Tetrahydrobiopterin Deficiency in Autism Spectrum Disorder

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Tetrahydrobiopterin is an essential cofactor for critical metabolic pathways, including those involved in the production of monoamine neurotransmitters and nitric oxide. Cerebrospinal fluid studies suggest that tetrahydrobiopterin concentrations in the central nervous system (CNS) may be lower in children with autism spectrum disorder (ASD) as compared to typically developing children. Clinical trials, including double-blind placebo controlled studies, suggest that oral tetrahydrobiopterin supplementation is therapeutic in children with ASD. Despite these previous studies, no clinical description of children with ASD and CNS tetrahydrobiopterin deficiency has been published. A series of six patients with ASD who were found to have CNS tetrahydrobiopterin deficiency is described. Most (83%) had global developmental delay while two (33%) had slow regression into an autism phenotype and only one (17%) had epilepsy. The pattern of metabolic abnormalities was not consistent with a primary disorder of pterin production. Overall, this case series suggests that children with ASD can have a CNS deficiency in tetrahydrobiopterin and that this deficiency is probably not a primary disorder of tetrahydrobiopterin production, but rather most likely secondary to reduced precursor availability, reduced recycling and/or increased utilization due to other multifactorial abnormalities associated with ASD such as abnormalities in CNS folate and/or oxidative stress.

Key Words: Tetrahydrobiopterin, autism spectrum disorder, mitochondrial disease, folate receptor alpha autoantibodies

INTRODUCTION

Autism spectrum disorder (ASD) is a neurodevelopmental disorder behaviorally characterized by impairments in communication and social interaction along with restrictive and repetitive behaviors. An estimated 1 out of 68 individuals in the United States are currently affected with ASD with the prevalence continuing to rise. Although several genetic syndromes, such as Fragile X and Rett syndromes, have been associated with ASD, empirical studies have estimated that genetic syndromes only account for a minority of ASD cases. Thus, the majority of ASD cases cannot be linked to a single gene or chromosomal disorder. Many studies have now suggested that cellular abnormalities not necessarily with the central nervous system (CNS) are associated within ASD, suggesting that systemic abnormalities may play a role in some children with ASD. Over the last decade, publications have started to implicate physiological systems that transcend specific organ dysfunction, such as immune dysregulation and abnormalities in various metabolic systems.
Abnormalities in pterin metabolism have been associated with ASD, although still little is known about the biological basis of this association. The pterin pathway is responsible for the production of the two better-known pterins: tetrahydrobiopterin (BH$_4$) and neopterin (Figure 1). Neopterin is associated with immune activation. Several immune modulators, such as TNF-alpha and IFN-gamma, increase activity of the first enzyme in the pterin production pathway, guanosine-5'-triphosphate (GTP) cyclohydrolase I, resulting in an increase in 7,8-dihydronopterin triphosphate, the precursor to neopterin and tetrahydrobiopterin. Evidence for changes in neopterin in ASD is mixed with some studies reporting elevated neopterin levels in urine$^8$ and blood$^9$ and other studies reporting reduced neopterin levels in urine, plasma$^{10}$ and cerebrospinal fluid (CSF).$^{11}$

Perhaps BH$_4$ is the more important pterin in relation to ASD because of its role as an essential cofactor for several critical metabolic pathways, particularly those responsible for the breakdown of phenylalanine and the production of monoamine neurotransmitters and nitric oxide.$^{12}$ Abnormal BH$_4$ metabolism is associated with neurometabolic disease. A deficiency in BH$_4$ synthesis or recycling can result in neurological disorders including phenylketonuria type IV$^{13}$ and dopamine-responsive dystonia,$^{14}$ all of which demonstrate accompanying changes in CSF monoamine neurotransmitter metabolites in addition to changes in CSF BH$_4$ concentrations. CNS BH$_4$ deficiency with associated abnormalities in CSF monoamine neurotransmitter metabolites has also been reported as a secondary consequence of cerebral folate deficiency syndrome.$^{15}$ Some abnormalities in BH$_4$ metabolism may be somewhat specific to ASD as reduced CSF BH$_4$ concentrations without changes in CSF monoamine neurotransmitter metabolites have been reported in children with ASD$^{15,16}$ and several clinical trials have shown that children with ASD have a favorable response to BH$_4$ supplementation.$^{17,19}$

The reason for reduced BH$_4$ CSF concentrations in children with ASD is not known, but it has been suggested that BH$_4$ overuse and poor recycling could contribute to the deficiency.$^{16}$ Indeed, BH$_4$ can act as an excellent antioxidant, but requires recycling through a folate-dependent salvage pathway once it is oxidized.$^{12}$ The mechanism behind the therapeutic response to treatment with BH$_4$ in ASD is unclear as only three studies have measured CSF BH$_4$ concentrations prior to treatment$^{19,21}$ and only two studies have examined the relationship between CSF BH$_4$ concentrations and treatment response.$^{19,21}$ One study found a borderline significant correlation between CSF BH$_4$ concentration before treatment and improvement in social interactions with BH$_4$ supplementation$^{19}$ and biomarkers used in another study suggested that nitric oxide metabolism was associated with the therapeutic response to BH$_4$ supplementation.$^{19}$ One study that examined changes in dopamine metabolism using positron emission tomography with BH$_4$ treatment showed a change in D$_2$ receptor binding as a result of BH$_4$ treatment but did not correlate this change with treatment response.$^{20}$

Despite the evidence for abnormalities in BH$_4$ metabolism in ASD, there is only limited knowledge as to whether such abnormalities are associated with a specific ASD phenotype. In fact, no clinical description of children with ASD and CNS BH$_4$ deficiency has been published. Here the results from a series of children with ASD and CSF examinations were reviewed to determine the characteristics of patients with CNS BH$_4$ deficiency.

