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Pregnancy and Fertility Amongst Women with the MTHFR C677T Polymorphism: An Anthropological Review

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by

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ABSTRACT

Effects of the MTHFR C677T allele on reproduction include multiple complications. The frequency of births with neural tube defects, Down Syndrome, or recurrent pregnancy loss of CC, CT, and TT mothers was measured using data collected from existing literature. Total participants n=2605 (n=1111 cases, n=1494 controls). Results show that folic acid supplementation did not prevent the incidence of NTD pregnancy in CT women. Furthermore, CT women are more fecund overall, producing more pregnancies than CC and TT women. While CT case women did have higher incidences of RPL, NTD pregnancy and DS pregnancy, this is due to the overall greater number of pregnancies. This higher fecundity may be the natural selective mechanism explaining the high frequency of this otherwise negative polymorphism.

LITERATURE REVIEW & THEORY

Introduction

The methylenetetrahydrofolate reductase (MTHFR) gene controls the metabolism of homocysteine into methionine and is an imperative part of 1-carbon metabolism (Friso et al., 2002). The C677T mutation is a single nucleotide polymorphism (SNP) at the 677th position which has been studied extensively since its discovery in the 1990s. The SNP is associated with deleterious health effects and negative effects on reproduction, and was first reported in Eurasian populations. Since then, high frequencies of the mutation have been reported in East Asian countries, the Mediterranean, Western Europe, Mexico, and Central America. This paper will review the known effects of the mutation and its geographic spread, and the evolutionary theory and hypotheses regarding its ongoing selection.

Later, I will quantify the mutation's response to natural selection by calculating differential fertility using previously collected data regarding the birth outcomes of women with the C677T mutation. Data was collected in cases where women with the C677T mutation gave birth to a child with Down Syndrome or neural tube defects, or in instances where women who suffered with RPL were able to conceive. By calculating how the T-allele influences pregnancy outcome, this aspect of the phenotypic response to natural selection can be quantified. This is first and foremost an evolutionary question. I wish to assist in finding the reason that allows this allele to continue to maintain its frequency in populations.

Furthermore, the populations which report the highest frequencies of the mutation are also the populations that are the least studied. These central American populations are commonly

Native and Indigenous populations in states of epidemiological transition. Clinical literature habitually omits populations that are not able to be reached through biomedical practitioners, either through physical distance or monetary disparity. Moreover, the literature frequently disregards the role of women in pregnancy and delivery, focusing instead on the fetus and the outcome of the birth. The goal of this project is to determine the role of genotype in determining the outcome of a birth (in regard to neural tube defects and Down Syndrome births) or the ability to conceive (in regard to recurrent pregnancy loss), and to demonstrate the need for research that combines reproductive justice theory, a framework that advocates for Native and Indigenous sovereignty, evolutionary genetics, and bioanthropology. Applied anthropology is a field that is simultaneously interdisciplinary and designed to incorporate theory into action. Our discipline is uniquely prepared to meet the needs of this research gap.

Biochemistry

The 5,10-methylenetetrahydrofolate reductase (MTHFR) enzyme is a crucial part of the 1-carbon cycle, as it is responsible for the conversion of 5,10-methylenetetrahydrofolate to 5-methyltetrahydrofolate (Liew & Gupta, 2015). 5-methylTHF is the principal circulatory folate present in the body and is the main methyl donor for the recycling of homocysteine to methionine, a predecessor to S-adenosylmethionine (SAM, a universal methyl donor). Not only is this step important for the continuation of one carbon metabolism, but it is also crucial to keep homocysteine levels low due to its innate toxicity. Failure to produce an adequate amount of methyl groups results in an abundance of total serum homocysteine and S-adenosylhomocysteine (SAH), an inhibitor of methylation reactions (Nazki et al., 2014).

This role in methyl group synthesis is also instrumental to epigenetic DNA methylation. The methionine produced by the re-methylation of homocysteine by 5-methylTHF is a substrate for SAM, which is the primary universal methyl donor responsible for DNA methylation processes. The production of methyl donors through one carbon metabolism is the principal source for methylation reactions conducted by SAM. DNA and histone methylation are crucial to cell maintenance: DNA repair, membrane transport, gene and chromosome inactivation and regulation are all functions facilitated through methylation processes. Abnormal or reduced epigenetic methylation has long been associated with carcinogenesis and neurological diseases. Furthermore, methyl tags are imperative to embryonic development and are heritable. Proper methylation is highly influential to trophoblast growth, imprinting, and epigenesis (Clement et al., 2020; Menezo et al., 2016). After being erased early in embryonic development, inherited methyl tags are reinstated in postnatal follicular development in females and in prenatal life in males (Servy et al., 2018).

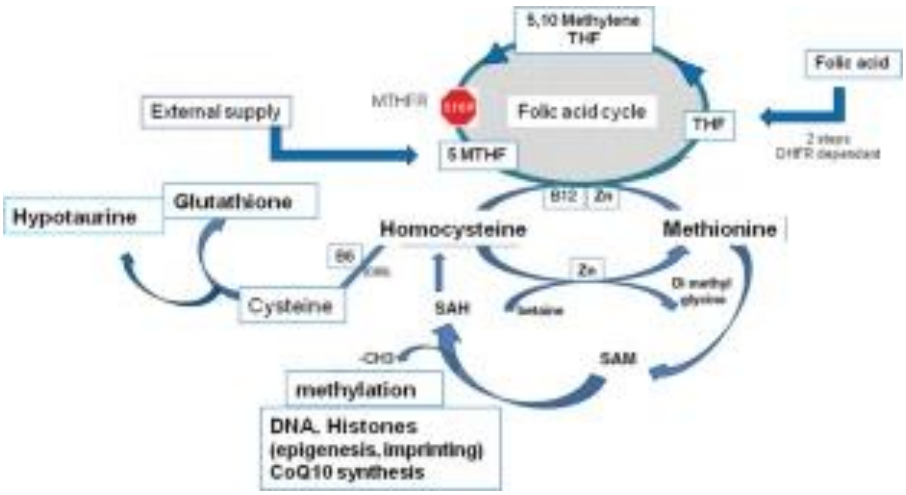

Figure 1. Folate and 1-Carbon cycle interaction. Reused from (Servy et al., 2018).The copyright disclosure can be found in Appendix A.

The MTHFR C677T mutation is found on the MTHFR locus on chromosome 1 and occurs due to a single nucleotide substitution at the 677th position. This substitution results in an

amino acid change from alanine to valine, which codes for a thermolabile variant of the MTHFR enzyme where performance is hindered above 37° Celsius. It is estimated that at 37° Celsius, MTHFR activity in homozygous individuals is reduced by 50-60% compared to wild-type individuals, while increased body temperature causes further reduction in performance (Liew & Gupta, 2015). In heterozygous individuals, the reduction of enzymatic performance is reported at varying levels; due to the co-dominant nature of the mutation, heterozygous individuals experience a lessened reduction in enzymatic function.

Such a reduction in enzymatic performance prevents the recycling of homocysteine into methionine. The accumulation of homocysteine can result in hyperhomocysteinemia, a condition associated with neural tube defects, cardiovascular disease, uteroplacental circulation, recurrent pregnancy loss, thrombosis, deficient cell division, and the production of inflammatory cytokines (Félix et al., 2004; Friso et al., 2002; I. W. Hwang et al., 2017; K. R. Hwang et al., 2017; R. M. Lee et al., 2004; Liew & Gupta, 2015; Nair et al., 2012; Sukla & Raman, 2012). In addition to hyperhomocysteinemia, individuals with the T-allele will also experience a reduction in the amount of 5-methyltetrahydrofolate. Servy and colleagues estimate this decrease in 5-methylTHF generation by 17-75% compared to the amount of 5-methylTHF converted in individuals with the wild-type allele.

As 5-methylTHF is the primary circulatory folate in the body, its diminution likely affects fecundity, embryonic growth and development, and the mitigation of congenital defects. Low levels of 5-mythlTHF can also lead to DNA hypomethylation and increased risk of DNA strand breakage, due to the role of methylenetetrahydrofolate in thymidylate synthesis. Decreased thymidylate synthesis often causes the misincorporation of uracil, which can lead to

DNA strand breakage and chromosomal breakage (Nazki et al., 2014). Folate deficiency additionally decreases the efficacy of the conversion of homocysteine to methionine.

Because fewer methyl groups are synthesized, homocysteine accumulation further inhibits the methylation process. Such an accretion of homocysteine can instigate abnormal and reduced DNA methylation by decreasing the amount of available methionine (and SAM), which has long been associated with carcinogenesis and neurological diseases (Friso et al., 2002; Servy et al., 2018; Tang et al., 2014). While it is currently recommended that pregnant women take a supplemental form of folic acid and is sometimes effective in meeting the adequate level of folates required, such a dose is not always a sufficient treatment for women with the MTHFR C677T allele. In fact, it can be harmful (Clément et al., 2020; Servy et al., 2018).

Synthetic folic acid must be metabolized into tetrahydrofolate prior to being methylated to 5,10 MTHF and then converted to 5-methylTHF (Figure 1). Both of these actions are difficult to complete for individuals with the T-allele; though the mechanism is unknown, heterozygous and (to a greater extent) homozygous individuals do not easily metabolize synthetic folates to tetrahydrofolate (THF) (Servy et al., 2018). Furthermore, any metabolized THF that enters the 1-carbon cycle is not easily converted later from 5,10-MTHF to 5-mthylTHF due to the thermolabile MTHFR enzyme. When left to accrue, this can develop into unmetabolized folic acid syndrome (UMFA), a harmful side effect due to its disruption of immune function and potential to trigger tumorigenesis.

Moreover, UMFA increases homocysteine circulation and the accumulation of folic acid blocks both dietary-derived methylfolate and 5-mthylTHF from entering the 1-carbon cycle (Clement et al., 2020). It has recently been suggested that supplementation with 5-mthylTHF itself is more effective for CT and TT individuals than synthetic folic acid, though the latter

remains the conventional recommendation (Clement et al., 2020; Servy et al., 2018). Furthermore, testing for the MTHFR C677T mutation is not commonly done prior to the detection of fertility problems, meaning that the typical woman endeavoring to conceive is unaware of the potential harm of folic acid supplementation.

