Exploration of Methylenetetrahydrofolate Reductase Gene Polymorphisms and Preliminary Links to Neurodevelopmental Disorders

Honors Thesis

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Abstract

The methylenetetrahydrofolate reductase (MTHFR) gene encodes an enzyme involved in folate metabolism, specifically the production of 5-methyltetrahydrofolate — the metabolically active form of folate, a crucial nutrient for fetal development. Mutations in the *MTHFR* gene lead to folate deficiency, which contributes to multiple developmental complications, such as neural tube development deficits. Preliminary research shows a correlation between MTHFR polymorphisms and the onset of neurodevelopmental disorders, more specifically Attention Deficit Hyperactivity Disorder (ADHD) and Autism Spectrum Disorder (ASD). These disorders can impact an individual's ability to perform daily activities, such as work and maintaining social relationships. This thesis explores the biochemical, genetic, and physiological mechanisms for MTHFR polymorphisms, as well as directing future research towards investigating a causal relationship through transgenic models in mice as well as the possible therapeutic benefits of 5-MTHF supplementation. Continued investigation into the relationship between MTHFR polymorphisms and neurodevelopmental disorders can further inform targeted interventions.

Introduction

Folate metabolism is a necessary pathway responsible for nucleotide synthesis and methylation of DNA sequences (Spellicy et al., 2012). These processes are especially essential during fetal development. Nucleotide synthesis contributes to DNA formation, while DNA methylation is utilized for epigenetic modification of genetic information. More specifically, nucleotide synthesis is key for the process of neural tube development in fetuses, in which the rapidly dividing cells during this development require a large number of nucleotides to maintain DNA synthesis (Imbard et al., 2013). A folate deficiency, therefore, would create a shortage in nucleotides that are critical to DNA synthesis, which can hinder neural tube development. Folate deficiencies can also create a deficiency in different neurotransmitters, such as dopamine and norepinephrine which are crucial to daily function (Miller, 2008). Abnormal DNA methylation has been associated with the development of Autism Spectrum Disorder (ASD) and other neurological disorders (reviewed in Araszkiewicz et al., 2025).

The methylenetetrahydrofolate reductase (MTHFR) gene has recently become a focus of research for its mutational correlation with the onset of neurodevelopmental disorders. The mechanisms pertaining to the development of disorders like ASD are still not fully understood. This uncertainty contributes to a fundamental lack of knowledge required for therapeutic treatments, whether holistic, pharmacological, or even behavioral, as well as a general lack in biomedical knowledge surrounding these disorders.

Currently, there is no causal relationship determined between the *MTHFR* gene polymorphisms and neurodevelopmental disorders. This thesis reviews the various genetic, biochemical and physiological connections between the two, and considers the potential for a causal relationship through this explanation. Then, this thesis will evaluate various methods for

providing further support, specifically through transgenic mice models that test different concentrations of metabolites under different genotypes, as well as testing the effect of supplementation on these models to treat folate deficiencies caused by *MTHFR* gene polymorphisms.

Further efforts toward unraveling the *MTHFR* gene polymorphisms and their connections with the development of psychiatric and neurodevelopmental disorders are critical to expanding current biomedical knowledge surrounding these disorders. Further research will also prove critical in public health by providing insight into future treatment options that may significantly improve quality of life for those affected individuals.

Section I: Biochemistry, Molecular Genetics, and Physiology

Within human metabolism, there are many different pathways that provide nutrients essential to protein synthesis and function — among them, the folate metabolism pathway is crucial for the performance of cell division and growth, the production of genetic material, and the maintenance of recently divided cells (Carboni, 2022). These mechanisms of rapid cell division are especially necessary in fetal development, and as will be discussed in this thesis, the formation of the neural tubes. Folate is a water-soluble vitamin (Vitamin B9) and must be obtained through diet. It is eventually converted to its active form, 5-methyltetrahydrofolate (5-MTHF), which is then utilized in the synthesis of amino acids and other products.

Folate metabolism begins with the conversion of folic acid into dihydrofolate (DHF) through the dihydrofolate reductase enzyme (DHFR) (Nazki et al., 2014). This enzyme then reduces DHF to tetrahydrofolate (THF). THF is then methylated through serine hydroxymethylase 1, which converts serine to glycine and provides the methyl group, to 5,10-

methylenetetrahydrofolate (5,10-MTHF). MTHFR is utilized for the NADPH-facilitated reduction of 5,10-MTHF to 5-MTHF (Araszkiewicz et al., 2025). 5-MTHF is the biochemically active form of folate, which circulates around the bloodstream. It is then used in the conversion of homocysteine into methionine through the methionine synthase enzyme, which uses vitamin B12 as a cofactor and homocysteine and 5-MTHF as substrates. 5-MTHF is converted into THF in this process, forming a folate cycle (Tate et al., 2024). Methionine is an amino acid used in the synthesis of proteins. It also plays a crucial role in the methylation of DNA in epigenetics via the conversion of methionine to SAM, which is known as S-adenosylmethionine (reviewed in Araszkiewicz et al., 2025). SAM donates the methyl group needed to methylate DNA. A simplified version of this pathway is illustrated in Figure 1.

