Genetics and Headache: The Role of the MTHFR Gene in Migraine

S Stuart¹, H Cox¹, R Lea¹, C Bellis, M Carless², TD Dyer², J Charlesworth^{2,3}, E Matovinovic¹, S Macgregor⁴, J Curran², J Blangero² and LR Griffiths^{1,†}

†Corresponding author

¹ Genomics Research Centre, Griffith Health Institute, Griffith University, Gold Coast Campus, Building. ²Department of Genetics, Southwest Foundation for Biomedical Research, San Antonio, USA. ³Menzies Research Institute, University of Tasmania, Australia. ⁴Statistical Genetics Laboratory, Queensland Institute of Medical Research, Australia

G05, GRIFFITH UNIVERSITY QLD 4222

Phone: +61(07) 5552 8664

Fax: +61(07) 5552 9081

E mail: l.griffiths@griffith.edu.au

Key words: methylenetetrahydrofolate reductase gene, migraine with aura, Norfolk Island population

Abstract

Migraine is a common neurological disorder and is characterized by debilitating head pain and an assortment of additional symptoms which can include nausea, emesis, photophobia, phonophobia and occasionally visual sensory disturbances. A number of genes have been implicated in the pathogenesis of this disease, including genes involved in regulating the vascular system. Of particular importance is the methylenetetrahydrofolate reductase gene (MTHFR) and the role it plays in migraine with aura (MA). MA has previously been shown to have a significant co-morbidity with stroke making the vascular class of genes a priority for migraine studies. In this report we outline the importance of the MTHFR gene in migraine and also discuss the use of a genetic isolate to investigate associated genetic variants. From this study, three MTHFR SNPs showing association with migraine in the Norfolk Island population have been discovered, thus reinforcing the importance of the role MTHFR plays in migraine susceptibility. Further studies will continue to build a gene profile of variants involved in the complex disease migraine and improve understanding of the underlying genetic causes of this disorder.

Introduction

Migraine: A complex disease

Migraine is a debilitating neurovascular disease which is associated with pulsating head pain accompanied by nausea, vomiting, photophobia, phonophobia and sometimes visual sensory disturbances. It affects approximately 12% of the population with affected individuals being predominantly female [1]. Migraine results in a significant cost to the economy each year mostly because of lost productivity in the work place, but it also has a large personal impact on sufferers. According to classification criteria of the International Headache Society migraine is sub-divided into two major categories namely migraine with (MA) and migraine without (MO) aura. Migraine with aura (MA) is categorized by a preceding motor and/or visual disturbance lasting 20 to 30 minutes prior to the onset of a migraine attack [2]. It is hypothesized that cortical spreading depression originating in the occipital region of the brain is responsible for the symptoms experienced [3].

While twin and family studies have shown that both common and rare forms of migraine have a significantly heritable component, environmental interactions still play an important role [4-6]. As with other complex diseases, migraine is thought to be caused by an intricate interaction of multiple, small to medium effect genetic variants influenced by environmental factors. Rare forms of migraine such as familial hemiplegic migraine (FHM) have better understood genetic causes with well defined causal variants [7-9]. On the other hand underlying genetic mechanisms for common forms of migraine are still poorly understood. Both MA and MO exhibit genetic heterogeneity with large phenotypic variance.

To try and investigate the most plausible candidate genes involved in common migraine pathogenesis, criteria utilizing a combination of physiological functionality in conjunction with regions of genomic association are used. Thus far three different groups of genes have been identified and investigated on this basis. These are genes involved in neurological, vascular or hormonal pathways [10-12]. Under the broad category of neurological genes include those involved in expressing or control of ion channels (CACNA1A, KCCN3, K Na-ATPase), the synthesis and/or release and binding of neuropeptides (CGRP protein, glutamate) and the dopamine and serotonergic pathways. The second category of genes include those involved in blood pressure regulation, expression of endothelial cells, vasoconstriction and vasodilation (MTHFR, ACE) [10]. Many of the vascular type genes associated with migraine also exhibit an overlap with genes playing a role in elevated risk of stroke and heart disease. This is of particular relevance to migraine with aura which has been shown to have a significant co-morbidity with risk for stroke and depression. The third sets of genes seem to be most relevant to females which in some part explains the sex biased distribution of affected patients. Genes associated with females and menstrual migraine include those governing estrogen and progesterone and fall into the bracket of hormonal pathways (ESR1, PGR) [13].