**METHODS**

As part of a medically based autism clinic, patients with neurodevelopmental disorders, particularly ASD, underwent a CSF examination when indicated. Here the results to 40 children with ASD and CSF examinations were reviewed to determine the patients that demonstrated BH$_4$ deficiency as determined by the age-dependent laboratory normative values. The laboratory only provides the normal range of values, so percentiles of normative values were not available. All children met the Diagnostic and Statistical Manual of Mental Disorders – Fourth Edition – Text Revision$^1$ criteria for ASD and had previously been diagnosed with ASD by a developmental pediatrician, pediatric neurologist or clinical psychologist. Review of each child’s medical record was obtained through an Institutional Review Board approved protocol. The CSF findings of three of the cases reported herein were published in a study focused on peripheral biomarkers of CSF BH$_4$ concentration.$^{16}$ The previous study did not concentrate on the clinical history of individuals with CNS BH$_4$ deficiency as is done here.

**RESULTS**

Six of 40 (15%) cases of children with ASD demonstrated below normal CSF BH$_4$ concentrations. These cases are listed in Table 1 along with important clinical information and laboratory values. All cases demonstrated normal serum phenylalanine levels thereby ruling out phenylketonuria type IV and all cases demonstrated normal CSF neurotransmitter levels. Only one child (case 6) demonstrated a frankly low neopterin level, while three other children demonstrated very low normal neopterin levels (cases 2, 3 and 5) and the other two cases demonstrated low normal neopterin levels (cases 1 and 4). This suggests that the low BH$_4$ concentrations in general were not due to a primary defect in the pterin production pathway (Figure 1) but could be due to other factors that indirectly affect CNS pterin concentrations.

The majority of the cases reported (5/6; 83%; cases 1-5) manifested global developmental delay. Two (2/6; 33%; cases 2 and 6) manifested a slow regressive phenotype with one child (case 2) having this regression after global developmental delay was well established. Epilepsy was only seen in one case and electroencephalograms were normal in all of the cases except for the case with epilepsy. MRI was normal in the majority (5/6; 83%) of the cases. Standard genetic testing was unremarkable in the majority (5/6; 83%) except for one male with a partial Xq duplication including MECP2. Two (33%) patients demonstrated mitochondrial disorders (cases 1 and 3), including a patient with the partial...
Xq duplication (case 3) and a patient with severe complex I and CoQ10 deficiency (case 1). Folate receptor autoantibodies were positive in the three patients who were tested (cases 1, 2 and 5). Although none of the cases demonstrated frank cerebral folate deficiency, two of the patients, both without folate receptor alpha autoantibody testing, demonstrated low normal 5-methyltetrahydrofolate concentrations in the CSF (cases 4 and 6).

Table 1. Characteristics of patients with autism and tetrahydrobiopterin deficiency.

<table>
<thead>
<tr>
<th>Case #</th>
<th>Workup</th>
<th>Genetic</th>
<th>Mitochondrial</th>
<th>Folate Receptor Autoantibodies</th>
<th>EEG</th>
<th>MRI</th>
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<tr>
<td>1</td>
<td>CoQ10 &amp; Severe Complex I Deficiency</td>
<td>CMA &amp; mtDNA NL</td>
<td>5MTHF (40-150)</td>
<td>0</td>
<td>14</td>
<td>305</td>
</tr>
<tr>
<td>2</td>
<td>NL</td>
<td>CMA NL</td>
<td>BH4 (7-65)</td>
<td>100</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>3</td>
<td>Lactate &amp; alanine increased</td>
<td>Xq+ inc MECP2</td>
<td>5HIAA (74-345)</td>
<td>124</td>
<td>12</td>
<td>8</td>
</tr>
<tr>
<td>4</td>
<td>NL</td>
<td>CMA NL</td>
<td>HVA (233-928)</td>
<td>42</td>
<td>12</td>
<td>20</td>
</tr>
<tr>
<td>5</td>
<td>NL</td>
<td>CMA NL</td>
<td>3OMD (0-150)</td>
<td>65</td>
<td>15</td>
<td>9</td>
</tr>
<tr>
<td>6</td>
<td>NL</td>
<td>CMA NL</td>
<td>NL</td>
<td>53</td>
<td>19</td>
<td>5</td>
</tr>
</tbody>
</table>

Abbreviations: 3OMD: 3-O-Methyl-dopa; 5HIAA: 5-Hydroxyindoleacetic acid; 5MTHF: 5-methyltetrahydrofolate; Abnl: Abnormal; BH4: Tetrahydrobiopterin; CMA: Chromosomal microarray; EEG: electroencephalogram; FTT: failure to thrive; GERD: gastroesophageal reflux disease; HVA: Homovanillic acid; LP: Lumbar puncture; mo: months old; MRI: magnetic resonance imaging; mtDNA: mitochondrial DNA; NL: Normal; ROM: recurrent otitis media; Rt: Routine. Note BH4 normal range is not given as it is age dependent and may be slightly different for each age range.