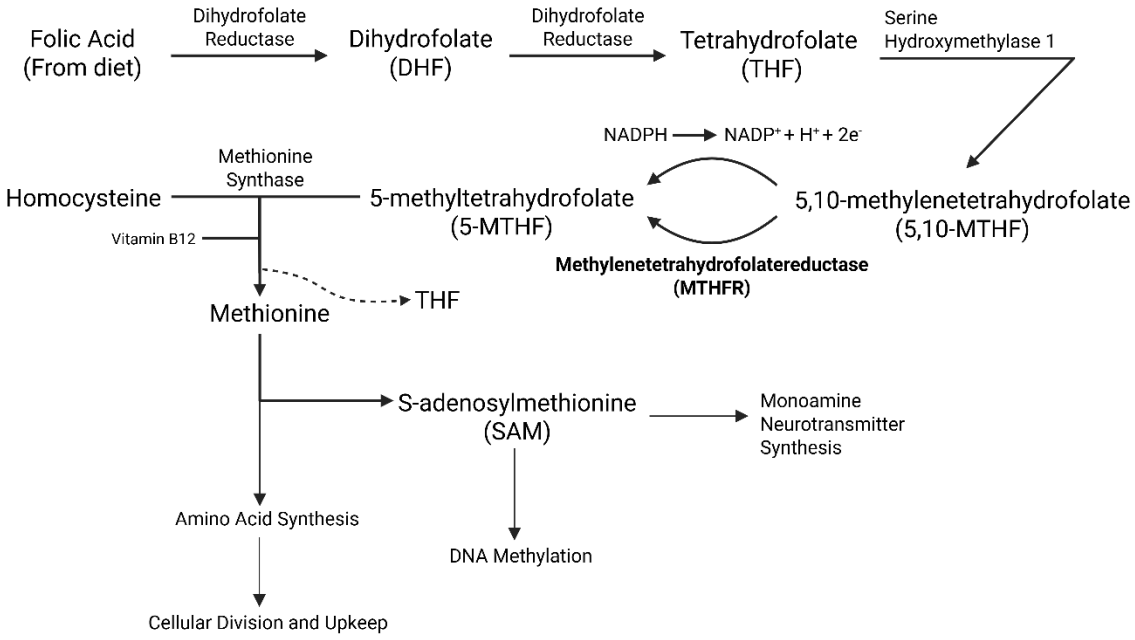

Figure 1: Modified folate metabolism detailing metabolic processes and functions of methionine and SAM.

Schematic of folate metabolism highlighting conversion steps from folic acid to DHF and THF, reduction to 5,10-MTHF and 5-MTHF (via MTHFR), and downstream synthesis of methionine and S-adenosylmethionine (SAM).

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MTHFR provides a crucial regulatory role within folate metabolism – it is the final enzyme required to form 5-MTHF, the biochemically active form of folate, which can then be converted to SAM and homocysteine. Deficiencies in MTHFR reduce available 5-MTHF, leading to downstream decreases in methionine and SAM. Deficiencies in methionine and SAM will have far-reaching impacts on DNA methylation and formation, as well as protein formation and cellular maintenance and neurotransmitter synthesis (Nazki et al., 2014).

As explained by Singh and Munakomi (2025), SAM is a crucial nutrient in the formation of the neural tube in embryos via methylation-dependent processes. More specifically, SAM is utilized in the closure of the neural tube. The neural tube is derived from the ectoderm – the ectoderm being one of three layers in the embryo, the others being the mesoderm and the endoderm. In a process called neurulation, a collection of neuroepithelial cells elongate and form the neural plate. This plate then folds in a process called induction and further elongates, forming neural folds. These folds are pulled together and form the primitive neural tube, signaled by the sonic hedgehog signaling pathway. The primitive neural tube continues to develop with secondary neurulation and eventually becomes the brain and the spinal cord (Singh & Munakomi, 2025). Accordingly, adequate SAM is required for proper neural tube closure.

According to Hasler et al. (2023), SAM also plays a role in preventing neural tube defects (NTDs). SAM's ability to methylate DNA has been shown to be crucial in the prevention of the development of NTDs — DNA methylation can inhibit the transcription of certain genes, which can allow for the fine-tuned control of protein production in specific stages of neurulation and the general development of the neural tube. Abnormal DNA methylation, which can potentially be caused by deficiency in SAM or other related imbalances, has been linked with the

development of ASD (Hasler et al., 2023). This suggests that improper DNA methylation can lead to the transcription of the improper genes or transcription of genes at the wrong time, which can interrupt the systematic flow of neurulation.

Given that SAM is derived from methionine, limited methionine can inhibit methylation-dependent processes of neurulation. Methionine is vital to the development and maintenance of new cells, especially neuroepithelial cells. A deficiency in methionine results in a deficiency in protein synthesis, since it is the initiating amino acid in protein translation. Decreased protein formation results in a decrease in bioavailability of molecular resources, which are used in cell division and maintenance. Consequently, diminished cell division and maintenance will be seen. Neuroepithelial cells, especially during primary neurulation, undergo highly regulated and rapid cell division, which is core to the process in the development of the neural plate and eventual closure of the neural tube.

