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The Near Universal Presence of Autism Spectrum Disorders in Children With Smith–Lemli–Opitz Syndrome

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Smith–Lemli–Opitz syndrome (SLOS) is an autosomal recessive condition caused by a defect in cholesterol synthesis. Affected children often have malformations and mental retardation. Autistic behaviors also are evident. The purpose of the present study was to determine the prevalence of autism spectrum disorders (ASDs) in children with SLOS. Fourteen children, 3–16 years old, were evaluated using three different methods to document autistic symptoms: (a) parent interview, (b) direct observation, and (c) a behavior checklist. Blood sterols were also measured at regular intervals. Each subject was determined to have Autistic Disorder, Pervasive Developmental Disorder, not otherwise specified (PDD NOS), or no diagnosis on the autism spectrum, based on DSM-IV criteria. Correlations among variables were calculated, and blood sterol levels were compared between diagnostic groups. Approximately three-fourths of the children with SLOS (71–86% depending on the evaluation method) had an ASD, about 50% diagnosed with Autistic Disorder and the rest with PDD NOS. The children's baseline cholesterol, 7-dehydrocholesterol (7-DHC), and 8-dehydrocholesterol (8-DHC) levels, and cholesterol levels following supplementation did not correlate with the presence or severity of autistic symptoms. These results suggest that most children with SLOS have some variant of autism. SLOS appears to have the most consistent relationship with autism of any single gene disorder. Therefore, a link between cholesterol metabolism and autism is suggested. With further study, these findings, together with knowledge of the genetic and biochemical defects in SLOS, will likely provide valuable insights into the causes of autism in general. © 2006 Wiley-Liss, Inc.

Key words: SLOS; autism spectrum disorders; cholesterol metabolism; sterol; neurosteroids; standardized assessments; dietary treatment

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INTRODUCTION

Children with Smith–Lemli–Opitz (or RSH) syndrome (SLOS) are impaired in their abilities to convert 7-dehydrocholesterol (7-DHC) to cholesterol in the final step of cholesterol biosynthesis, causing low plasma and tissue cholesterol concentrations and increased plasma and tissue 7-DHC and its metabolite 8-dehydrocholesterol (8-DHC) [Tint et al., 1994]. SLOS is caused by deficiency of the final enzyme in the cholesterol synthetic pathway, 7-dehydrocholesterol reductase (DHCR7). Affected individuals are homozygotes or compound heterozygotes for mutations in the gene encoding the SLOS enzyme, *DHCR7* [reviewed in Witsch-Baumgartner et al., 2001]. The SLOS phenotype includes malformations, motor delay with hypotonia, oral motor dysfunction, mental retardation, and difficult behavior [Kelley and Hennekam, 2000]. Manifestations of SLOS appear to be caused not only by the cholesterol deficiency, but also by potentially toxic accumulations of 7-DHC and 8-DHC throughout the body, including the brain. As the critical role of cholesterol synthesis in the development and functioning of the central nervous system (CNS) has emerged [Dietschy and Turley, 2004], it has become

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increasingly clear that the developmental and behavioral difficulties found in individuals with SLOS are secondary to cholesterol deficiency (or related excess of cholesterol precursors) within the CNS and possibly elsewhere in the body [Porter, 2003]. Cholesterol supplementation has been proposed for the past 10 years as a treatment for SLOS. Despite cholesterol's inability to cross the blood-brain barrier, there is anecdotal evidence that cholesterol supplementation can improve many areas of functioning in individuals with SLOS (e.g., mobility, caloric intake, growth) [Irons et al., 1997]. However, we have shown that dietary cholesterol supplementation does not improve developmental functioning [Sikora et al., 2004].

