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Effects that the methylenetetrahydrofolate gene mutation (both the C677T and A1298C polymorphisms) have on both men and women's fertility abilities and subsequent fetal development, as well as what nutritional changes can possibly do to aid in reversing these supposed negative effects.

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Abstract

The variants (C677T and A1298C) in the methylenetetrahydrofolate reductase (MTHFR) gene on have perceived negative effect on male and female fertility and fetal development, and nutrition changes may aid in reversing these negative effects. This research project aimed to identify the possible association with and connection between nutrition and fertility in both male and female individuals who have either of the two most common MTHFR gene polymorphisms, 677C>T and 1298A>C. These two polymorphisms are of particular interest because they are associated with the most decreased activity of the MTHFR enzyme. Although it is still unclear the impact that an MTHFR genetic polymorphism may or may not have on an individual's everyday life, and its contributions to current or preexisting health conditions remain unclear it is relatively simple to see that a majority of the clinical tests and research pertaining to the MTHFR gene and the issues it potentially causes reveal disparate results.

Methylenetetrahydrofolate gene polymorphisms are becoming increasingly common in the general population, so it is difficult to determine correlation verses causation when considering its exact influence on human health. Nutrients that can act as methyl donors related to these genes, including folate (vitamin B9) and vitamin B12, play integral roles in the phenotypic expression of related gene mutations in methylation pathways (Shiao, P et al., 2018). A healthy diet is generally classified as a high intake of fruits and vegetables, wholegrains, nuts and legumes, fish and other seafood, and milk and other dairy products, and decreases the risk of manifestations of the MTHFR genetic mutation (Shiao, P et al., 2018). Additionally, healthy eating involves limiting salt, saturated fat, and empty calories from sugar and alcohol as additional dietary parameters (Shiao, P et al., 2018).

Key Words

A1298C, C677T, fertility, fetal development, folate, folic acid, homocysteine, methionine methylenetetrahydrofolate, methylated folate, neural tube defect, nutrition, polymorphisms

Introduction

The Methylenetetrahydrofolate reductase (MTHFR) is a key enzyme in folate and homocysteine metabolism. The MTHFR enzyme catalyzes the reduction of 5, 10-methylenetetrahydrofolate to 5-methyltetrahydrofolate, and this therefore provides the methyl group for the re-methylation of homocysteine to methionine (Li, et al., 2005). This reaction is catalyzed by methionine synthase and uses vitamin B12 as a cofactor. Methionine is the precursor of S-adenosylmethionine (SAM), the methyl group donor necessary for DNA synthesis, cell division, tissue growth, among other reactions. In addition, methionine is essential for DNA methylation as it plays an important role during critical periods of growth and development (Torres-Sanchez, Lopez-Carillo, Blaco-Munoz & Chen, 2014).

5-methylenetetrahydrofolate is the primary form of folate found in blood, and the multistep process that converts the amino acid homocysteine to another amino acid, methionine is essential. Reduced MTHFR activity results in an increased requirement for folic acid to maintain normal homocysteine remethylation to methionine. In the absence of sufficient folic acid, intracellular homocysteine accumulates, methionine resynthesis is reduced, and remethylation reactions are interrupted. Folate is also essential for various cellular processes such as synthesis of DNA, RNA, methylation, and embryonic developmental processes including the cardiovascular system (Kedar, Chandel, 2019). In an individual without this mutation, folic acid and other B vitamins break down homocysteine and alter it into other substances the human body needs. This should lead to very reduced amounts of homocysteine left in the bloodstream.

The MTHFR gene has the seemingly common reputation of being considered a "blame gene" when many individuals who test positive for either variant tend to consider any or all of their medical issues, complications, conditions, and maladies to be in direct correlation with the gene mutation. While there may be a level of causal correlation, lifestyle, nutrition, and many other important factors additionally serve a larger role in one's overall health than a mutation on the MTHFR gene does. According to Klug (2009), “Mutations have a wide range of effects on organisms depending on the type and location of the nucleotide change within the gene and the genome,” and the MTHFR gene mutation variants are no exception to this statement. The MTHFR mutation is a mutation in a metabolic enzyme. According to Dr. Alan Snow, a biology coordinator, scientific researcher, and Professor of Instruction at the University of Akron, Wayne College, “The gene seems to be gaining more awareness. With any genetically linked disease, environmental influences will be a focus of interest to those groups doing the research.” The purpose of this study was to examine the perceived negative effects that the MTHFR gene mutation, both the C677T and A1298C polymorphisms, have on both men and women’s fertility abilities and subsequent fetal development, as well as what nutritional changes can possibly do to aid in reversing these supposed negative effects.

The MTHFR Gene: Definition, Mutation

A gene mutation is defined as an alteration in DNA sequence. Any base-pair change in any part of a DNA molecule is considered a mutation. A mutation may comprise a single, base-pair substitution, a deletion, or insertion of one or more base pairs, or a major alteration in the structure of a chromosome (Klug, 2009). Mutations may or may not bring about a detectable change in phenotype and genotype of an organism. These mutations are termed silent mutations (Kumar, Dorbrovolsky, Dhawan et al., 2018). The extent to which a mutation changes the

characteristics of an organism depends on where the mutation occurs and the degree to which the mutation alters the function of the gene product (Klug, 2009). Severe MTHFR deficiency results most frequently from missense mutations; however, nonsense, splice site and deletion mutations have also been described. Twenty-four different mutations have been described, and these occur at different locations from those in mild MTHFR deficiency. Severe deficiency is defined as <20% residual enzymatic activity in cultured fibroblasts or as a plasma homocysteine level more than 10-fold greater than normal (Nguyen & Kant, n.d.) A missense mutation reflects a change in a base pair that could alter the coding sequence and results in coding for a different amino acid, thus altering the function of a given protein that may alter the physiology of the cell entirely (Kumar, Dorbrovolsky, Dhawan et al., 2018). In mild MTHFR deficiency, the most commonly associated variant is the CT sequence change at nucleotide 677, called MTHFR C677T (standard nomenclature c.665C>T). This change results in a missense change, an alanine to valine substitution, at codon 222. Second common MTHFR variant is the A>C transition at nucleotide position 1298, also known as A1298C (standard nomenclature c.1286). This change results in a missense glutamine to alanine substitution at codon 429. (Nguyen & Kant, n.d.). It is possible to have one variant and be heterozygous or have two mutations and be homozygous.

Around 10% of the population is homozygous for the MTHFR gene (Voet, Voet, & Pratt, 2019). Prevalence of the mutation varies widely according to ethnic origin but appears to be approximately 33-40% heterozygotes and 10% homozygotes in the North European Caucasian populations (Holmes, Chilcott, Cohen & Cohen, 1999). Yet with these statistics in mind, it is supposed by many medical professionals that more individuals than previously thought have an MTHFR gene mutation. However, many people do not have a reason to be tested for it, and therefore may not even have an observable phenotype.