Neural tube defects have wide-ranging clinical implications and generally result from improper neurulation or incomplete closure of the neural plate. These defects are present in about one to ten per 1000 births and are generally classified as open or closed, depending on whether neural tissue is covered by other tissue (Imbard et al., 2013). They are commonly lethal, especially in defects that directly impact cerebral tissue. NTD development risk has been associated with folate availability in the mother of the fetus. Because these defects correlate strongly with folate metabolism, genetic mutations like MTHFR have become a major focus in research.

Methionine deficiency therefore has far-reaching implications in neural tube development, as decreased amino acid availability impacts neuroepithelial cell division, key to neurulation. Imbard et al. (2013) describe that conditions such as spina bifida are directly

associated with NTDs caused by decreased cell division and growth. This mechanism linking methionine deficiency to neurulation interruptions provides the basis for preliminary association between the *MTHFR* gene and various neurodevelopmental disorders. Given the dependence of these processes on folate metabolism, *MTHFR* gene polymorphisms have become a central focus in researching the onset of NTDs and related neurodevelopmental disorders.

***MTHFR* Gene Polymorphisms and ASD**

The *MTHFR* gene is located on chromosome one of the human genome (*MTHFR* gene, n.d.) and follows autosomal inheritance patterns. Figure 2 shows the molecular structure of the MTHFR protein. There are two primary polymorphisms currently being studied for their implications in various diseases. The 677C>T mutation is more common within the population, with 30-40% of the population carrying at least one polymorphism (Araszkiewicz et al., 2025). This conservative missense mutation results in a substitution of alanine for valine in the 222nd position (A222V), both of which are nonpolar amino acids. The 1298A>C polymorphism is less common, resulting in a nonconservative missense mutation of glutamate for alanine (E429A), with glutamate being a negatively charged amino acid and alanine being a neutral amino acid, thereby possibly affecting electrostatic interactions between amino acids in the protein.

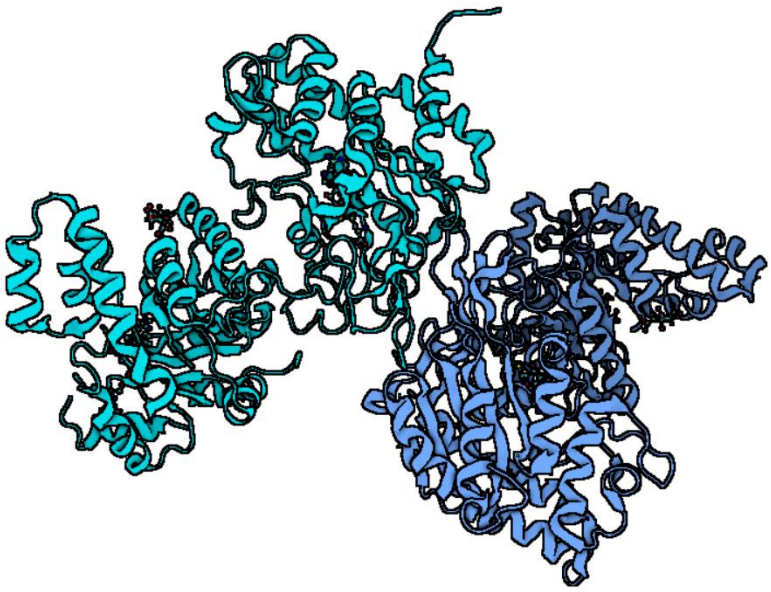

Figure 2. Molecular representation of the MTHFR protein.

The MTHFR protein is comprised of two separate chains, highlighted by teal and light indigo. Secondary structures such as alpha helices and beta-pleated sheets are visible.

Adapted from *Froese, D.S., Kopec, J., Rembeza, E. et al.* (2018). *Structural basis for the regulation of human 5,10-methylenetetrahydrofolate reductase by phosphorylation and S-adenosylmethionine inhibition. Nat Commun* **9**, 2261. https://doi.org/10.1038/s41467-018-04735-2. PDB ID: 6FCX. *Created in BioRender. Gibson, R. (2025). https://BioRender.com/z5q3nbh.*

The mechanistic effects of the 677C>T polymorphism are better characterized, as this mutation occurs within the catalytic domain of the MTHFR enzyme (Araszkiewicz et al., 2025). It causes a 35% decrease in the efficiency of the enzyme per mutated allele. While the 677C>T polymorphism is a conservative missense mutation, the decrease in efficiency can be attributed to the location of the substitution. Due to valine's branched structure, which is slightly bulkier in comparison to alanine, the shape of the enzyme may be slightly altered, which can drastically affect the function and efficiency of the enzyme. The 1298A>C polymorphism causes a 39% decrease in enzyme efficiency in homozygous individuals (Wan et al., 2018). Since the 1298A>C

polymorphism is not located on the catalytic region of the enzyme, it can be inferred that it will have a lower effect on enzyme activity compared to the 677C>T polymorphism.

