

The methylene tetrahydrofolate reductase gene variant c677t is not associated with migraine in twelve sudanese pedigrees with migraine

Abstract

Introduction: High homocysteine level is one of the incriminated factors in the vascular dysfunction theory in migraine. Homocysteine is metabolized by the methylenetetrahydrofolate reductase *MTHFR* enzyme. C667T variant of the *MTHFR* gene reduce the enzymatic activity and is thought to be associated with migraine.

Aims: The aim of this study is therefore to determine whether the C677T variant was associated with migraine in Sudanese migraineurs families.

Patients and methods: 162 subjects from twelve clinically diagnosed migraine families with a positive family history of migraine were included. The *MTHFR* variant was genotyped in 106 migraineurs and 56 unaffected relatives.

Results: Genotype distribution and allele frequencies were determined for 106 migraine patients and 56 pseudo controls. The genotype frequencies in the migraine group (51.85 %, 11.72 %, 1.85 % for C/C, C/T and T/T, respectively) showed very low homozygosity of the mutant TT allele. The 95% confidence interval Risk Ratio and Odd Ratio for the C and T (CT+TT) allele were found to be 1.16 and 1.2 respectively. However the C and T allele frequencies for the case and control groups did not deviate from Hardy-Weinberg expectations ($P > 0.4$ and 0.6 respectively).

Conclusion: No association was found between *MTHFR* gene variant and migraine in Sudanese families. The lack of association could be due to different factors; the ethnic variation of the Sudanese subject may have constituted a major factor, the selection of family based approach rather than case control could be another reason although analysis using the fBAT software package is intended to overcome this obstacle. The comorbidity of migraine with aura and migraine without aura within the studied families may have played a role as well.

Keywords: *mthfr* gene variant, homocysteine, migraine with aura (ma), migraine without aura(mo)

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Introduction

No definitive pathophysiology exists for migraine yet, nevertheless, many theories were postulated with the most ancient one being the vascular theory which was developed by H. Wolff during the 1940s.¹ According to Lauritzen, Wolff described migraine as a vasospastic disorder that is initiated by vasoconstriction in the cranial vasculature. The vasoconstriction is then followed by dilatation of intracranial or extracranial blood vessels. Dilation of the richly innervated meningeal blood vessels activates the trigeminal sensory nerves that surround these blood vessels, initiating pain. The activated trigeminal nerves release vasoactive neuropeptides that further contribute to dilation and worsening of the pain.²

High homocysteine level is one of the incriminated factors in the vascular dysfunction theory.³ Elevated serum homocysteine is believed to affect the functions of endothelial cells and alter blood coagulation as well.⁴ Homocysteine is also postulated to act as an excitatory amino acid and to cause temporary thrombosis of cerebral blood vessels, reducing oxygen into the brain.^{5,6} Homocysteine is metabolized by Methylene-tetrahydrofolate reductase (*MTHFR*) enzyme which catalyses the conversion of homocysteine to methionine thus reducing its level in the blood. The human *MTHFR* gene is located in chromosome 1p36.⁷ A variant in the *MTHFR* gene

called (C677T) result due to modification of the amino acid sequence, alanine (Ala) for valine (Val). The resultant variant decrease but not abolish *MTHFR* enzyme activity with Ala/Val retain 65% and Val/Val retain 30% of enzyme activity.⁸ This lead to mild elevation in plasma homocysteine levels particularly when intake of folate is low.⁹ Hyperhomocysteinemia have been reported in patients with migraine.³ Mild hyperhomocysteinemia is also reported to carry an increased risk for vascular disease and may lead to disturbed circulation in cerebral, coronary, and peripheral vessels.¹⁰

The association of the C677T variant with MA was reported in Japanese, Turkish and Dutch population.¹¹⁻¹³ Two recent meta-analysis studies investigated the association between migraine and *MTHFR* C677T variant; Markus Schürks et al.¹⁴ performed a systemic review and meta-analysis in 2010.¹⁴ The studies were performed in Caucasians, Japanese, Turkish, and in north Indians. They found that the pooled effect estimates among all studies suggest that the T allele of the *MTHFR* 677C>T polymorphism is associated with an increased risk for migraine. The association was most pronounced for carriers of the homozygous mutant TT genotype supported by recently meta-analysis that reviewed 15 studies and concluded that there is a significant association between this genetic variant and MA in Caucasian population and overall migraine in non-Caucasian population.¹⁵ With the strong association with migraine in different

populations, the MTHFR gene seemed a plausible candidate gene to investigate in Sudanese population. The aim of this study is therefore to determine if the C677T variant was associated with migraine in Sudanese migraineurs families.