Etiology

Etiology can be easily defined as the study of a root cause. Past research and studies considered whether the MTHFR gene mutation was caused by maternal stress during pregnancy, or simply a consequence of heredity, whether it be a dominant or recessive gene mutation. A MTHFR genetic mutation is simply an error in the gene that causes it to malfunction. While we still do not have a significant amount of conclusive research, geneticist are well aware of the genetic combinations which can lead to either a homozygous or heterozygous mutation of the MTHFR gene, involving either the C677T variant or the A1298C variant (*This is illustrated in Appendix A, photo 1).*

Pathophysiology

Pathophysiology seeks to study physiological processes on the cellular level. Around 10% of the population is homozygous for a mutation in the MTHFR. The mutation does not affect the enzyme's reaction kinetics but instead increases the rate at which its essential flavin cofactor dissociates. Folate derivatives that bind to the enzyme decrease the rate of flavin loss, thus increasing the enzyme's overall activity and decreasing the homocysteine concentration. The prevalence of the MTHFR mutation in the human population suggests that it has (or once had) some selective advantage, nevertheless, this is yet a matter of speculation. (Voet, Voet, & Pratt, 2019). Several mutations and polymorphisms in MTHFR enzyme genes had results in defective or diminished enzyme function. MTHFR enzyme deficiency will cause higher homocysteine levels. Without functional MTHFR metabolic enzyme, homocysteine cannot be converted to methionine. Therefore, homocysteine builds in the bloodstream, and the amount of methionine is subsequently reduced. A slight decrease in MTHFR enzyme activity may result in

serious outcomes, particularly in arterial diseases such as peripheral neuropathy, due to microangiopathy, stroke, thrombosis, and coronary heart disease (Yalcin, Kosar, 2019).

Testing

MTHFR mutation testing is debated in the healthcare field. Because MTHFR polymorphism is only one of the many factors contributing to the overall clinical picture, the utility of this testing is currently ambiguous. After the MTHFR genetic test is completed, the blood sample will be sent away for evaluation, and then lab results will be sent to the patient *(This is illustrated in Appendix A, photo 3)*. Genetic counseling should consider the clinical reason for which the test was performed (Hickey, Curry & Toriello, 2013). Currently, guidelines by the American College of Medical Genetics (ACMG), American Academy of Family Physicians (AAFP), and the American College of Obstetrics and Gynecology (ACOG) do not endorse testing for MTHFR polymorphisms or plasma homocysteine testing for routine risk assessment or in specific settings, including evaluation of thrombosis risk or recurrent pregnancy loss (Levin & Varga, 2016).

Yet, several alternative medicine providers and websites purport the value of MTHFR genetic testing and recommend specific supplementation regimens to ameliorate the effects of enzyme deficiency to cure or prevent medical disorders. This has led to a great deal of controversy, as highlighted in the popular press (Levin & Varga, 2016). Typically, if a blood test shows higher than normal levels of homocysteine, a specific MTHFR genetic blood test will not even be completed unless there are other factors which lead medical practitioners to believe there is an MTHFR mutation. Predictably, the actual MTHFR genetic test is administered to patients when a close relative is diagnosed with an MTHFR mutation in conjunction with a high blood homocysteine lab result. As a screening method, the most common and cost-effective laboratory

test is quantitation of plasma homocysteine levels. These levels are known to fluctuate with environment including diet, underlying comorbidities, and interplay with other proteins associated with homocysteine and folate levels. Therefore, a definitive diagnosis requires MTHFR genotyping for common variants (Nguyen & Kant, n.d., year). If homocysteine levels are normal, even if there is an MTHFR mutation variant present, then nothing needs to be done clinically. To address a common misconception pertaining to testing, MTHFR polymorphism testing is frequently ordered by physicians as part of the clinical evaluation for thrombophilia. It was previously hypothesized that reduced enzyme activity of MTHFR led to mild hyperhomocysteinemia, which led to an increased risk for venous thromboembolism, coronary heart disease, and recurrent pregnancy loss. There is growing evidence that MTHFR polymorphism testing has minimal clinical utility and, therefore should not be ordered as a part of a routine evaluation for thrombophilia (Hickey, Curry & Toriello, 2013). A medical geneticist may be asked to evaluate a patient who has tested positive, either heterozygous or homozygous, for an MTHFR polymorphism. The geneticist should assess the information provided to the patient's family by the previous provider, including interpretation pertaining to causality for current symptoms. It is imperative that the geneticist ensure that patients have perceived thorough and appropriate evaluations for their symptoms because it is not uncommon that medical problems are incorrectly attributed to positive MTHFR status (Hickey, Curry & Toriello, 2013). In summary, there have been conflicting data regarding the association among MTHFR polymorphisms, hyperhomocysteinemia, and recurrent pregnancy loss.

Diagnosing

To expound on the validity of this genetic mutation, E72.12 is a valid billable ICD-10 diagnosis code for MTHFR deficiency. It is found in the 2020 version of the ICD-10 Clinical

Modification (CM) and can be used in all HIPAA-covered transactions from Oct 01, 2019 - Sep 30, 2020 (ICD-10, 2019). The MTHFR gene mutation meets a HIPPA transaction standard, so it can flow from the healthcare provider to the payer with essentially no human interaction. Both medical doctors and naturopaths are licensed to order for an MTHFR genetic blood test and subsequently can interpret the results.

C677T Variant

Since it's genetic identification in 1995 (Agodi, et al., 2011), the most extensively studied folate-related genetic variant is C→T substitution at base pair 677 in the gene producing MTHFR. Individuals who are homozygous for the C677T variant (TT) exhibit lower specific activity of MTHFR and reduced enzyme stability, as well as elevated blood homocysteine concentrations, especially in response to folate deficiency. The association of the TT MTHFR genotypes with elevated plasma homocysteine exists primarily in individuals with poor folate status, suggesting a reduced ability to adapt to folate deprivation. There is evidence of somewhat lowered MTHFR enzyme activity in individuals who are heterozygous, but the clinical significance of having a heterozygous genotype is poorly understood. Increased risk for Down Syndrome, and pre-eclampsia have been reported for CT individuals. But in most studied to date, the CT genotype is associated with elevated health risk only when present in combination with at least one other risk factor.