With a decrease in the availability of 5-MTHF, methionine and SAM levels drop significantly. DNA methylation and protein synthesis will be affected systematically. It is important to mention that other systems may be impacted by MTHFR polymorphisms as well, such as hyperhomocysteinemia in the cardiovascular system due to the buildup of homocysteine throughout the body, which can lead to premature coronary artery disease or recurrent venous thrombosis (reviewed in Araszkiewicz et al., 2025). Other neurological deficits have been observed as well, such as an elevated risk in the development of schizophrenia and bipolar disorder (Wan et al., 2018).

The downstream effects of *MTHFR* gene polymorphisms are foundational to understanding the conceptual link of these polymorphisms to neurodevelopmental disorders. Decreased enzyme efficiency will drop the levels of methionine and SAM due to a lack of availability of 5-MTHF, which will severely impact cellular division. This is especially prevalent in neurulation and can cause improper folding of the neural plate and improper closure, leading to NTDs. These NTDs have been directly associated with an increased risk in development of ASD with four times increase in risk (Hasler et al., 2023).

Mechanisms of MTHFR Polymorphisms and ADHD Development

The mechanisms underlying Attention Deficit Hyperactivity Disorder are quite different in comparison to ASD. One of the hallmark mechanisms of ADHD is the dysregulation of monoamine neurotransmitters, more specifically dopamine and norepinephrine (da Silva et al., 2023). Dopamine receptors D1 and D2 are present in regions of the cranium responsible for

learning, reward pathways and other systems. A deficit in dopamine results in decreased stimulation of these receptors, which could present symptoms such as hyperactivity, impulsiveness, and inattentiveness. Other symptoms such as chronic depression and anxiety can also be seen in patients with ADHD. These symptoms are developed during adolescence and adulthood, whereas NTD and ASD onset most often occur during fetal development.

Dopamine deficits can be attributed to a deficit in SAM, which is important in the donation of a methyl group to a dopamine precursor (Miller, 2008). SAM is also used in the synthesis of other monoamine neurotransmitters, like norepinephrine. This is directly related to a lack of availability of 5-MTHF, since 5-MTHF is a requirement for the synthesis of methionine. The MTHFR polymorphisms operate under this mechanism. Decreased efficiency in the formation of 5-MTHF results in lower amounts of methionine and SAM, which slows dopamine and norepinephrine synthesis. Lower levels of neurotransmitters, specifically dopamine, decreases the frequency of neural firing due to weakened graded potentials, which results in decreased activity in different dopaminergic systems.

Within the broad category of dopaminergic systems are the mesolimbic and mesocortical systems (Cox & Lee, 2016). Neurons from the mesolimbic system synapse into the amygdala, hippocampus and nucleus accumbens. The function of this system is primarily in motivation and reward signaling. Separate from the mesolimbic system is the mesocortical system, neurons of which synapse into the frontal cortex. Primarily, the mesocortical system is utilized for attention, planning and memory. Symptoms of ADHD can be traced back to their respective systems — inattention and short-term memory issues result from dopamine deficiencies in the mesocortical system, whereas impulsive and depressive symptoms result from dopamine deficiencies in the mesolimbic system.

As outlined, development of both ASD and ADHD originates from the same regulatory mechanism but branch at key points. Both are a result of decreased MTHFR enzyme efficiency accompanied by decreased SAM production. The difference comes in the use of the methyl group that SAM provides. The proposed mechanism illustrates that ASD results from insufficient DNA methylation and amino acid synthesis, which complicates neural tube development and can lead to NTDs. The proposed mechanism for ADHD development, however, illustrates that ADHD results from decreased monoamine neurotransmitter synthesis, which results in decreased activation of the mesolimbic and mesocortical neural systems. Figure 3 summarizes the various downstream effects of MTHFR polymorphisms and associations with the development of ADHD and ASD.

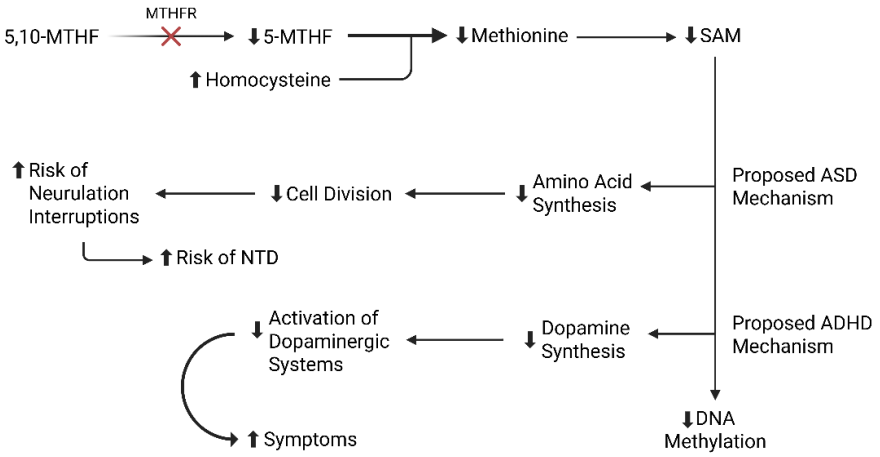

Figure 3. Proposed mechanistic pathways linking MTHFR gene polymorphisms and neurodevelopmental disorders.