Health Effects

The majority of research conducted on the effects of the C677T mutation have taken place in Europe and Asia. Nevertheless, the effects of the mutation remain largely unstudied in populations outside Eurasia. The available literature show that the mutation is associated with a number of adverse effects ranging from vascular disorders, neurological ailments, psoriasis, psychiatric disorders, multiple forms of cancer, and a number of adverse effects on pregnancy and fertility. (J. Chen et al., 1996a; Hernández-Vásquez et al., 2017; Lykke et al., 2012; Michalek et al., 2017; Stonek et al., 2007; Yang et al., 2014; D. Zhang et al., 2015).

Cardiovascular disease, coronary artery disease, and arterial hypertension have all been associated with hyperhomocysteinemia, an effect that commonly occurs with the MTHFR C677T mutation due to its effect on 1-carbon metabolism (Nazki et al., 2014). A phenotypic increase in the levels of circulatory homocysteine due to the MTHFR C677T mutation has also been linked to migraine with auras. Homocysteine can perform as a stimulant amino acid in the pathophysiology of migraines, instigating a temporary thrombotic event or vasodilation of cerebral vasculature, thus diminishing the amount of oxygen able to reach the brain and allowing the migraine to occur (A. Liu et al., 2010). It is also suggested that migraine susceptibility is enhanced in individuals with the T-allele due to vascular endothelial dysfunction, which can activate fibers of the trigeminal nerve (A. Liu et al., 2010).

These pathways leading to migraines ultimately rely on an upstream event of hyperhomocysteinemia in order to activate downstream neurological symptoms witnessed in individuals with migraines with aura. While many repeated studies have found an association between migraine with aura and the MTHFR C677T allele in young and middle-aged adults, some report that no association exists in older populations though this may be due to selective survival as both phenotypes are associated with lowered longevity (Scher et al., 2013). Similarly, increased homocysteine, decreased methionine (and therefore, decreased methylation), and anomalous folate circulation have also been associated with tumorigenesis and carcinogenesis as well as numerous types of cancer (Tang et al., 2014). Due to folate's role in nucleotide synthesis, individuals with lower measures of circulating folate due to the MTHFR C677T mutation may also experience increased double-strand breaks in DNA which further increases cancer risk (Stumbryte et al., 2018).

In a meta-analysis of over thirty-five thousand individuals, high homocysteine coupled with low folate was associated with increased risk of carcinogenesis overall and showed heterozygous advantage. Cancer risk was only associated with homozygous TT individuals and homozygous wild-type CC individuals (D. Zhang et al., 2015). Tang and colleagues found that Asian individuals are at higher risk of developing cancer in general, though rates of stomach and esophageal cancer are particularly high among this demographic group (Tang et al., 2014). This association was later confirmed in Chinese populations after another meta-analysis found increased risk of development of esophageal cancer, and again in the Han Chinese ethnic group when the polymorphism was significantly associated with risk of esophageal squamous cell carcinoma in individuals with low serum circulatory folate (Huang et al., 2013; Yang et al., 2014).

The mutation has been found to be a strong risk factor for stomach cancer in Chinese populations, but this risk is not duplicated in other populations (Nazki et al., 2014). In lung cancer patients, CT individuals with Stage III or Stage IV cancer have a lower rate of survival than earlier stage patients (Stumbryte et al., 2018). CT and TT individuals were also shown to have a more rapid disease progression to death in patients with non-small-cell lung cancer (Alberola et al., 2004; Hong et al., 2013). The wildtype CC genotype was also found to have a better response to gemcitabine/platinum chemotherapy, which is one of the most commonly used treatment regiments for patients of non-small-cell lung cancer. This is again due to the lowered efficacy of DNA methylation under the conditions of the mutant T-allele (Hong et al., 2013). Other treatments, such as a combination of pemetrexed with pemetrexed plus carboplatin have shown to be much more effective for carriers of the T-allele, and can significantly lengthen the period of progression-free survival (Smit et al., 2009; Tiseo et al., 2012).

The association of the mutation with risk of colorectal cancer and leukemia has also been examined expansively. These have returned population-dependent results, with no consistently recurring patterns across multiple populations. Acute lymphoblastic leukemia (ALL) was found to have no association with the mutation in two Brazilian populations nor in two Western European populations but was found to be associated with the mutation in a Korean population (Nazki et al., 2014). Three more studies (one in a Greek population and two in Brazilian populations) found that the mutation decreased the risk of developing leukemia (Nazki et al., 2014). In the case of colorectal cancers, methyl status may also play a role in these inconsistent results. If folate is high in combination with low methyl levels, risk of cancer may be lower. However, if folate is low in combination with low methyl, the risk of colorectal cancer is increased (J. Chen et al., 1996b; Ma et al., 1997).

The risk of head and neck carcinogenesis may only be associated with the MTHFR C677T mutation when alcohol abuse is involved, but there is variance in the reported level of abuse required to observe such an association (Nazki et al., 2014).The mutation has also been associated with an increased risk of schizophrenia (Muntjewerff et al., 2006). In a meta-analysis of eighteen studies, Zhang and colleagues found that homocysteine levels at or above 5 µmol/l is associated with a 70% increase risk of developing schizophrenia. The homozygous TT genotype was also found to carry a 36% higher risk of developing schizophrenia over the wild-type CC genotype (Yanling Zhang et al., 2013). Psoriasis has also been found to be associated with the mutation as a result of the hypomethylation seen in individuals with the T-allele (Liew & Gupta, 2015). However, this pattern of association has only been found in Chinese and Czech populations, and was not repeated in Austrian or Malaysian populations (Liew & Gupta, 2015; Vasku et al., 2009).

Most recently, it has been theorized that individuals with the MTHFR C677T mutation in a state of hyperhomocysteinemia suffer more severe health outcomes from the COVID-19 virus (Karst et al., 2020). Karst and colleagues hypothesize that the health outcomes associated with the C677T mutation such as hyperhomocysteinemia, atherosclerosis, and hypertension all worsen COVID-19 outcomes. Furthermore, they postulate that worldwide differences in severity of the disease can be attributed to the mutation as well. Countries like Italy, China, and the UK have well-documented, high frequencies of the C677T polymorphism and also faced some of the most severe epidemiological consequences of the pandemic. No clinical study has been conducted on this relationship between the mutation, homocysteine levels, and COVID-19 severity yet due to the ongoing nature of the pandemic. It is recommended for now that homocysteine levels are assessed in patients facing severe symptoms from the COVID-19 virus (as this is less time-

consuming than genetic testing for the mutation) and dietary changes are made in patients with hyperhomocysteinemia (Karst et al., 2020).

Effects on Reproduction

Different aspects of reproduction in men and women have been reported to be affected by the MTHFR C677T mutation. Due to hypomethylation and reduced folate, the mutation has been associated with male and female infertility (Milenkovic et al., 2020; Tara et al., 2015; Turgal et al., 2018; Vani et al., 2012), recurrent pregnancy loss (Mukhopadhyay et al., 2009; Wolski et al., 2017; Xu et al., 2019) spontaneous abortion (Stangler Herodež et al., 2013; Zetterberg et al., 2002), thrombophilia (Karakantza et al., 2008; Mehandjiev et al., 2019), pre-term birth (Hiltunen et al., 2011; Tiwari et al., 2015), low birth weight (H. A. Lee et al., 2013; Wu et al., 2017), poor outcomes with assisted reproductive treatment (ART) (Enciso et al., 2016), chromosomal abnormalities such as Down Syndrome (Kedar & Chandel, 2019; Vraneković et al., 2010), pregnancy complications (Lykke et al., 2012; Pogliani et al., 2010; Sultana et al., 2020), and congenital defects including congenital heart defects (Z. Li et al., 2015; Zidan et al., 2013) and, perhaps most notably, neural tube defects (NTD) (Blom et al., 2006; Van Der Put & Blom, 2000; Y. Wang et al., 2015b).

Male infertility was found to be associated with the T-allele in population specific studies, though this has not been replicated in all instances of study. In an Iranian population, low semen quality was associated with both the CT and TT genotypes, and it was observed that supplementation with vitamin B12 can restore the quality of sperm (Najafipour et al., 2017). Another Iranian study found that hypomethylation due to presence of the MTHFR C677T mutation male partners can cause recurrent miscarriage (Tara et al., 2015). This association

between low semen quality and the T-allele was replicated in a Chinese population and is thought to be due to hyperhomocysteinemia (L. Liu et al., 2012). Again in a North Indian population, the presence of the T-allele increased the risk of male infertility two-fold, where the mechanism was postulated to be hypomethylation due to the importance of methylation in spermatogenesis (Naqvi et al., 2014).

Hyperhomocysteinemia has also been considered as the mechanism behind MTHFR C677Tand male infertility in Indian populations (Dhillon et al., 2007; Ferlin et al., 2007). However, no association was found in a South Indian population, nor in the Netherlands (Ebisch et al., 2003; Vani et al., 2012). General infertility in female patients has also been reported in association with the MTHFR C677T mutation. In a case-control study of 225 females with unexplained infertility undergoing *in-vitro* fertilization, the T-allele was significantly associated with unexplained infertility (Milenkovic et al., 2020). Difficulty getting pregnant through assisted reproductive treatment (ART) has also been reported in couples with the C677T polymorphism.

Other infertility problems in women such as recurrent pregnancy loss and spontaneous abortions have also been reported in association with the MTHFR C677T mutation, however the mechanism causing the observed outcomes remain unknown (Mehandjiev et al., 2019). The mutation was found to be significantly associated with recurrent pregnancy loss in an endogamous North Indian population, a highly heterogenous North Indian population, a meta-analysis representing the broader Chinese population, and in a central Eastern Chinese population (H. Chen et al., 2016; Mukhopadhyay et al., 2009; Nair et al., 2012; Xu et al., 2019). Conversely, no association with recurrent pregnancy loss was found in a Yamato Japanese

population, a Polish population, and the South Korean population (K. R. Hwang et al., 2017; Makino et al., 2004; Wolski et al., 2017).

Spontaneous abortion is not as widely investigated in the absence of other fetal concomitant pathologies, nonetheless it was significantly associated with the T-allele in a Slovenian population. However, it was discovered that male probands with heterozygote or homozygous C677T mutations primarily contribute to the spontaneous abortion event (Stangler Herodež et al., 2013). A Swedish study found the composite presence of the C677T and MTHFR A1298C mutation increased the risk of spontaneous abortion by 14 times due to the impact of hyperhomocysteinemia (Zetterberg et al., 2002). Deciduous and intervillous thrombophilia pathologies have also been examined in cases of pregnancy loss.

