Research article

The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura

Rod A Lea[1,3,4], Micky Ovcaric[1], James Sundholm[1], John MacMillan[2] and Lyn R Griffiths*[1]

Address: [1]Genomics Research Centre, School of Health Science, Griffith University, Queensland, Australia, [2]Queensland Clinical Genetics Service, Royal Children's Hospital, Queensland, Australia, [3]Institute for Molecular Systematics, School of Biological Sciences, Victoria University of Wellington, New Zealand and [4]The Institute of Environmental Science and Research Ltd. Wellington, New Zealand

Email: Rod A Lea - Rod.Lea@vuw.ac.nz; Micky Ovcaric - m.ovcaric@griffith.edu.au; James Sundholm - james_sundholm@hotmail.com; John MacMillan - John_MacMillan@health.qld.gov.au; Lyn R Griffiths* - l.griffiths@griffith.edu.au

* Corresponding author

Abstract

Background: The C677T variant in the methylenetetrahydrofolate reductase (MTHFR) gene is associated with increased levels of circulating homocysteine and is a mild risk factor for vascular disease. Migraine, with and without aura (MA and MO), is a prevalent and complex neurovascular disorder that may also be affected by genetically influenced hyperhomocysteinaemia. To determine whether the C677T variant in the MTHFR gene is associated with migraine susceptibility we utilised unrelated and family-based case-control study designs.

Methods: A total of 652 Caucasian migraine cases were investigated in this study. The MTHFR C677T variant was genotyped in 270 unrelated migraine cases and 270 controls as well as 382 affected subjects from 92 multiplex pedigrees.

Results: In the unrelated case-control sample we observed an over-representation of the 677T allele in migraine patients compared to controls, specifically for the MA subtype (40% vs. 33%) (χ^2 = 5.70, P = 0.017). The Armitage test for trend indicated a significant dosage effect of the risk allele (T) for MA (χ^2 = 5.72, P = 0.017). This linear trend was also present in the independent family-based sample (χ^2 = 4.25, $P_{adjusted}$ = 0.039). Overall, our results indicate that the T/T genotype confers a modest, yet significant, increase in risk for the MA subtype (odds ratio: 2.0 – 2.5). No increased risk for the MO subtype was observed (P > 0.05).

Conclusions: In Caucasians, the C677T variant in the MTHFR gene influences susceptibility to MA, but not MO. Investigation into the enzyme activity of MTHFR and the role of homocysteine in the pathophysiology of migraine is warranted.

Background

Migraine is a debilitating neurovascular disease that affects approximately 12% of the Caucasian population. It is characterised by nausea and vomiting, photophobia and phonophobia, neurological disturbances and severe recurrent headache. Pharmaceutical treatments for

migraine exhibit variable efficacy among patients and there is no laboratory-based diagnostic test available. At present, migraine is clinically diagnosed based on criteria specified by the International Headache Society (IHS). The IHS has defined two major classes of migraine. These are migraine without aura (MO), which accounts for ~70% of all migraine in the population, and migraine with aura (MA), which comprises ~25% of all migraine [1]. The two subtypes have substantial symptomatic overlap, but MA sufferers experience distinguishing neurological disturbances (the aura) that usually precede the headache phase of an attack [1].

Strong familial aggregation of migraine indicates a significant genetic component for the disease [2]. Heritability is estimated to be between 40% and 60%, indicating that the disease is partly explained by non-genetic determinants [3,4]. Thus, migraine is a multifactorial (complex) disease of which the genetic aetiology is likely to be comprised of a number of modest effect susceptibility genes that perhaps act in combination. It is likely that both migraine subtypes (MO and MA) have some genetic determinants in common although different modifying factors (including genetic and lifestyle triggers) may contribute to the variable expression of the clinical end-point.