"Idiopathic" autism is a complex neurological disorder with an increasing prevalence in the United States and around the world [Yeargin-Allsopp et al., 2003]. Over the past 10 years, the prevalence of autism has increased from 1 in 1,000 to 1 in 150–200 individuals. A growing number of genetic disorders are associated with autism, including Fragile X syndrome, tuberous sclerosis, Angelman syndrome, duplication of 15q11-q13, Rett syndrome, Down syndrome, and Cohen syndrome [Cohen et al., 2005]. The word autism has been used rather indiscriminately to describe a heterogeneous group of individuals with core deficits in socialization, communication, and repetitive/stereotypic behavior. However, the DSM-IV-TR [APA, 2000] distinguishes Autistic Disorder from other types of Pervasive Developmental Disorders, including Asperger's Disorder, Childhood Disintegrative Disorder, Rett's Disorder, and Pervasive Developmental Disorder, not otherwise specified (PDD NOS). For clarity, we use the term autism spectrum disorders (ASDs) herein, instead of the term autism or PDDs, to refer to all Pervasive Developmental Disorders.

A large percentage of individuals with SLOS display numerous signs and symptoms of ASDs [Tierney et al., 2000; Cohen et al., 2005]. Opitz [1999] first reported autistic behavior in individuals with SLOS, while Tierney et al. have also written about the relationship between autistic behavior and SLOS [Tierney et al., 2000, 2001]. Children with SLOS not only exhibit core symptoms of autism, but also secondary characteristics such as sleep problems, feeding problems, and sensory issues [Tierney et al., 2000]. Tierney et al. [2001] reported that 53% of their subjects with SLOS (9 of 17) met the Autism Diagnostic Interview-Revised (ADI-R [Lord et al., 1999]) algorithm criteria for the clinical diagnosis of autism. These same nine individuals also met DSM-IV [APA, 1994] diagnostic criteria for autism. The authors further noted that only 22% of subjects who began cholesterol supplementation before the age of 5 met criteria for autism, while 88% of subjects beginning cholesterol supplementation after age 5 met the criteria. Tierney et al. [2001] cited the use of informant (usually parent) report rather than subject examination as a limitation of their study. They recommended that future investigation of the relationship between SLOS and autism should rely on direct observation.

The purpose of the present study was to build on the work by Tierney et al. [2001] by documenting the prevalence of ASDs in a different group of children with SLOS and by gathering information in three ways, using reliable and valid diagnostic methods and the latest published diagnostic criteria. In addition, the utility and results of the three different methods for assessing autistic behaviors could be compared. We also sought to characterize the pattern of autistic behaviors in children with SLOS, the relationship between autism and cholesterol and 7- and 8-DHC, and the effects of cholesterol supplementation. We hypothesized that if individuals with SLOS are carefully evaluated prospectively using a standardized instrument, the Autism Diagnostic Observation Schedule-Generic (ADOS-G [Lord et al., 2000]), nearly all would show ASD manifestations, with a significant proportion falling into the autism category. In addition, we hypothesized that most individuals in our sample of children with SLOS also would meet DSM-IV-TR diagnostic criteria for Autistic Disorder based on a DSM-IV Checklist, and parents' completion of an autism-specific questionnaire, the Gilliam Autism Rating Scale (GARS [Gilliam, 1995]). In other words, we hypothesized that there would be good agreement between three different instruments used to assess autism.

METHODS

Patients. Fourteen children diagnosed with SLOS (seven males, seven females) ages 3–16 (M = 7.1, SD = 3.5) participated in this study, as part of participation in a longitudinal study of SLOS (see Table I). SLOS was confirmed in all by biochemical analysis. All patients live with their parents and attend either public or private educational activities. All had received continuous cholesterol supplementation for a minimum of 2 years, and some up to 8 years, at the time of their evaluation (M = 4 years, 9 months, SD = 2 years, 5 months). For most patients, supplementation includes egg yolks consumed on their own or mixed with other foods. Some use crystalline cholesterol in oil, aqueous crystalline cholesterol, butterfat, heavy cream, or milk to provide supplemental cholesterol.

