MTHFR genetic testing: Controversy and clinical implications

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Background

A polymorphism is a variant within a gene that does not necessarily affect its function, unlike a pathogenic mutation. Genetic testing for two common polymorphisms in the methylenetetrahydrofolate reductase gene (*MTHFR*), 677C>T and 1298A>C, is being accessed by general practitioners (GPs) and alternative medicine practitioners (based on in-house records from referrals), and promoted through some pharmacies in Western Australia (based on the authors' personal communication). Due to the large, varied and often conflicting data reported on *MTHFR*, these polymorphisms have been weakly associated with multiple conditions, including autism, schizophrenia, cardiac disease, fetal neural tube defects, poor pregnancy outcomes and colorectal cancer.

Objectives

The aim of this review is to explain the difficulty in translating inconclusive results – and results of uncertain clinical relevance – of genetic-association studies on common polymorphisms into clinical practice. We will also explore why testing for polymorphisms needs to be reconsidered in a diagnostic clinical setting.

Discussion

On the basis of the available scientific evidence, we propose that there are very limited clinical indications for testing for the 677C>T and the 1298A>C polymorphisms in the *MTHFR* gene, and that testing is not indicated as a non-specific screening test in the asymptomatic general population.

The *MTHFR* gene is responsible for the production of the enzyme methylenetetrahydrofolate reductase (MTHFR).[1] Numerous studies have reported associations of *MTHFR* polymorphisms with an array of conditions including autism, schizophrenia, cardiac disease, fetal neural tube defects, poor pregnancy outcomes (eg preterm birth) and colorectal cancer.[1–4] Over the past 24 months, Genetic Services of Western Australia has seen an increase in referrals for *MTHFR* polymorphism testing and counselling. Referrals have been received from general practitioners (GPs), obstetricians and alternative health practitioners. In addition, there have been cases of clients self-referring after a family member has had testing, or after a pregnancy loss or pregnancy with a neural tube defect (based on in-house records). Providers ordering testing for *MTHFR* polymorphisms are generally using the Medicare Benefits Schedule (MBS) item number 73308.[5,6] However, this MBS item number is not specifically for *MTHFR* testing. It has the descriptor 'Characterisation of the genotype of a patient for Factor V Leiden gene mutation, or detection of the other relevant mutations in the investigation of proven venous thrombosis or pulmonary embolism'.[7]

Scientific evidence and review

Folate (the salt of folic acid) is a vitamin the body needs for day-to-day functioning. It is required at higher doses during pregnancy, when it has an important role in preventing the formation of neural tube defects.[8] Folate deficiency can cause glossitis, diarrhoea, gastrointestinal lesions, anaemia and poor growth.[9,10]

The enzyme MTHFR is involved in the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, which is the primary form of circulating folate in the blood.[11] 5-methyltetrahydrofolate is involved in the remethylation of homocysteine to methionine, which is then converted to S-adenosylmethionine. This S-adenosylmethionine is then able to serve as a methyl donor in many varied methyl donor reactions throughout the cellular system.[10,11]

There are two predominant *MTHFR* polymorphisms, 677C>T and 1298A>C. In the general population, 60–70% of individuals

will have at least one of these variants, 8.5% will be homozygous for 677C>T or 1298A>C, and 2.25% will be compound heterozygous. Overall, 10% of the population will be homozygous or compound heterozygous for these two polymorphisms.[4,12]

The *MTHFR* polymorphisms in the homozygous or compound heterozygous form only reduce enzyme production mildly and are thus of limited pathogenicity.[12] The 677C>T polymorphism in the homozygous form may result in mild homocystinuria due to decreased *MTHFR* activity.[13] The 1298A>C polymorphism does not cause homocystinuria in a heterozygous or homozygous form, but may affect enzyme activity when inherited with the 677C>T polymorphism.[13]

Severe MTHFR deficiency (<20% of the enzyme) results in the clinical picture of homocystinuria. This is a severe, autosomal recessive genetic condition that can present with early, significant neurological defects, or with gait abnormalities and psychiatric disorders later in life.[12] This condition is not caused by the 1298A>C or the 677C>T *MTHFR* polymorphisms.

Neural tube defects

The important role of folate in the prevention of neural tube defects is well known.[14] Intuitively, therefore, polymorphisms that interfere with folate metabolism could potentially result in an increased risk of neural tube defects. However, in practice, studies both supporting and refuting this hypothesis exist.[1,15]

Given the prevalence of the *MTHFR* polymorphisms (60–70% of the population) and the frequency of neural tube defects (approximately 1 in 1000 in Australia),[14] it is unlikely that the polymorphisms alone have a significant role in the formation of neural tube defects. While there may be some increased risk of neural tube defects in individuals with the homozygous 677C>T variant,[11] the level of risk has not been quantified and the impact of environmental factors, such as folate supplementation, has a more significant role. This has been demonstrated through a significant drop in neural tube defects when population-level fortification of folate occurs,[14,16] with a decrease in the incidence of neural tube defects by about 70%.[8]

Practically, it would seem to be more useful to measure maternal serum folate levels and supplement as appropriate, rather than test for the presence or absence of the polymorphisms. Alternatively, measuring levels of homocysteine rather than *MTHFR* polymorphisms may give useful information regarding the risk of neural tube defects.[17] Regardless of the mother's *MTHFR* polymorphism status, 400 µg of folate daily for women of reproductive age is recommended.[16] Thus, knowing a couple's *MTHFR* polymorphism status has no effect on their pregnancy-related management in terms of the prevention of primary or recurrent neural tube defects.

