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Autism: The role of cholesterol in treatment

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Abstract
Cholesterol is essential for neuroactive steroid production, growth of myelin membranes, and normal embryonic and fetal development. It also modulates the oxytocin receptor, ligand activity and G-protein coupling of the serotonin-1A receptor. A deficit of cholesterol may perturb these biological mechanisms and thereby contribute to autism spectrum disorders (ASDs), as observed in Smith-Lemli-Opitz syndrome (SLOS) and some subjects with ASDs in the Autism Genetic Resource Exchange (AGRE). A clinical diagnosis of SLOS can be confirmed by laboratory testing with an elevated plasma 7DHC level relative to the cholesterol level and is treatable by dietary cholesterol supplementation. Individuals with SLOS who have such cholesterol treatment display fewer autistic behaviours, infections, and symptoms of irritability and hyperactivity, with improvements in physical growth, sleep and social interactions. Other behaviours shown to improve with cholesterol supplementation include aggressive behaviours, self-injury, temper outbursts and trichotillomania. Cholesterol ought to be considered as a helpful treatment approach while awaiting an improved understanding of cholesterol metabolism and ASD. There is an increasing recognition that this single-gene disorder of abnormal cholesterol synthesis may be a model for understanding genetic causes of autism and the role of cholesterol in ASD.

Introduction

Autism spectrum disorders (ASDs) are defined by core abnormalities in reciprocal social interaction and communication, and by the presence of restrictive or stereotyped interests and behaviours (Muhle, Trentacoste, & Rapin, 2004). In the majority of cases, specific underlying causes cannot be identified. However, a number of factors are being investigated including genetic, infectious, metabolic and environmental, with specific causes known in less than 10% to 12% of cases (Muhle et al., 2004). With an incidence of 1 in 150 (Centers for Disease Control, 2007), ASDs manifest in early childhood and persist throughout life in majority of the cases. Evidence of a genetic contribution in ASDs which includes increased concordance in monozygotic compared to dizygotic twins, increased recurrence risk in siblings, and cognitive, language, and behavioural disturbances in close relatives (Muhle et al., 2004). ASDs are associated with specific heritable disorders such as fragile X syndrome, phenylketonuria, tuberous sclerosis and with sterol abnormalities as observed in Smith-Lemli-Opitz syndrome (SLOS) (Smith, Lemli, & Opitz, 1964).

SLOS and its association with cholesterol

SLOS is an autosomal recessive disorder due to an inborn error of cholesterol metabolism that is caused by mutations of the 7-dehydrocholesterol (7DHC) reductase gene (DHCR7) (Tint et al., 1994), present on chromosome 11q12–13 (Wassif et al., 1998, Fitzky et al., 1998). SLOS is not uncommon, with an estimated incidence among individuals of European ancestry of 1 in 20,000 to 1 in 60,000 births and a carrier frequency of at least 1% (Tabin & McMahon, 1997). In people with SLOS, this enzyme functions abnormally and, as a result, there is not enough cholesterol produced in the body and 7DHC accumulates (Figure 1).

SLOS is characterized by a broad spectrum of phenotypic abnormalities including developmental delay, the characteristic facial anomalies of hypertelorism (wide-set eyes), posteriorly rotated ears, a high arched palate, a prominent nasal bridge, upturned nares and 2–3 toe syndactyly (webbing). The lower the plasma cholesterol level, the more severe are the phenotypic abnormalities in SLOS with malformations of the heart, brain, lungs, and genitals frequently found in more severe cases (Tabin & McMahon, 1997).

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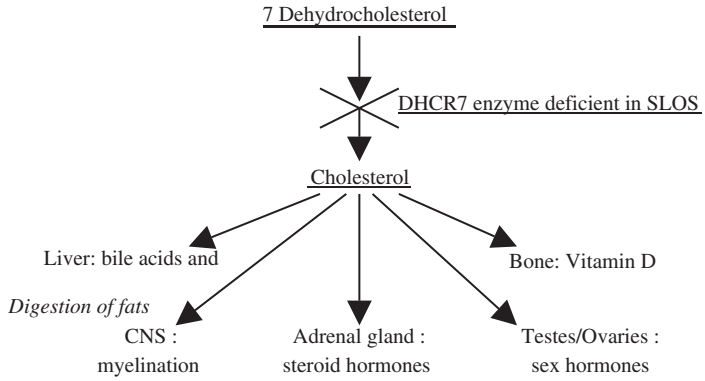

Figure 1. The pivotal role of cholesterol.

