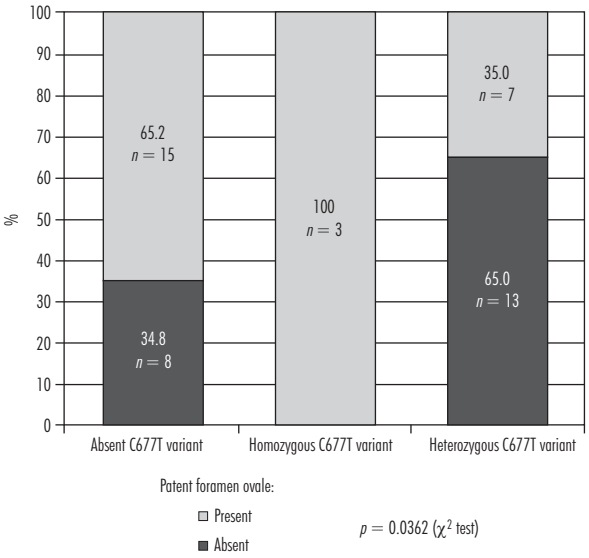

Fig. 1. Prevalence of patent foramen ovale as related to presence of homozygous or heterozygous C677T variant of *MTHFR* gene in patients with migraine with aura

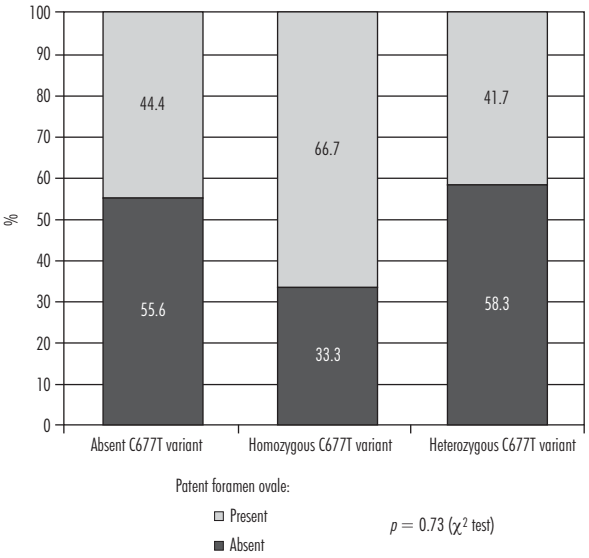

Fig. 2. Prevalence of patent foramen ovale as related to presence of homozygous or heterozygous C677T variant of *MTHFR* gene in patients with migraine without aura

ciated with greater risk of migraine development regardless of its type, although the differences were non-significant for the Caucasian population alone. There was no agreement among the analysed studies regarding the probability of development of a particular type of migraine among patients having the C677T variant of the *MTHFR* gene. It was suggested that the presence of that variant increases the risk for migraine with aura but not all studies confirmed that supposition.

TT increases the risk of migraine with aura in the Japanese population but the same genotype increases the risk of migraine without aura in the Turkish population. Such an association was not confirmed in Caucasians. Schürks *et al*. explain divergent results of the analysed studies with the biological heterogeneity of migraine, different methods of its diagnosis and divergent populations of patients [25].

The current study did not reveal significant differences in prevalence of the C677T variant of the *MTHFR* gene between patients with migraine and controls. This finding might be explained by the relatively small studied samples. Thus, a study on larger groups is required to find potential differences in the Polish population.

Hyperhomocysteinaemia, being one of the vascular risk factors, might also play a role in a pathomechanism of migraine. The endothelial damage caused by homocysteine impairs the release of nitric oxide, and the decreased bioavailability of nitric oxide may result in abnormal reactions between the vessel wall, platelets and macrophages, changing the coagulative properties of blood and affecting the neurovascular functions [26].

The reports on the significance of homocysteine in the migraine pathomechanism are equivocal. Israeli authors studied serum concentration of homocysteine in 78 migraineurs and 126 healthy subjects and found significantly increased homocysteine concentration in one patient only [27]. Scher and colleagues think that the risk of migraine with aura is associated with homozygosity in the C677T variant of the *MTHFR* gene independently of other vascular risk factors [22]. On the other hand, de Tommaso *et al*. [14] believe that hyperhomocysteinaemia might promote neuronal factors predisposing to the development of migraine.

The most recent study by Oterino *et al*. [28], performed among 228 patients with migraine with aura, 199 patients with migraine without aura and in 310 controls, showed that patients with migraine with aura had significantly greater serum concentration of homocysteine, and that the C677T variant of the *MTHFR* gene was the best genetic indicator of homocysteine concentration.

Our study showed that hyperhomocysteinaemia was more common in migraineurs with the C677T variant of the *MTHFR* gene (particularly in homozygous patients), which might suggest the possible increased risk of migraine in patients with confirmed hyperhomocysteinaemia and with the C677T variant of the *MTHFR* gene.

Migraine can be a risk factor for ischaemic stroke, which might be related to the paradoxical embolism due to the presence of PFO, especially among patients with migraine with aura. Domitrz *et al*. [12] studied 62 patients with migraine with aura, 60 patients with migraine without aura, and 65 healthy volunteers, and noted that PFO was much more prevalent among patients with migraine with aura (53%) than in patients with migraine without aura (25%) or in healthy subjects (25%).

Rigatelli [29] reviewed papers published between 1988 and 2008, related to migraine and PFO, and concluded that a high proportion of subjects with PFO suffer from migraine, especially from migraine with aura, and these are persons with an increased risk of paradoxical embolism. The current study showed that PFO was more commonly found in patients with migraine with aura who had the C677T variant of the *MTHFR* gene. This finding should be replicated in a larger group of patients but it may suggest an association between migraine with aura and PFO, as well as a possible common genetic background for both these conditions.

It is suggested that patients with migraine with aura more often have white matter lesions found in MRI. Recent studies show, however, that an association between paradoxical embolism and lesions seen in MRI is rather unlikely. Italian authors analysed the possible relationship between PFO-related shunt and white matter lesions in 87 patients with migraine with aura. The presence of shunt was noted in 45% of patients, but subjects with or without shunt did not differ regarding the number and size of lesions detected in MRI [30]. The same findings were reported by Bosca *et al*. [31], who did not find an association between vascular lesions seen in MRI and presence of the C677T variant of the *MTHFR* gene.

Preliminary conclusions

1. Frequency of the C677T variant of the *MTHFR* gene was similar in patients and controls, which seems to confirm the results of other authors.

2. Significantly higher prevalence of PFO in patients with migraine with aura (with homozygous recessive genotype of *MTHFR*) might suggest their common genetic basis.
3. Hyperhomocysteinaemia was significantly more frequent in migraine patients with the C677T variant, which could be an additional risk factor of this disease.

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Disclosure

Authors report no conflict of interest.

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