Fertility and poor pregnancy outcomes (other than neural tube defects)

Hyperhomocysteinaemia in pregnancy has been associated with poor pregnancy outcomes,[18,19] yet studies have produced conflicting results.[20] In 2012, Bergen et al reported that high homocysteine levels increase the risk of prematurity, small size for gestational age and preeclampsia, although the *P* value was not significant for any of these associations (except for folate in the lowest quintile being associated with prematurity).[21] However, even if homocysteine was conclusively linked with poor pregnancy outcomes, the *MTHFR* polymorphisms have been shown to result in only mildly elevated hyperhomocysteinaemia.[13] Therefore, on the basis of the available evidence, genetic testing for either of the two *MTHFR* polymorphisms would not appear to provide any useful information as a substitute for, or in addition to, measuring serum homocysteine levels in this clinical situation.

MTHFR polymorphisms have been associated with a higher risk of preterm labour,[22] but the findings have also been contradicted in different populations.[23,24] Therefore, on the basis of association studies, *MTHFR* polymorphisms are not useful in predicting pregnancy outcomes[25–28] or for the management of pregnancies. While sufficient folate is an important factor in preventing neural tube defects, increased folate levels in the presence of *MTHFR* polymorphisms do not seem to be associated with helping women undergoing in vitro fertilisation (IVF) treatment to achieve pregnancy.[29] Therefore, testing for the polymorphisms may result in increased anxiety during pregnancy without any clinical benefit.

Thrombophilia

While homozygosity for the 677T>C *MTHFR* polymorphism is linked to an increase in homocysteine level, it is not clearly linked to an increase in thrombophilic events.[26,30] *MTHFR* polymorphisms do not increase the risk of thromboembolic disease when found in a heterozygous state.[30] Supplementation with folic acid and vitamin B12 does not significantly decrease the risk of thrombotic events.[30]

Cardiovascular disease

Increased levels of homocysteine have been associated with cardiovascular disease.[10,31] The link was first hypothesised when children with severe homocystinuria were found to have vascular lesions.[32] However, a meta-analysis of studies has found that the association was not as strong as previously believed.[32] As stated above, while homozygosity for the 677C>T polymorphism is linked to a small increase in homocysteine levels, the increased risk of ischaemic heart disease and stroke is more closely related to the serum levels of homocysteine rather than the presence of the *MTHFR* polymorphisms. Importantly, there seems to be no increased risk of mortality from cardiovascular disease to *MTHFR* 677C>T homozygotes.[11]

Mental illness

Recent media attention regarding the tenuous link between *MTHFR* polymorphisms and depression and anxiety has garnered much interest from individuals living with these complex conditions. For example, a recent online newspaper article purports to provide an 'easy' fix for affected individuals, advising them that vitamin supplementation will 'cure' them of symptoms.[33]

Although some studies found evidence that *MTHFR* polymorphisms were associated with an increased risk of bipolar and schizophrenic disorders,[2] other studies have found no convincing link.[34–36] In one study, an increased rate of 677C>T homozygotes was found in a population with major depressive disorder. However, no differences in vitamin B6, vitamin B12 or homocysteine levels were found in 677C>T patients with a major depressive disorder, compared with those who were unaffected. The same study found folate levels in control subjects were higher than in participants who were depressed, but this difference was not found with statistical correction for demographics.[35] There is no convincing evidence linking mental illness and *MTHFR* polymorphisms.

Marketing to consumers

The ethics of nutritional companies and alternative practitioners offering consumers genetic testing has been debated for some time, with concerns about the clinical validity of the results. The issues involve the role of single nucleotide polymorphisms in individual genes in influencing complex nutritional conditions and, most importantly, how consumers will use the information derived from this testing.[37] The position of the Academy of Nutrition and Dietetics in the US is that:

> *There is insufficient evidence regarding C677T polymorphism in the MTHFR gene to modify current folate recommendations from those provided in the Dietary Reference Intakes.*[38]

Two examples of websites offering *MTHFR* 'information' are *MTHFR* Support Australia[39] and *MTHFR*.net from the US.[40]

Recommendations for clinical practice

Folate plays a vital role in cellular health, and the *MTHFR* gene has an important role in the folate pathway. However, we propose that there is no statistically significant evidence that the 677C>T and the 1298A>C polymorphisms have a clinically important impact on this pathway. The American College of Medical Genetics and Genomics has issued a detailed guideline that discourages testing for the two common polymorphisms in the *MTHFR* gene.[11] The Academy of Nutrition and Dietetics states no dietary interventions are needed, even in individuals with homozygous 677C>T mutations who have elevated levels of homocysteine. As the 677C>T and 1298A>C polymorphisms occur at high rates in the general population, and there are no clinically significant interventions that could be offered to carriers of the variants in heterozygous, homozygous or compound heterozygous states, it is not useful to offer genetic testing for these variants.

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