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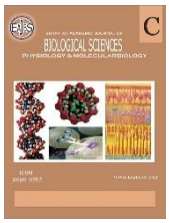

Supplementary Therapy for DNA Methylation in Autism

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ABSTRACT

Epigenetics plays a crucial role in various clinical diseases, such as autism, by mediating the impact of environmental variables on genomic regulation. Autism spectrum disorder (ASD) is a neurological disorder influenced by both genetic and environmental factors that affect the developing brain. DNA methylation, histone tail modifications, and non-coding RNA activity can change the function of genes without altering nucleotide sequences. Genes and environment combine to produce the etiology of ASD. One of the main areas of ASD research now being studied is the effects of epigenetic factors on gene expression, such as DNA methylation. Autistic patients exhibit evidence of oxidative stress and impaired methylation, which may reflect the effects of toxic exposure on sulfur metabolism that may lead to cellular damage in the brain and altered expression of epigenetic genes. This review paper summarizes the findings of the supplementary therapy studies of ASD, showing that supplements, including B9, B12, B6, D, E, C, glutathione, omega-3, and choline, are highly effective in modifying methylation in autism, improving many nutrient and metabolic problems, and resulting in significant improvements in symptoms.

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental condition marked by limited interests, repetitive activities, and difficulties in social interactions (Tian &Yang, 2022). It is also a neurobiological condition impacted by environmental and genetic variables affecting the growing brain (Hodges *et al.*, 2020). ASD is becoming more widely acknowledged as a public health concern, which has increased in 20 years from 2–5/10,000 to 1/68 children (Bio *et al.*, 2014). Some environmental risk variables from preconception to early infancy that may be involved in the presentation of ASD were found in a recent 10-year meta-analysis (Ng *et al.*, 2017). Aging parents, obesity, immune system issues, prenatal exposure to air pollution, early birth, low birth weight, and cesarean delivery are some of these factors. However, the exact causal link between environmental exposure and the neurodevelopmental disruption at the root of ASD remains unclear (Grossi *et al.*, 2018). In certain cases, prenatal, perinatal, and postnatal environmental variables, such as supplements, may alter genetic risk (Wang *et al.*, 2017). A study indicated that comparing the peripheral blood levels of methionine (Met), s-adenosylmethionine (SAM), s-adenosylhomocysteine (SAH), and the SAM/SAH ratio are significantly abnormal in ASD individuals, supporting the link between ASD and poor methylation (Guo *et al.*, 2020).

Epigenetic Insights into Autism:

In several clinical diseases, epigenetics is becoming more significant in mediating the impact of environmental variables on genomic regulation (Portela & Esteller, 2010). Epigenetic mechanisms, including DNA methylation, histone tail modifications, and non-coding RNA activity, can change the function of genes without altering nucleotide sequences (Gibney & Nolan, 2010). DNA methylation is one example of an epigenetic mechanism that operates at the gene-environment interface and is crucial to the development of the human brain (Lasalle, 2013). The family of enzymes known as DNA methyltransferases (Dnmts) catalyzes the methylation of DNA by moving a methyl group from S-adenyl methionine (SAM) to the fifth carbon of a cytosine residue, where it forms 5 mC (Guo *et al.,* 1994). ASD was shown to have changed plasma levels of the metabolites homocysteine, S-adenosyl methionine, and methionine, which are necessary for DNA methylation processes. This indicates a metabolic profile compatible with a decreased ability to methylate DNA (James *et al.,* 2004).

Unveiling DNA Methylation in Autism:

There are several ways to search for evidence of aberrant DNA methylation in ASD, including genetic abnormalities in the epigenetic machinery and alterations in DNA methylation that are both locus-specific and genome-wide. Since global DNA methylation regulation is dynamic during the embryonic stages and during the initial postnatal period, which coincides with the peak time of synaptogenesis, epimutations in DNA methylation are obtained at any point in life (Tremblay & Jiang, 2019). The alteration of cytosine the most researched DNA alteration and epigenetic mark in the mammalian genome is 5-methylcytosine (5mC) (Wu *et al.,* 2017). The majority of somatic cells exhibit 5mC at 5mCpG sites, where it occurs in symmetrical CG dinucleotides (CpG), and less frequently at 5mCpH sites (where H might be A, C, or T). Nonetheless, 5mCpH methylation is as abundant in neurons as 5mCpG, indicating that neurons' epigenomes differ from those of other cell types in a distinctive way (Lister *et al.,* 2013). A CpG island is a genetic area that has been enriched in CpGs. These CpG islands are longer than 200 base pairs, have a minimum of 50% CG content, and are commonly linked to regulatory elements, which include most of the genome's promoters (Bird, 2002).