In a Japanese study of 243 patients, an association between the CT and TT genotypes was found present with thrombophilia leading to pregnancy loss (Mehandjiev et al., 2019). Advanced thrombophilic pathologies that lead to spontaneous abortion are thought to be related to hyperhomocysteinemia, as high methionine levels are needed for proper tissue growth in early stages of development (Mehandjiev et al., 2019). Thrombophilia and homocysteine levels were again linked to the mutation in a Greek population where the presence of the homozygous TT genotype resulted in the increased risk of placental abruption by 4.8 times even with normal folate levels, and folate and vitamin B_{12} supplementation (Karakantza et al., 2008).

Difficulty to conceive has also led individuals with the MTHFR C677T mutation to seek ART. Heterozygous individuals were found to be overrepresented within the pool of subfertile patients seeking ART in a German population due to recurrent miscarriage or failure of embryo implantation, and homozygous TT individuals were significantly associated with failure to implant healthy embryos without chromosomal abnormalities under care of ART (Enciso et al.,

2016). In a Swedish study examining pregnancy outcomes following IVF, there was no association between heterozygosity or homozygosity and successful pregnancy outcome suggesting that pregnancy is not dependent on these MTHFR variations in the study population (Murto et al., 2015). Folic acid supplementation also played no role in determining successful pregnancy outcome, regardless of MTHFR C677T genotype, which indicates alternative folate supplementation strategies may provide a better substitute for achieving pregnancy (Murto et al., 2015).

After conception, other pregnancy complications may also impede the gestation of women with the MTHFR C677T mutation. In a North Indian population, severe pre-eclampsia has been significantly associated with the MTHFR C677T variant, and a 4 times greater risk of developing pre-eclampsia was found when the T-allele is present (Sultana et al., 2020). A large meta-analysis also reported that this connection was present between pre-eclampsia and the C677T mutation in European and East Asian populations (X. M. Wang et al., 2013). Results of the Danish Birth Cohort study also chronicle a significant association between the mutation and severe pre-eclampsia in the Danish population (Lykke et al., 2012). These studies cite hyperhomocysteinemia and oxidative stress caused by the mutation as the driving force behind the pre-eclamptic phenotype. Contrarily, the C677T mutation was associated with a decreased risk of developing pre-eclampsia during gestation in a Mexican Maya-Mestizo population (Canto et al., 2008). This again suggests geographically localized selection and adaptation to the effects of the mutation.

Congenital anomalies and defects have also been observed in association with the MTHFR C677T mutation. In the Korean population, pre-term birth was significantly associated with the mutation, however this result was not repeated amongst the pre-term births of the

Finnish Birth Cohort study (Hiltunen et al., 2011; I. W. Hwang et al., 2017). In a northeast Indian population, pre-term delivery and low birth weight were both significantly associated with the mutation (Tiwari et al., 2015). Pre-term birth is one of the most common contributors to infant mortality, holds substantial consequences for long-term adverse health and cognition outcomes, and also places a heavy financial burden on families due to cost of neonatal care. Low birth weight is also a determinant of long-term health and a leading cause of infant mortality (Tiwari et al., 2015).

The etiology of the relationship between pre-term birth or low birth weight and the MTHFR C677T mutation is largely unknown due to the multifactorial and complex nature of the pathologies. However, an Indian study and a separate Korean study both performed Mendelian randomization analysis investigating the causality between maternal homocysteine concentration, maternal C677T genotype, and offspring birth weight. Each found that maternal genotype predicts homocysteine level and furthermore, that the T-allele predicts higher homocysteine concentration and lower birth weight (H. A. Lee et al., 2013; Yajnik et al., 2014). Maternal genotype has also been found to increase risk of congenital heart disease (CHD) in a central-eastern Chinese population and a North Egyptian population (Z. Li et al., 2015; Zidan et al., 2013). Congenital heart disease, including all of the arrays of malformations that constitute this diagnosis, is of polygenic inheritance and therefore its connection with the C677T mutation is not completely elucidated. It is hypothesized that hyperhomocysteinemia may be a leading factor in CHD (Zidan et al., 2013).

Perhaps the two most commonly occurring birth outcomes associated with the MTHFR C677T mutation are Down Syndrome and neural tube defects. Down syndrome is the most frequently occurring chromosomal abnormality, resulting in an additional 21st chromosome as

indicated by its clinical name, Trisomy 21. In about 95% of cases, this additional chromosome results from maternal non-disjuncture, a malfunction in chromosome segregation during meiosis which transpires prior to conception in oocyte maturation (Kedar & Chandel, 2019). Part of the etiology of this process may rely on proper folate metabolism, leading to the interest in the investigation of the MTHFR C677T mutation. This proposed etiology and association were found in two Indian populations and an Italian population, though no association between the C677T mutation and Down Syndrome was found in a Croatian population (Kedar & Chandel, 2019; Rai et al., 2006; Scala et al., 2006; Vraneković et al., 2010).

The relationship between the C677T mutation and the formation of NTD is thought to be due to the mutation's role in folate metabolism and increased homocysteine as well. In China, both the heterozygous CT and homozygous TT genotypes are risk factors for developing NTD pregnancy (Y. Wang et al., 2015b; Yan et al., 2012; Zhang et al., 2019). The homozygous TT genotype was also found to confer risk of NTD in an Indian population (Deb et al., 2011). In Turkey, the C677T mutation alone was not associated with risk of NTD; in combination with the other common MTHFR mutation, the A1298C polymorphism, there was an increased risk of NTD, however (Boduroğlu et al., 2005).

Moving east into Europe, similar findings were reported in an English population where it was found that the combined C677T and A1298C genotypes were associated with significant risk of NTD pregnancy development (Caroline L. Relton et al., 2004). In a different English population, however, C677T alone was significantly associated with the risk of NTD pregnancy (C. L. Relton et al., 2004). An association between the MTHFR C677T mutation and NTD was found in the Netherlands, and was attributed to elevated homocysteine (Van Der Put & Blom, 2000). Though the studies were exclusive to pregnancies resulting in spina bifida, no association

with the mutation was found in a German population (Koch et al., 1998), but an association between the mutation and spina bifida was found in an Italian population (De Franchis et al., 1998). In France, the C677T mutation was reported as a small risk factor for the development of NTD pregnancies, although it was noted that folate level was more of a determinant than genotype (Candito et al., 2008). This same conclusion was drawn in a European-descendant population in Canada (Christensen et al., 1999). In the highly homogenous Irish population, both the CT and TT genotypes are associated as significant risk factors for the development of NTD (Kirke et al., 1996, 2004).

Very little study on the relationship between the C677T mutation and NTD has been completed in Central and South America. While no association between the mutation and NTD were found in Mexican Mestizo parents (Dávalos et al., 2000), it appears that the mutation's contributions to hyperhomocysteinemia and low B_{12} levels do effect NTD risk in Brazil (Félix et al., 2004). Considering the very high levels of the mutation in Mexico and Central America, and the very high prevalence of NTD in these areas (Dávalos et al., 2000), this is a relationship that is worth much further exploration.

Distribution of the Allele and Natural Selection

Much of the origins, historical population frequency, and spread of the mutation remains widely unknown. Based on publicly available ancient genome data from the Population Genomics and Genetics Single Nucleotide Variant database (www.pggsnv.org), the mutation does not appear to be present in Neanderthal or Denisovan populations and is not recorded in the modern human genome until 7000 BC (unpublished data, Liu et al. 2021). However, very little ancient DNA data is publicly available, and much of the accessible data is from European

populations. It is probable that the evolutionary force primarily responsible for the modern frequency of the mutation is natural selection, as it is found repeatedly in multiple large populations.

In modern populations, the C677T mutation is virtually absent in Native populations of Africa, the South Pacific, and South India. There are very few studies on the populations of South America, the USA, and Canada. High frequencies clustered in the native peoples of Europe, North-East Asia, the Middle East, the Mediterranean, Mexico, and Central America. All of these former listed regions with the exception of Central America and Mexico have repeatedly reported frequencies ranging from 20-40%, with some populations sometimes measuring frequencies above 40%. Much more recently and to a far lesser extent, the range and effect of the mutation has begun to be measured in Mexico and Central America. While there are now a variety of studies which list allele frequency in Mexico and Central America ranging between 45-90%, research in this geographical area began much later than in Eurasia and the clinical manifestations of the allele remain widely unstudied (Reyes et al., 2021).

Though the frequency of the mutation is high in both European and Native and Indigenous groups of the New World, it is unlikely that the mutation was first introduced during colonial times. Prior to colonization, the C677T mutation was already in high frequency in Central and South American populations, though the A1298C variant may have been introduced in this way (Binia et al., 2014). It is possible, however, that the effect of colonization impacted the frequency of the mutation. It has been hypothesized that a bottleneck event occurred during and after initial colonization due to the devastation of Native and Indigenous populations from afflictions such as measles, smallpox, and influenza (Contreras-Cubas et al., 2016).

Natural Selection Hypotheses

Because of the numerous deleterious health effects and potentially detrimental effects on fertility (and therefore, evolutionary fitness), it is astounding that the C677T mutation remains so widespread in human populations. For that reason, research groups have searched for evidence of selective advantage in the populations where the allele is found in high frequencies. This has led to an abundance of hypotheses as to the origin, increase, and sustainment of the allele frequency around the world. These hypotheses include evidence of heterozygote advantage by way of enhanced cognition performance in elderly Chinese individuals (Tsai et al., 2011), homozygote advantage of TT fetuses of mothers receiving supplemental folate (Mayor-Olea et al., 2008a), heterozygote advantage in survival of chemotherapy due to heightened abilities to process heavy metals (Cui et al., 2011; Krawczyk et al., 2014; X. Li et al., 2014), and homozygous advantage when adequately consuming folate by way of lower risk of developing acute lymphoid leukemia (Robien & Ulrich, 2003). More general hypotheses include an increase in the T-allele due to gene-environment interaction (Lucock et al., 2012), UV-B radiation as the selective force of natural selection rather than UV-A radiation (Lijun et al., 2012), and the ability to normally metabolize homocysteine notwithstanding a thermolabile enzyme in the presence of a folate-rich diet (Ojeda-Granados et al., 2017).