Decreased MTHFR activity (red X) results in decreased reduction of 5,10-MTHF to 5-MTHF, resulting in decreased methionine and S-adenosylmethionine synthesis. Downstream effects include decreased amino acid and neurotransmitter synthesis, which increases the risk of NTD development and decreased activation of dopaminergic systems.

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Section II: Testing, Experiments and Therapeutic Options

The proposed mechanisms underlying the development of ADHD and ASD remain correlational rather than causational primarily due to inconsistencies across studies and insufficient replication of data. This section will outline current approaches to determining the presence of MTHFR polymorphisms, experimental design and research that will aim to provide further evidence towards a relationship, as well as further explore treatment options that target the deficits in 5-MTHF and SAM.

MTHFR polymorphisms are primarily detected through polymerase chain reaction (PCR) techniques on genetic material (Ulvik & Ueland, 2013). These reactions amplify isolated segments of DNA and identify key genetic variants (Khehra et al., 2025). More specifically, PCR determines the presence of 677C>T and 1298A>C polymorphisms with high precision. The process begins by isolating target DNA; restriction enzymes cleave DNA at defined sites to generate analyzable fragments. Taq polymerase is then added, and the temperature of the solution fluctuates between extreme heat (95°C) and 55-72°C, allowing for rapid strand separation and replication until an adequate amount of DNA is reached. Finally, this DNA is analyzed for the presence of certain polymorphisms, usually through gel electrophoresis, which separates genetic material based on molecular weight and allows for visualization of distinct polymorphic variants.

Despite the diagnostic precision of PCR-based MTHFR testing, its clinical application remains controversial. Long and Goldblatt (2016) illustrate that MTHFR polymorphism testing has been used as a non-specific screening test for individuals, including those who are asymptomatic. Due to the high prevalence of MTHFR polymorphisms in the population, an overinterpretation of positive results can cause unnecessary concern, especially with

confounding factors such as folate intake. Consequently, clinical testing is best reserved for individuals presenting with symptoms suggestive of neurodevelopmental dysfunction.

When applied appropriately, such testing can provide key bioinformatic data that informs individualized treatment. More precisely, under the proposed mechanisms, treatment of these disorders through proper supplementation aimed at restoring methylation cycles can provide potentially longer-term benefits in comparison to nonspecific pharmacologic interventions. As research continues to define the relationship between MTHFR polymorphisms and neurodevelopmental disorders, genetic testing can be pivotal to targeted therapeutic options.

Proposed Experiment to Provide Further Support to ADHD Development

Establishing a relationship between MTHFR polymorphisms and attention-deficit/hyperactivity disorder (ADHD) requires experimental models capable of demonstrating the mechanistic sequence linking genetic variations with downstream biochemical and neurological outcomes. Specifically, these experiments should show *MTHFR* gene polymorphisms leading to decreased MTHFR enzyme efficiency, lowering the concentration of circulating 5-MTHF. This reduction would result in lower SAM and methionine levels, decreasing dopamine synthesis and subsequently decreased dopaminergic system input. Supporting this chain through experimental methods would not only validate the proposed mechanisms of neurodevelopmental disorders but also guide targeted treatments designed to replenish homeostatic balance in an affected individual.

Murine models are particularly suited for these experiments — mice have highly homologous MTHFR enzyme sequences and can be engineered to contain 677C>T polymorphisms (Reagan et al., 2022). Utilizing transgenic models of mice can determine the

effect the 677C>T polymorphism has on efficiency of the MTHFR enzyme, as well as levels of homocysteine, SAM and methionine. Additionally, the use of micro-dialysis can measure dopamine concentrations in the mesolimbic and mesocortical systems of the brain (Darvesh et al., 2011). Correlating reductions in these biochemical and neurochemical baselines across genetically modified models and consistently replicating these findings would provide strong evidence for a deeper relationship between MTHFR dysfunction and ADHD.

The genetic variation setup will include two separate groups of mice. The control group of mice (CM) will possess two wild-type MTHFR alleles. The transgenic group (TM) will have one wild-type allele and one 677C>T allele. Both groups will be housed under identical environmental conditions set at room temperature with minimal environmental stimulation and a standardized diet containing adequate amounts of folic acid to reduce confounding variables. Initial measurements of circulating 5-MTHF, methionine and SAM should be collected via venous blood draw and analyzed biochemically. Initial measurements of dopamine in the mesocortical and mesolimbic systems of the brain will be quantified through in-vivo micro-dialysis, establishing baseline levels of these metabolites for intergroup comparison.

Microdialysis is an in-vivo analytical technique that utilizes a probe setup to analyze extracellular concentrations of neurotransmitters in the brain (Darvesh et al., 2011). A probe with a semi-permeable membrane is inserted into the target region and perfused with physiologic fluid. Small molecules such as dopamine diffuse across the membrane and are collected over time for biochemical analysis, providing specific neurochemical profiles.

Following the initial analysis of metabolic levels, mice should be allowed to reproduce and produce offspring within their groups. CM mice will produce homozygous wild-type mice, TM mice will yield a mix of wild-type, heterozygous and homozygous mutant offspring.