Recent research has indicated that 58 significantly methylated regions (DMRs) were identified using the 450 K Bead Array, which comprised brain-specific microRNAs and loci linked to GABAergic system genes, namely *ABAT* and *GABBR1*. A targeted, Next-Generation Bisulfite Sequencing was used to validate the selected DMRs. Three co-methylation modules that are strongly connected with ASD were found using weighted gene correlation network analysis. These modules enrich regions of the genome underlying genes related to the nervous system, GABAergic system, and immunological system (Nardone *et al.,* 2017). Through the remethylation of hemimethylated DNA during genome replications, DNMT1 is known to contribute to the maintenance of DNA methylation (Lyko, 2018). One gene or allele incorrect methylation is profound and affects the brain. A prevalent form of mental impairment, fragile X syndrome is brought on by aberrant methylation of a trinucleotide repetitive expansion in the *FMR1* gene on the X chromosome (Verkerk, *et al.,* 2017).

Numerous investigations on DNA methylation, gene circuits, and gene sequences offer important mechanistic insights into ASD. It will be essential to comprehend the pathways at the center of this "perfect storm" to improve the diagnosis and treatment of ASD (Ciernia & LaSalle, 2016). The Significance of Dietary Factors in ASD Human disease is influenced by wide factors, including genetic, psychological, environmental, and behavioral traits. Diet and disease are closely related (Negger, 2014). According to these findings, autism is associated with a unique deficiency in

antioxidant and methylation capabilities, which may lead to cellular damage and altered expression of epigenetic genes (Melnyk, *et al.,* 2012) & (Hendren *et al.,* 2016). The rise in research on this subject in recent years indicates an interest in identifying a DNA methylation signature as a biomarker with therapeutic value to improve ASD care. However, it is currently unclear if blood-level DNA methylation biomarkers can be used to diagnose or assess the severity of ASD symptoms (Stoccoro *et al.,* 2023).

The Crucial Supplements in DNA Methylation:

Numerous nutritional and metabolic markers, including those indicating vitamin deficiency, elevated oxidative stress, decreased energy transfer capacity, sulfation, and detoxification, were statistically significantly different in the autistic condition. Variations in the severity of autism were substantially correlated with multiple biomarker groupings. These deficiencies in nutrition and metabolism are likely reversible with dietary supplements, and the majority of the findings are consistent with previously published research. (Adam *et al.,* 2014). For healthy fetal growth, prenatal vitamin consumption is advised both before and throughout pregnancies. Although DNA methylation and other epigenetic variables can be influenced by nutrient levels, the connections between prenatal vitamin consumption by mothers and DNA methylation have not received much attention. First-month prenatal vitamin use may be associated with decreased placental global DNA methylation as well as reduced DNA methylation in brain-related pathways in the placenta and cord blood (Dou *et al.,* 2022). ASD supplements are important in regulating methylation processes, which are necessary for epigenetic alterations and gene expression. Particular nutrients are necessary for the embryo's early growth and are important for methylation reactions. The network of metabolic pathways known as one-carbon metabolism controls the synthesis of nucleotides, the metabolism of amino acids, and epigenetic activities including DNA methylation and demethylation (Clare *et al.,* 2019).

Commonly occurring comorbidities, gastrointestinal disorders are believed to be both an additional sign of ASD and a factor in the manifestation of social and behavioral symptoms. The nutritional and metabolic state of children with autism can be improved with oral vitamin and mineral supplementation, which also improves methylation (Adam *et al.,* 2011). Therefore, the majority of individuals with ASD apply nutritional therapies to reduce gastrointestinal and behavioral symptoms, both with and without therapeutic supervision (Karhu *et al.,* 2020).

Research has indicated that folate (vitamin B9) plays a critical role in preserving appropriate DNA methylation processes since it is an essential component of one-carbon metabolism, which has been noted in autism (Williams, 2012). MTHF-5 form: physiologically active foods do contain trace levels of 5-MTHF, the main physiological form of folate present in blood and umbilical cord blood. It is readily available and doesn't need to be metabolized like a food element. Specifically, methyl folate, 5-MTHF, or (6S)-5-MTHF, also known as 5-methyltetrahydrofolate, has been assessed as a superior substitute for folic acid administration (Carboni, 2022). A lack of folate can cause issues with DNA methylation, which can change how genes are expressed and raise the risk of long-term health conditions and developmental defects (Zeisel, 2009). Research has indicated that sufficient consumption of folate is crucial throughout crucial developmental stages, including pregnancy, to create appropriate DNA methylation patterns and prevent neural tube anomalies (Coppedè, 2012). As a result, the World Health Organization (WHO) advises folic acid (FA) intake throughout the first three months of pregnancy to avoid neural tube abnormalities (Henderson, 2018).