Canto and colleagues found that in Maya-Mestizo women in particular, the presence of the T allele reduces the risk of preeclampsia, resulting in more completed pregnancies and full-term births and therefore, increasing the chance of passing the mutation to the future generation (Canto et al., 2008). In Native and Indigenous populations within Mexico, Binia and colleagues also found that carriers for the MTHFR C677T mutation may have better blood pressure control with vitamin B_{12} supplementation, a finding that is notable due to the increasing rate of

hypertension in Mexico (Binia et al., 2014). Some clinical studies show beneficial associations between the mutation and enhanced chance of survival from disease for heterozygous individuals, such as one paper showing that CT individuals have protection against chronic HBV infection amongst young West African adults in Benin and Togo (Bronowicki et al., 2008).

These proposals are typically population specific, and do not demonstrate a universal benefit. It is possible that due to the widespread geographic frequency of the allele, the mutation may favor local specialization of benefits within different populations. I hypothesize that natural selection is the active evolutionary force working upon the allele. While both natural selection and genetic drift are forces that may lead a population to derive a high frequency of a mutation, the MTHFR C677T mutation is found in multiple large populations across multiple continents and is a balanced polymorphism, which suggests that the force at work here is natural selection. It should also be clarified that natural selection will not allow a deleterious allele to continue in high frequency unless the allele bestows a beneficial phenotype where it evolved. In short, some (currently unknown) factor of the phenotype bequeathed by this mutation must be considered beneficial in order for natural selection to maintain its presence.

Orr defines fitness as the ability of an organism, population, or species to survive and reproduce within the environment they find themselves in to pass on genetic material to future generations (Orr, 2009). Change in fitness in response to natural selection requires that some of the differences in fitness must be due to genetic content. In this case, the genetic basis is known, and it is likely that natural selection is occurring, but the response to natural selection has not yet been measured. The purpose of this project is to detect natural selection operating via differential selection of the three genotypes in women throughout the world. We will do this considering the participants' age and folate consumption.

MATERIALS AND METHODS

IRB Approval

I requested approval for this project from the USF IRB board. The study was given the number 002190 and was determined not to meet the specifications of human-subject research. I include the letter stating that this project is not human research in Appendix C.

Data Collection

To test the null hypothesis that genotypes do not affect reproductive outcome, previously collected data were gathered using a scoping review methodology primarily through Web of Science, accessed through the USF Library system. Previously collected data from peer-reviewed, case-control studies concerning pregnancy complications, birth outcomes, and fertility of women with and without the MTHFR C677T mutation were obtained through a number of search terms yielding a total of eighty-eight articles (Figure 2). A secondary search by title of the reference sections of these eighty-eight articles yielded an additional twenty-three studies, bringing the total to one hundred and ten articles. Inclusion criteria designated that each study must include the maternal genotype, maternal age, folate supplementation status of the participants (by vitamin/supplement of synthetic folic acid, dietary intake, or both if applicable), and the birth outcome of each offspring listed by maternal genotype.

Thirty-five articles were removed from the original list due to missing one or more relevant variables, twenty-seven were removed for not listing or being related to a birth outcome, twelve were removed for using previously published data, three articles were removed for having

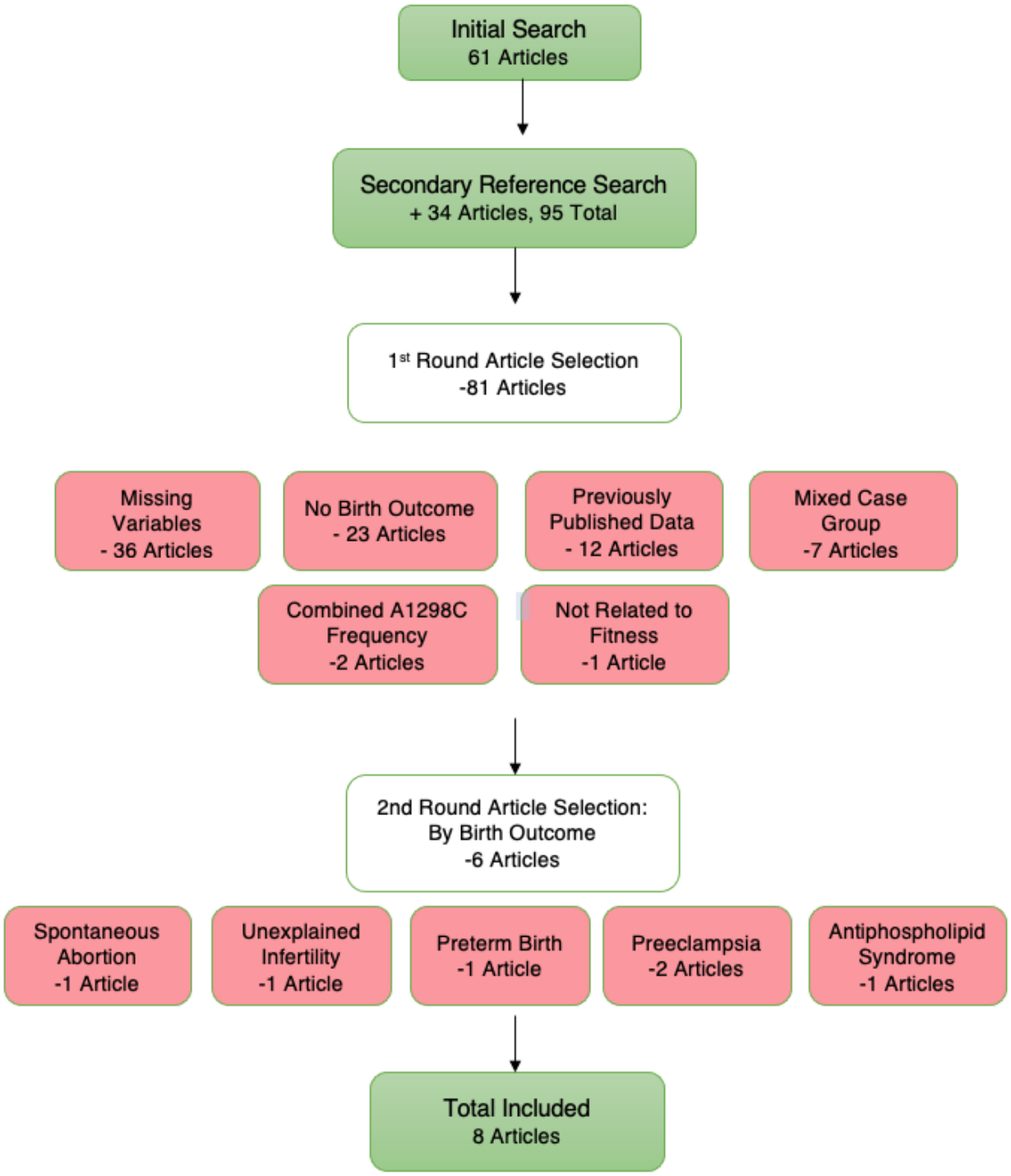

Figure 2: Data Selection Methodology

a case group with mixed birth outcomes, two were removed for listing combined frequencies with the MTHFR A1298C allele, and the last article was removed because the birth outcome was asthma and allergy unrelated to death of the offspring (and therefore, unrelated to fitness). This

left a total of twenty-nine relevant articles that meet inclusion criteria and listed all the required variables (Figure 2). The literature search yielded studies where the observed outcome was Down Syndrome, recurrent pregnancy loss, unexplained female infertility, preterm birth, preeclampsia, neural tube defect (NTD), and spontaneous abortion.

After grouping the data together by birth outcome, those groups without enough participants with comparable data were excluded. Articles included for analysis were studies where the observed birth complication was Down Syndrome (DS), recurrent pregnancy loss (RPL), and neural tube defects, (NTD). These birth outcomes were chosen due to the frequency at which they appeared in the literature: they returned the greatest amount of data that would allow for the multivariate analysis needed to understand the relationship between the mutation and pregnancy outcomes. I was able to amass a large number of total participants. In total, the sample size is n = 2605 individuals (1111 cases and 1494 controls). In all of the included literature, each study is investigating the association of the MTHFR C677T mutation (by way of maternal genotype) in pregnant women and the birth outcome of the offspring.

The control women were defined in the literature as healthy women who were primiparous (at minimum) and gave birth absent of any obstetrical complications. They were recruited either during pregnancy or while at a routine follow-up appointment with their healthcare provider shortly after giving birth (Table 1). Control participants were recruited at the same facilities that case participants were identified and treated. The case women for the Down Syndrome group were defined as women who are less than 35 years old, were pregnant at the time of recruitment into the study and gave birth to a child with Down Syndrome; the case women comprising the RPL group were defined as women with a history of two or more unexplained instances of loss of pregnancy and were pregnant at the time of study (Table 2).

Exclusion criteria for the RPL group specified that women were ineligible for the case study if they had uterine abnormalities, presence of protein C, protein S, antithrombin deficiency, known reason for recurrent miscarriage, or if she or her partner had chromosomal abnormalities.

The case women of the NTD group had a broader range of inclusion criteria. Half of the literature included only mothers who were pregnant at the time of recruitment into the study and then gave birth to a child with ambulatory spina bifida (Christensen et al., 1999) or spina bifida occulta (Caroline L. Relton et al., 2004), and excluded mothers who were also pregnant at the time of study, but whose child was born with a syndromic NTD (Table 2). The other half of the included literature incorporated women who were pregnant at the time of recruitment into the study and who then gave birth to a child with any form of NTD, syndromic or non-syndromic (Deb et al., 2011; Y. Wang et al., 2015a). These include mothers whose child was born with anencephaly, encephalocele, myelomeningocele, spina bifida, spinal dysraphism, or a combination of one or more of these NTDs (Table 2).

Maternal age was reported for each included study, but many were reported as ranges. In order to optimize the data analysis, the average age of the participants from each study was taken and converted to a discrete variable (Table 3). The ethnicity of the participants was also collected as a separate variable. Due to the differences in the mutation's frequency by ancestral gene pool, population frequency by ethnicity is a common approach to cataloguing allele frequencies, especially within heterogenous populations (Candito et al., 2008; Christensen et al., 1999). Reporting results by ethnicity can also aid in measuring change of the frequency within the population over time. If ethnicity was not reported within the original data and could not be found through government population demographic websites, nationality was used as a proxy.