The pathophysiology of migraine is not completely understood and continues to be rigorously investigated. For MA, a dramatic reduction in cerebral blood flow is associated with the depolarisation wave that propagates across the brain cortex (cortical spreading depression; CSD) [5]. The characteristic head pain that is common to both MA and MO may arise due to dilation of cerebral blood vessels following activation of the trigeminovascular system (TVS). The CSD can activate the TVS, providing a possible link between migraine aura and headache [6]. Therefore, biochemical factors that have the potential to disrupt vascular endothelial function and cerebral blood flow, leading to CSD and/or affecting the TVS, are important targets for involvement in migraine susceptibility [7].

Homocysteine, a highly reactive amino acid, has been shown to produce endothelial cell injury in both experimental animal and cell culture studies [8,9]. The pathophysiological consequences of such homocysteine-related endothelial injury may include impaired release of nitric oxide (NO) [10]. In turn, altered bioavailability of NO may cause abnormal reactions between the vessel wall, platelets, and macrophages [11] leading to significant alterations in vascular function and the coagulant properties of the blood [12,13]. Thus, it is within reason that homocysteine-related endothelial dysfunction may be involved in the initiation and maintenance of a migraine episode. In support of this, studies have also demonstrated that the firing rate of trigeminal neurons respond-

ing to pain increases with the application of D, L-homocysteic acid, a substance that mimics the effect of homocysteine, [14,15]. In humans, homocysteine is metabolised by methylenetetrahydofolate reductase (MTHFR). This enzyme catalyses the reduction of 5,10-methylenetetrahydofolate to 5-methylenetetrahydofolate, which is the circulatory form of folate and carbon donor for re-methylation of homocysteine to methionine [16].

The human MTHFR gene has been localised to chromosome 1p36 and consists of 11 exons spanning ~17 kb [17]. A common MTHFR gene variant (C677T) alters the amino acid sequence, substituting alanine (Ala) for valine (Val) at position 222. Individuals with the Val residue may exhibit significantly reduced MTHFR enzyme activity whereby, compared to baseline levels, the mean activity is 65% in the Ala/Val heterozygote and 30% in the Val/Val homozygous state [16]. The latter enzymatic form can lead to mild elevation in plasma homocysteine levels particularly if dietary folate intake is low [16,18]. Mild hyperhomocysteinaemia is known to increase risk of cardiovascular disease (CVD) including coronary, cerebral, and peripheral vascular disease [16,18,19]. More specifically, meta-analyses have concluded that the homozygous TT genotype of the MTHFR gene variant (C677T) is a modest, yet significant, genetic risk factor for ischaemic stroke, another severe neurovascular disease (odds ratio (OR): 1.5 – 2) [18,20]. Interestingly, the migraine disorder itself is also associated with an increased risk of ischaemic stroke, particularly with MA [21-25]. Indeed, the CSD, which is characteristic of MA, as well as changes in cerebral blood flow and headache, can also occur during a stroke episode [26]. Based on the comorbidity of migraine and stroke and the role homocysteine seems to play in disturbing in the cerebrocirculatory system, it is possible that the MTHFR C677T variant may represent an important genetic determinant for the vascular pathophysiologies underlying both neurovascular conditions. The objective of our study was to determine whether the MTHFR C677T variant is associated with migraine susceptibility in Caucasians utilising unrelated and family-based case-control designs.

Methods
Disease diagnosis
Our study protocol was approved by the Griffith University Ethics Committee for Experimentation on Humans and all subjects gave informed consent. Migraine patients were shown to exhibit phenotypic variation, which included differences in age of onset, frequency and severity of attacks, environmental triggers and medication response. However, all affected individuals were diagnosed as having either MA or MO by an experienced clinical neurologist using the IHS criteria (MA = criteria 1.2.1 and MO = criteria 1.1) [1]. The small number of patients

experiencing both subtypes of migraine was phenotyped as being affected with MA. To reduce confounding, any volunteer that reported being affected with known migraine comorbid conditions such as mental illness (e.g. depression) and CVD (e.g. stroke) were not included in the study.