Folate regeneration is linked to vitamin B12, which transforms it into an active form. Due to its role in the synthesis of myelin, deficiencies in this vitamin hurt brain

development and cognitive function (Gusso *et al.,* 2023). The ideal methyl B12 treatment decreased clinician-rated symptoms of ASD, which were connected with improvements in markers of methionine metabolism and cellular methylation capacity, according to a study on subcutaneous B12 administration in children with autism (Blencowe *et al.,* 2010). Since it absorbs the methyl group from 5-methyl tetrahydrofolate (folic acid) and forms methylcobalamin, which in turn releases the methyl group needed to convert homocysteine into methionine, vitamin B12 (cobalamin) is essential to the methionine cycle (Chen *et al.,* 2023). Vitamin B12 deficiency can cause issues with methionine metabolism, which lowers SAM levels and damages DNA methylation (Kumar *et al.,* 2012).

Numerous studies have been published on the advantages of high-dose vitamin B6 supplements for autistic children and adults (Adams et al., 2006). In more than 100 metabolic processes, including one-carbon metabolism, which is critical for DNA synthesis, repair, methylation, and defense against oxidative stress, vitamin B6 (pyridoxine) is a necessary cofactor (Current, 2010).

Vitamin D deficiency or metabolic abnormalities have been linked to autism because vitamin D is essential for neuronal growth and development (Bouillon *et al.,* 2008). The vitamin D system controls about 3% of the human genome and performs pleiotropic activities (Fetahu, 2014). The importance of vitamin D in maintaining the typical epigenetic landscape highlights the hormone's pivotal function in physiology (Wang *et al.,* 2022).

Studies have shown that all players involved in the vitamin E, vitamin C, and glutathione networks are impaired with ASD (Pangrazzi *et al.,* 2020). Low steady-state amounts of vitamin E radicals and ascorbate are found in the cells, and vitamin loss or intake is prevented when the vitamin E, vitamin C, and glutathione systems work together harmoniously. Strong antioxidants, like highly reactive compounds produced by metabolic processes not only in brain tissue but also in various organs, can be neutralized and eliminated by vitamin C (Pangrazzi *et al.,* 2020).

Small investigations of omega-3 fatty acid supplements in children with ASD have shown trends toward reduced hyperactivity (Amminger *et al.,* 2007). It is well established that omega-3 polyunsaturated fatty acids (n-3 PUFAs) have anti-inflammatory properties and can change the way genes are expressed in cells. New research suggests that changing epigenetic markers like DNA methylation is one of the processes underlying this process (Hussey *et al.,* 2016).

Choline is necessary for the proper functioning of the nervous system (acetylcholine synthesis), the metabolism of methyl groups (homocysteine reduction), cell membrane signaling (phospholipids), and lipid transport (lipoproteins) (Gabis *et al.,* 2019). Additionally, choline has been linked to abnormalities in learning, memory, cognitive function, and sensory processing in people with ASD (Olson *et al.,* 2020). Choline is known to aid in the synthesis of methionine, an important amino acid, and to contribute to brain growth (Agam *et al.,* 2020). Therefore, supplements have been shown to be beneficial in modifying methylation in autism, resolving numerous dietary and metabolic issues, and producing notable symptom improvements.

Conclusion

In autism disorder, oxidative stress and reduced methylation are associated with epigenetics, potentially as a result of toxic exposure. It is unclear, nevertheless, exactly how environmental exposure and the neurological disturbance at the core of ASD are causally related. Peripheral blood levels, including Met, SAM, SAH, and the SAM/SAH ratio, are significantly abnormal in ASD individuals, supporting the link between ASD and poor methylation. Thus, more research is necessary to determine whether these indices may be used as biomarkers for ASD diagnosis and treatment targets. Supplements significantly contribute

to human diseases like autism, with abnormalities like vitamin deficiency and oxidative stress that cause cellular damage and altered gene expression. Reversible deficiency can be addressed with prenatal vitamin intake and dietary supplementation, which are advised before and throughout pregnancies. Vitamins play a significant role in preserving appropriate DNA methylation processes and are essential for epigenetic alterations and gene expression. They have been recommended for autistic children and adults, as they are essential for neuronal growth and development.

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