Table 1: Control Data by Group

Source	Geographic Location	Ethnicity	CC	CT	TT	p	Q	HWE	National Folate Fortification	Individual Folate Supplementation	Dietary Folate	Maternal Age	Diagnosis Group
Christensen et al.	SE Canada	European Descendant	44	36	10	0.6889	0.3111	Yes	Yes	No		3.5	NTD
Deb et al.	N. India	Multiethnic: Majority Punjabi	149	64	9	0.8128	0.1872	Yes	No	Yes. Amount varied.	High	1	NTD
Wang et al.	N. China	Han Chinese	96	45	159	0.395	0.605	No	No	Yes. Amount varied.		1	NTD
		NTD Group:	289	145	168								
Makino et al.	Japan	Yamato	29	32	15	0.5921	0.4079	Yes	No	None	High	1	RPL
Wolski et al.	Poland	Polish	201	164	35	0.7075	0.2925	Yes	No	400mg/day		2.5	RPL
		RPL Group	230	196	50								
Rai et al.	India	Multiethnic/ Gujarati	124	39	2	0.8697	0.1303	Yes	No		Low	1.5	DS
Coppede et al.	Italy	Italian	39	54	18	0.5946	0.4054	Yes	No		High	2	DS
		DS Group:	163	93	20								

Table 2: Case Data by Group

Source	Geographic Location	Ethnicity	CC	CT	TT	p	q	HWE	National Folate Fortification	Individual Folate Supplementation	Dietary Folate	Maternal Age	Diagnosis Group
Christensen et al.	SE Canada	European Descendant	24	27	11	0.6048	0.3952	Yes	Yes	None		4	NTD
Deb et al.	N. India	Multiethnic: Majority Punjabi	80	25	6	0.8333	0.1667	Yes	No	Yes. Amount varied.	High	1	NTD
Relton et al.	N. England	English	31	36	15	0.5976	0.4024	Yes	No	None		5.5	NTD
Wang et al.	N. China	Han Chinese	31	73	40	0.4688	0.5313	Yes	No	Yes, amount varied		1	NTD
		NTD Group:	166	161	72								
Makino et al.	Japan	Yamato	56	55	14	0.668	0.332	Yes	No	None	High	2.5	RPL
Wolski et al.	Poland	Polish	165	153	41	0.6727	0.3273	Yes	No	400mg/day		2.5	RPL
		RPL Group	221	208	55								
Coppede et al.	Italy	Italian	20	43	16	0.5253	0.4747	Yes	No		High	2	DS
Rai et al.	India	Multiethnic/ Gujarati	97	40	12	0.7852	0.2148	No	No		Low	1.5	DS
		DS Group:	117	83	28								

Table 3: Age Range Reclassification to Discrete Variable

Age:	Group
20-22.49	1
22.5-25	1.5
25.1-27.49	2
27.5-30	2.5
30.01-32.49	3
32.5-35	3.5
35.1-37.49	4
37.5-40	4.5
40.1-42.49	5
42.5-45	5.5
45.1-47.49	6
47.49-50	6.5
50.1-52.49	7

The recorded ethnicities of participants can be found in Tables 1 and 2, and the geographic spread of the data collected can be found on Figures 3 and 4. These figures show the frequency of the two alleles in a pie chart. It is clear that all of the data come from the Northern Hemisphere. The T allele is virtually absent in Africa and the South Pacific, and the few studies which have been done in South American Indigenous groups do not report it (Reyes et al., 2021).

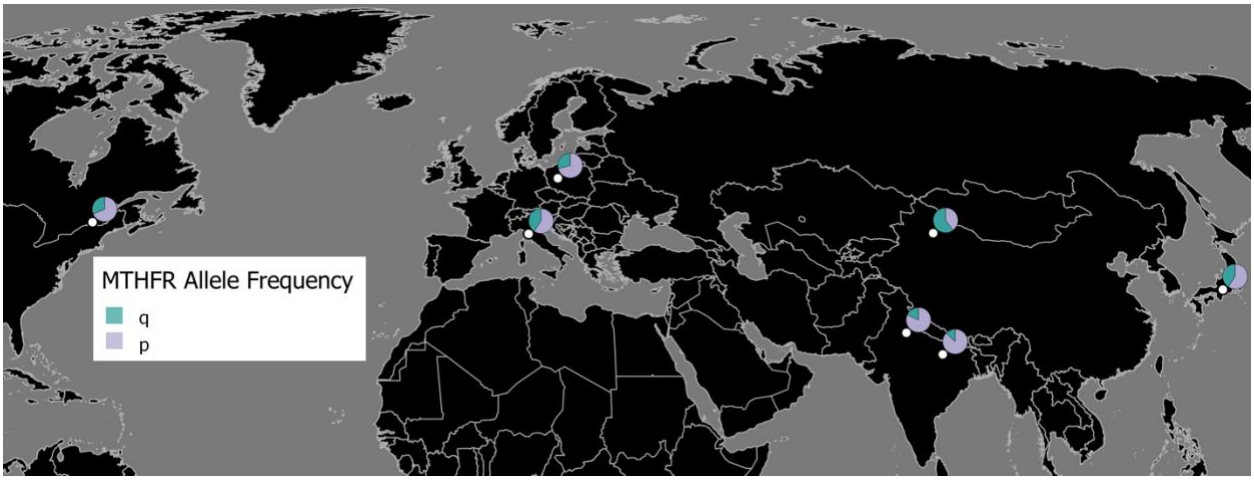

Figure 3: Geographic Spread of Control Data

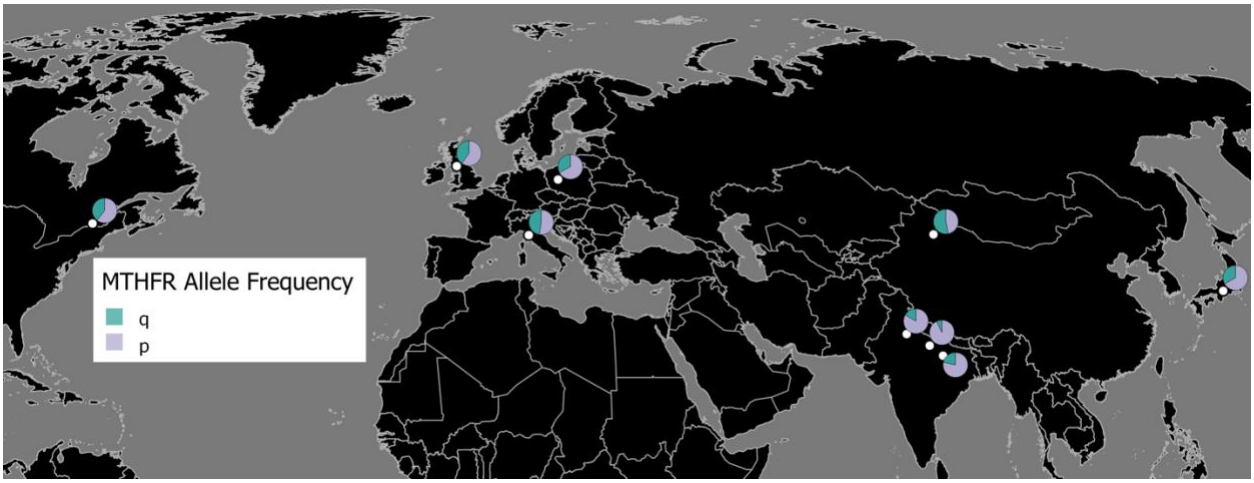

Figure 4: Geographic Spread of Case Data

Data Analysis

All analyses were conducted using Statistical Analysis System (SAS) Copyright © 2016 by SAS Institute Inc., Cary, NC, USA. Case data, that is women who gave birth to a child with DS or NTD or who conceived after suffering from RPL, were conducted separately, in three data subsets. These data are not comparable, as the birth outcome in group is different. At first, control data from each diagnosis group was done separately. Upon realizing that similar results were returned, the control data from all studies included were grouped together, as they all meet the same inclusion criteria (see *Data Collection)* and analysis was conducted on the full controls data set. After completing descriptive statistics and examining our data groups using simple frequency distributions, several logistic regression models were computed for to each data set. We determined that the best fitting model was ordinal logistic regression. Here the dependent variable is number of births by CC, CT, and TT genotypes. The following variables were included as independent predictor variables: presence/absence of folate supplementation, presence/absence of folate-supplemented foods, and maternal age (as explained above).

The general null hypothesis is that the model does not explain variation of the dependent variable. Each model was evaluated with three built-in inferential tests (the likelihood ratio, score test, and Wald test) to determine if the logistic regression provided an improvement over the baseline intercept-only value. The parameters of each model were then individually evaluated by looking at their own p value and the confidence limits of their respective odds ratio (Peng et al., 2002). The odds ratio of a single predictive variable explains its effect on the dependent variable, holding all other predictive variables constant. This is an iterative process that requires entering variables, removing them, and evaluating each model for each data subset (controls and cases for each birth outcome). Odds ratios for the dependent variable cannot be calculated in

logistic regression. To determine the odds and relative risk lent by maternal genotype in the birth outcome, a Chi square test, odds ratio, and relative risk was computed for each diagnosis group to compare the probability of giving birth to a child without congenital defects (in the NTD and DS groups) or to compare the probability of achieving pregnancy (in the RPL group). Cases and controls were included within the same odds ratio; since an odds ratio is determining the odds that an event will occur, both women who had a negative birth outcome (cases) and those who had no obstetrical complications (controls) must be included in the same test.

Upon realizing that the same results were obtained for each individual control data subsets (the data were telling the same story), the analysis was repeated for the entire control dataset. Because all controls meet the same inclusion criteria and had almost the same variables measured across all studies, all of the control data were comparable and could be grouped into one large dataset. The same progression of analysis was completed with the large control dataset: logistic regression with the three inferential statistic tests, the Wald chi-square test for significance, and the odds ratio of the predictor variables (maternal age and folate status).

RESULTS

Down Syndrome Group

DS Case Subset

Table 4a: Frequencies of Down Syndrome Case Subset by Age and Genotype

Table of Genotype by Age				
Genotype	**Age**			
	1.5	**2**	**2.5**	**Total**
CC	97 17.6	111 20.15	31 5.63	239 43.38
CT	40 7.26	119 21.6	88 15.97	247 44.83
TT	12 2.18	16 2.9	37 6.72	65 11.8
Total	149 27.04	246 44.65	156 28.31	551 100

Table 4b: Chi-Square Results of Down Syndrome Case Subset Demonstrating Significance

Statistic	DF	Value	Prob
Chi-Square	4	78.7649	<.0001
Likelihood Ratio Chi-Square	4	80.1582	<.0001

This data subset includes only women who gave birth to a child with Down Syndrome (Coppede et al., 2006; Rai et al., 2006). In these studies, age groups 1.5, 2, and 2.5 were represented. Table 4a shows the number of Down Syndrome (DS) births by maternal age and genotype. 274 participants were deleted due to missing values for one or more variables. The Chi-square and likelihood ratio tests reject the null hypothesis of no association between genotype and age (Table 4b). Here, the outcome is DS birth, maternal genotype is the dependent variable, and age is an independent predictor variable. These data suggest that the outcome of pregnancy is affected by maternal genotype. No independent variable contributed significantly to the model including age, where the point of reference is the TT genotype (Table 4c).