Methods

162 subjects from twelve clinically diagnosed migraine families with a positive family history of migraine were included. Individual DNA samples were genotyped using a candidate gene selected from previous studies based on pathophysiological pathways that have been postulated in the development and progression of migraine.¹⁴ The marker is the C677T variant in the MTHFR gene which is located in chromosome 1p36.3 with primer 5'-TGA AGG AGA AGG TGT CTG CGG GA-3' (forward), 5'-AGG ACG GTG CGG TGA GAG TG-3' (Reverse).¹⁶

In a total volume of 25 µL solution containing 50ng genomic DNA, 1 µL of 10mM of each primers and 1 unit/µL of Taq polymerase, 3.5 µL ddH₂O, 17.5 µL Master mix (1.5 µL of 25 mM MgCl₂, 1µL of 10mM dNTPs, 2.5 µL of 25mM KCl buffer, 12.5 µL ddH₂O) for 30 cycles, 94 °C for 3min (initial denaturation), 94 °C for 1min, 61 °C for 2 min (annealing), 72 °C for 1 min (extension) and 72 °C for 10 min (final extension). In order to distinguish the alleles, all the amplicons (The PCR products) were separated by 2% agarose gel stained with ethidium bromide.

For the primer C677T MTHFR gene polymorphism was investigated by PCR, as previously described by Frosst et al.⁸ The generated 198 bp PCR product was digested by the *Hinf* I restriction enzyme (Inqaba), resulting in 175 and 23 bp fragments in the homozygous T state, and 198, 175, and 23 bp fragments in heterozygotes. The wild type remains undigested, preserving the original 198 bp fragment.

Data analysis

We used the family based association test (FBAT), including the Haplotype Based Association Test (HBAT) to determine if any HTTLPR or C677T alleles or haplotypes of these alleles are associated with migraine in our sample. We adopted FBAT because it is unaffected by population admixture or stratification, and is useful in cases where multiple comparisons must be made and is ideal for

studying the association of a given gene in a complex disorder.¹⁷ Vassar stat online software package was also used.

Results

Genotype distribution and allele frequencies were determined for 106 migraine patients and 56 pseudo controls (Figure 1). Table 1 displays the frequency distributions observed for the MTHFR C677T variant for the affected and non affected individuals from the selected families. The genotype frequencies in the migraine group (51.85 %, 11.72 %, 1.85 % for C/C, C/T and T/T, respectively) showed very low homozygosity of the mutant TT allele (Figure 2).

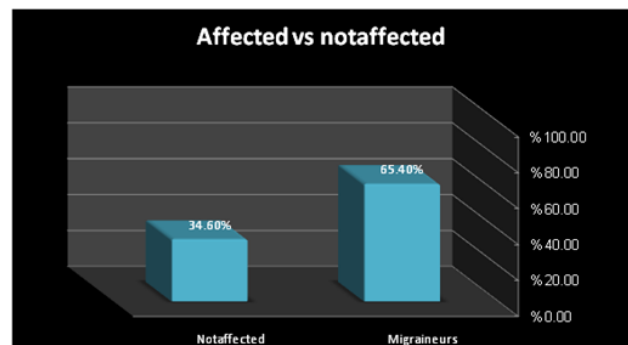


Figure 1 The distribution of migraineurs and nonmigraineurs.

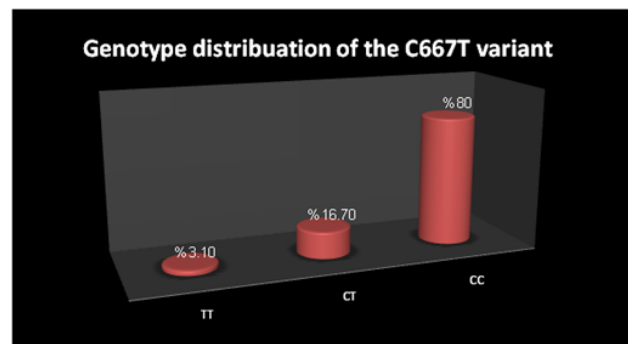


Figure 2 Genotype distributions among migraineurs and nonmigraineurs.