The SLOS behavioural phenotype includes many features found in ASD, including social and language impairments as well as repetitive and stereotyped behaviours. In one study with 17 SLOS subjects who were administered the Autism Diagnostic Interview – Revised (ADI) (Lord, Rutter and Le Couteur, 1994), 53% met DSM-IV and the ADI algorithm diagnostic criteria for autism (Tierney, Nworkoro, & Kelly, 2001). Another recent study reported that approximately three quarters of the children with SLOS had some variant of ASD, suggesting the most consistent relationship of any single gene disorder with ASDs (Sikora et al., 2006). Other behavioural symptoms include repeated self-injury (89%), self-biting (54%), head banging (48%), and opisthokinesis (54%) (a highly characteristic upper body movement in which there is an arched backwards diving motion) (Tierney et al., 2001). Cognitive functioning in SLOS individuals range from borderline intellectual functioning to profound mental retardation (Smith et al., 1964). These physical and behavioural features of SLOS are summarized in Table I and seen in Figures 2 and 3.

Although SLOS is associated with ASDs (Tierney et al., 2001, Sikora, Petit-Kekel, Penfield, Merkens, & Steiner, 2006), the incidence of SLOS and other sterol disorders among individuals with ASDs is not known. In blood samples of ASD subjects from multiplex families (families in which more than one child in the immediate family had ASD) ($n = 100$) obtained from the Autism Genetic Research Exchange specimen repository, no sample had sterol levels consistent with SLOS, but 19/100 had total cholesterol levels lower than 100 mg/dl, which is below the fifth centile for children above two years of age (Tierney et al., 2006) These findings suggest that there may be other disorders of sterol metabolism or homeostasis associated with ASDs, in addition to SLOS (Geschwind et al., 2001). Also, the fact that 20% ASD children had hypocholesterolemia warrants further study since hypocholesterolemia and its predicted effects on neurosteroid metabolism and related cholesterol dependent biomechanisms may offer insights into the causes and treatment of ASD.

The cause of hypocholesterolemia seen in SLOS and some individuals with ASDs is decreased cholesterol synthesis rather than low cholesterol from gastrointestinal disturbances or abnormal diets. Individuals with decreased dietary intake or increased intestinal losses of cholesterol will have *higher* serum levels of cholesterol precursors, especially lathosterol (Lund, Sisfontes, Reihner, & Bjorkhem, 1989). In contrast, subjects with ASDs with cholesterol levels below 100 mg/dl, similar to SLOS subjects, have intrinsically reduced cholesterol synthesis and *lower* levels of lathosterol (Tierney et al., 2006).

Cholesterol related etiological pathways in ASDs: Lessons from SLOS

There is an increasing recognition that this single-gene disorder of abnormal cholesterol synthesis may be a model for understanding genetic causes of autism and the role of cholesterol in ASDs (Tabin & McMahon, 1997). Current research suggests that alteration in cholesterol production secondary to SLOS impacts dendrite differentiation, brain receptor functioning, myelination, steroid hormone synthesis and subsequently, the final development of the brain and central nervous system. Therefore, it is possible that disruption of these components may result in the presentation of various ASD features.

The presence of ASDs in individuals with SLOS is frequent (1: 2) and is much higher than in the general population (1:150), and it is among the highest when compared to other single-gene disorders, indicating that a genetic defect in SLOS could be responsible for the ASD phenotype present in SLOS (Tierney et al., 2001). The high prevalence of ASDs in SLOS reported by two independent studies (Tierney et al., 2001, Sikora et al., 2006) using different methodologies suggests that these conditions might share etiological mechanisms. Also, high prevalence of

Table I. Phenotypic characteristics of SLOS.