Table 4c: Results of DS Case Subset Logistic Regression Demonstrating Genotype Contribution to Outcome

Analysis of Maximum Likelihood Estimates						
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	**CC**	1	0.4025	0.1226	10.7744	0.0010
Intercept	**CT**	1	2.1852	0.1993	120.1930	<.0001

Here, TT women have the lowest predicted fecundity. CC women have slightly greater fecundity than do TT women ($p = 0.001$), while CT women are predicted to have the highest fecundity ($p < 0.0001$).

DS Control Subset

Table 5a: Frequency of DS Control Subset by Age and Genotype

Table of Genotype by Age				
Genotype	**Age**			
	1.5	**2**	**2.5**	**Total**
CC	124	113	66	303
	18.42	16.79	9.81	45.02
CT	39	243	0	282
	5.79	36.11	0	41.9
TT	2	75	11	88
	0.3	11.14	1.63	13.08
Total	165	431	77	673
	24.52	64.04	11.44	100
Frequency Missing = 118				

Table 5b: Chi-Square Results of Down Syndrome Control Subset Demonstrating Significance

Statistic	DF	Value	Prob
Chi-Square	4	186.416	<.0001
Likelihood Ratio Chi-Square	4	224.736	<.0001

The DS controls subset includes the women recruited within the DS studies who did not give birth to children with Down Syndrome. These women were defined as controls within the source studies and gave birth to a child with no obstetrical complications (Coppede et al., 2006; Rai et al., 2006). In this group, 118 participants were deleted due to missing values and only age groups 1.5, 2, and 2.5 are represented in this sample (Table 5a). Here, the dependent variable is again maternal genotype and age is an independent predictor variable. Age contributed to the

model in addition to maternal genotype (Table 5b), where the point of reference is the TT genotype.

Table 5c: Results of DS Control Subset Logistic Regression Demonstrating Genotype Contribution to Outcome

Analysis of Maximum Likelihood Estimates						
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	**CC**	1	6.5419	0.9230	50.2306	<.0001
Intercept	**CT**	1	9.0107	1.0008	81.0673	<.0001
Age		1	-3.6103	0.5281	46.7408	<.0001

The model is highly significant ($X^2 = 186$, df = 4, $p < 0001$) and shows that CT women are predicted to have a greater fecundity than CC or TT women ($p < 0.001$), though CC women are also predicted to have higher fecundity than TT women, who are modeled to have the lowest fecundity. For all women, the effect of age is to lower predicted fecundity by 3.6 units, where the unit is a categorical variable constructed for this study. As age increases by one unit, fecundity will decrease by 3.61.

Odds Ratios

A Chi square test, odds ratio, and relative risk was computed to compare the probability of giving birth to a child with DS to determine the risk lent by different maternal genotypes. All DS case and control individuals minus those missing data for any variables are included in the odds ratio estimates (Coppede et al., 2006; Rai et al., 2006). Upon initial comparison, no significant relationships were found between variables (data not shown). Due to the size of the data set, the data was then broken up into the discrete age categories. At this level of analysis, several associations were found, but no patterns emerged. There does not appear to be any

systematic difference between birth outcomes (mothers who gave birth to a child with or without DS) by maternal genotype.

Recurrent Pregnancy Loss Group

Table 6a shows the number of pregnancies within the group of women who previously suffered from RPL by maternal age and genotype; in other words, the case group (Makino et al., 2004; Wolski et al., 2017). Results indicate that genotypes and age are not independent (Table 6b). Table 7a shows the number of pregnancies within the control group, which is comprised of women who achieved pregnancy without history of RPL or spontaneous abortion and gave birth to a healthy child in the absence of obstetrical complications (Makino et al., 2004; Wolski et al., 2017). Within the control group, an interaction between genotype and age is also present, even though the age categories are not the same as those of the case participants (Table 7b). At this point, it was concluded that the interaction among age and genotype was equal in both cases and controls.

Table 6a: Frequency of RPL Case Subset by Age and Genotype

Genotypes	Age			
	1.5	**2**	**2.5**	**Total**
CC	104	75	247	426
	9.56	6.89	22.7	39.15
CT	208	6	240	454
	19.12	0.55	22.06	41.73
TT	59	3	146	208
	5.42	0.28	13.42	19.12
Total	371	84	633	1088
	34.1	7.72	58.18	100

Table 6b: Chi-Square Results of RPL Case Subset Demonstrating Significance

Statistic	DF	Value	Prob
Chi-Square	4	127.9820	<.0001
Likelihood Ratio Chi-Square	4	131.1489	<.0001

Table 7a: Frequency of RPL Control Subset by Age and Genotype

Genotypes	Age			
	2	**2.5**	**6.5**	**Total**
CC	107	241	94	442
	9.43	21.23	8.28	38.94
CT	34	286	156	476
	3	25.2	13.74	41.94
TT	15	137	65	217
	1.32	12.07	5.73	19.12
Total	156	664	315	1135
	13.74	58.5	27.75	100

Table 7b: Chi-Square Results of RPL Control Subset Demonstrating Significance

Statistic	DF	Value	Prob
Chi-Square	4	71.2675	<.0001
Likelihood Ratio Chi-Square	4	69.9533	<.0001

Table 8a: Frequency of RPL Cases and Controls with Folate Supplementation by Genotype

Genotypes	Sample Size
CC	85
CT	32
TT	15

We tested if there was a difference in genotype frequency between cases and controls and found that there was no significant difference between the two subsets (data not shown; $X^2 = 0.0123$; df = 2; p=0.99). For that reason, the case and controls data subsets were merged into one dataset to investigate the null hypothesis that maternal genotype does not affect the ability to become pregnant. With this larger sample, folate supplementation was held constant, so participants whose access to folate was unknown were eliminated. That decreased the sample size considerably (Table 8a). To obtain odds ratios for these participants, I performed an odds ratio calculation using the *proc freq* procedure of SAS for two genotypes and for cases and controls. I show in Table 8b (where none of the included women supplemented with folate) and 8c (where all included women supplemented with folate) the number of participants by genotype and case or control categories. Remember that case women are those who suffered

from RPL prior to becoming pregnant, while control women did not have any history of RPL and had a normal pregnancy.

Table 8b: Frequency by CC and CT Genotype of RPL Cases and Controls with No Folate Supplementation

No Folate Supplementation			
Genotypes			
	Cases	**control**	**Total**
CC	56	29	85
			72.65%
CT	0	32	32
			27.35%
Total	47.86	52.14	
			n=117

The chi-square is highly significant (X^2 = 40.43, df = 1, $p < 0.0001$). The odds ratio is not computed due to the 0 value for CC cases (Table 8b). With supplementation (Table 8c), CT women recorded as cases are not significantly different from that of CC women. I then proceeded to compute the odds ratio of CC and TT women in cases and control groups, controlling for supplementation. Results are shown in Tables 8d and 8e. As we had seen in the previous comparison, TT women are only predicted to be cases when supplementing (X^2 = 22.45, df = 1, $p < 0.0001$). Otherwise, TT women are more likely to be controls, or women who did not suffer from RPL (X^2 = 1.98, df = 1, p = 0.158).

Table 8c: Frequency by CC and CT Genotype of RPL Cases and Controls with Folate Supplementation

Individual Folate Supplementation. Genotypes	**Cases**	**control**	**Total**
CC	165	201	366
			53.59%
	45.08%	54.92%	
CT	153	164	317
			46.41%
Total			
	46.56%	53.44%	n=683

Table 8d: Frequency by CC and TT Genotypes of RPL Cases and Controls with No Folate Supplementation

No folate supplementation. Genotypes	Group Cases	Control	Total
CC	56 100	29 65.91	85 85
TT	0 0	15 34.09	15
Total	56	44	100

The results of the comparison between CC and TT participants also indicates that both heterozygous CT and homozygous TT individuals who receive folate supplementation have a higher probability of being cases.

Table 8e: Frequency by CC and TT Genotypes of RPL Cases and Controls with Folate Supplementation

Folate Supplementation Genotypes	Group Cases	Control	Total
CC	165	201	366 82.81
TT	41	35	76 17.19
Total	206 46.61	236 53.39	442 100

These analyses indicates that folate supplementation in women who have had multiple recurrent pregnancy losses may be helpful in completing a pregnancy.

Neural Tube Defect Group

NTD Case Subset

The NTD case subset includes mothers who gave birth to a child with one or more neural tube defects (Christensen et al., 1999; Deb et al., 2011; Caroline L. Relton et al., 2004; Y. Wang et al., 2015b). Here, the modeled outcome is the birth of children with neural tube

Table 9a: Frequency of NTD Case Subset by maternal genotype.

Response Profile		
Ordered Value	**Genotype**	**Total Frequency**
1	CC	252
2	CT	239
3	TT	94

defects by the dependent variable of maternal genotype and the independent variable folate supplementation. Participants without reported information for their folate supplementation and age categories were excluded, which reduced the sample sizes (Table 9a). This was done because of the known importance of folate in preventing NTD.

Table 9b: Results of NTD Case Subset Logistic Regression Demonstrating Genotype, Insolation, and Folate Supplementation Contribution to Outcome

Analysis of Maximum Likelihood Estimates						
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	**CC**	1	0.6465	0.163	15.7274	<.0001
Intercept	**CT**	1	2.7545	0.1983	192.989	<.0001
Folate Supplementation		1	-1.02	0.1984	26.4391	<.0001

Table 9c: Log Odds Ratio Estimate of Folate Supplementation's Effect in Birth Outcome

Odds Ratio Estimates			
Effect	**Point Estimate**	**95% Wald Confidence Limits**	
Folate Supplementation	0.361	0.244	0.532

In this subset, TT women are predicted to have the lowest fecundity, meaning they are modeled to have the fewest instances of birth of children with NTDs. CC women are predicted to have slightly higher fecundity than TT women, indicating that the risk of giving birth to a child with NTD is slightly higher ($p < 0.0001$), while CT women are predicted to be much more fecund than TT women ($p < 0.0001$). This indicates that CT women are predicted to have the highest instances of NTD birth when maternal genotype is the dependent variable. Folate supplementation was predicted to lower the log odds of giving birth to a child with one or more NTD. I calculated an odds ratio of 0.361 with a 95% confidence interval of 0.244-0.532,

indicating that the log odds of giving birth to a child with NTD is 36% lower when supplementing with folate (holding all other variables constant).