Study design and samples

To test for association of the MTHFR C677T variant to migraine, we utilised a combination of traditional and modern case-control study designs. In general, the case-control approach remains a practical and powerful strategy for detecting genetic variants that confer a modest effect on disease susceptibility provided its shortcomings are addressed appropriately [27,28]. For this study we initially implemented the popular *unrelated* case-control approach to compare genotype and allele frequencies. This was followed by *family-based* case-control tests for association utilising a) a transmission disequilibrium-type test (TDT-type) in nuclear pedigrees [29] and b) a statistical method for comparing related cases to unrelated controls [30]. The difference between this latter design and the traditional unrelated case-control design is that, provided relatedness is accounted for, multiple cases from a single affected family(s) can be included in the analysis. This can result in substantial information gain over the use of family-based controls [30].

The unrelated case-control panel examined in this study is a *clinic-based* sample comprised of 270 unrelated migraine cases and a matched group of 270 unrelated non-migraine controls. Of this unrelated case group, 68% were female, 63% were phenotyped with MA, and 90% of patients had a known family history of migraine or at least one affected first degree relative. The unrelated control group was carefully matched with the case group for the variables; age (± 5 years), gender and ethnicity (Caucasian and of British descent). The control group was recruited from the same geographical location as the affected group (east coast of Australia). We also utilised an independent family-based sample comprised of 92 affected pedigrees. Of the 432 subjects in this pedigree sample, 290 (67%) were female, 382 (88%) were diagnosed with migraine (MA and/or MO) and 295 (68%) were affected with MA. Overall, our study included a total of 652 migraine cases, of which 465 (71%) suffered from MA.

Genotyping

Genomic DNA was isolated from whole blood by a standard salting out procedure. DNA fragments containing the C677T MTHFR variant were amplified by PCR using the oligonucleotides published by Kowa *et al.* (2000) [31]. The sense primer sequence was 5'-TGA AGG AGA AGG TGT CTG CGG GA-3', and the antisense sequence was 5'-AGG ACG GTG CGG TGA GAG TG-3'. PCR conditions were as follows; 1.75 mM of $MgCl_2$, 1 × standard PCR buffer, 0.2 mM of dNTPs, 0.2 µM each of forward and reverse primers, 0.16 g/l of BSA, 1 unit of *Taq* polymerase, 40 ng of genomic DNA, mixed to final volume of 25 µl with sterile distilled water. The thermal cycle parameters included: 1 cycle at 95°C for 3 min for an initial denaturation, followed by 35 cycles of denaturation for 1 min at 94°C, primer annealing for 1 min at 65°C, primer extension for 2 min at 72°C and a final extension for 10 min at 72°C [31]. This amplification reaction resulted in the synthesis of a 198-bp fragment.

The C to T substitution at nucleotide 677 in the MTHFR gene introduces a restriction site for the *Hinf*I enzyme. This enzyme was used to distinguish the alleles by cleaving the mutant fragment into 175-bp and 23-bp fragments. For the digestion reaction, 7 units of *Hinf*I, 2 µl of NE buffer II and 2.3 µl of sterile water were added to each PCR product mix for a final volume of 20 µl. Samples were digested overnight at 37°C and then fractionated by using a 5% ultra-high-resolution agarose gel, stained with ethidium bromide and visualised under ultraviolet light. To confirm results, the DNA samples were also genotyped using an ABI-310 Genetic Analyser, which is capable of distinguishing DNA fragments that differ by as little as 2 bp. For this technique, the reverse PCR primer was labelled with 5-carboxyfluorescein (FAM) fluorescent dye. Following initial PCR and restriction fragment length polymorphism digest, 1 µl of each digested PCR product was added to 12 µl of formaldehyde/size standard mix (Perkin-Elmer). This mixture was denatured for 2 minutes at 95°C before running on the ABI-310 Genetic Analyser.

An independent laboratory technician, blinded to the initial genotyping processing, performed quality control checks for the MTHFR gene variant by repeating the PCR and genotyping of a random selection of 100 subjects. All genotype discrepancies were rectified or excluded from subsequent data analysis.