The frequency of MTHFR C677T polymorphism varies among racial and ethnic groups. Approximately 12% of Caucasian and Asian populations are TT homozygotes, and up to 50% are CT heterozygotes (Moyers & Bailey, 2001). Cloning of MTHFR led to identification of a common variant, C677T that results in a thermolabile enzyme with only 35-50% residual activity in homozygous mutant (TT) persons. This prevalent genotype (10-15% of many North American

and European populations) is the most common genetic risk factor for hyperhomocysteinemia. Hyperhomocysteinemia, or low methionine synthesis, which results from mild MTHFR deficiency or inadequate dietary folate, may contribute to several pathologic states through various mechanisms such as direct toxic effects or through indirect effects such as a disruption in methylation or an increase in oxidative or endoplasmic reticulum stress. The well-studied pathologic states associated with homocysteine metabolism include vascular diseases and neural tube defects (Li, et al., 2005). According to a study published in 2016, a high proportion of individuals afflicted with cleft lip, male infertility, recurrent pregnancy failures and the chromosomal disorder, Down Syndrome, have MTHFR C677T homozygosity as a risk factor but the extent of risk is modulated depending upon the levels of vitamin B12, folates, and homocysteine. Thus, the genetic disease burden is influenced by the level of micronutrients. It is recommended that supplementation of both folates and vitamin B12 should be considered for prospective mothers during pregnancy (Raman, 2016). The homozygous mutated subjects have higher homocysteine levels while the heterozygous mutated subjects have mildly raised homocysteine levels compared with the normal, non-mutated controls.

Hyperhomocysteinemia is an emerging risk factor for various cardiovascular diseases and with the increasing significance of this polymorphism given the morbidity and the mortality impact on the patients, further prevention strategies and nutritional recommendation with supplementation of vitamin B12 and folic acid which reduces plasma homocysteine level would be necessary as part of future health education. A study completed in 2014 sought to specifically examine the MTHFR C677T polymorphism, and its epidemiology, metabolism, and associated diseases. The researchers specifically studied the C677T variant and its correlation with the following: vascular diseases, psoriasis, neurological and psychiatric diseases, diabetes mellitus,

breast cancer, cervical cancer, oral cancer, acute lymphoblastic leukemia, bladder cancer, lung cancer, stomach cancer, and colorectal cancer. Though many studies suggested the role of the MTHFR C677T polymorphism in diseases, some studied stated otherwise. The interpretation of the conclusions to the findings of these studies would be more significant if the sample sizes for all the individual studies could be increased (Liew & Gupta, 2014). A clinical study begun in 2016 sought to test the effects of folate intake on MTHFR C677T polymorphism. The study postulated that a diet containing antioxidants, especially folate, is beneficial for are overweight or obese adult women with this genetic alteration because it possesses anti-inflammatory function, and acts on and oxidative stress which plays an important gene function (Riberiro, 2016). The researchers acknowledged that the C677T polymorphism of the MTHFR gene is associated with several biochemicals imbalances, such as changes in folic acid serum levels, some inflammatory markers, elevation of the oxidative stress, and increasing the risk of developing non communicable diseases. They wanted to know that if a diet containing folate as a main antioxidant nutrient could reduce not only the oxidative stress, but also has many other benefits for individuals with this genetic alteration, like the anti-inflammatory function, which could help restore the altered serum levels and minimize or avoid the development of future diseases (de Carvalho Libosa, 2017).

A1298C Variant

There has been a significantly smaller amount of data published concerning the A1298C MTHFR gene variant as compared to the C677T polymorphism. However, the body of research regarding a second MTHFR polymorphism that involves an A→C substitution at base pair 1298, which causes a glutamate to alanine substitution in the MTHFR protein, is rapidly growing. Analysis of small control populations from case control studies suggests that the frequency of the

A1298C allele may range from 23%-45% (Moyers & Bailey, 2001). Most findings thus far suggest the variant is benign unless it is present in combination with the C677T mutation. There is no evidence that the A1298C genotype along elevates plasma homocysteine or interacts with plasma folate, although there is one report that links homozygosity for the A1298C variant with elevated risk for early-onset coronary artery disease, independent of homocysteine (Moyers & Bailey, 2001). Specific activity of MTHFR is reported to be lower in individuals who have both variants, especially when double heterozygous C677T/A1298C. In these individuals, MTHFR activity may be reduced by nearly two-thirds compared with individuals with the doubly homozygous 677CC/1298AA genotype (Moyers & Bailey, 2001).

Fertility Effects

The worldwide prevalence of infertility is high, affecting approximately one in ten couples worldwide (Murto, et al., 2015). Infertility can be caused by female factors, male factors, a combination of male and female factors, or it can remain unexplained. It has been suggested that dietary deficiencies and specific genotypes may be associated with infertility. This has been discussed in particular with relation to MTHFR variations (Murto, et al., 2015). Hyperhomocysteinemia is also associated with neural tube defects, the cause of a variety of severe birth defects, including spina bifida (defects in the spinal column that often result in paralysis) and anencephaly (the invariably fatal failure of the brain to develop), which is the leading cause of infant death due to congenital anomalies. Hyperhomocysteinemia is readily controlled by ingesting the vitamin precursors of the coenzymes that participate in the homocysteine breakdown, namely B6 (pyridoxine), B12, and folate. Folate, especially, alleviates hyperhomocysteinemia; its administration to pregnant women dramatically reduces the incidence of neural tube defects in steps of embryogenesis, women of childbearing age are encouraged to

consume adequate amounts of folate even before they become pregnant. Despite this, a clinical trial indicate that vitamin therapy in individuals with hyperhomocysteinemia does not reduce their incidence of cardiovascular disease. (Voet, Voet, & Pratt, 2019). Women homozygous for C66C→T should be counseled that they have modestly increased risk to have offspring with neural tube defect. This risk is increased further if the fetus is also homozygous (Hickey, Curry & Toriello, 2013). There have been conflicting data regarding the effects of mild hyperhomocysteinemia in women during pregnancy. Elevated homocystinemia has been observed more frequently in women with preeclampsia, placental abruption, and pregnancy loss. Data regarding the association of elevated homocysteine and recurrent pregnancy loss have been conflicting. It is plausible that hyperhomocysteinemia may be a consequence, rather than a cause, of recurrent pregnancy loss (Levin & Varga, 2016).

There has been a considerable effort to explore the effects on the MTHFR gene with regards to male fertility. It was commonly supposed that female MTHFR gene mutations contributed largely to fetal abnormalities and complications with conception, but there have been multiple scholarly studies and clinical trials designated to studying how male fertility can be affected by an MTHFR gene mutation. A study was begun in 2018 to try to identify genetic causes of impaired sperm production and male infertility, focusing on the possible role of the MTHFR C677T genetic mutation. The researchers reasoned that if the nutritional intake or metabolism of the folate was related to male infertility, then that cause of infertility could be curable. Results for the study have not yet been posted (National Institutes of Health Clinical Center, 2018). A scholarly study was completed in an effort to examine both MTHFR gene mutation variants' effect from male partners with regards to recurrent miscarriage couples and concluded that the MTHFR gene composition of male partners of recurrent miscarriage couples

may contribute to increased risk of miscarriage (Tara, et al., 2015). A study regarding the effect of folate and B12 vitamin intake on semen parameters and fertility of men with MTHFR polymorphism was conducted in 2017. The effect of the two polymorphisms on male infertility and serum total homocysteine levels, in addition to the effect of the vitamin B family intake on sperm parameters were assessed. Their findings suggested that the T allele of MTHFR C677T is accompanied by the highest susceptibility to infertility and increased total serum and total homocysteine levels and daily consumption of vitamins B9 and B12 had a critical effect on sperm parameters and the fertility of men with different genotypes of MTHFR polymorphism, especially genotypes with T allele. (Najafipour, Moghbelinejad, Aleyasin & Jaliland, 2017). There was also a similar study completed with an emphasis on the A1298C polymorphism and how it related to idiopathic male infertility. The researchers wanted to see if the A1298C mutation is an additional risk factor for male infertility specifically in a male population located in India, and found that homozygous (C/C) A1298C polymorphism of the MTHFR gene was present at a statistically higher significance in idiopathic infertile men. Therefore, they concluded that A1298C genotype is an additional genetic risk factor for male infertility (Singh, Singh & Raman, 2010).