NTD Control Subset

This subset of NTD Controls is comprised of women who gave birth to a child in the absence of any obstetrical complications, where the children born did not have any NTDs or any other congenital defect (Christensen et al., 1999; Deb et al., 2011; Y. Wang et al., 2015b). The participants included in the control subset are included in Table 10a, which shows the number of births by maternal genotype. Here, the modeled outcome is the birth of children without NTDs by the dependent variable of maternal genotype and the independent variable folate supplementation. Again, participants without recorded folate supplementation or age values were removed, which reduced the number of participants included. The logistic regression model for this group of controls is shown in Table 10b.

Table 10a: Frequency of NTD Control Subset by Genotype

Response Profile		
Ordered Value	**genotype**	**Total Frequency**
1	CC	289
2	CT	145
3	TT	178

Table 10b: Results of NTD Control Subset Logistic Regression Demonstrating Genotype, Insolation, and Folate Supplementation Contribution to Outcome

Analysis of Maximum Likelihood Estimates						
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	**CC**	1	0.2398	0.2035	1.3876	0.2388
Intercept	**CT**	1	1.4402	0.2134	45.5655	<.0001
Folate Supplementation		1	1.2778	0.28	20.8194	<.0001

Figure 10c: Log Odds Ratio Estimates of Folate Supplementation's Effect on Birth Outcome

Odds Ratio Estimates			
Effect	**Point Estimate**	**95% Wald Confidence Limits**	
Folate Supplementation	3.589	2.073	6.213

Once again, TT women are predicted to have the lowest fecundity. CC women are predicted to have slightly higher fecundity than TT women (a non-significant difference, $p = 0.239$) while CT women are predicted to have much greater fecundity than do TT women ($p > 0.0001$). Furthermore, folate supplementation was a significant predictor in this model. As folate increases by one unit, the predicted fecundity of all women increases by 1.28. I calculated an odds ratio of 3.589 with a 95% confidence interval of 2.073-6.213 indicating that the log odds of giving birth to a child without NTD or any other congenital defect is 3.5 times greater when mothers take a folate supplement (Table 10c).

Odds Ratio Estimates

I then calculated chi-square tests and odds ratios to quantify the differences seen between the maternal genotypes in the previous logistic regression models. Here, cases and controls of two genotypes are considered at the same time as both the negative and positive outcome must be present to calculate an odds ratio. Without considering any independent variables, there are significant differences between the effects of the maternal genotypes. The incidence of NTD birth is significantly different between the three genotypes.

Table 10d: Frequency of CC and TT Genotypes of the NTD Group with Cases and Controls

	Case	**Control**	**Total**
CC	86	193	279
TT	66	19	85
Total	152	212	364

When comparing CC and TT cases (those who gave birth to a child with NTD) vs CC and TT controls (those whose child was born without congenital defects), we find that 77.65% of TT women gave birth to a child with NTD. These genotypes are significantly different (X^2 = 58.729, df = 1, $p < 0.0001$).The odds ratio of 0.13 with a 95% confidence interval of (0.0725-0.2268) indicates that that CC women are 13% less likely to have an NTD than TT women. The relative risk of 3.1 with a 95% confidence interval of 2.04-4.63 indicates that the control group is comprised of a far greater amount of CC women than TT women. These results indicate that TT women are more likely to give birth to an NTD child than CC women.

The differences between CC and CT women within the NTD group are also significant. The odds ratio of 0.32 indicates that CC women are 32% less likely than CT women to have a child with NTDs. CT and TT women are also significantly different in the instance of NTD birth. The odds ratio of 3.27 with a 95% confidence interval of 2.31-4.64 indicates that CT women have 3.2 times higher odds of giving birth to a child with NTD than TT women.

Table 10e: Odds Ratio and Relative Risk, CT vs. TT Women from NTD Literature

Odds Ratio and Relative Risks			
Statistic	**Value**	**95% Confidence Limits**	
Odds Ratio	**0.3915**	0.2210	0.6936
Relative Risk (Column 1)	**0.7422**	0.6337	0.8692
Relative Risk (Column 2)	**1.8956**	1.2415	2.8944

When considering folate supplementation, interesting relationships become apparent. I considered the NTD source material where individuals did supplement with folate separately from those that did not. When considering women who did not supplement with folate (Christensen et al., 1999; Caroline L. Relton et al., 2004), TT women had 3.9 times higher odds of giving birth to a child without NTD than did CC women (OR = 3.9394, 95% CI = 1.2489-11.5049).

Table 10f: Chi-Square Tables Demonstrating CT Women's Higher Probability for NTD Birth When Supplementing with Folate

	Case	Control	Total
CC	148	245	393
CT	187	109	296
Total	35	364	689

	Case	Control	Total
CT	187	109	296
TT	88	168	256
Total	275	277	552

When women were supplementing with folate, we found that the effects of folate supplementation do not seem to reduce the number of NTD births in CT women. The relative risk value for case women of 0.5961 indicate that CC women are much less likely to have a child with NTD than are CT women when supplementing with folate (R = 0.5961, 95% CI = 0.5110-0.6954). Again, CT women were much more likely to give birth to children with NTDs than TT women (R=1.8378, 95% CI = 1.5194-2.2231). However, there was no distinguishable difference between the probability of having a child with NTD between CC and TT women who took folate supplements. In the logistic regression models, we found that folate supplementation reduced the likelihood of giving birth to a child with NTD. Here, we found that folate supplementation may aid in the prevention of NTD for TT women to a greater extent than it does for CT women.

Preliminary Conclusions

Through these initial tests, I have concluded the following:

1. In all cases presented within this study, TT women have the lowest predicted fecundity followed by CC women.
2. Except for one comparison, CT women always had a significantly higher number of pregnancies. This was true even when controlling by age, folate supplementation, or any other independent predictor variable. Sometimes these women had more than twice the number of pregnancies than CC women did.
3. The results were virtually identical for control and case groups.

Therefore, I proceeded to perform the same progression of analyses for all control women by combining the three control subsets into one large dataset. These are comparable data: all participants were women who did not experience RPL and gave birth to a child with no adverse health conditions present. The aim of this secondary analysis is to ask the following question: Is it possible that CT women have higher numbers of poor pregnancy outcomes (RPL, DS, and NTD) because these women also have higher numbers of pregnancies? In short, are CT women more fecund? If this is the case, then this may be the natural selective mechanism explaining the high frequency of this otherwise negative polymorphism.

Results of Control Dataset Analysis

This is the exploration of the full control dataset, which includes all control participants from each study included in this review (Table 1). Analysis was restricted to women whose age and folate status was known, which reduced sample size (Table 11a).

Table 11a: Sample Size of Control Dataset Where Age and Folate Supplementation Status are Known

Response Profile		
Ordered Value	**Genotypes**	**Total Frequency**
1	CC	178
2	CT	96
3	TT	24

Table 11b: Results of Control Dataset Logistic Regression Demonstrating Genotype and Age Contribution to Outcome

Analysis of Maximum Likelihood Estimates						
Parameter		**DF**	**Estimate**	**Standard Error**	**Wald Chi-Square**	**Pr > ChiSq**
Intercept	**CC**	1	2.0408	0.3587	32.3672	<.0001
Intercept	**CT**	1	4.216	0.4364	93.3109	<.0001
Age		1	-1.3097	0.264	24.6034	<.0001

Once again, TT women are likely to have the lowest fecundity, CC women are predicted to have greater fecundity over TT women, while CT women are likely to have the highest fecundity, holding age constant (Table 11b). In summary, the data analysis indicates that CT women are likely to be more fecund than either CC or TT women. If CT women have greater numbers of birth defects or are more likely to suffer from RPL, it is also because these women have a greater overall number of pregnancies.

DISCUSSION

The results show that CT women are more fecund than CC and TT women, yet also more commonly suffer from RPL or give birth to a child with NTD and DS. The current clinical literature emphasizes that the TT genotype will face most drastic effects of the mutation by way of higher homocysteine, lower methylation status, and lower levels of 5-methylTHF, and generally states that CT women face these effects to a significantly lower degree (Friso et al., 2002; Liew & Gupta, 2015; Servy et al., 2018). The results found within this study demonstrate that the effects leveraged by the mutation are still enough to contribute lowered fertility and poor birth outcomes. Moreover, this pattern signals that some aspect of the phenotype portrayed in CT individuals is being selected for. Greater fecundity of CT women may explain part of this selective force, though this is not a conclusive statement. The results of this study also show that CT women are more likely to suffer from RPL or give birth to a child with NTD or DS. I have concluded that this greater number of fertility and pregnancy issues arise as a result of the increase in total pregnancies in CT women (Table 11b).

The results of this study also show that folate supplementation decreased the odds of pregnancy resulting in NTD for TT women but did not decrease the probability of pregnancy resulting in NTD for CT women (Table 9b, 10f). It also appears to increase the probability of a successful pregnancy in women who have suffered from RPL, but otherwise was not a significant contributor to the fecundity of other groups within this study. While supplementation with folic acid is the current recommended practice upheld by the CDC and most clinical physicians, there is a growing body of literature that suggests that supplementation with 5-

methylTHF is just as effective for wild-type individuals and is safer and more effective for heterozygous or homozygous individuals (Clément et al., 2020; Crider et al., 2011; Lucock & Yates, 2005, 2009; Plumptre et al., 2015; Servy et al., 2018). The CDC's website cites that folic acid is the only supplement known to prevent NTD and should therefore continue the recommendation that all pregnant women and those trying to become pregnant should supplement with folic acid.