Migraine with Aura

Approximately 20% of migraine attacks involve a preceding neurological disturbance known as an aura [1]. Numerous studies have linked cortical spreading depression with the visual scintillations typically experienced during aura and recently MRI has been used to confirm these findings in addition to describing underlying physiological mechanisms [3]. Cortical spreading depression(CSD) is a wave of neuronal and glial depolarization/ neuronal hyperexcitability

followed by a long lasting suppression of neural activity [14]. CSD is thought to activate the trigeminal nerves thus resulting in the pain associated with migraines. This electrophysiological event has been linked to aura in the human visual cortex and is thought to be partly responsible for the sensory and motor disturbances experienced during MA attacks. Furthermore a change in blood flow in the occipital lobe has been shown to correspond with the aura attack. This could be as a result of increased and decreased neuron firing and merely a side effect of energy metabolism or alternatively the altered blood flow may be a contributing factor towards triggering attacks. This in conjunction with the fact that MA patients also have an elevated risk for stroke has made genes involved in vascular pathways good candidates for genetic studies [15-17].

Given the role of neuronal hyperexcitability in CSD any genes which may alter the electrophysiological signaling of neurons are also good candidate genes to examine. This idea has led to an interest in ion channel genes involved in channelopathies. Familial hemiplegic migraine (FHM; OMIM 141500) is a rare hereditary subtype of migraine with aura caused by mutations in ion channel genes. Three main genes have been implicated in the pathogenesis of FHM namely CACNA1A (P/Q calcium channel), ATP1A2 (P type Na^+/K^+ ATPase) and SCN1A (Na^+ channel α subunit), with a possibility of a fourth locus at 14q32 [12]. Previous studies have shown that FHM mutations are not found in typical migraine with aura, suggesting that other ion channels may be involved. A recent and exciting study by Lanfreniere et al has highlighted the role that the TRESK potassium channel gene may play in common migraine with aura.

TRESK and Common Migraine with Aura

TRESK encodes for K2P channels which are expressed throughout the central nervous system, including the trigeminal ganglion neurons [18]. They play an important role in controlling resting membrane potential and neuronal excitability [19], and therefore an alteration in expression could feasibly lower the threshold for CSD. A large cohort of both case-control individuals as well as families were studied, which identified a number of variants in the TRESK gene. The most notable variant identified was a frame shift mutation (F139WfsX24) which segregated perfectly in a family affected with typical MA. All eight affected individuals carried the mutation while all eight unaffected individuals did not. The mutation was shown to suppress wild type channels and was thus classified as being a dominant-negative mutation inherited in a dominant fashion. Drug induced inhibition of TRESK activity has previously been associated with an increase in headache frequency and/or intensity [20]. This adds to the growing body of evidence that TRESK plays a role in MA susceptibility. Lanfreniere and colleagues have highlighted the need to investigate other variants in the TRESK gene which may be associated with MA and have also suggested the potential role of agonist mediated drugs as a therapeutic target [18].

Vascular Genes Involved in Migraine Susceptibility

MTHFR

The MTHFR gene maps to chromosomal location 1p36.3 and has been studied extensively to try and identify associations between variants and increased risk for disease [21]. Numerous associations have been made for a variety of diseases, many of which are neural and/or vascular and may therefore be involved in pathways overlapping with migraine pathogenesis. The methylenetetrahydrofolate reductase (MTHFR) gene encodes for the MTHFR enzyme which is

involved in the amino acid and purine biosynthesis pathway as illustrated in figure 1. The MTHFR enzyme catalyses the reduction of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate which is needed for the conversion of homocysteine to methionine [22, 23]. Folate is also needed to drive the pathway therefore a lack of dietary folate and/or reduced MTHFR enzymatic activity can result in an increase of homocysteine levels in the blood plasma. Hyperhomocysteinemia has been associated with a variety of metabolic disorders and increased risk for complex diseases including heart disease and stroke as well as migraine with aura [24-26].

