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Abnormalities of Cholesterol Metabolism in Autism Spectrum Disorders

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Abstract

Although Smith-Lemli-Opitz Syndrome (SLOS), a genetic condition of impaired cholesterol biosynthesis, is associated with autism [Tierney et al., 2001], the incidence of SLOS and other sterol disorders among individuals with autism spectrum disorders (ASD) is unknown. This study investigated 1) the incidence of biochemically diagnosed SLOS in blood samples from a cohort of subjects with ASD from families in which more than one individual had ASD and 2) the type and incidence of other sterol disorders in the same group. Using gas chromatography/mass spectrometry, cholesterol and its precursor sterols were quantified in one hundred samples from subjects with ASD obtained from the Autism Genetic Resource Exchange (AGRE) specimen repository. Although no sample had sterol levels consistent with SLOS, 19 samples had total cholesterol levels lower than 100 mg/dL, which is below the 5th centile for children over age 2 years. These findings suggest that, in addition to SLOS, there may be other disorders of sterol metabolism or homeostasis associated with ASD.

Keywords

Smith-Lemli-Opitz syndrome; hypocholesterolemia; Autism Genetic Resource Exchange; Asperger disorder; pervasive developmental disorder

Smith-Lemli-Opitz syndrome (SLOS, MIM 270400) is an autosomal recessive malformation syndrome caused by a deficiency of the last step of cholesterol biosynthesis, 7-dehydrocholesterol reductase (DHCR7) [Tint et al, 1994]. Principal abnormalities include a typical facial appearance, microcephaly, hypotonia, cleft palate, hypogenitalism, 2–3 toe syndactyly, and a characteristic behavioral profile including autism, usually accompanied by mental retardation. The enzymatic deficiency manifests biochemically in blood and all tissues as a reduced level of cholesterol and increased levels of 7-dehydrocholesterol (7DHC) and its isomer, 8-dehydrocholesterol (8DHC), although a few very mildly affected patients have normal plasma cholesterol levels despite increases in 7DHC and 8DHC. This

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sterol abnormality in turn affects the synthesis and metabolism of various sterol-derived compounds, including bile acids, adrenal steroids, neurosteroids, and the structure of sterol-rich membranes, such as myelin, the plasma membrane, and various subcellular organelles.

SLOS is a relatively common genetic disorder with an estimated incidence among those of European ancestry of approximately 1 in 50,000 births. SLOS has a wide spectrum of clinical and biochemical severity, from functionally normal individuals to malformed fetuses that die in utero [Lowry and Yong, 1980; Kelley and Hennekam, 2000]. Although the combined carrier frequency for several common DHCR7 null alleles of about 1.25% predicts an incidence of 1 in 25,000 births in European-derived populations, about 50% of conceptuses appear to be lost early in pregnancy [Kelley and Herman, 2001]. Most SLOS individuals who survive the newborn period are compound heterozygotes for a common DHCR7 null mutation and a milder missense mutation, whereas the most mildly affected SLOS individuals carry two missense alleles with residual DHCR7 activity. Various genetic disorders such as fragile X syndrome, Joubert syndrome, Prader-Willi syndrome, tuberous sclerosis, and Down syndrome may present with autism spectrum disorders [Folstein and Piven, 1991].

Individuals with SLOS children have a high incidence of autism [Tierney et al., 2001]. Among 17 patients with SLOS administered the algorithm questions of the Autism Diagnostic Interview-Revised (ADI-R; Lord et al., 1994), 53% met criteria for autism. Furthermore, of 9 patients who began cholesterol supplementation before age 5.0 years, 22% satisfied the criteria for autism, whereas, of 8 not supplemented with cholesterol before age 5.0 years, 88% met the criteria for autism at age 4.0–5.0 years. Many parents of SLOS patients have reported changes in their children's abnormal behaviors—including autistic behaviors—within days of supplementation, before there is a change in the plasma cholesterol or 7DHC level. This suggests that the behavioral change follows changes in the levels of cholesterol-derived steroid precursors or other compounds rather than the level of cholesterol, which cannot cross the blood-brain barrier.

At the mild extreme of the biochemical spectrum, SLOS patients may have no evident dysmorphism, although many will have developmental delay, severe behavioral disturbances, or autism. We therefore hypothesized that individuals with ASD who have undiagnosed SLOS or another sterol disorder may not be rare, and that other disorders of cholesterol metabolism may similarly manifest as ASD. We designed a study to determine 1) the incidence of SLOS in a cohort of subjects from mostly multiplex ASD families (multiplex = 2 or more first-degree relatives with ASD), and 2) whether or not other disorders of sterol metabolism might present as ASD. Determining the prevalence of individuals with ASD who have SLOS or other sterol disorders and their specific clinical findings could help identify which subjects with ASD should be tested for a sterol disorder. In addition, the study of such individuals might lead to the discovery of mechanisms of ASD of more general importance to research and treatment of ASD in patients lacking defects in cholesterol metabolism.