Statistical data analysis
Unrelated case-control association analyses
Genotype and allele frequencies for the MTHFR C677T variant were calculated from observed genotype counts. As a statistical control for systematic genotyping error and population stratification, the expected genotype proportions according to the Hardy-Weinberg law were calculated and compared to observed genotypes. Genotype and allele frequencies were initially assessed for association with migraine using conventional contingency table analyses incorporating the standard chi-squared test for independence. This analysis produces a χ^2 statistic with one or two degrees of freedom and corresponding *P*-values for allele and genotype distributions, respectively. To assess the dosage effect of possessing zero, one or two copies of

the risk allele (T) (i.e. an additive effect), the Armitage test for linear trend in proportions was performed on the genotype frequency data. Genetic risk magnitudes (effect size) were estimated by calculating ORs with 95% confidence intervals.

Family-based association analyses
Our study of the MTHFR C677T variant also involved family-based association analyses of 92 migraine pedigrees. Mendelian inheritance of the MTHFR alleles in pedigrees was checked using the PEDMANAGER program. The Family Based Association Test (FBAT) was performed on the pedigree data using the software package of Laird *et al.* (2000) [29]. This is a transmission disequilibrium test (TDT) type approach for assessing association between DNA variant alleles and disease phenotype under the standard genetic models (dominant, recessive and additive). Unlike the classic TDT, which was designed for specific pedigree structures (ie. parent-child triads), the FBAT can appropriately utilise data from multiple nuclear families derived from a single multiplex pedigree to test for association [29].

We also utilised a powerful statistical test developed by Slager and Schaid (2001) [30], which involves comparing genotype frequencies of genetically related cases (sampled from multiplex affected families) with the unrelated control group frequencies. This method is based on the Armitage test for trend and includes a variance estimate that appropriately accounts for the non-independence of related cases. Thus, this method allows assessment of the dosage effect conferred by the risk allele whilst maintaining the correct type I error rate [30]. For this analysis we used the GENEHUNTER program to estimate the IBD-sharing probabilities [32] and the S-PLUS function of Slager and Schaid (2001) [30] to calculate the association test statistics for the pedigree data. Unaffected family members can be used as a comparative group and may possibly reduce false positives arising from population stratification. However, for our pedigree sample there was only 50 individuals *not* diagnosed with migraine and many of these individuals (n = 33) were labelled as phe-

notype "unknown". Therefore these familial controls were considered inappropriate for comparison.

It is unclear to what extent the MA and MO subtypes are influenced by the same genetic aetiologies. Therefore, we performed stratified analyses of the migraine subtypes (MO subtype and MA subtype), in addition to comparisons of all migraineurs grouped together (MO+MA). Analysis of the group of individuals with co-occurring MA and MO was not conducted due to small numbers. The conventional α-level of 0.05 was specified as the significance threshold for all analyses performed. We chose not to correct for multiple comparisons but opted instead to establish the credibility of a positive association by verification in an independent sample.

Results
Unrelated case-control analysis
Table 1 displays the frequency distributions observed for the MTHFR C677T variant for the unrelated case and control groups. Genotype and allele frequencies were determined for 268 migraine patients and 269 controls. The observed genotypes for the case and control groups did not deviate significantly from Hardy-Weinberg expectations ($P > 0.1$). The genotype frequencies in the migraine control group (43%, 48%, 9% for C/C, C/T and T/T, respectively) are very similar to an independent control group (n = 249) utilised for case-control studies of hypertension conducted in our laboratory (42%, 48%, 10% for C/C, C/T and T/T, respectively). Furthermore, the frequency of the T allele in our control group (33%) is also consistent with the proportion of this allele in the general Caucasian population (34%) [18,33], giving us confidence that the unrelated non-migraineurs used in this study constitute a reliable (unbiased) comparative panel.