Collecting data on circulating concentrations of these metabolites will point to potential deficiencies characteristic of neurodevelopmental disorders. Following gestation, both offspring and parental groups should continue to be monitored for persistent metabolic and neurotransmitter abnormalities.

Complementary to neurochemical analysis, behavioral assays that test attention and neurocognitive function may also be used. These assays would focus on the performance of complex tasks that would measure attention span and short-term memory. One such assay is the multiple-choice serial reaction test, in which the mouse must observe and identify the locations of different objects. The accuracy and speed of identification under a regulated amount of time is the basis for data collection in attention and vigilance (Rodriguiz & Wetsel, 2006). Under ideal conditions, the TM mice group would produce results consistent with symptomatic ADHD, including decreased attention span and decreased short-term memory in comparison to the CM group. Figure 4 outlines theoretical data that would be indicative of a linkage between MTHFR polymorphisms and the relative concentration of key metabolites.

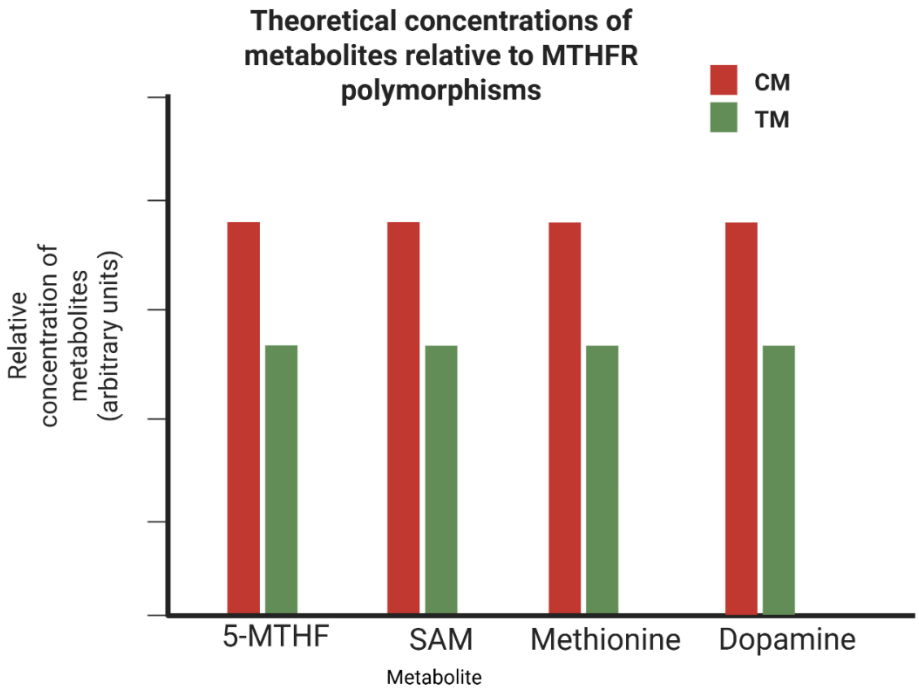

Figure 4. Theoretical concentrations of metabolites relative to MTHFR polymorphisms.

Predicted concentrations of folate-cycle metabolites analyzed through biochemical and neurochemical assays. Red bars represent the control group, green bars represent the transgenic group. When these trends are paired with empirical data that demonstrates increased ADHD onset in the presence of MTHFR polymorphism, they further support a relationship between MTHFR activity, metabolite availability and ADHD development, supporting the proposed mechanistic model.

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After data collection and analysis, the CM and TM mice groups should be separated into further groups to examine the effects of 5-MTHF supplementation on metabolite levels. The CM group should be separated into the CM- and the CM+ groups, the CM- group pertaining to the reception of a placebo medication and the CM+ group receiving 5-MTHF supplementation. The TM mice groups will be separated into similar groups, TM- and TM+. The CM- group will provide normal functionality and will act as a negative control for result comparison. The CM+

group isolates whether 5-MTHF produces non-specific metabolic or behavioral changes in wild-type animals, allowing treatment effects to be distinguished from genotype-specific rescue. TM- will be another negative control utilized to analyze the effect of 5-MTHF on the TM+ group.

Groups will be administered treatment over a course of one week within the same environment, and metabolite levels should be analyzed before and after the one-week period. Behavioral assays should also be performed after the one-week period to determine behavioral changes in each experimental group. Results should be analyzed relative to pre-treatment baselines to evaluate whether 5-MTHF supplementation restores metabolic and behavioral deficits associated with the MTHFR polymorphism. Figure 5 illustrates theoretical data of 5-MTHF supplementation and effects on each experimental group.