Methods

Our study was performed in compliance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) and standards established by the Institutional Review Board of the Johns Hopkins Medical Institutions, Cure Autism Now, Smith-Lemli-Opitz Advocacy and Exchange, and AGRE.

Because SLOS is an autosomal recessive disorder, ASD cases due to SLOS should occur at a higher frequency in multiplex families than in singleton families. Moreover, because

SLOS is a nonprogressive metabolic disorder with no known predilection for acute brain injury, regression should not be a characteristic of ASD due to SLOS. Therefore, 100 AGRE samples were selected with the inclusion criteria: 1) 2 or more children with ASD in the same sibship, 2) age ≥ 2.0 years, 3) no loss of developmental milestones, and 4) ADI-R long version results available. Only 1 sample per AGRE family was analyzed, and 1 subject was a monozygotic twin. AGRE used ADI-R data to derive 3 affected status categories: autism, not quite autism (NQA), and broad spectrum (BS). NQA is 1 point away from meeting autism criteria on any or all of the social, communication, and/or behavior domains, and either meets criteria on the “age of onset” domain or meets criteria on all 3 domains, but does not meet criteria on the “age of onset” domain. BS shows patterns of impairment within the spectrum of pervasive developmental disorders [AGRE, 2005].

The samples were obtained in stages. The first 51 samples (cohort 1) met the requirement that there be at least one affected female in the family to increase selection bias for an autosomal recessive condition. However, because there were insufficient autism families to provide 100 samples while retaining the female sib requirements, the 49 additional samples included individuals from families that did not have an affected female (cohort 2). In the second stage, a greater number of individuals with the diagnoses of BS and NQA were chosen. This, in effect, broadens the spectrum of diagnoses and represents better the spectrum of ASD in the general population.

Serum samples from the 100 selected ASD individuals (22 females and 78 males) were analyzed in the Kennedy Krieger Institute Clinical Mass Spectrometry Laboratory (CMSL). Cholesterol and all post-squalene sterol precursors, including 7DHC, were identified and quantified by dual column gas chromatography FID + mass spectrometry [Kelley, 1995]. Repository samples that had been divided into 25 μ L aliquots and stored at -70°C were transferred to CMSL and stored at -20°C for ≤ 2 weeks before analysis.

Results

No sample had an abnormally increased level of 7DHC consistent with the diagnosis of SLOS or abnormal level of any other sterol precursor of cholesterol. However, 19 of the 100 samples had a total cholesterol level lower than 100 mg/dL, which is below the 5th centile for 7,499 youths, age 4–19 years, in the CDC's National Health and Nutrition Examination Survey (NHANES, 2006). There was a significant difference (t-test $p < .001$) between the mean $\pm$ SD of cholesterol levels of the 19 samples below 100 mg/dL (89.1 ± 11.1) and the other 81 samples (138.3 ± 32.8). Patients with a cholesterol level below 100 mg/dL in the first group had a statistically lower level of lathosterol (0.199 $\mu\text{g/mL}$) compared to the full group 1 cohort (0.461 $\mu\text{g/mL}$; $p < 0.05$). When expressed relative to cholesterol, the difference was 0.028% vs. 0.050%, which was just above the $p = 0.05$ level.

The low cholesterol (<100 mg/dL) group included 16 males and 3 females. Of those 19 individuals in the low cholesterol group, 31.5% met criteria for a broad spectrum diagnosis other than autism, while, of the other 81 individuals, 10 % met the same criteria.

Discussion

These results indicate that SLOS is an uncommon cause of ASD in individuals without clinical signs of SLOS. Because we found no SLOS patients among the 100 patients selected to have a greater representation of autosomal recessive genetic disorders, unrecognized SLOS probably represents less than 1% of autism and, more likely, less than 0.2%. This is consonant with the epidemiology of SLOS and the distribution of DHCR7 alleles. As described by Kelley and Herman [2001], at least 80% of DHCR7 mutations are null