Physical features	Behavioural features
Microcephaly	Autism spectrum disorder
Ptosis (drooping of eyelids)	Social impairment
Low-set and small ears	Communication disorder
Hand or foot malformations (2–3 toe syndactyly, clinodactyly)	Repetitive and ritualistic behaviours
Soft cleft palate or bifid uvula	Mental retardation
Failure to thrive/feeding difficulties	Self-injury
Malformations of heart, brain, and lungs	Opisthokinesis (backward arched motion)
Malformations in gastrointestinal and genital tracts	Anxiety
Growth retardation	Attention deficit hyperactivity disorder
Hypotonia	Severe sleep disturbance
	Sensory hyper reactivity

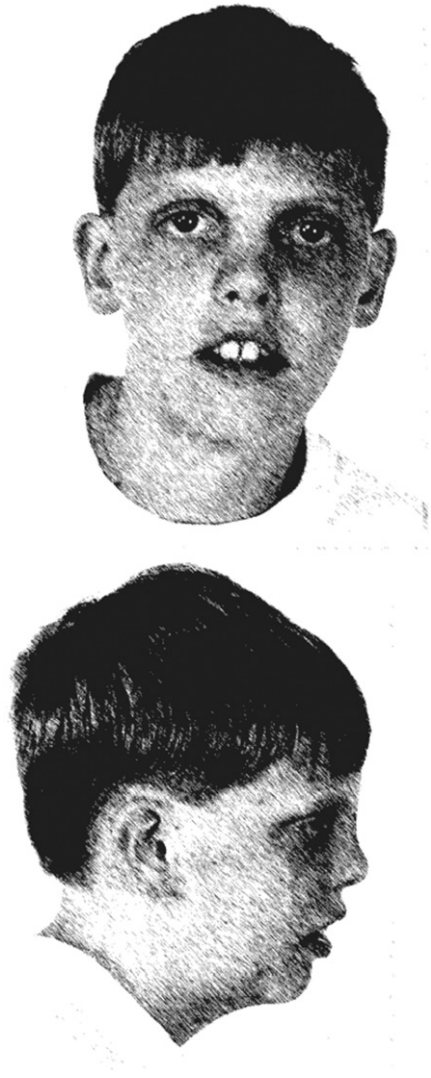

Figure 2. Physical features of SLOS.

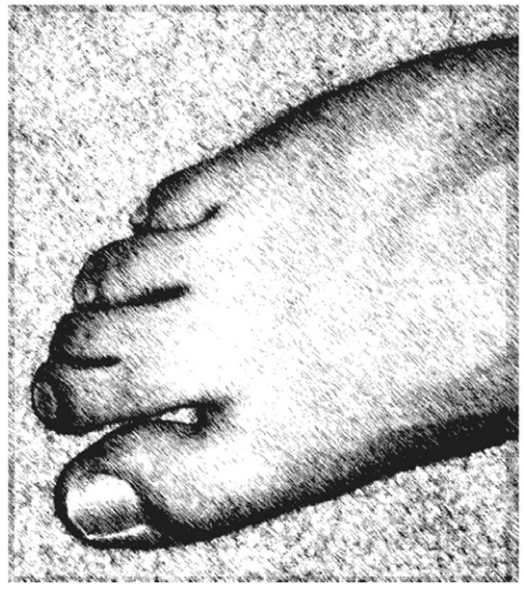

Figure 3. Toe syndactyly in SLOS.

abnormally low cholesterol blood levels in an ASD population is a finding of potential significance regarding the possible role of non-SLOS cholesterol disorders in the etiology of ASDs as well (Smith et al., 1964). Such sterol abnormalities, which involve multiple genes, may impact a common neurodevelopmental pathway that results in behaviours observed in ASDs.

Below are hypotheses regarding the role of sterol function in ASD and SLOS

Cholesterol is necessary for normal embryonic and fetal development. CNS abnormalities, similar to those observed in individuals with ASD, have been noted in individuals with SLOS. These are considered to result in a disruption in the hedgehog patterning protein signalling pathway, secondary to inadequate cholesterol availability (Tabin & McMahon, 1997). The SLOS mouse model has exhibited hippocampal abnormalities, commissural deficiencies, and hypermorphic development of serotonin (5-HT) neurons that may help explain the ASD behavioral phenotype seen in individuals with SLOS (Waage-Baudet et al., 2003).

Cholesterol is a precursor of neuroactive steroids. The defect in cholesterol synthesis in SLOS may lead to abnormal neurosteroid production (Marcos, Guo,

Wilson, Porter, & Shackleton, 2004). These neuroactive steroids modulate neurotransmitter receptor activity. Thus, a deficit in neurosteroids may be associated with both anxiety and mood disorders (Pisu & Serra, 2004). There may be lower dehydroepiandrosterone (DHEA) and DHEA-sulfate (DHEA-S) levels in adults with ASDs (Strous et al., 2005).