Nutrition Effects

Folates are essential water-soluble vitamins that are required for one-carbon biosynthetic and epigenetic processes. To obtain folates, humans rely on dietary sources such as beans, and other legumes, citrus fruits and juices, whole grains, dark green leafy vegetables, milk, and liver. The synthetic form of folic acid can additionally be consumed as nutritional supplements or fortified foods (Laanpere, 2010). It has a more stable chemical nature and better bioavailability than naturally occurring folates *(This is illustrated in Appendix A, photo 5)*. Folic acid supplements have also been shown to result in higher serum folate levels in comparison with

natural folates. Mammals cannot synthesize folic acid, so it must be provided in the diet or by intestinal microorganisms (Voet, Voet, & Pratt, 2019). The MTHFR enzyme gene mutation inhibits the method by which the human body is able to process folic acid and other vital B vitamins. Therefore, changing the supplementation of this nutrient is a potential focus in countering the supposed negative effects the MTHFR mutation can have. The metabolism of folic acid and vitamin B12 is interrelated and both vitamins, along with the flavoprotein MTHFR, participate in the synthesis of methionine from homocysteine (Torres-Sanchez, Lopez-Carillo, Blaco-Munoz & Chen, 2014). Folate is necessary for DNA, RNA and protein synthesis in addition to its significant role in energy production and normal cell division. Consequently, it is also important for oocyte quality, oocyte maturation, embryo implantation and normal pregnancy. Folate deficiency is usually caused by poor dietary intake or malabsorption, but there are also several micronutrients, including vitamins B2, B6, and B12, which are needed for folate metabolism. Insufficient levels of these vitamins impair metabolism, thereby causing functional folate deficiency accompanied by high levels of homocysteine.

Previous studies have shown that higher folate and lower homocysteine levels in follicular fluid are associated with better embryo quality and pregnancy rate after fertility treatment (Murto, et al., 2015). Preceding studies have demonstrated that infertile women tend to have a relatively high folic acid intake, but how nutritional supplements affect infertility treatment outcome among women with different genotypes has not been well investigated (Murto, et. al., 2015). Theoretically, it is possible that women with a genotype resulting in low serum folate levels would benefit from high folic acid intake, as this would compensate for the low folate concentrations in blood, which could possibly improve pregnancy outcome (Murto, et. al., 2015). Folate deficiency can occur in humans due to poor dietary intake or malabsorption.

Functional folate deficiency may be caused by insufficient levels of micronutrients necessary for folate metabolism, such as vitamins B2, B6, B12, Fe, and Zn, or it may arise from inefficient folate utilization due to defects in folate-metabolizing genes, such as the MTHFR gene (Laanpere, 2010). For individuals with the MTHFR gene mutation, it is highly suggested that they take the bioavailable form of folate (methylated folate) in order to help their bodies more readily absorb it. A non-exhaustive folate-rich food list would include lentils, beans, peas, bok choy, banana, cantaloupe, raspberries, sunflower seeds, spinach, asparagus, and more. Patients with *MTHFR* gene polymorphisms such as C677T can lower homocysteine levels via folate supplementation. Patients with *MTHFR* gene variants and hyperhomocysteinemia may also have low magnesium levels. Therefore, magnesium supplementation and low methionine intake may also benefit these patients (Nguyen & Kant, n.d.). It was demonstrated that a mutation of MTHFR C677T increased plasma total homocysteine concentration and decreased folate. Natural foods can improve homocysteine levels, but the effect of certain foods remains undetermined. Zeng, et al. (2017) examined the association between food groups and homocysteine, and to explore the correlations between homocysteine and dietary folate/vitamin for genotype-specific populations in China. They concluded that homocysteine levels were influenced by food groups to varying degrees, which were based on gender and MTHFR C677T genotypes. Homocysteine levels were closely correlated with folate for males (CC, CT, TT) and the female TT group, but it was more closely correlated with vitamin B12 for female CT/CC groups (Zeng, et al., 2017).

Implications of Daily Life with the Gene

As previously mentioned, there have been a number of medical issues and health problems associated with having an MTHFR gene mutation, and data have correlated more

issues relating to homozygous mutations (when an individual has a copy of both variant mutations). Health conditions and issues which have been associated with the MTHFR gene mutation include: cardiovascular and thromboembolic disease, depression, anxiety, migraines, nerve pain, recurrent miscarriages, pregnancies with defects such as acephaly or neural tube defects, colon cancer, schizophrenia, bipolar disease, preeclampsia, hearing loss, chronic fatigue, glaucoma, ataxia, anemia, scoliosis, homocystinemia, microcephaly, to reference a small range of associated issues. Health care practitioners often suggest supplementation with folic acid, vitamins B6 and B12, methionine, 5-THF for patients who have an MTHFR enzyme deficiency. One important thing to mention is that patients should be counseled that it is important to provide their MTHFR genotype status to any physician who is considering starting them on types of chemotherapy whose activity depends on intracellular concentration of folate (Hickey, Curry, & Toriello, 2013).

Reviewing Relevant MTHFR Clinical Studies and Scholarly Research

The potential associations between MTHFR genotype status and a number of medical complications have been evaluated using methodologies such as case-control, cohort, Mendelian randomization, and meta-analysis. A modest positive association has been found between the MTHFR polymorphism and many different medical complications, including, but not limited to: thromboembolic disease, stroke, aneurysm, peripheral artery disease, migraine, hypertension, recurrent pregnancy loss, male infertility, hypertension, recurrent pregnancy loss, risk for offspring with neural tube defects, certain cancers, neuropsychiatric disease, and chemotherapy toxicity. Conversely, many other studies looking at similar complications found no statistical association (Hickey, Curry, & Toriello).