mutations. Given the previous estimate of a carrier frequency of 1.25% for null alleles, this predicts a prevalence of milder missense mutations of 0.2 to 0.3%, and thus a collective carrier frequency of approximately 1.5% in European-derived populations. The small percentage of SLOS patients whose physical findings are so mild that they could escape recognition until the age when a diagnosis of ASD could be made, most have been compound heterozygotes for two mild missense mutations or, occasionally, a null allele with a unique high residual activity missense allele. Therefore, one can estimate a birth incidence of SLOS mild enough to be “misdiagnosed” as ASD of approximately 1 in 400 – 500,000 births ($0.003 \times 0.003 \times 0.25$). If the incidence of non-regressive autistic spectrum disorder is estimated to be 1 in 1,000 births, the incidence of SLOS in autism is about 0.2%, in agreement with the high estimate from our biochemical screening of the AGRE samples. Thus, even using an incidence of non-regressive autism that may underestimate the current incidence of autism, our results indicate that routine screening of ASD for disorders of cholesterol biosynthesis is not indicated. Moreover, our clinical experience with close examination of biochemically minimally affected SLOS patients, whom we find are not truly normal physically, is that the most efficient way to screen for SLOS-caused ASD is a careful physical examination by a geneticist, who at the same time could assess the patient for other genetic conditions that can present as ASD.

The detection of a high incidence of hypocholesterolemia in our patient population is a finding of potential clinical significance regarding the possible role of non-SLOS cholesterol disorders in the etiology of ASD. A limitation of our study is that we lack information regarding parental lipoprotein profiles, which could help identify conditions such as hypobetalipoproteinemia, which are common but not known risk factors for autism. A major limitation of our study sample is the lack of clinical information about diet and other conditions, such as chronic diarrhea, medications, and the use of alternative therapies, that could underlie the hypocholesterolemia found in 19% of our subjects. However, studies indicate that individuals with decreased dietary intake or increased intestinal losses of cholesterol will have increased levels of cholesterol precursors, especially lathosterol (Lund et al., 1989), which we did not find in comparing the patients with cholesterol levels below 100 mg/dL vs. the full cohort. Rather, individuals with a cholesterol level below 100 mg/dL had a statistically lower level of lathosterol compared to the entire cohort, indicating that the cause of the hypocholesterolemia was decreased cholesterol synthesis rather than increased cholesterol losses from gastrointestinal disturbances of abnormal diets. Instead, the data argue that the ASD patients with low cholesterol levels have intrinsically reduced cholesterol synthesis, similar to patients with Smith-Lemli-Opitz syndrome.

Several studies of ASD patients have reported increased incidences of chronic diarrhea, “fungal overgrowth,” and other intestinal disorders, which could be associated with hypocholesterolemia caused by bile acid and cholesterol malabsorption. However, in view of the rapid behavioral response of SLOS children to cholesterol supplementation, including partial remission of autistic spectrum behaviors, genetic or acquired hypocholesterolemia could be a contributing factor to the development of ASD. Our results, therefore, warrant further study. Many SLOS experts have speculated that the rapid behavioral response of SLOS patients to cholesterol supplementation is mediated by changes in neurosteroid levels, which are known potent modulators of behavior via modulation of GABA receptor synthesis and the responsiveness of receptors to GABA [Kelley and Herman, 2001]. Indeed, the behavioral profile of SLOS [Tierney et al., 2001] has several features, such as excessive anxiety, that would be expected in a CNS GABA-deficiency syndrome.

In summary, we have shown that, although ASD is a very common behavioral profile in SLOS, SLOS is a rare cause of ASD, accounting for no more than 1% of ASD. However, the unexpected finding that up to 20% of children from a sample of mostly multiplex ASD

sibships have substantial hypocholesterolemia warrants further research, since the study of hypocholesterolemia and its predicted effects on neurosteroid metabolism may offer important insights into the cause and treatment of ASD.

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TABLE I

Characteristics of participants under study

Cholesterol Group	Subjects (n)	Age (y) (Mean±SD)	Diagnoses
All subjects	100	9.5±3.0	Autism: 83 BS: 14 NQA: 3
<100 mg/ dL	19	9.1±1.7	Autism: 13 BS: 6 NQA: 0
≥100 mg/ dL	81	9.6±3.3	Autism: 70 BS: 8 NQA: 3
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<u>1st cohort subjects</u>	51	10.4±3.6	Autism: 46 BS: 3 NQA: 2
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<u><100 mg/ dL</u>	9	9.0±2.0	Autism: 7 BS: 2 NQA: 0
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<u>≥100 mg/ dL</u>	42	10.6±3.8	Autism: 39 BS: 1 NQA: 2
<hr/>			
<u>2nd cohort subjects</u>	49	8.6±2.0	Autism: 37 BS: 11 NQA: 1
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<u><100 mg/ dL</u>	10	9.1±1.5	Autism: 6 BS: 4 NQA: 0
<hr/>			
<u>≥100 mg/ dL</u>	39	8.5±2.1	Autism: 31 BS: 7 NQA: 1

BS, broad spectrum; NQA, not quite autism.