The clinical consequences of elevated homocysteine plasma levels include endothelial cell injury, spontaneous trigeminal cell firing and alterations in coagulant properties of blood [27]. Spontaneous trigeminal cell firing leading to inflammation in the meninges and dialation of cerebral vessels is thought in part to cause the pain associated with migraine [28]. Thus homocysteine dysfunction can clearly increase patient propensity for developing migraine. Oxidative damage to the vascular endothelium via formation of superoxide anions (auto-oxidation of homocysteine) may also increase the likelihood of migraine and other vascular disorders such as stroke [29]. Physiological studies have demonstrated these relationships, making it clear that genetic variants altering enzyme activity or substrate pathways can increase the risk for developing migraine and other vascular diseases.

Variants within the MTHFR gene which result in decreased enzyme activity may therefore be associated with migraine and should be studied. Two variants in particular within this gene have been examined in previous studies. These are an A>C change occurring at position 1298 of the

MTHFR gene and a C>T change at position 677 [10]. The A1298C variant results in decreased MTHFR activity to a somewhat lesser degree than the C677T variant. It has been associated with neural tube defects and cardiovascular disease, but its role in migraine pathogenesis remains unexamined [30, 31].

The question of whether or not the C677T variant is associated with migraine generated some controversy. Six previous studies found a significant association between the MTHFR C667T variant and migraine with aura [32-37], while two conflicting studies found no association [38, 39]. Hinsanori Kowa et al. conducted a study in a Japanese patient cohort in 2000 to examine the association between the MTHFR C667T variant and migraineous headaches (the T/T genotype has been found to increase homocysteine levels in the blood). The study found a significant association between the T/T genotype and migraine sufferers. The homozygous transition was found in 20.3% of migraine sufferers compared to 9.6% in controls, and occurred in a remarkably high frequency in individuals suffering from MA (40.9%). Findings were concluded to be highly significant with an odds ratio of 6.5 [32].

A different study group examining Spanish patients suffering from migraine with aura also found a significant association with the T/T genotype. Patients were recorded as having elevated homocysteine blood plasma levels and were therefore also considered to be at risk of stroke and other vascular anomalies. While the odds ratio in this cohort was only 2.34, findings were still considered to be significant and authors concluded that the homozygous mutation is associated with MA in the population group they were studying [36]. Similarly Scher et al. and other studies found significant association between the T/T genotype and migraine with aura (odds ratios were

in the region of 2.05). A subsequent large meta-analysis which pooled data from all previous studies regarding the association between C677T found that migraine but only MA and not MO is associated with the C to T transition [40]. This significant study has provided compelling evidence that the MTHFR gene plays a critical role in MA pathogenesis.

Angiotension I Converting enzyme

Angiotension I-converting enzyme catalyses the conversion of angiotension I to angiotension II, which acts as a vasoconstrictor. ACE also plays a role in the inactivation of bradykinin, a strong vasodilator, thus having an overall powerful vasoconstrictory effect on blood vessels [41]. A modest risk factor for vascular disease involving the angiotension I-converting enzyme(ACE) I/D polymorphism has also been implicated as a risk factor for migraine [42]. It has been suggested that the D/D genotype could have a synergistic effect in individuals carrying the MTHFR T/T genotype and result in a much greater propensity towards developing migraine. As with the MTHFR gene, ACE seems to play an important role in the pathogenesis of migraine with aura, rather than in migraine without aura. Studies by Paterna et al first suggested a role for the ACE D/D genotype in migraine and later an investigation in a Japanese cohort showed a relationship between the D allele and MA [43-45]. A recent study in an Australian cohort confirmed an over-representation of the D/D genotype in patients experiencing MA and furthermore suggested that the MTHFR T/T genotype acts in combination with the D allele in increasing migraine susceptibility [46].