Contingency table analysis of the MTHFR C677T frequency data indicated that the risk allele (T) was over-represented in the migraine group compared to the control group (38% vs. 33%), although the P value for this comparison did not reach statistical significance ($\chi^2 = 3.36$, $P = 0.066$). The proportion of the T allele in the MA sub-

Table 1: Distribution of MTHFR C677T (genotype and allele) frequencies in unrelated migraine and control groups.

| Group | Genotypes | | | | Alleles | |
	C/C	C/T	T/T	N (genotypes)	C	T
MO+MA	104 (39%)	125 (46%)	39 (15%)	268	333 (62%)	203 (38%)
MA*	64 (38%)	72 (43%)	32 (19%)	168	200 (60%)	136 (40%)
MO	40 (40%)	53 (53%)	7 (7%)	100	133 (66%)	67 (34%)
Control	117 (43%)	129 (48%)	23 (9%)	269	363 (67%)	175 (33%)

*genotype distribution comparison (Trend $\chi^2 = 5.72$, $P = 0.017$), T/T genotype vs. C/C (OR = 2.54, 95% CI: 1.37 – 4.71).

Table 2: Distribution of MTHFR C677T genotypes in family-based migraine case and unrelated control groups.

	Genotypes				Alleles	
Group	C/C	C/T	T/T	N (genotypes)	C	T
Related-MO+MA	112 (35%)	167 (52%)	40 (13%)	319	391 (61%)	247 (39%)
Related MA*	84 (34%)	132 (53%)	31 (13%)	247	300 (61%)	194 (39%)
Unrelated Control	117 (43%)	129 (48%)	23 (9%)	269	363 (67%)	175 (33%)

*genotype distribution comparison (Trend χ^2 = 4.25, *P* = 0.039 adjusted for relatedness in affecteds), T/T genotype vs C/C (OR = 1.88, 95% CI: 1.01 − 3.52).

group however, was shown to be significantly higher than in the control group (40% vs. 33%) (χ^2 = 5.70, *P* = 0.017). Further comparison of the genotype data indicated a difference in frequency distributions, with the homozygous T/T genotype being over-represented in the migraine group (15% vs. 9%). Stratified analyses indicated that this difference was specifically attributed to the MA subtype group, with the T/T frequencies being significantly higher in cases compared to controls (19% vs. 9%) (χ^2 = 10.37, *P* = 0.006). Interestingly, there was also a significant dosage (additive) effect of the risk allele for MA as assessed by the Armitage test for linearity (χ^2 = 5.72, *P* = 0.017). To determine the magnitude of the increased risk of migraine conferred specifically by the homozygous T/T genotype, odds ratios were calculated after dichotomising the genotype frequency data into risk "T/T" and baseline "C/C" genotype groups. Comparing the total migraine group against controls under this grouping scheme produced an OR of 1.91 with a 95% CI of 1.07 – 3.04 (*P* = 0.027), and the MA subtype comparison produced an OR of 2.54 (95% CI: 1.37 – 4.71, *P* = 0.0025). There was no association detected for the C677T variant under a dominant grouping scheme. Furthermore, no genotypic association was indicated for the MTHFR gene variant and the MO subtype (*P* > 0.1), nor was there any gender-specific associations observed for this variant (results not shown).

Family-based case-control analysis

In genetic association studies it is important to substantiate positive findings in independent samples. Therefore, we also genotyped the MTHFR C677T variant in an independent sample of migraine pedigrees. The frequency of the risk allele in the affected members of the pedigree sample (0.39) was similar to that observed in the unrelated case group (0.38) and greater than that of the unrelated control group (0.33). Genotypes for the family-based case groups (Table 2) conform to Hardy-Weinberg equilibrium expectations (*P* > 0.05).

Our initial association analysis of the pedigree data involved calculation of TDT-type statistics using the FBAT

program [29]. The program automatically decomposed the 92 multiplex pedigrees into 174 nuclear families for this analysis. Based on the results of the unrelated case-control analysis we assessed the family data under both *additive* and *recessive* inheritance models and considered "MO+MA" and "MA subtype" as disease phenotypes. Encouragingly, the results under both disease models suggested an association, indicating over-transmission of the 677T allele from parents to migraine-affected offspring. However, the TDT-type statistics produced were not significant for either migraine phenotype tested (*P* > 0.10). It is important to note that, due to the relatively low degree of heterozygosity for this MTHFR gene variant (∼0.48), less than 30 of all 174 nuclear families examined had heterozygous parents that could provide allele transmission information to affected offspring for this association analysis. That is, inheritance phase was indiscernible in many families and therefore the power to detect the expected association of the MTHFR C677T variant and migraine, as detected in the unrelated case-control analysis, was severely diminished. This FBAT result is not really surprising and exemplifies the substantial analytical inefficiencies that may occur when testing for association of a bi-allelic variant by allele transmission from parental controls [28,34].