Table 1 Genotype distributions of migraineurs and their unaffected relatives

Case study	Genotype frequency (C/C)	Genotype frequency (C/T)	Genotype frequency (T/T)	Allele frequency (C)	Allele frequency (T)
Control (n=56)	28.4% (n=46)	4.9% (n=8)	1.23% (n=2)	0.89	0.12
Migraine (n=106)	51.8% (n=84)	11.7% (n=19)	1.85 % (n=3)	0.88	0.12

The 95% confidence interval Risk Ratio and Odd Ratio for the C and T (CT+TT) allele were found to be 1.16 and 1.2 respectively. However the C and T allele frequencies for the case and control groups did not deviate from Hardy-Weinberg expectations ($P > 0.4$ and 0.6 respectively). This analysis was done by the Vassarstat online software analysis. When we used family based analysis software fBAT, the program identified 80 nuclear families within the twelve pedigrees, an additive mode of inheritance and the estimated frequencies for the C and T alleles of 0.9 and 0.11 respectively. It should be noted that in the analysis using the Vassarstat software, we combined the CT and TT into the T allele since it has been reported that having one or two genotype of the mutant variant will lead to reduction in expression of the MTHFR protein.⁸

Discussion

Migraine is a complex neurological disorder with a strong familial

aggregation suggesting significant genetic involvement.¹⁸ Several candid genes were investigated for an association with migraine in different populations. A common polymorphism from the MTHFR gene, the C677T, has been reported to be associated with migraine mainly migraine with aura.³ Individuals carrying the C677T variant (rs1801133) in the MTHFR gene have been shown to have decreased enzyme activity that eventually leads to hyperhomocystinemia and possibly resulting in vascular dysfunction. Several studies linked MTHFR gene variant and migraine, in particular migraine with aura. Of all the candidate genes linked to migraine, the MTHFR gene was the most cited. Two recent meta-analysis studies investigated the association between migraine and MTHFR C677T variant; Markus Schürks et al.¹⁴ in 2010 and Zienab Saman et al.¹⁵ They both came to conclusion that there is a significant association between this genetic variant and MA.

In our study group we could not find an association between the *MTHFR* variant and migraine among the Sudanese families. The lack of association is possibly due to ethnic variation of the Sudanese population. The families under study come from different ethnic groups in Sudan; Afroasiatics (Ja'afra, Jaa'lia, Shawiga, Kwahla) and Nilosaharan (halfaween and Mahhs). In addition, ethnic variation may affect health behaviour and life style factors such as dietary habits and strategies of diet fortification with folic acid. In consistent with our results, the two largest studies in C677T gene variant have shown negative results Todt et al.¹⁹ and Kaunisto et al.²⁰ In addition to the different population studied, there is also a significant variation in migraine case identification that added further variability in the reported studies. Our clinical study that preceded this genetic study revealed that both migraine with aura and migraine without aura co-exist in the same family and even in the same individual (in press).

In agreement to our findings, The T-allele frequency of the C677T *MTHFR* polymorphism has been found to vary among different populations and ethnic groups, with a lower frequency among Africans.^{21,22} It is worth noting that we have co-analyzed the C677T *MTHFR* gene variant with the 5-HTTLPR variant of the serotonin transporter gene using the haplotype family based association test (hBAT) in the fBAT software, the resultant haplotypes if we could call it haplotype can form a basis for future work involving one or both of these genes.

Conclusion

No association was found between *MTHFR* gene variant and migraine in Sudanese families. The lack of association could be due to different factors; the ethnic variation of the Sudanese subject may have constituted a major factor, the selection of family based approach rather than case control could be another reason although analysis using the fBAT software package is intended to overcome this obstacle. The comorbidity of migraine with aura and migraine without aura within the studied families may have played a role as well and finally the small number of the study group could have affected the results. Never the less, our study is a preliminary one since it was the first in Sudan.