Our results suggest that this is not a universal truth for all populations. A large, blind study comparing the efficacy of folic acid versus 5-MTHF in preventing NTD should be completed in order to better understand the relationship between folates and NTD. Furthermore, the CDC should reevaluate the literature and consider amending their statement on folic acid supplementation. Their website cites literature that is outdated and seemingly biased. Neither their web page devoted to the MTHFR mutation and folate supplementation, nor the citations referenced show any clinical literature that has demonstrated the negative affects leveraged by folic acid supplementation or the efficacy of 5-MTHF supplementation. Their current statement simply no longer matches the current literature and reads as follows:

> "You might have read or heard that folic acid is not safe if you have one or two copies of the MTHFR C677T variant. This is not true. Even if you have one or two copies of the MTHFR C677T variant, your body can safely and effectively process the different types of folate, including folic acid." (CDC, https://www.cdc.gov/ncbddd/folicacid/mthfr-gene-and-folic-acid.html)

Limitations

The use of previously conducted data is generally considered a limitation due to the limited knowledge that is made public. Furthermore, many of the studies first discovered through

the literature search could not be included due to poor study design. Thirty-five articles failed to include folate supplementation status or report maternal age and thus could not be included (Figure 2). Seven more articles could not be included because the study design included multiple birth outcomes within a single case group.

Maternal age and folate supplementation status were included in this study as independent predictor variables due to the known ways that folate status and maternal age can impact pregnancy and fertility. For example, it is generally accepted that lower folate can make conception more difficult or may result in adverse birth outcomes and increasing maternal age may lead to the same results. However, the most common variable missing from literature was the folate status of the women included. Less commonly, maternal age was not included. The results concluded in this study further show that genotype cannot be considered in contributing to the birth outcomes studied alone, and maternal age and folate supplementation status should always be included to fully understand the interactions at play.

A significant observation in the NTD Group data collection was individuals who gave birth to a child with syndromic NTD are often excluded as part of the study design. Frequently, studies on the association of NTD and the mutation are limited only to spina bifida or spina bifida occulta and exclude all other forms of NTD or any offspring who are deceased due to syndromic NTD. The result of these exclusions is an underreporting of the totality of the way the MTHFR C677T mutation can contribute to NTD. It also impacted the data collected in this paper; only half of the articles included in this study allowed for the inclusion of participants who gave birth to children with syndromic NTD.

Lastly, the clinical nature of this literature leads to the frequent misclassification of ethnicity. Ethnicity is often listed in racial terms (e.g., “Caucasian, Black, White”) or nationality

(e.g., “Polish” or “Czech”) rather than the historical or ancestral ethnic group of individuals. These terms are not descriptive and tell us nothing about the historical allele frequency within the population or whether to expect the allele frequency to be high or low in that group. In some instances, when ethnicity was simply listed using a broad racial, literature had to be excluded due to the highly heterogenous nature of the location from whence they came. For example, a South African paper documenting the allele frequency and instance NTD within the population cited the participant population as Black rather than one of the many ethnic groups found in South Africa (Ubbink et al., 1999). Furthermore, these terms continue the false narrative of “race-based medicine,” or the idea that certain racial groups are predisposed to certain health conditions based on race alone. As we fundamentally agree that race is a constructed identity and is in no part inherently biological, clinicians must report ethnicity data more responsibly.

Relevance to Applied Anthropology

The current literature includes a plethora of studies on the association of the mutation with male infertility and fetal death but fails to focus on the facilitator and carrier of the pregnancy. One of the goals of this study was to place focus on this erased population by only collecting data pertaining to mothers and carriers of the pregnancy. It is important to note that because all data collected for the use in this paper are from previously conducted clinical studies, the data most-likely only represent cis-gendered women. Though many biologists and biological anthropologists alike agree that chromosomal or genital-based sex assignment at birth is not what defines one’s sex or gender identity, none of the reference papers included in this review make any mention of including patients other than those traditionally considered women.

That is, only those who were assigned the female sex at birth can be assumed to have been included in the reference data. It should be noted that people of many genders experience

pregnancy and are also more likely to have inadequate access to health care, face discrimination from health care providers, and may not seek care due to fear of stigma (Moseson et al., 2020). While it was a goal of this study to encompass more experiences than solely those of cis-gendered women, it was not possible in this setting. Nonetheless, in my future applied dissertation research among the Bribri and Cabecar of Costa Rica, I intend to foster an environment which celebrates and includes genders outside the Western binary.

Additionally, the clinical nature of the data available has excluded Native and Indigenous populations. As previously noted, the highest frequencies found in the world are recorded in Mexico and Central American Indigenous populations. Sadly, there are extremely limited data available for the clinical ramifications of the T-allele in Indigenous populations, particularly in Mexico and Central America due to the isolated nature of many Indigenous communities. Isolated communities have limited access to fertility clinics and biomedical hospitals, which largely exclude them from the available literature. Moreover, these populations have not been acknowledged in the history of the research of this polymorphism. Most of the studies on the clinical outcomes of this mutation have focused on European (Mayor-Olea et al., 2008b) or East Asian (Y. Wang et al., 2015b) populations. We do not know if Native American communities in their ancestral lands with their own diet suffer of pathology related to the mutation. However, their absence from the clinical literature is a loud reminder of unequal representation, particularly of Native women.

Furthermore, times of epidemiological transition in Mexican and Central American indigenous communities may amplify the effects of the mutation. As neoliberalism expands and the need to enter the cash economy increases, it is a concern that Native and Indigenous folks with the mutation may see worsened health outcomes. Facets of assimilated Western life that

contribute to the morbidity of chronic diseases, such as a sedentary lifestyle and loss of traditional diet, will only intensify the effects of the mutation. Furthermore, common health outcomes such as cancer, obesity, cardiovascular disease, and infertility are often compounding and require long-term healthcare. These are chronic conditions which in Western society are frequently perceived as resulting from one's own poor lifelong health skills.

For example, the development of obesity in an individual in the United States is not judged through the consideration of the economic or systemic barriers that may decrease access to healthy foods, a safe neighborhood to walk or run in, or the time an individual must spend to generate enough income to stay alive. The development of obesity in an individual is instead judged as that individual's fault regardless of all other factors. It is concerning to think that this assumption may be passed onto other individuals who are being forced by neoliberalism to abandon their sovereignty and live a lifestyle that may be damaging to their health.

Applied research to investigate the role of the mutation in the Native and Indigenous communities of Mexico and Central America must be completed. Again, I iterate that these populations show the highest frequencies of this mutation anywhere in the world, yet the phenotypic effects of the mutation have been largely unstudied within these populations (Reyes et al., 2021). Individuals have a right to autonomy over their own health and should be aware of the ways this mutation may be affecting their lives.

CONCLUSIONS

Since its discovery in the late 1990s, the MTHFR C677T mutation has been the focus of many geneticists, physicians, and biologists due to its wide global distribution and many associated health effects, caused by hyperhomocysteinemia, lower methylation, and lower levels of circulatory folate. Evolutionary theory leads us to predict that populations have evolved and maintained high frequencies of a deleterious allele in their homelands because somehow in their niche the allele is not deleterious. The results of this study indicate that the observed higher fecundity of CT women may explain part of this evolutionary advantage. However, the relationship between folates and adverse birth outcomes must be further explored.

This study has closed a fraction of the gap in knowledge at the nexus of population genetics, reproductive justice, Indigenous rights, and evolutionary biology. Nevertheless, before more applied work can be completed, baseline investigatory work such as this review was necessary to first quantify the way populations react to selection.

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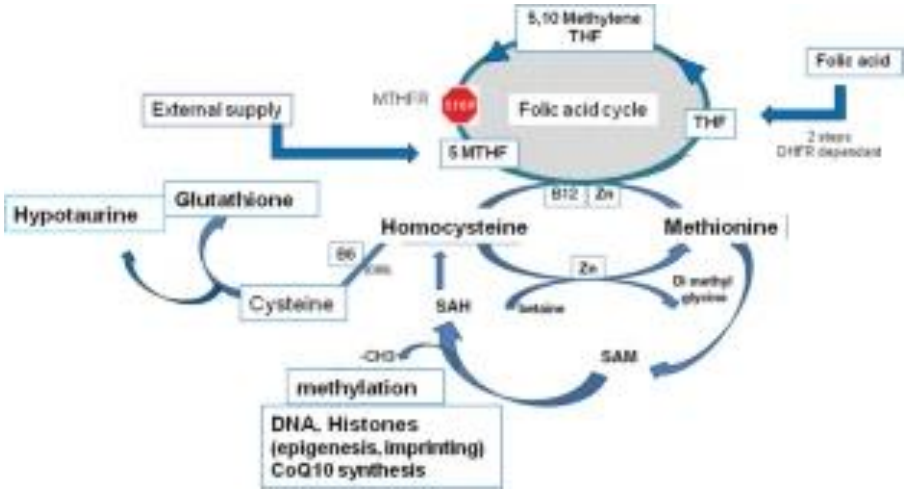
APPENDICES

Appendix A: Copyright License



Reprinted by permission from Springer Nature Customer Service Center GmbH: Springer Nature. Journal of Assisted Reproduction and Genetics. MTHFR isoform carriers. 5-MTHF (5-methyl tetrahydrofolate) vs. folic acid: a key to pregnancy outcome: a case series. Servy, E. J., Jacquesson-Fournols, L., Cohen, M., & Menezo, Y. J. R. Copyright © 2018.

Appendix B: Complete List of Articles from Which Data was Collected

Christensen, B., Arbour, L., Tran, P., Leclerc, D., Sabbaghian, N., Platt, R., Gilfix, B. M., Rosenblatt, D. S., Gravel, R. A., Forbes, P., & Rozen, R. (1999). Genetic polymorphisms in methylenetetrahydrofolate reductase and methionine synthase, folate levels in red blood cells, and risk of neural tube defects. *American Journal of Medical Genetics*, *84*(2), 151–157. https://doi.org/10.1002/(SICI)1096-8628(19990521)84:2<151::AID-AJMG12>3.0.CO;2-T

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Wang, Y., Liu, Y., Ji, W., Qin, H., Wu, H., Xu, D., Turtuohut, T., & Wang, Z. (2015b). Variants in MTHFR gene and neural tube defects susceptibility in China. *Metabolic Brain Disease*, *30*(4), 1017–1026. https://doi.org/10.1007/s11011-015-9662-4

Wolski, H., Barlik, M., Drews, K., Klejewski, A., Kurzawińska, G., Ozarowski, M., Łowicki, Z., & Seremak-Mrozikiewicz, A. (2017). Contribution of inherited thrombophilia to recurrent miscarriage in the Polish population. *Ginekologia Polska*, *88*(7), 385–392. https://doi.org/10.5603/GP.a2017.0072

Appendix C: IRB Determination of Not Human Subjects Research

Notification of Not Human Research Determination

To:	Lorena Madrigal
Link:	STUDY002190
P.I.:	Lorena Madrigal
Title:	The C677T mutation and fertility
Description:	The IRB reviewed this submission and assigned a determination of not human subjects research.