The Effects of Vitamin Supplementation and MTHFR

The results of a recent clinical trial verifies that the C677T MTHFR variant which results in reduced enzyme levels does in fact have a direct correlation with homocysteine levels in the blood and pathogenesis of migraine with aura. The results strengthen the hypothesis pertaining to the role of homocysteine in MA susceptibility by showing that vitamin supplementation leading to decreased homocysteine levels also brought about a decrease in the frequency and severity of migraines in MA sufferers [47]. Homocysteine can be alternatively metabolized through a B6 dependent pathway [48] and therefore supplementation with folic acid, B6 and B12 can decrease homocysteine levels. The pilot study by R Lea et al showed that a combination of folic acid, B6 and B12 supplementation reduced the homocysteine levels in all patient's blood samples and that this correlated with a reduction in the frequency and severity of MA attacks. Furthermore response to treatment was directly correlated to MTHFR C677T genotype, with T/T homozygotes showing the smallest response to supplementation. It was suggested that dose should be dependent on genotype and that T/T individuals should receive the highest doses [47]. This is what is referred to as personalized medication, a concept that has been growing in popularity.

New Variants in MTHFR Associated with Migraine Found in the Norfolk Island Population

To investigate variants associated with migraine, a recent pedigree based genome wide association study (pGWAS) was undertaken in the Norfolk Island population as outlined in Cox et al 2011 [49]. The Norfolk Island population is a genetically isolated population which has been very well characterized in terms of pedigree structure, risk for cardiovascular disease, as well as migraine [50-52]. A sub-sample of 285 related individuals (135 males; 150 females)

descended from the population founders were genotyped for 620,901 genome wide markers (mean spacing 4.7kb) in accordance to the manufacturer's instructions on Illumina Infinium High Density (HD) Human610-Quad DNA analysis BeadChip version 1. Of these related individuals include 76 migraine cases (22 males; 54 females). Association between SNPs and migraine was tested using measured genotype analysis [53] embedded in a variance components-based linkage model [54] and annotated using the Whole Genome Association Study Viewer (WAGViewer) program and NCBI Build 37.1. Results for this study have pinpointed three SNPs showing highly significant association with migraine in this unique population cohort. These results are summarized in Table 1 [55]. This finding strengthens the hypothesis that the MTHFR gene plays a pivotal role in migraine pathogenesis.

The first SNP identified, rs6696752 has no previous associations of clinical significance and hasn't been reported to show association with migraine in any previous publication [56]. The remaining two SNPs have never been reported to show association with migraine, but have been implicated in other diseases. The second SNP, rs4846048, has been associated with age of menopause onset in Caucasian females [57] as well as increased risk for colorectal cancer [58] and lean body mass [59]. The third SNP, rs2274976 has also been associated with some disorders including, early onset ischemic stroke [60] and a significant increased risk for nonsyndromic cleft lip and/or palate [61]. Of particular relevance is the overlap that rs2274976 shares with stroke risk as this disorder has been shown to exhibit co-morbidity with MA as discussed previously.

Conclusion

Due to the complex nature of migraine, it has been difficult for researchers in this area to elucidate a complete list of susceptibility variants or even all the genes involved in the pathogenesis of this disease. Matters have been further complicated by gene-gene interactions and gene-environment interactions which often mask the true underlying causes. In recent years much progress has been made in this field and new susceptibility loci are constantly identified. Many new promising genes are emerging such as TRESK [18], ESR1 and MTHFR [12] and warrant further detailed investigation. Further studies will continue to build a gene profile of the complex common migraine disorder and provide new drug targets.

Acknowledgements.

This research was supported by funding from the National Health and Medical Research Council (NHMRC) of Australia, from a Medical Bioinformatics Genomics Proteomics Program (MGBPP) grant. Hannah Cox was supported by a NHMRC Biomedical Postgraduate Scholarship. Rod Lea is partially supported by a Corbett Research Fellowship. The SOLAR statistical genetics computer package is supported by a grant from the US National Institute of Mental Health (MH059490). Lastly, our appreciation to the Norfolk Islanders who volunteered for this study.