A study conducted in 1999 aimed to assess the potency of the C677T MTHFR mutation as an independent genetic risk predictor for recurrent pregnancy loss. However, the results of their expansive clinical tests allowed them to conclude that the C677T MTHFR mutation was not a risk predictor in women with a history of miscarriages. This study also examined hyperhomocysteinaemia, and its connection to the MTHFR gene mutation. Slight increases in plasma homocysteine levels can be caused by nutritional deficiencies of folate or vitamin B12, or by functionally deficient intermediates in the homocysteine metabolic pathway. A case-control study had shown mildly elevated levels of homocysteine to be present in a high proportion of women with a history of recurrent miscarriages, suggesting that this was an independent risk factor for recurrent fetal loss (Holmes, Chilcott, Cohen & Cohen, 1999).

There was also a study conducted in 2014, and this important research evaluated the effects of dietary intake of vitamin B12 and folate during pregnancy and their interactions with maternal polymorphism of MTHFR (677C>T; 1298A>C) on intrauterine development. They suggested that MTHFR polymorphism 677C>T is associated with low birth weight, and women with the 1298A>C variant showed an increased risk of placental vasculopathies. According to their findings, in relation to 677C>T, 49.78% of women were carriers of the heterozygous genotype (677CT) and 34.63% were homozygotes (677TT). For the 1298A>C polymorphism, 81.66% of women were carriers of the 1298AA genotype. They concluded that the C677T polymorphism has a high occurrence frequency and can theoretically increase the susceptibility of the population to the adverse reproductive effects caused by nutritional deficiencies, such as those related to the metabolism of methyl groups (Torres-Sanchez, Lopez-Carillo, Blaco-Munoz & Chen, 2014). In the offspring, low maternal vitamin B12 status during pregnancy is associated

with increased risk of neural tube defects. Therefore, the current standard for prenatal care promotes preventative use of folic acid supplementation, but not the vitamin B12.

Folic acid deficiency is believed to be responsible for approximately 50-70% of spina bifida occurrence based on evidence presented by two large trials and studies whose results were included in the spina bifida research (what are those two clinical trials). A study published 2013 analyzed the role of maternal C677T mutation in MTHFR gene on spina bifida development in Japanese newborns and found a strong link between spina bifida and the MTHFR gene mutation have been strongly correlated for years. The study concluded that it is not necessary for Japanese women to undergo genetic screening C677I mutation of the MTHFR gene as a predictive marker for spina bifida prior to pregnancy, because the TT genotype is not a risk factor for having an affected infant (Kondo, Fukda, Matsuo, Shinozaki, & Okai, 2013).

The background of a study published in 2019 sought to evaluate the influence of MTHFR enzyme gene polymorphism on maternal risk for Down Syndrome and observed the impact of this polymorphism on folate, homocysteine, and vitamin B12 concentrations and their association with pregnancy outcome. The study conclusion suggested that the identification of MTHFR genotype adequate folate and vitamin B12 intake during the preconception and pregnancy period could help protect against congenital malformations and improve pregnancy outcomes. It also strongly concluded that MTHFR genetic polymorphisms have been associated with chromosome damage and maternal risk of birth of a child with Down Syndrome (Kedar, Chandel, 2019).

Several studies have shown that low folate levels are associated with depression in the general population, and some clinical trials also show that folate may have a therapeutic effect on depression. However, a study in 2011 sought to examine whether high folate intake, in the form

of supplements, during pregnancy might offer protection against depression during pregnancy and postpartum. The researchers also tested whether there was a main effect of MTHFR C677T genotype on change in depression scores, and carried out and analysis of folic acid supplementation and depression stratifying genotype. Their findings showed that there was no strong evidence that folic acid supplementation reduced the risk of depression during pregnancy and up to 8 months after pregnancy. However, they did find evidence to suggest that folic acid supplements during pregnancy protected against depression for up to 21 months post-partum, and that effect was more pronounced in those with the MTHFR C677T TT genotype. They thus concluded that low folate was unlikely to be an important risk factor for depression during pregnancy and for postpartum depression but may be a risk factor for depression outside of pregnancy, especially among women with the MTHFR C677T TT genotype (Lewis, Araya, Leary, Smith, Ness, 2011).

Multiple studies have been completed pertaining to the MTHFR gene involving the use of mice as study subjects. One study completed in 2005 sought to study the effect of mild MTHFR enzyme deficiency, low dietary folate, or both on reabsorption rates, on length and weight, and on the incidence of heart malformations in murine embryos. The results of the controlled study suggested that mild MTHFR deficiency, low dietary folate, or both, increase the incidence of fetal loss, fetal growth retardation, intrauterine growth issues, and congenital heart defects. The data supports the benefit of folic acid supplementation in pregnant women, particularly those with an MTHFR deficiency. This study also supports results from previously completed studies stating that the common MTHFR polymorphism or low dietary folate may increase the risk of intrauterine developmental delays, fetal loss, and other pregnancy complications; other studies did not show this association, however. The relation between folate

and congenital heart defects (CHD) was also found to be unclear. Two studies that examined the effect of maternal MTHFR mutant genotype on offspring concluded that it was associated with increased risk of heart defects one study of both maternal and offspring genotypes did not reach this conclusion (Li, et al., 2005). Limitations on the sample size of the aforementioned clinical studies could contribute to the inconsistent results.

A study in 2006 took place in order to examine the effects of changes in various lifestyle habits and lifestyle related biological cardiovascular disease risk markers on changes in total homocysteine (tHcy) in relation to MTHFR (C677T) genotype. The results of the study concluded that none of the studied lifestyle changes, which included smoking, physical activity, dietary habits, and coffee, tea, and alcohol consumption was significantly associated with changes in tHcy, overall and in the MTHFR genotype subgroups (Husemoen, Thomsen, Fenger & Jorgensen, 2006).

In 2015, a study was published which sought to study folic acid intake, folate status, and pregnancy outcomes after infertility treatment in women with different infertility diagnoses in relation to MTHFR 677C>T, 1298A>C and 1793G>A polymorphisms. The results showed that women in the infertility group used significantly more folic acid supplements and had better folate status than fertile women, but pregnancy outcome after fertility treatment was not dependent on folic acid intake, folate status of MTHFR gene variations. They concluded that high folic acid intakes and MTHFR gene variations seem not to be associated with helping women to achieve pregnancy during or after fertility treatment (Murto, et. al., 2015).

The purpose of a 2015 study was to determine whether 5-MTHF is more effective than folic acid supplementation in treatment of recurrent abortion in different MTHFR gene C677T and A1298C polymorphisms. The results did not support any beneficial effect of 5-MTHF versus

folate supplementation in women with recurrent miscarriages with any MTHFR C677T and/or A1298C polymorphism (Hekmatdoost, et al., 2015).