Measures. At the present time, the ADOS-G is considered the "gold standard" of autism assessment, used in a wide variety of research, clinical, and educational settings [American Academy of Pediatrics, 2001]. The ADOS-G is a semi-structured, standardized, play-based assessment measure [Lord

TABLE I. Demographic Information for Each Participant

Patient	Age	Gender	Length of cholesterol supplementation	SLOS severity score[a]	Biochemical severity score[b]	IQ/DQ
14	16	M	Unknown	6	0.192	54
63	9	F	2 years	10	0.093	65
28	7	F	6 years, 7 months	17	0.076	64
03	5	M	6 years, 8 months	11	0.261	23
55	3	M	2 years, 9 months	15	0.032	29
02	8	F	7 years, 11 months	17	0.386	29
20	11	F	7 years, 6 months	33	0.470	28
41	5	F	5 years, 8 months	17	0.017	62
77	3	M	2 years, 8 months	5	0.005	35
06	7	M	7 years, 6 months	17	0.155	75
64	10	F	2 years	10	0.110	70
33	5	M	3 years, 3 months	6	0.072	53
21	7	F	8 years, 10 months	10	0.045	51
62	4	M	2 years, 2 months	30	0.226	31

[a]Based on a 0–100 scale, non-linear. Individuals receive a score of 0–10 on 10 different malformations, and the scores are summed. Many scores of 0 are obtained. Higher scores indicate both more malformations and greater severity of malformations.
[b]Biochemical severity score = Baseline 7-DHC + 8-DHC/Total sterols.

et al., 2000]. The ADOS-G consists of a set of activities that assess social interaction, communication, play skills, and repetitive and stereotyped behaviors. These activities provide planned opportunities to elicit autistic behaviors that can then be coded and analyzed. The ADOS-G is divided into four separate modules, each having its own protocol and taking 30–60 min to administer. Each module is aimed at a specific level of expressive language ability. The use of different modules reduces possible biasing effects of variability in language skills [Lord et al., 2000]. Only one module is administered to each individual depending on the level of expressive language.

Scoring of the ADOS-G occurs immediately after its administration. Each item is scored on a 0–3 scale (0, no evidence of abnormal behavior to 3, markedly abnormal behavior) scores [Lord et al., 2000]. The ADOS-G algorithms contain those items with the highest inter-rater reliabilities that discriminated among Autism, ASD, and non-spectrum individuals in the standardization sample. Each module has a different algorithm. Items used in the algorithms are divided into four areas: Communication, Social Interaction, Play/Creativity, and Restricted/Repetitive Behaviors or Interests. Cutoff scores in the domains of Communication, Social Interaction, and Combined (Communication + Social Interaction), allow an individual to be placed in a(n) Autism, ASD, or non-spectrum category in each of these three areas. No cutoff scores are available in the domains of Play/Creativity or Restricted/Repetitive Behaviors or Interests due to limited variability in scores across the autism categories. The authors report good to excellent reliability of the items, domains, and classification categories [Lord et al., 2000].

Validity studies were conducted by carrying out several analyses. Correlation matrices were generated for all items on each module for all domains. Inter-correlations that were above 70 for two or more items within a module and overlapped in conceptualization were removed from the algorithm [Lord et al., 2000]. A fixed-effects analysis of variance (ANOVA) was then carried out to compare samples of autism and non-spectrum individuals. Items that did not show significant differences were excluded from the algorithm. Further analyses were conducted to compare three groups (i.e., Autism, PDD-NOS, and non-spectrum) for each of the items that had been retained in the algorithm.

The GARS [Gilliam, 1995] is a behavior checklist developed for use in individuals aged 3–22. The questionnaire consists of 56 items, each describing a different behavior often seen in individuals with autism, divided into four scales: Social Interaction, Communication, Stereotyped Behaviors, and Developmental Disturbances. Those completing the form are asked to rate the frequency of each behavior based on a 4-point scale. The scores for each scale are then summed and converted to standard scores (mean = 10, standard deviation = 3), based on the normative sample of 1,092 individuals previously diagnosed with autism. These four standard scores are then combined into a summary score called the Autism Quotient (AQ; mean = 100, standard deviation = 15), which is then used to predict the probability that a child has autism. The AQ is broken down into seven different categories, ranging from a "Very Low" to a "Very High" probability of autism. A score of 90 or above suggests that the child is "probably autistic." The manual for the GARS claims adequate reliability and validity. The GARS is widely accepted within the autism community as an accurate measure of a child's behavior in schools, clinics, and on research projects. In the present study, the GARS was completed by one or both parents.