Images and Graphics

Figure 1

Table 1

Rs Number	NCBI Build 37.1 Position	Region of Gene	Minor/Major Allele	MAF	P Value
rs6696752	11835935	Intronic	T/C	0.301	0.021
rs4846048	11846252	3 Prime UTR	G/A	0.309	0.030
rs2274976	11850927	Exonic (non-synonomous)	A/G	0.084	0.018

References

1. Lipton, R.B. and M.E. Bigal, *The epidemiology of migraine*. The American journal of medicine, 2005. **118 Suppl 1**: p. 3S-10S.
2. Olesen, J., *Preface to the Second Edition*. Cephalalgia, 2004. **24**(1 suppl): p. 9-10.
3. Hadjikhani, N., et al., *Mechanisms of migraine aura revealed by functional MRI in human visual cortex*. Proceedings of the National Academy of Sciences of the United States of America, 2001. **98**(8): p. 4687-92.
4. Gervil, M., et al., *Migraine without aura: A population-based twin study*. Annals of Neurology, 1999. **46**(4): p. 606-611.
5. Mulder, E.J., et al., *Genetic and environmental influences on migraine: a twin study across six countries*. Twin research : the official journal of the International Society for Twin Studies, 2003. **6**(5): p. 422-31.
6. Stewart, W.F., et al., *Familial risk of migraine: variation by proband age at onset and headache severity*. Neurology, 2006. **66**(3): p. 344-8.
7. Ophoff, R.A., et al., *Genetic heterogeneity of familial hemiplegic migraine*. Genomics, 1994. **22**(1): p. 21-6.
8. Ophoff, R.A., et al., *Familial hemiplegic migraine and episodic ataxia type-2 are caused by mutations in the Ca²⁺ channel gene CACNL1A4*. Cell, 1996. **87**(3): p. 543-52.
9. Barrett, C.F., et al., *Familial hemiplegic migraine*. Advances in genetics, 2008. **63**: p. 57-83.
10. Colson, N.J., et al., *The role of vascular and hormonal genes in migraine susceptibility*. Molecular genetics and metabolism, 2006. **88**(2): p. 107-13.
11. Fernandez, F., N.J. Colson, and L.R. Griffiths, *Pharmacogenetics of migraine: genetic variants and their potential role in migraine therapy*. Pharmacogenomics, 2007. **8**(6): p. 609(14).
12. Maher, B.H. and L.R. Griffiths, *Identification of molecular genetic factors that influence migraine*. Molecular genetics and genomics : MGG, 2011. **285**(6): p. 433-46.
13. Colson, N., F. Fernandez, and L. Griffiths, *Genetics of menstrual migraine: the molecular evidence*. Current pain and headache reports, 2010. **14**(5): p. 389-95.
14. de Almeida, R.F., et al., *Migraine with persistent visual aura: response to furosemide*. Clinics, 2009. **64**(4): p. 375-6.
15. Olesen, J., et al., *Timing and topography of cerebral blood flow, aura, and headache during migraine attacks*. Annals of Neurology, 1990. **28**(6): p. 791-8.
16. Geraud, G., et al., *[Positron emission tomographic studies of migraine]*. Revue neurologique, 2005. **161**(6-7): p. 666-70.
17. Etminan, M., et al., *Risk of ischaemic stroke in people with migraine: systematic review and meta-analysis of observational studies*. BMJ, 2005. **330**(7482): p. 63.
18. Lafreniere, R.G., et al., *A dominant-negative mutation in the TRESK potassium channel is linked to familial migraine with aura*. Nature medicine, 2010. **16**(10): p. 1157-60.
19. Enyedi, P. and G. Czirjak, *Molecular background of leak K⁺ currents: two-pore domain potassium channels*. Physiological reviews, 2010. **90**(2): p. 559-605.
20. Ferrari, U., et al., *Calcineurin inhibitor-induced headache: clinical characteristics and possible mechanisms*. Headache, 2005. **45**(3): p. 211-4.