A recent study published in 2018 sought to analyze DNA methylation changes during a randomized-controlled-trial for dietary supplementation with broad spectrum vitamins, minerals, and amino acids in humans. This was of particular interest because it sought to connect nutrition deficiencies with DNA methylation patterns during gametogenesis, fertilization, and in-utero development. The results of the study showed that micronutrient supplementation is unlikely to have a substantial biological effect on DNA methylation over 10 weeks, however, the trend toward hypermethylation that the researchers observed was predicted to become more marked with longer exposure periods. It is important to mention, however, that a subset of individuals demonstrated elevated plasma folate and B12 levels after micronutrient supplementation in the treatment group. The researchers investigated if this was due to a genetic polymorphism in the MTHFR gene, and found the C677T genotype was weakly associated with folate change. When analyzed together, MTHFR genotypes did not appear to have a significant effect on baseline homocysteine or folate levels, in the treatment or placebo group. The researchers ended the study by providing suggestions for future studies. They wrote that the results suggested that interaction of both MTHFR genotypes is a determining factor of DNA methylation patterns. This is likely to indicate that compound heterozygosity is driving the observed effects and future studies need to incorporate appropriate haplotyping methods for these variants and should also consider increasing the sample size and incorporating the effects of genotype on epigenetic analysis, as it is likely that both diet and MTHFR genotype interact to determine epigenetic outcomes (Stevens, et al., 2018).

Insufficient folate status disrupts DNA methylation and integrity and increases blood homocysteine levels. Elevated levels of follicular fluid homocysteine correlate with oocyte immaturity and poor early embryo quality, while MTHFR gene variants are associated with lower ovarian reserves, diminished response to follicular stimulation, and reduced chance of live birth after in vitro fertilization. Embryos carrying multiple MTHFR variants appear to have a selective disadvantage; however, the heterozygous MTHFR C677T genotype in the mother and fetus provides the greatest chance for a viable pregnancy and live birth, possibly due to a favorable balance in folate cofactor distribution between methyl donor and nucleotide synthesis. The results of previous studies clearly emphasize that imbalances in folate metabolism and related gene variants may impair female abilities as well as compromise implantation and the chance of a live birth (Laanpere, 2010).

A study was published in 2010 which wanted to investigated whether pregnancy-induced changes in total homocysteine (tHcy) are associated with folate and vitamin B12 nutritional status, genetic C677T polymorphism in the MTHFR enzyme, and gestation outcome at a time when folic acid supplementation started to be recommended in the Spanish health care system. Although tHcy seems to be physiologically low in the Spanish population and unrelated to folate and B12 nutritional status, C677T MTHFR genotype, and some pregnancy complications, they supported the statement that appropriate folate concentration may be important throughout pregnancy to prevent abnormalities associated with altered status (e.g. neural tube defects). According to the study, supplementation with folic acid seems to achieve this purpose because diet alone may be insufficient. In addition, a poor vitamin B12 status, as measured by plasma levels, may indicate that supplementation of both vitamins is needed (Ubeda, Reyes, Gonzalez-Medina, Alonso-Aparte, Varela-Moralas, 2010).

A 2008 study decided to test the notion that folic acid supplement use is recommended in pregnancy to reduce the risk of neural tube defects, but concerns have been raised that increasing folic acid intake may select for embryos with genotypes that increase disease risk in the offspring. The researchers measured the MTHFR genotype of mothers and their offspring, maternal supplement intake, intake of folate and vitamin B12 from natural foods and maternal blood folate and B12 status at 19 weeks of gestation. They found no evidence to support the concern that folic acid fortification or supplement use in pregnancy results in selection of deleterious genotypes (Haggarty, et al., 2008).

It is imperative to consider the results, conclusions, and suggestions of the aforementioned scholarly studies. In relating the information contained in the studies to the purpose of this composition, it is rather straightforward to see that we still have a great deal to learn about the MTHFR gene and its implications on men and women's fertility capabilities and how nutritional changes can reverse the negative effects. The results of clinical studies, scholarly research, and long-term subject studies have been variable. There is a significant amount of gray area on the topic of effects that MTHFR enzyme mutation variants C677T and A1298C has on human health, as it relates to fertility and nutrition.

Discredit of Unreputable Online Articles

The media tends to largely distort the true information regarding the MTHFR gene mutation and its effect on individuals. Many articles and online websites repeatedly include the phrase "studies have shown," but often authors are merely selecting portions of the scholarly studies which will support their claims and statements. This leads to a slippery slope where individuals suspect their everyday health complications are directly connected to MTHFR variant mutations, and we can see from the completed research that it is not yet appropriate to

directly connect certain medical conditions to MTHFR metabolic enzyme capabilities. Taking it back to a concept mentioned earlier, the MTHFR gene mutation has the seemingly common reputation of being considered a "blame gene." This means that many individuals who test positive for either variant of the mutation tend to consider any or all of their medical issues, complications, conditions, and maladies to be in direct correlation with the gene mutation. And online articles are doing nothing but helping confirm their notions and fears. Some articles suggest and offer *treatment*, while others offer a *cure*. This is completely inaccurate and false. According to Dr. Alan Snow, the biology coordinator and professor of instruction at the University of Akron, Wayne College, with regards to the validity of true symptoms which may present as a result of the MTHFR mutation, “the key here would be medical identified symptoms as evidenced by the research. Often the layperson is bombarded with symptoms that are not uncommon as part of drug treatment ads. Symptoms can often be correlated but not always causative. It’s relatively hard to assign those symptoms to the disease as they are associated with so many other ailments.” Dr. Snow also advised that, “A person must be aware that most information today is circulated with purpose and that purpose might be to make someone scared enough to try (buy) a drug or medicine advertised to treat or prevent but most of these are money makers for the producer and have little to no affect as advertised.” While many online articles are useful and mostly accurate, it can be difficult to distinguish between what can be trusted and taken seriously as opposed to information which has been pieced together to become an erroneous source. With regards to a controversial topic such as MTHFR gene mutations where conclusions are largely uncertain, sifting through information is a large task, and avoiding unreliable, unscholarly sources is essential.

Conclusion

The purpose of this investigation was to examine the current body of knowledge related to this controversial topic. Therefore, a direct conclusion cannot be stated or made. Klug (2009) accurately summarizes gene mutations by writing, "Mutation is a source of genetic variation and the basis for natural selection. It is also the source of genetic damage that contributes to cell death, genetic diseases, and cancer." According to a geneticist from Metro Health Hospital in Cleveland Ohio (name undisclosed for privacy) who was interviewed regarding the MTHFR gene, "there is a lot of controversy over this gene and its effects on health." Clearly, healthcare professionals have much more to learn about the MTHFR gene. Humans have about 30,000 genes, many of which are still to be identified and understood in terms of their function (Acharya & Sankaran, 2005, p.13). According to a 2009 academic study, examining the effects of folate on fetal growth among women with infants with different MTHFR genotypes have not produced consistent results (Kordas et al., 2009). In a statement taken from genetic testing and researching company, *23andMe,* "Some websites have spread the idea that having one or two copies of an MTHFR variant can lead to dozens of negative health consequences. There are a couple problems with this claim. First, it's unlikely that variants in a single gene could cause dozens of unrelated health problems. Second, the C677T and A1298C variants are very common: in some ethnicities, more than 50 percent of people have at least one copy of one of these variants. Most disease-causing genetic variants are not this common. Another claim about MTHFR is that people who carry an MTHFR variant should avoid foods that are fortified with folic acid. However, there's no evidence that individuals with an MTHFR variant should reduce their folic acid intake. Over the past two decades, scientists have examined associations between the MTHFR C677T and A1298C variants and more than 600 medical conditions.