A DSM-IV Checklist was created using the 12 specific diagnostic criteria for Autistic Disorder. A structured series of questions about each of the diagnostic criteria were asked of each parent. Responses were recorded, and subsequently the DSM-IV Checklist was completed by one of the authors (D.M.S.), based solely on parent responses. Each criterion was scored as either met or not met, and the total scores in the areas of communication, socialization, and repetitive/restricted behavior were calculated. Independent review of scores by a colleague with expertise in ASDs occurred for each subject, and consensus was reached if there was disagreement.

Procedure. The Institutional Review Board at Oregon Health & Science University gave approval to conduct the study. Parents of all patients gave their informed consent for participation. Patients had been recruited informally, via word of mouth from their primary care physicians, direct contact after reading a published article, or as current patients seen through the OHSU Metabolic Clinic. Every parent seen over the past 2 years agreed to participate; therefore, the sample is very representative of our population as a whole. Subjects from across the United States participating in our NIH-supported longitudinal study of SLOS undergo repeated week-long inpatient evaluations in the General Clinical Research Center (GCRC) every 3–12 months depending on age and distance from the center. During one such visit, 14 children with SLOS participated in the ADOS with one of the authors (either D.M.S. or K.P.K.). Both authors had received training in the administration and scoring of the ADOS and had reached an inter-rater reliability of over 85%. The appropriate module was used, based on the expressive language skill demonstrated by the subject, and was scored immediately after administration (see Table II). During the same evaluation, parents were interviewed regarding specific DSM-IV diagnostic criteria for Autistic Disorder and the DSM-IV Checklist was completed. Parents also independently completed the GARS.

Plasma sterol concentrations were measured by capillary-column gas chromatography on a Perkin Elmer gas chromatograph (model 8500 or AutoSystemXL) with a CP-Wax 57 column (25 M, 0.32 mm ID; 0.25 μm film; Chrompack Co., Rariton, NJ). Internal standard (5α-cholestane) and authentic cholesterol standards were used for calibration.

RESULTS

All statistical analyses were completed using SPSS Version 13.0. Specific scores for each patient for the ADOS domains are listed in Table II. In the Communication domain, 12 out 14 subjects scored in the autism category, while the remaining 2 scored in the non-spectrum category. In the Social Interaction domain, eight patients scored in the autism category, four in the ASD category, and two in the non-spectrum category. The exact same distribution occurred in the Combined domain. Results from the ADOS suggest that 57% of subjects fell in the autism category, and 86% of subjects fell in the autism and ASD categories combined. Only 14% of subjects scored in the non-spectrum category.

Specific AQs from the GARS and total scores from the DSM-IV Checklist are listed in Table III. According to the GARS manual, AQs of 90 or above are indicative of autism [Lecavalier, 2005]. Only 3 of the 14 subjects (21%) in our study obtained AQs above 90, even though 8 out of 14 subjects scored in the autism category on the ADOS. Even if the cutoff score for autism is decreased from 90 to 80, as recommended by South et al. [2002], only two additional patients move into the autistic range (for a total of 36%).

TABLE II. ADOS Modules, Scores, and Associated Categories (Autism, ASD, Non-Spectrum) for Each Participant

Patient	Module	Communication	Social	Combined	Play	Restricted behavior
14	3	2[a]	3[a]	5[a]	1	0
63	3	3[b]	4[c]	7[c]	1	0
28	3	4[b]	4[c]	8[c]	1	2
03	1	8[b]	12[b]	20[b]	1	4
55	1	8[b]	12[b]	20[b]	2	2
02	1	4[b]	10[b]	14[b]	4	5
20	1	7[b]	12[b]	19[b]	4	5
41	2	5[b]	8[b]	13[b]	0	0
77	1	7[b]	13[b]	20[b]	2	4
06	3	3[b]	4[c]	7[c]	1	0
64	3	1[a]	3[a]	4[a]	1	0
33	1	7[b]	11[b]	18[b]	3	2
21	2	7[b]	8[b]	15[b]	2	2
62	1	5[b]	6[c]	11[c]	4	2

[a]Non-spectrum (below cutoff for both autism and ASD).
[b]Autism (at or above cutoff for both ASD and autism).
[c]ASD (at or above cutoff for ASD, but below for autism).