21. Goyette, P., et al., *Human methylenetetrahydrofolate reductase: isolation of cDNA mapping and mutation identification*. Nature genetics, 1994. **7**(4): p. 551.
22. Heux, S., et al., *The methylenetetrahydrofolate reductase gene variant (C677T) as a risk factor for essential hypertension in Caucasians*. Hypertension research : official journal of the Japanese Society of Hypertension, 2004. **27**(9): p. 663-7.
23. Frosst, P., et al., *A candidate genetic risk factor for vascular disease: a common mutation in methylenetetrahydrofolate reductase*. Nature genetics, 1995. **10**(1): p. 111-113.
24. Graham, I.M. and P. O'Callaghan, *Vitamins, homocysteine and cardiovascular risk*. Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy, 2002. **16**(5): p. 383-9.
25. Taylor, L.M., Jr., et al., *The association of elevated plasma homocyst(e)ine with progression of symptomatic peripheral arterial disease*. Journal of vascular surgery : official publication, the Society for Vascular Surgery [and] International Society for Cardiovascular Surgery, North American Chapter, 1991. **13**(1): p. 128-36.
26. Mizrahi, E.H., et al., *Further evidence of interrelation between homocysteine and hypertension in stroke patients: a cross-sectional study*. The Israel Medical Association journal : IMAJ, 2003. **5**(11): p. 791-4.
27. Hering-Hanit, R., et al., *Evidence for activation of the coagulation system in migraine with aura*. Cephalalgia : an international journal of headache, 2001. **21**(2): p. 137-9.
28. Parsons, A.A. and P.J. Strijbos, *The neuronal versus vascular hypothesis of migraine and cortical spreading depression*. Current opinion in pharmacology, 2003. **3**(1): p. 73-7.
29. Das, U.N., *Folic acid says NO to vascular diseases*. Nutrition, 2003. **19**(7-8): p. 686-92.
30. van der Put, N.M., et al., *A second common mutation in the methylenetetrahydrofolate reductase gene: an additional risk factor for neural-tube defects?* American journal of human genetics, 1998. **62**(5): p. 1044-51.
31. Weisberg, I.S., et al., *The 1298A-->C polymorphism in methylenetetrahydrofolate reductase (MTHFR): in vitro expression and association with homocysteine*. Atherosclerosis, 2001. **156**(2): p. 409-15.
32. Kowa, H., et al., *The homozygous C677T mutation in the methylenetetrahydrofolate reductase gene is a genetic risk factor for migraine*. American journal of medical genetics, 2000. **96**(6): p. 762-4.
33. Kara, I., et al., *Association of the C677T and A1298C polymorphisms in the 5,10 methylenetetrahydrofolate reductase gene in patients with migraine risk*. Brain research. Molecular brain research, 2003. **111**(1-2): p. 84-90.
34. Lea, R.A., et al., *The methylenetetrahydrofolate reductase gene variant C677T influences susceptibility to migraine with aura*. BMC medicine, 2004. **2**: p. 3.
35. Scher, A.I., et al., *Migraine and MTHFR C677T genotype in a population-based sample*. Annals of Neurology, 2006. **59**(2): p. 372-5.
36. Oterino, A., et al., *MTHFR T677 homozygosis influences the presence of aura in migraineurs*. Cephalalgia : an international journal of headache, 2004. **24**(6): p. 491-4.
37. Oterino, A., et al., *Thymidylate synthase promoter tandem repeat and MTHFD1 R653Q polymorphisms modulate the risk for migraine conferred by the MTHFR T677 allele*. Brain research. Molecular brain research, 2005. **139**(1): p. 163-8.
38. Todt, U., et al., *MTHFR C677T polymorphism and migraine with aura*. Annals of Neurology, 2006. **60**(5): p. 621-2; author reply 622-3.