Despite thousands of scientific publications, the evidence linking MTHFR to most of these health conditions is inconclusive or conflicting. For example, some studies report an increased risk of heart disease for individuals with two copies of the C677T variant, while other studies report no association with heart disease. The same is true for cancer, blood clots, and many other well studied health conditions" (*23andMe*, 2020). From both the research presented in this study and beyond, it is rather effortless to see that we simply cannot draw distinct conclusions regarding the effect that nutrition can have on men and women's fertility abilities, specific to individuals with either variant of the MTHFR genetic mutation. The MTHFR gene mutation is becoming increasingly common in the general population, but at this time, it is somewhat difficult to determine correlation versus causation when considering its exact influence on human health, especially in regard to the topic of nutrition and fertility. Therefore, the point of the study was to examine the way that nutrition can reverse the negative effects (particularly fertility difficulties) caused by the MTHFR genetic mutation, and it remains unclear at this time whether or not nutrition can even serve a role, because even the negative effects which have been correlated with the MTHFR gene remain under research.

Appendix A

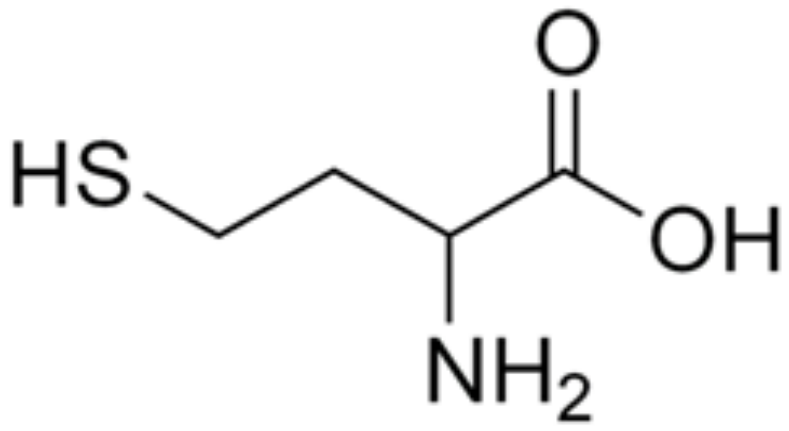
Photo #1 (Mutation Possibilities Representation)

Mutation Representation (possibly schematic)

M^N N=normal	1) M^NM^N (homozygous, normal)
M^C C=C677T	2) M^NM^C (heterozygous, single mutation)
M^A A=A1298C	3) M^NM^A (heterozygous, single mutation)

4) M^CM^A (heterozygous, double mutant)
5) M^CM^C (homozygous for C677T)
6) M^AM^A (homozygous for A1298C)

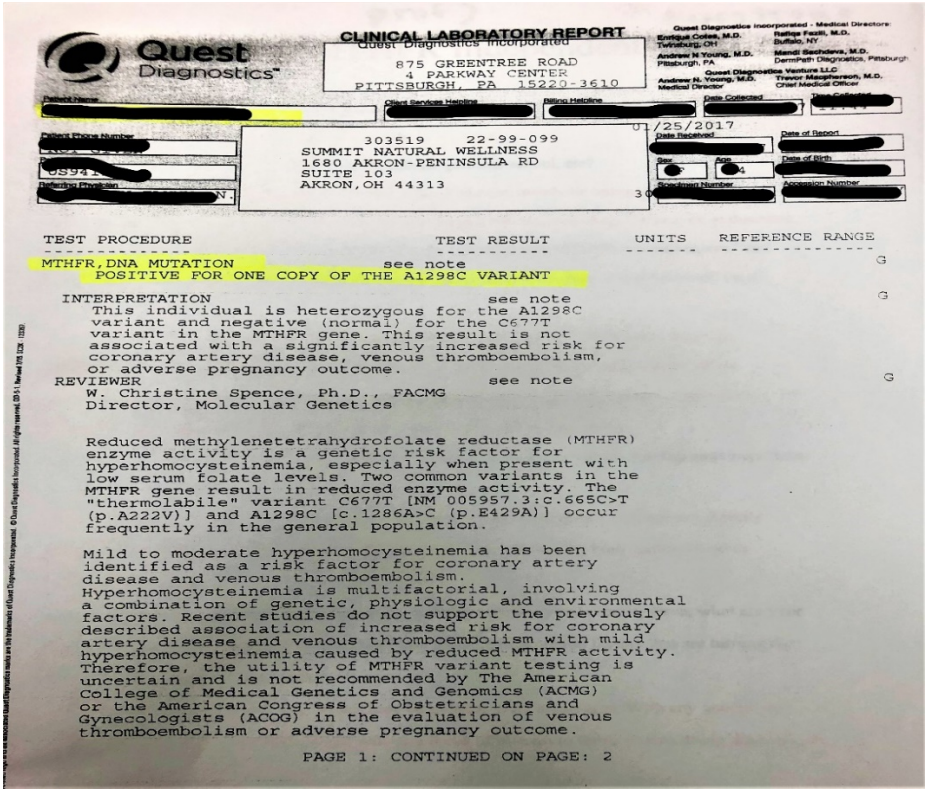
Photo #2 (Homocysteine Chemical Formula)



Homocysteine

blog.baucominstitute.com

Photo #3 (Sample Positive MTHFR A1298C Variant Lab Result)



Quest Diagnostics

CLINICAL LABORATORY REPORT

Quest Diagnostics Incorporated
875 GREENTREE ROAD
4 PARKWAY CENTER
PITTSBURGH, PA 15220-3610

[redacted]