Cholesterol is required for the growth of myelin membranes. In mice created without ability to synthesize cholesterol in myelin-forming oligodendrocytes, it was shown that cholesterol availability to oligodendrocytes is a rate-limiting factor for brain maturation (Saher et al., 2005).

Cholesterol can be a modulator in oxytocin receptor functioning (Gimpl, Wiegand, Burger, & Fahrenholz, 2002). Oxytocin has been found to be associated with social functioning (Hollander et al., 2007). Therefore, in individuals with mutation in the gene for the oxytocin receptor, a low cholesterol level may affect the oxytocin receptor function and this in turn could result in abnormal social functioning in individuals with SLOS and individuals with ASD from other etiologies.

Cholesterol is a modulator of the ligand binding activity and G-protein coupling of the serotonin1A (5-HT1A) receptor (Chattopadhyay, Jafurulla, Kalipatnatpu, Pucadyil, & Harikumar, 2005). Dysfunction in the serotonin system due to abnormal cholesterol metabolism may lead to the social and behavioural problems seen in individuals with SLOS and ASDs. Cholesterol is an important constituent of the lipid rafts of the serotonin transporter (Allen, Halversnon-Tamboli and Rasenick, 2007) and disruption of lipid rafts by cholesterol-interfering agents produces a 50% decrease in the transport rate of the GABA transporter, 5-HT transporter (5-HTT), and glutamate transporters (Saher et al., 2005). The mouse model of SLOS supports the link between cholesterol abnormalities and abnormal serotonergic neuron development (Waage-Baudet et al., 2003). Clinically, SLOS may present with debilitating irritability, sleep disturbance and obsessive compulsive disorder. While speculative, it is possible that such symptoms, while sometimes responsive to SSRIs, might also improve with cholesterol supplementation. Unfortunately, there are no data so far to suggest that individuals with ASD who respond to SSRIs might also have cholesterol abnormalities.

Assessment approaches

Professional organizations such as the American Academy of Neurology and the Child Neurology Society are recommending screening for biochemical and genetic disorders that are associated with ASDs (Filipek et al., 2000). The recommended diagnostic tests to evaluate a child for recognized causes of ASDs (in the presence of mental retardation) include a lead level, high-resolution chromosome study, and DNA probe for fragile X. Tests to be considered include EEG, quantitative urinary organic and plasma amino acids, and carbohydrate deficient glycoprotein analysis, based upon additional symptoms and clinical findings (Zimmerman, 2005).

A clinical diagnosis of SLOS can be confirmed by biochemical testing. An elevated plasma 7DHC level relative to the cholesterol level establishes the diagnosis. Furthermore, although the majority of individuals with SLOS have lower than normal cholesterol levels, there are a number of individuals diagnosed clinically and biochemically with SLOS who have normal total cholesterol levels and only mildly elevated levels of 7DHC. Thus, a normal value for plasma cholesterol does not exclude the diagnosis of SLOS. The specific test for SLOS – analysis of the ratio of 7DHC to cholesterol – is required.

Cholesterol treatment approaches

The recognition of the biochemical cause of SLOS not only resulted in discovery of the diagnostic marker, the ratio of plasma levels of 7DHC to total cholesterol, but also offered a potential treatment. The current standard treatment for SLOS is to begin dietary cholesterol supplementation as soon as this condition is diagnosed. Cholesterol is supplied in natural form (single egg yolk, cream, liver) or as purified cholesterol; the starting dose for purified cholesterol is 40 to 50 mg/kg/day up to 150 mg/kg/day for maintenance treatment. Tube feeding is often required in infants and younger children because of feeding difficulties. Medical and surgical management of gastroesophageal reflux may be needed. During severe acute illness (e.g. infections) or following major surgical procedures, patients with SLOS may develop overt adrenal insufficiency requiring fresh frozen plasma as a source of cholesterol.

Ironically, multiple lines of evidence suggest statins, a class of medications used to lower cholesterol in those with abnormally high levels, may improve DHCR7 activity resulting in increased cholesterol levels in individuals with mild DHCR7 deficiency (Jira et al., 2000), in human fibroblasts from mildly affected individuals (Wassif et al., 2005) and in a SLOS mouse model (Correa-Cerro et al., 2006). The mechanism by which statins increase cholesterol is hypothesized to result from increased expression of a DHCR7 allele that encodes a mutant enzyme with residual enzymatic function and is supported by *in vitro* experiments using SLOS fibroblasts (Wassif et al., 2005).