To overcome this information loss we implemented a simple statistical method that was designed to compare genotype frequencies from family-based case groups (sampled from multiple affected families) to frequencies from unrelated control groups (as used in the unrelated case control analyses). This statistical method correctly adjusts for correlation of genotypes in the family data and has been successfully utilised to implicate the luteinizing hormone β gene in prostate cancer susceptibility [30]. Table 2 summarises the genotype data of the MTHFR C677T variant obtained from all migraineurs in the sample of 92 migraine pedigrees. As observed from the genotype frequency distributions in the unrelated case group (Table 1), the T/T genotype was over-represented in both case groups from the family-based sample (MA+MO and

MA subtype groups) compared to the unrelated control group (13% vs. 9%). Ignoring the inherent non-independence in the family-based case sample data, we observed differences between the frequency distributions of these groups that approached statistical significance (χ^2 = 5.36, P = 0.069; χ^2 = 5.71, P = 0.058, respectively). If weights are assumed to be equal to the number of high-risk alleles (i.e. there is a gene dosage effect) the unadjusted Armitage trend statistic was significant for both MO+MA and MA-subtype groups (χ^2 = 5.36, P = 0.021 and χ^2 = 5.67, P = 0.017). Importantly, this linear trend in proportions remained significant for the MA-subtype after adjusting for the relatedness in the case groups (χ^2 = 4.25, $P_{adjusted}$ = 0.039). Further risk assessment of the MA-subtype produced an OR of 1.88 (95% CI: 1.01 – 3.52) after accounting for familial relationships.

Discussion

The multifactorial nature of the common forms of migraine has so far prevented researchers from clearly identifying the predisposing genetic mechanisms. The investigation of candidate genes utilising case-control association designs is a powerful and direct strategy for detecting disease susceptibility genes of modest effect [27,30]. To date there have been numerous such studies reporting positive associations of candidate gene variants and migraine. Notably, polymorphisms within the dopamine 2 receptor (*DRD2*), dopamine beta-hydroxylase (*DBH*), and recently, insulin receptor (*INSR*) genes have been implicated in migraine susceptibility [35-37]. These genes are not only physiologically plausible candidates, but importantly, the specific associations have been verified in independent migraine samples. Despite this, the functional significance of these susceptibility gene variants and the role they play in migraine pathophysiology is yet to be determined.

In addition to disturbances in neurological pathways, migraine pathophysiology is partly explained by changes in vascular tension and altered cerebral blood flow [5,6]. Indeed, it is possible that vascular disturbances may act as a trigger for downstream neurological manifestations. Hence, molecular mechanisms that have the potential to affect vascular endothelial function should be targeted for involvement in migraine susceptibility. An elevation in circulatory homocysteine levels is thought to disrupt endothelial cells and alter the coagulant properties of the blood [13]. Interestingly, a recent meta-analysis indicated that mild hyperhomocysteinemia may by a "causal" risk factor for cerebro- and cardio-vascular disease and suggested that lowering homocysteine levels by simple dietary folate supplementation may reduce CVD risk [18]. Experiments on animal models suggest that hyperhomocysteinemia may also increase susceptibility to migraine by heightening cerebral artery sensitivity [15]. In addition,

elevated levels of homocysteine have been reported in patients affected with MA [38]. It has been shown that the 677T allele in the MTHFR gene is directly correlated with decreased enzymatic activity and the T/T genotype is indirectly associated with mild hyperhomocysteinaemia possibly leading to vascular disease [16,18].