39. Kaunisto, M.A., 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 : an international journal of headache, 2006. **26**(12): p. 1462-72.
40. Rubino, E., et al., *Association of the C677T polymorphism in the MTHFR gene with migraine: a meta-analysis*. Cephalalgia : an international journal of headache, 2009. **29**(8): p. 818-25.
41. Sharma, P., *Meta-analysis of the ACE gene in ischaemic stroke*. Journal of neurology, neurosurgery, and psychiatry, 1998. **64**(2): p. 227-30.
42. Rigat, B., et al., *An insertion/deletion polymorphism in the angiotensin I-converting enzyme gene accounting for half the variance of serum enzyme levels*. The Journal of clinical investigation, 1990. **86**(4): p. 1343-6.
43. Paterna, S., et al., *Migraine without aura and ACE-gene deletion polymorphism: is there a correlation? Preliminary findings*. Cardiovascular drugs and therapy / sponsored by the International Society of Cardiovascular Pharmacotherapy, 1997. **11**(4): p. 603-4.
44. Paterna, S., et al., *Angiotensin-converting enzyme gene deletion polymorphism determines an increase in frequency of migraine attacks in patients suffering from migraine without aura*. European neurology, 2000. **43**(3): p. 133-6.
45. Kowa, H., et al., *Association of the insertion/deletion polymorphism of the angiotensin I-converting enzyme gene in patients of migraine with aura*. Neuroscience Letters, 2005. **374**(2): p. 129-31.
46. Lea, R.A., et al., *Genetic variants of angiotensin converting enzyme and methylenetetrahydrofolate reductase may act in combination to increase migraine susceptibility*. Brain research. Molecular brain research, 2005. **136**(1-2): p. 112-7.
47. Lea, R., et al., *The effects of vitamin supplementation and MTHFR (C677T) genotype on homocysteine-lowering and migraine disability*. Pharmacogenetics and genomics, 2009. **19**(6): p. 422-8.
48. Wang, X., et al., *Efficacy of folic acid supplementation in stroke prevention: a meta-analysis*. Lancet, 2007. **369**(9576): p. 1876-82.
49. Cox H, L.R., Bellis C, Carless M, Dyer T, Blangero J, Griffiths L, *Variants in the human potassium channel gene (KCNN3) are associated with migraine in a high risk genetic isolate*. Journal of Headache and Pain, 2011.
50. McEvoy, B.P., et al., *European and Polynesian admixture in the Norfolk Island population*. Heredity, 2010. **105**(2): p. 229-34.
51. Bellis, C., et al., *Linkage mapping of CVD risk traits in the isolated Norfolk Island population*. Human Genetics, 2008. **124**(5): p. 543-52.
52. Bellis, C., et al., *Linkage disequilibrium analysis in the genetically isolated Norfolk Island population*. Heredity, 2008. **100**(4): p. 366-73.
53. Boerwinkle, E., R. Chakraborty, and C.F. Sing, *The Use of Measured Genotype Information in the Analysis of Quantitative Phenotypes in Man .1. Models and Analytical Methods*. Annals of Human Genetics, 1986. **50**: p. 181-194.
54. Blangero, J., et al., *Quantitative trait nucleotide analysis using Bayesian model selection*. Human Biology, 2005. **77**(5): p. 541-559.
55. H., C., *Chapter 7: A systematic analysis of putative migraine susceptibility genes in the norfolk 'mutany on the bounty' pedigree implicates receptor gene (ESR1)*, in *Genomics Research Centre 2011*, Griffith University: Brisbane.

56. NCBI. 2011 20/06/2011]; Available from: <http://www.ncbi.nlm.nih.gov>.
57. Liu, P., et al., *Association analyses suggest multiple interaction effects of the methylenetetrahydrofolate reductase polymorphisms on timing of menarche and natural menopause in white women*. Menopause, 2010. **17**(1): p. 185-90.
58. Levine, A.J., et al., *Genetic variability in the MTHFR gene and colorectal cancer risk using the colorectal cancer family registry*. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology, 2010. **19**(1): p. 89-100.
59. Liu, X., et al., *The MTHFR gene polymorphism is associated with lean body mass but not fat body mass*. Human genetics, 2008. **123**(2): p. 189-96.
60. Giusti, B., et al., *Early-onset ischaemic stroke: analysis of 58 polymorphisms in 17 genes involved in methionine metabolism*. Thrombosis and haemostasis, 2010. **104**(2): p. 231-42.
61. Bufalino, A., et al., *Maternal polymorphisms in folic acid metabolic genes are associated with nonsyndromic cleft lip and/or palate in the Brazilian population*. Birth defects research. Part A, Clinical and molecular teratology, 2010. **88**(11): p. 980-6.