Although Sikora and colleagues (Sikora et al., 2004) found that the developmental status of SLOS did not improve over time with cholesterol supplementation; some reports suggest that cholesterol supplementation improves growth, speech articulation and neurodevelopmental status (Irons et al., 1997). Of subjects with SLOS who began cholesterol supplementation before the age of 5 years (9/17), 22% (2/9) met the criteria for autism at age 4 to 5 years, whereas, of the 8 subjects not supplemented with cholesterol before age 5 years, 88% met the criteria for autism at age 4 to 5 years (Tierney et al., 2001). Although these findings are clearly preliminary, they suggest that cholesterol supplementation may prevent the onset of ASDs in some children with SLOS.

In addition, open label studies with cholesterol treatment in children with SLOS have demonstrated statistically significant reductions in the number of autistic behaviours and infections. Also, increased growth, weight gain, improved sleep (Tabin & McMahon, 1997; Tierney et al., 2000); reduced irritability, hyperactivity, better affect and attention span have been found (Irons et al., 1995; Nwokoro & Mulvihill, 1997; Opitz, 1999). Individuals with SLOS treated with cholesterol have been reported to be more sociable, including initiating hugs and being more interactive (Irons et al., 1995; Ryan et al., 1998). Other behaviours shown to improve with cholesterol supplementation include aggressive behaviours (Nwokoro & Mulvihill, 1997; Ryan et al., 1998), self injury (Irons et al., 1995), temper outbursts, trichotillomania and tactile defensiveness (Nwokoro & Mulvihill, 1997).

To date, no effect of cholesterol supplementation on cognitive development in pre-pubertal children has been shown (Tabin & McMahon, 1997). It is possible that supplementation with cholesterol beginning in infancy or early childhood may improve cognitive outcomes in SLOS patients. Furthermore, no side effects have been reported with cholesterol treatment, which has been in use for about 15 years, and blood cholesterol levels tend to not increase significantly beyond the pre-supplementation cholesterol levels, so the risk for atherosclerosis does not appear to be increased.

Certain medications for treatment of behavioural and psychiatric disorders interfere with the activity of enzyme DHCR7, and thus lower the production of cholesterol. However, the benefits of these medications for behavioural treatment may outweigh the potential risks of lowering cholesterol synthesis. Mildly to moderately raised levels of 7DHC have been found in three psychiatric patients without SLOS who were treated with haloperidol (Nowaczyk & Tierney, 2004), with 7DHC levels directly proportional to the dose of haloperidol. 7DHC levels decreased to normal upon haloperidol discontinuation, although it is unknown if an increase in 7DHC levels would have a beneficial or a deleterious effect.

Considering that there have been several studies, as discussed above, showing improvement in various symptoms of ASDs with cholesterol supplementation, this ought to be considered as a helpful treatment approach while awaiting an improved understanding of cholesterol metabolism and ASD.

Conclusion

The importance of performing biochemical analyses in individuals with ASD for SLOS who have its relevant physical or behavioural features is essential for its early diagnosis (Nowaczyk & Waye, 2001). The study of phenotype-genotype relationships in single-gene disorders such as fragile X syndrome, Rett syndrome, and SLOS may provide insights as to how the disruptions of biological mechanisms observed in these disorders may result in the symptoms of ASD.

Current research on SLOS also leads to the question whether abnormalities in cholesterol metabolism not due to SLOS may exist in patients with 'typical' ASD. A cholesterol deficit might lead to: physical abnormalities; abnormal structure of sterol-rich membranes, such as myelin; impairment in the function of serotonin and other brain receptors; and disruption in the synthesis and metabolism of sterols, including neuroactive steroids. It is likely that in some forms of ASD, the symptoms may be due to interaction of components that are sterol dependent. Therefore, further study of SLOS and the consequent abnormal cholesterol conditions would help us understand etiological mechanisms and treatment of ASD in individuals without defects in cholesterol metabolism. Thus, treatment of SLOS with cholesterol supplementation might be a precursor to future innovative treatment approaches in individuals with autism.

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