The objective of our study was to determine the prevalence of the MTHFR allele (677T) in Caucasian migraineurs and to test for a relationship of this variant to migraine susceptibility using unrelated case-control and family-based association designs. Statistical analyses of the unrelated case-control data revealed that the C677T variant was associated with migraine. Stratification by migraine subtype indicated that this association was not arising in the MO case group but attributed specifically to MA. Analysis of both the unrelated and family-based case groups indicated a linear trend in the proportion of MA cases as the number of risk alleles increased, suggesting a gene dosage (additive) effect. This trend is consistent with observed correlations in MTHFR enzyme activity according to the number of copies of the Val residue corresponding to the 677T allele [16]. For both migraine case groups we also observed a significant over-representation of the T/T genotype in the migraineurs compared to controls, indicating the odds of being affected with MA (the effect size) to be approximately 2 – 2.5 times greater in those homozygous for the T allele compared with C/C homozygotes.

We are not the first researchers to have investigated the MTHFR C677T variant in relation to migraine susceptibility. A genetic association study by Kowa *et al.* (2000) [31], originally reported a positive association between the MTHFR C677T variant and migraine in a Japanese case-control cohort. These researchers indicated an increased risk of migraine (ie. MA+MO) in Japanese individuals possessing the homozygous T/T genotype. Stratified analyses specifically showed that the T/T genotype was significantly over-represented in these Japanese patients with MA compared to non-migraine controls (40% vs. 9.6%), producing an OR of ~6 [31]. These positive findings were reinforced by another recent migraine case-control study conducted in a Turkish population [40]. These researchers reported that the MTHFR C677T is associated with migraine and also indicated that the T/T genotype specifically increased risk of MA (OR ~10). It is important to note that the frequency of the MTHFR 677T allele, and indeed migraine prevalence, is known to vary substantially among different ethnic populations [33,39]. Thus, although our association study nicely supports the findings of both Kowa *et al.* (2000) [31] and Kara *et al.* (2003) [40], it cannot be considered a true replication of association. The differing ethnic backgrounds of the research subjects in the two previous studies may also partially explain

the much larger ORs reported compared to the more modest effect seen in the Caucasians of British descent that we investigated. Nevertheless, all three migraine association studies of the C677T variant show a similar overall trend, and interpreted together, provide compelling evidence that the homozygous T/T genotype of the MTHFR gene, perhaps leading to reduced MTHFR enzyme activity and mild hyperhomocysteinemia, increases susceptibility to migraine, particularly MA.

There is convincing epidemiological evidence indicating an association between migraine and stroke [23,24]. Although it is not conclusive, studies have indicated that this relationship may be specific to MA [26,41]. Interestingly, the CSD, which is characteristic of MA, as well as changes in cerebral blood flow and headache, can also occur during stroke [26]. It is possible that higher than normal levels of homocysteine may lead to temporary cerebral thrombosis and/or altered blood flow allowing less oxygen into the brain and manifesting the symptoms common to MA and ischaemic stroke [40]. Whilst our study was not designed to investigate the comorbidity of migraine and stroke, our findings lead us to suspect that the C677T variant in the MTHFR gene may represent a non-coincidental risk factor for both diseases. If this were true, certain genes identified as stroke risk factors, such as those with the potential to cause cerebral vascular damage, blood coagulation and flow alteration, should also be considered candidates for future studies into the molecular genetics of migraine.

Conclusion
Our study confirmed an association between the MTHFR C667T mutation and migraine susceptibility, specifically indicating the T/T genotype to be a modest risk factor for migraine with aura, but not migraine without aura, in Caucasians. Appropriately designed studies are now necessary to determine the impact of this MTHFR gene variant on homocysteine levels in migraineurs. Finally, dietary folate supplementation, as a modifier of homocysteine levels, should be investigated as a simple therapeutic option (a nutraceutical) for the long-term treatment of migraine.

Competing Interests
None declared.

Authors' contributions
All authors have contributed equally to this work.

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