Acknowledgments

None.

Conflicts of interest

The authors declare no conflicts of interest.

References

- Wolff H. Headache and other head pain. New York, USA: Oxford University Press. 1963.
- Lauritzen M. Cerebral blood flow in migraine and cortical spreading depression. *Acta Neurol Scand Suppl.* 1987;113:1–40.
- Evers SH, Husstedt I-W. Plasma homocysteine levels in primary headache. In: Edvinsson L. Headache Pathogenesis: Monoamines, Neuropeptides, Purines and Nitric Oxide. USA: Lippincott-Raven Publishers Olsen. 1997;215–218.
- Hering-Hanit R, Gadoth N, Yavetz A, et al. Is blood homocysteine elevated in migraine? *Headache.* 2001;41(8):779–781.
- Kara I, Sazci A, Ergul E, et al. Association of the C677T and A1298C polymorphisms in the 5, 10 methylenetetrahydrofolate reductase gene in patients with migraine risk. *Brain Res Mol Brain Res.* 2003;111(1–2):84–90.
- Oterino A, Valle N, Bravo Y, et al. MTHFR T677 homozygosity influences the presence of aura in migraineurs. *Cephalalgia.* 2004;24(6):491–494.
- Goyette P, Pai A, Milos R, et al. Gene structure of human and mouse methylenetetrahydrofolate reductase (MTHFR). *Mamm Genome.* 1998;9(8):652–656.
- Frosst P, Blom HJ, Milos R, et al. A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase. *Nat Genet.* 1995;10(1):111–113.
- Wald DS, Law M, Morris JK, et al. Homocysteine and cardiovascular disease: evidence on causality from a meta-analysis. *BMJ.* 2002;325:1202.
- Coull BM, Malinow MR, Beamer N, et al. Elevated plasma homocyst(e)ine concentration as a possible independent risk factor for stroke. *Stroke.* 1990;21(4):572–576.
- Kowa H, Yasui K, Takeshima T, et al. The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine. *Am J Med Genet.* 2000;96(6):762–764.
- Kara I, Sazci A, Ergul E, et al. Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk. *Brain Res Mol Brain Res.* 2003;111(1–2):84–90.
- Scher AI, Terwindt GM, Verschuren WM, et al. Migraine and MTHFR C677T genotype in a population-based sample. *Ann Neurol.* 2006;59(2):372–375.
- Markush RE, Karp HR, Heyman A, et al. Epidemiologic study of migraine symptoms in young women. *Neurology.* 1975;25(5):430–435.
- Samaan Z, Gaysina D, Cohen-Woods S, et al. Methylenetetrahydrofolate reductase gene variant (MTHFR C677T) and migraine: a case control study and meta-analysis. *BMC Neurol.* 2011;11:66.
- Schürks M1, Rist PM, Kurth T. MTHFR 677C>T and ACE D/I polymorphisms in migraine: a systematic review and meta-analysis. *Headache.* 2010;50(4):588–599.
- Horvath S, Xu X, Laird NM. The family based association test method: strategies for studying general genotype--phenotype associations. *Eur J Hum Genet.* 2001;9(4):301–306.
- Russell MB, Olesen J. Increased familial risk and evidence of genetic factor in migraine. *BMJ.* 1995;311(7004):541–544.
- Todt U, Freudenberg J, Goebel I, et al. MTHFR C677T polymorphism and migraine with aura. *Ann Neurol.* 2006;60(5):621–622.
- Kaunisto MA, Kallela M, Hämäläinen E, et al. Testing of variants of the MTHFR and ESR1 genes in 1798 Finnish individuals fails to confirm the association with migraine with aura. *Cephalalgia.* 2006;26(12):1462–1472.
- Krieger H, Morton NE, Mi MP, et al. Racial admixture in north-eastern Brazil. *Ann Hum Genet.* 1965;29(2):113–125.
- Arruda VR, Siqueira LH, Gonçalves MS, et al. Prevalence of the mutation C677 --> T in the methylene tetrahydrofolate reductase gene among distinct ethnic groups in Brazil. *Am J Med Genet.* 1998;78(4):332–335.