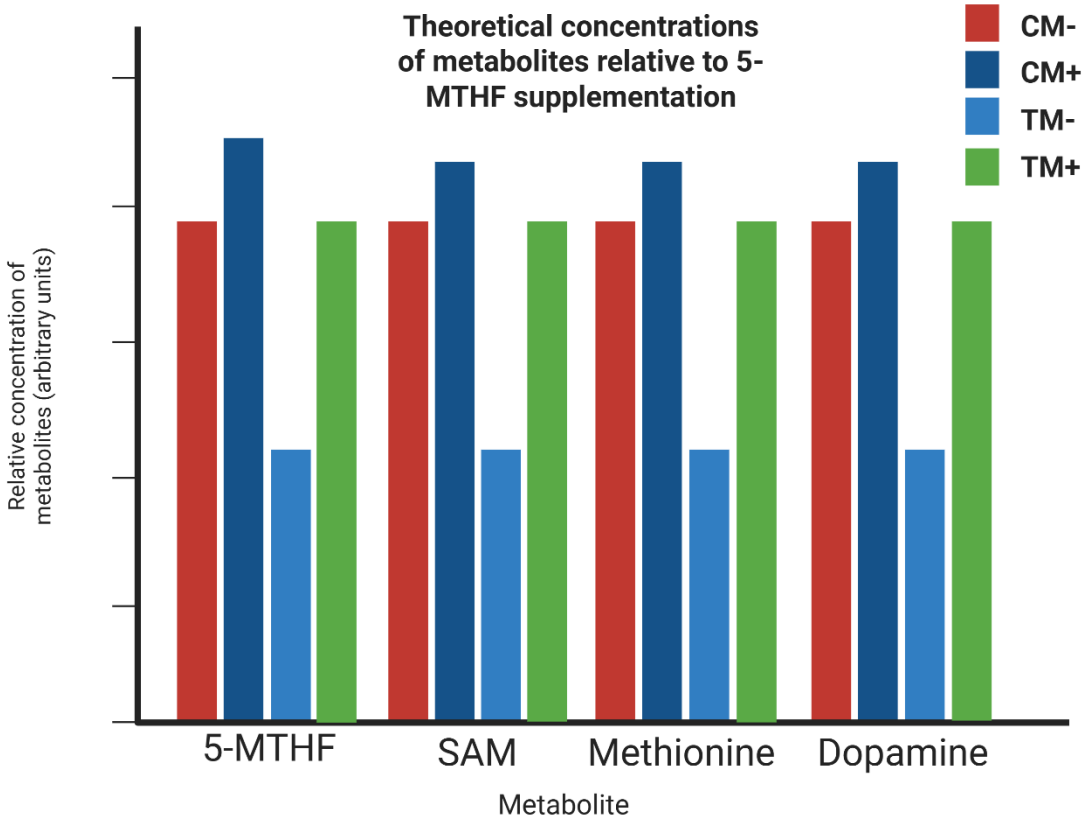

Figure 5. Theoretical concentrations of metabolites relative to 5-MTHF supplementation.

Predicted concentrations of folate-cycle metabolites analyzed through biochemical and neurochemical assays. Red bars represent CM-, navy blue bars represent CM+, sky blue bars represent TM-, and green bars represent TM+. When paired with Figure 4 and behavioral data, the links between MTHFR polymorphisms, ADHD development and manifestation and possible therapeutic effects of 5-MTHF are further supported.

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The outlined experimental methodology will provide relevant neurochemical and biochemical data that can then be analyzed to further support a link between MTHFR polymorphisms and neurodevelopmental outcomes. Figure 6 is a summary of expected results. When combined with Figure 4 and behavioral assays, 5-MTHF supplementation may alleviate ADHD-relevant phenotypes. With further support, attention can shift toward evaluating therapeutic strategies aimed at restoring folate-cycle homeostasis for more sustained benefit.

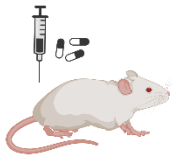
	Normal Conditions		Treatment
CM-	5-MTHF: Normal Methionine: Normal SAM: Normal Dopamine: Normal Behavior: Normal		5-MTHF: Normal Methionine: Normal SAM: Normal Dopamine: Normal Behavior: Normal
CM+	5-MTHF: Normal Methionine: Normal SAM: Normal Dopamine: Normal Behavior: Normal		5-MTHF: Slightly Elevated Methionine: Slightly Elevated SAM: Slightly Elevated Dopamine: Slightly Elevated Behavior: Normal
TM-	5-MTHF: Abnormally Low Methionine: Abnormally Low SAM: Abnormally Low Dopamine: Abnormally Low Behavior: Abnormal		5-MTHF: Abnormally Low Methionine: Abnormally Low SAM: Abnormally Low Dopamine: Abnormally Low Behavior: Abnormal
TM+	5-MTHF: Abnormally Low Methionine: Abnormally Low SAM: Abnormally Low Dopamine: Abnormally Low Behavior: Abnormal		5-MTHF: Normal Methionine: Normal SAM: Normal Dopamine: Normal Behavior: Normal

Figure 6: Summary of murine experiment.

Summary of expected results across all mouse groups. Normal values are shown in black, slight elevations in orange, and abnormal values in red. The TM+ group demonstrates restoration of metabolic and behavioral markers following treatment, while the TM- group shows no improvement. The CM+ group exhibits mild increases in metabolite levels, likely reflecting normal homeostatic responses to elevated 5-MTHF. The CM- group serves as the negative control. Color differences between CM and TM mice are for visual clarity only.