01/25/2017

303519 22-99-099
SUMMIT NATURAL WELLNESS
1680 AKRON-PENINSULA RD
SUITE 103
AKRON,OH 44313

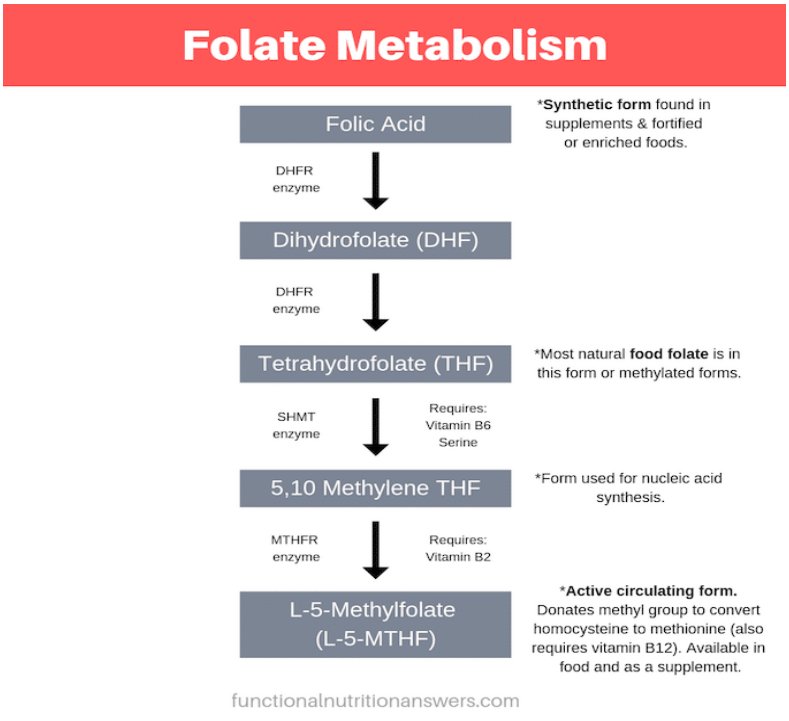
TEST PROCEDURE	TEST RESULT	UNITS	REFERENCE RANGE	
MTHFR,DNA MUTATION	see note POSITIVE FOR ONE COPY OF THE A1298C VARIANT			G
INTERPRETATION	see note This individual is heterozygous for the A1298C variant and negative (normal) for the C677T variant in the MTHFR gene. This result is not associated with a significantly increased risk for coronary artery disease, venous thromboembolism, or adverse pregnancy outcome.			G
REVIEWER	see note W. Christine Spence, Ph.D., FACMG Director, Molecular Genetics			G

Reduced methylenetetrahydrofolate reductase (MTHFR) enzyme activity is a genetic risk factor for hyperhomocysteinemia, especially when present with low serum folate levels. Two common variants in the MTHFR gene result in reduced enzyme activity. The "thermolabile" variant C677T [NM 005957.3:c.665C>T (p.A222V)] and A1298C [c.1286A>C (p.E429A)] occur frequently in the general population.

Mild to moderate hyperhomocysteinemia has been identified as a risk factor for coronary artery disease and venous thromboembolism. Hyperhomocysteinemia is multifactorial, involving a combination of genetic, physiologic and environmental factors. Recent studies do not support the previously described association of increased risk for coronary artery disease and venous thromboembolism with mild hyperhomocysteinemia caused by reduced MTHFR activity. Therefore, the utility of MTHFR variant testing is uncertain and is not recommended by The American College of Medical Genetics and Genomics (ACMG) or the American Congress of Obstetricians and Gynecologists (ACOG) in the evaluation of venous thromboembolism or adverse pregnancy outcome.

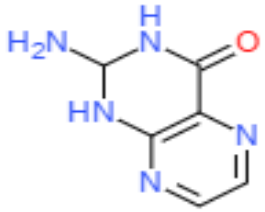
PAGE 1: CONTINUED ON PAGE: 2

Photo #4 (Folate Metabolism)

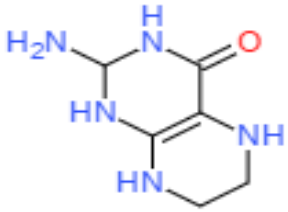


https://www.functionalnutritionanswers.com/folate-vs-folic-acid/

Photo #5 (Folic Acid and Folate Chemical Structures)



Folic Acid



Folate

https://www.foodsweeteners.com/folic-acid/

Appendix B

Chromosome: physical structure where most genes are located (Acharya & Sankaran, 2005, p. 1)

Epidemiology: The study of the distribution and determinants of health-related states and events in populations, and the application of this study to the control of health problems. Epidemiology is concerned with the traditional study of epidemic diseases caused by infectious agents, and with health-related phenomena (Venes, 2005, p. 722).

Etiology: The study of the causes of disease (Venes, 2005, p. 747).

Folic Acid: A member of the vitamin B complex (Venes, 2005, p. 684).

Gene: A unit of heredity (Acharya & Sankaran, 2005, p. 1).

Genome: Word used to describe the entire genetic material of a living organism (Acharya & Sankaran, 2005, p. 1).

Genotype: The total heredity information present in an organism; the pair of genes present for a particular characteristic or protein (Venes, 2005, p. 870).

Heterozygous: An individual with different alleles for a given characteristic (Venes, 2005, p. 994).

Homocysteine: An amino acid produced by the catabolism of methionine. There is evidence that a high level of homocysteine in the blood may be associated with an increased risk of developing atherosclerosis. Blood homocysteine levels may be lowered by eating foods rich in folic acid, such as leafy green vegetables and fruits, and by vitamin B6 or B12 supplementation (Venes, 2005, p.1007).

Homozygous: Produces by similar alleles, an individual developing from gametes with similar alleles and thus possessing like pairs of genes for a given hereditary characteristic (Venes, 2005, p. 1008).

Methionine: A sulfur-containing essential amino acid (Venes, 2005, p. 1353).

MTHFR: 5,10-methylenetetrahydrofolate reductase

Mutation: Permanent variation in genetic structure with offspring differing from parents in a characteristic. Differentiated from gradual variation through many generations; a change in a gene potentially capable of being transmitted to offspring (Venes, 2005, p. 1163).

Neural Tube Defects: A group of congenital structural disorders that result from a failure of the embryonic neural tube to close during development. Cranial fusions disorders, including

anencephaly, spinal fusion disorders including spina bifida, meninogocele, etc may result as a consequence of this failure. To reduce risk, U.S. Public Health Service recommends a daily folic acid intake for all fertile women of childbearing age (Venes, 2005, p. 1451).

Pathophysiology: The study of how normal physiological processes are altered by disease (Venes, 2005, p. 1606).

Phenotype: The expression of the genes present in an individual. This may be directly observable (e.g. eye color) or apparent only with specific tests (Venes, 2005, p. 1654).

Polymorphism: The property of crystalizing into two or more different forms; the occurrence of more than one form in a life cycle (Venes, 2005, p. 1727).

Quick Links Reference Guide

**While the below websites contain useful information, it is important to remember that words such as "proposed" or "suggested" and related jargon do not mean anything definitive in MTHFR informational articles. These articles have been chosen as summary articles which can aid individuals in gaining a better understanding of the gene mutation and the implications on health that it has been shown to have. The quick links are included to provide readers with reliable resources which contain basic information they may not fully understand, as this study seeks to build on preexisting knowledge one has of the MTHFR gene mutation. **

1) https://rarediseases.info.nih.gov/diseases/10953/mthfr-gene-mutation
2) https://ghr.nlm.nih.gov/gene/MTHFR

3) https://medlineplus.gov/lab-tests/mthfr-mutation-test/

4) https://blog.23andme.com/health-traits/our-take-on-the-mthfr-gene/

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