DISCUSSION

Several studies have implicated abnormal pterin metabolism is ASD, including abnormalities in neopterin and BH4 concentration. In addition, several clinical trials have shown that children with ASD have a favorable response to BH4 supplementation. To date, no studies have reported a case series of children with ASD and CNS BH4 deficiency. Here we review the results of 40 children with ASD who were seen in a medical-based autism clinic and had CSF examinations. Six of the 40 (15%) demonstrated below normal CSF BH4 concentrations suggesting that a subgroup of children with ASD may have a deficiency in CNS BH4 metabolism.

All of the children reported herein had a variety of clinical and laboratory characteristics. Most of the cases did demonstrate global developmental delay and only one had epilepsy, so patients with ASD and CNS BH4 deficiency might be defined by global developmental delay without epilepsy although further larger case series will be needed to define characteristics of a subgroup. Thus, it is clear that a collection of symptoms that define a syndrome of CNS BH4 deficiency with ASD does not seem apparent at this time and further study is needed.

One of the main questions is whether this CNS BH4 deficiency is primary or secondary. A primary CNS BH4 deficiency would be considered a deficiency in the pterin production pathway (Figure 1) where as a secondary CNS BH4 deficiency could be due to other factors that indirectly affect CNS pterin concentrations.

This series of patients did not show any classic neurometabolic abnormality associated with BH4 production (Figure 1) as CSF neurotransmitter concentrations were normal in all patients. Thus, the findings suggest that any BH4 deficiency was not so severe or chronic that neurotransmitter production was compromised, at least not severe enough that the enzymes responsible for
neurotransmitter production could not adapt. This suggests that classic neurometabolic disorders such as dopamine-responsive dystonia and other disorders of neurotransmitter metabolism, including those in which GTP cyclohydrolase I is affected, are most likely not the culprit in these cases. Other reasons for abnormally low CSF BH4 concentrations include a decrease in GTP, the precursor of the pterin production pathway, increased degradation of BH4 and/or reduced recycling of BH4.

The three cases that had folate receptor alpha autoantibodies testing (cases 1, 2 and 5) were positive for these autoantibodies. Previous it was reported that elevated folate receptor alpha autoantibody titers were related to depressed CSF BH4 concentrations, independent of a folate deficiency. This suggests that this might be one reason for low CSF BH4 concentrations, but folate may still have a role in the CNS BH4 deficiency in these cases. The CSF 5-methyltetrahydrofolate concentration was low normal in the two cases (cases 4 and 6) where folate receptor alpha autoantibody titers were not tested, suggesting that folate metabolism could have been compromised in these cases perhaps due to reduced transport of folate into the CNS by elevated folate receptor alpha autoantibodies. Reduced folate metabolism could compromise BH4 production through two mechanisms. First, GTP, the precursor to pterin production, is produced from the folate cycle. Abnormal folate cycle function, potentially due to limitation in folate concentrations in the CNS could result in a limitation in the production of GTP, the essential precursor to BH4. Second, folate is required for the dihydrofolate reductase dependent recycling of BH4 from BH2. Oxidation of BH4 as a result of reaction with reactive oxygen species is one of the primary reason for conversion of BH4 to BH2. Given that there is good evidence that children with ASD have high levels of oxidative stress, it is likely that, in children with ASD, BH4 commonly undergoes oxidation and needs to be recycled, suggesting that the dihydrofolate reductase folate-dependent recycling pathway is rather crucial in children with ASD.

In the case without autoantibody testing and normal CSF 5-methyltetrahydrofolate concentration (case 3), the patient demonstrated a genetic disorder and a possible mitochondrial disorder. Given that mitochondrial disorders are associated with high levels of oxidative stress and high levels of oxidative stress has been suggested to be a reason for low CSF BH4 concentrations, it is possible that high levels of oxidative stress could have resulted in the BH4 deficiency in this case.

Thus, this report suggests that some children with ASD may have abnormally low CSF BH4 concentrations, resulting in CNS BH4 deficiency. Most of the cases demonstrated global developmental delay without epilepsy. Overall, the pattern of metabolic abnormalities points to secondary factors, particularly abnormalities in CNS folate metabolism and oxidative stress that could combine to result in CNS BH4 deficiency in these patients. The clinical significance of this deficiency is not precisely clear at this time but further reports would be helpful in the future to clarify the cause and consequence of such a deficiency in ASD.

CONFICT OF INTEREST
None.

REFERENCES