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Future Therapeutic Avenues

Current therapeutic options for ADHD primarily target symptom management rather than underlying biochemical imbalances. Medications such as Adderall and Ritalin are prescribed to improve attentiveness in adolescents and adults by modulating dopaminergic activity within the synaptic cleft. Ritalin, in particular, can act as a weak agonist at the 5-HT_{1A} receptor, a serotonin-

type receptor (Faraone, 2018). While both medications can effectively alleviate short-term attention deficits, supplementation with 5-methyltetrahydrofolate (5-MTHF) and prenatal folate may provide more sustained improvement by addressing the root cause of the symptoms linked to MTHFR polymorphisms. Furthermore, supplementation with prenatal folate has been shown to reduce the incidence of NTD development, supporting its broader therapeutic potential.

Both Adderall and Ritalin belong to the psychostimulant drug class (Faraone, 2018). Adderall acts primarily by increasing the amount of dopamine and norepinephrine in the synaptic space between neurons by inhibiting reuptake transporters for these monoamine neurotransmitters, thereby allowing for a higher concentration of dopamine in the synaptic cleft. Ritalin inhibits the same reuptake transporters and has a similar effect as Adderall. These medications share similar mechanisms of action that increase mesocortical and mesolimbic activation above baseline. Although efficacious, the effects are transient and dependent on continued administration, limiting long-term efficacy (Hasler et al., 2023).

In contrast, 5-MTHF and prenatal folate supplementation aims to restore the disrupted folate cycle that would otherwise be interrupted by MTHFR polymorphisms. This approach targets the biochemical root of symptom development in ADHD and may concurrently reduce risk factors associated with autism spectrum disorder (ASD). With 5-MTHF supplementation, the remethylation of homocysteine to methionine resumes, normalizing methionine availability and enabling the proper synthesis of SAM. As SAM levels are restored, DNA methylation stabilizes, and the synthesis of monoamine neurotransmitters such as dopamine and norepinephrine increases.

Through the restoration of this cycle, dopamine synthesis will return to adequate amounts, potentially reducing the dopamine-deficient symptoms characteristic of ADHD. Unlike

psychostimulants, 5-MTHF supplementation works upstream to restore folate cycle metabolism and resuming the synthesis of neurotransmitters instead of simply inhibiting reuptake. This may produce prolonged synaptic dopamine availability and greater activation of the mesocortical and mesolimbic systems, improving attention, working memory and motivation.

Even though it is available, 5-MTHF supplementation is not a widely known treatment, partly due to limited awareness and reliance on Adderall or Ritalin as a first-line therapy. Enhancing public awareness of 5-MTHF supplementation through different outreach efforts can inform providers of the promising avenue of supplementation, as well as inform patients of alternative options to psychostimulants that often have side effects. 5-MTHF supplementation has no known side effects with appropriate usage, further highlighting its potential in the future for targeted therapy in individuals with ADHD.

Similarly, prenatal folate plays a critical role in limiting the development of NTDs. Folate levels in the serum of mothers naturally decline during gestation, which can have a consequent effect on available folate levels for the developing fetus (Tate et al., 2024). Inadequate folate levels impair the operation of the folate cycle, reducing the levels of methionine and SAM, which can lead to a higher risk of developing NTDs. Prenatal folate supplementation restores these pathways, thereby increasing nucleotide availability and reducing the risk of NTDs —and, by extension, certain neurodevelopmental conditions such as ASD. Together, 5-MTHF and prenatal folate supplementation represent promising therapeutic avenues aimed at mechanistic correction and moving beyond symptom management. With further research into the links between MTHFR polymorphisms and neurodevelopmental disorders, these interventions may help reduce the risk of NTDs, ADHD and ASD, while also providing a foundation for a deeper understanding of the underlying biochemical mechanisms driving these interconnected disorders.

Concluding Remarks and Future Directions

MTHFR polymorphisms are integral to understanding potential mechanisms underlying multiple neurodevelopmental disorders. A single deficiency in 5-MTHF resulting from mutations in the MTHFR enzyme causes extensive downstream effects on folate metabolism, human development, and neurological function. While the mechanisms underlying ADHD and ASD are not fully understood, further research into the roles these polymorphisms have in the development of these disorders may prove invaluable in advancing treatment.

Accordingly, the utilization of transgenic mice models represents a promising method for highlighting a possible relationship between MTHFR polymorphisms and homeostatic imbalances in folate metabolism that contribute to the development of ADHD and ASD. Research emphasizing restoration of homeostasis within the folate cycle—specifically supplementation with 5-MTHF and prenatal folate—can complement these investigations into linkage. As future efforts are made toward further understanding the actions of MTHFR polymorphisms, individuals affected by ASD and ADHD may be more effectively treated.

Together with the solidification of physiological and biochemical mechanisms, the profound impact a single point mutation has on metabolic homeostasis, neurocognitive function and development can be further elucidated. Neurodevelopmental disorders such as ASD and ADHD have significant effects on the quality of life in many individuals. Continued research into MTHFR polymorphisms and the development of targeted treatments has the potential to substantially improve the lives of those affected by neurodevelopmental disorders worldwide.

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