TABLE III. GARS and DSM-IV Checklist Scores for Each Participant

Patient	GARS autism quotient	GARS autism probability	DSM-IV checklist total	DSM-IV diagnosis	ADOS category
14	73	Low	3	No diagnosis	Non-spectrum
63	72	Low	3	No diagnosis	ASD
28	63	Very low	3	PDD NOS	ASD
03	85	Below average	7	Autistic disorder	Autism
55	78	Low	7	Autistic disorder	Autism
02	74	Low	8	Autistic disorder	Autism
20	106	Average	6	Autistic disorder	Autism
41	77	Low	4	PDD NOS	Autism
77	78	Low	5	PDD NOS	Autism
06	55	Very low	3	No diagnosis	ASD
64	65	Very low	2	No diagnosis	Non-spectrum
33	117	Above average	8	Autistic disorder	Autism
21	85	Below average	8	Autistic disorder	Autism
62	93	Average	6	Autistic disorder	ASD

While the ADOS-G and the GARS provide information used by clinicians in the diagnostic process, a medical diagnosis of an ASD is based on criteria outlined in the DSM-IV-TR [APA, 2000]. At times, an individual may receive a DSM-IV-TR diagnosis that differs slightly from his or her ADOS category, as the ADOS informs but does not limit the diagnosis. The DSM-IV-TR specifies that a minimum of six criteria, with two in the social, one in the communication, and one in the restricted/repetitive behavior areas are needed to make a diagnosis of Autistic Disorder. Using the DSM-IV Checklist, 7 out of 14 subjects (50%) scored at or above the cutoff for Autistic Disorder, which is very similar to the results of testing with the ADOS (8 out of 14). The remaining subjects obtained DSM-IV Checklist scores between 2 and 5. Because the DSM-IV-TR does not specify the number of symptoms needed to make a diagnosis of PDD NOS, it is left to clinical judgment to determine which of these remaining subjects would warrant a DSM-IV-TR diagnosis of PDD NOS. The DSM-IV-TR [APA, 2000] suggests that a diagnosis of PDD NOS "should be used when there is a severe and pervasive impairment in the development of reciprocal social interaction associated with impairment in either verbal or non-verbal communication skills OR with the presence of stereotyped behavior, interests, and activities. . ." (p. 84). Using those guidelines, an item analysis from the DSM-IV Checklist suggested that three out of the seven patients who did not meet criteria for Autistic Disorder met criteria for PDD NOS. More specifically, patients meeting criteria for PDD NOS had at least two symptoms in the social area, and at least one symptom in either the communication or restricted/repetitive behavior area. The remaining four patients did not appear to meet DSM-IV criteria for either Autistic Disorder or PDD NOS, based on parent report of behavior, even though they may have exhibited some autistic behaviors. Therefore, a total of 71% of our patients received DSM-IV-TR diagnoses of either Autistic Disorder or PDD NOS, based on parent report. This percentage is slightly lower than the percentage obtained using the ADOS, but significantly higher than the percentage using the GARS.

Of the 14 patients participating in the present study, only 3 had begun cholesterol supplementation after 5 years of age. Two of those children scored in the non-spectrum category on the ADOS, and one scored in the ASD category. None of them met DSM-IV diagnostic criteria for Autistic Disorder or PDD NOS by parent report.

Baseline cholesterol, 7-DHC, and 8-DHC levels, highest recorded cholesterol levels (using cholesterol supplementation), and cholesterol levels at the time of the autism evaluation for each subject are listed in Table IV. Correlations of cholesterol, 7-DHC, and 8-DHC levels with Communication and Social Interaction scores from the ADOS, the GARS AQ, and total scores from DSM-IV Checklist were computed. Although very high, significant correlations among ADOS scores (0.89–0.94) and significant correlations among ADOS and GARS scores (0.57–0.64) as well as ADOS and DSM-IV Checklist scores (0.79–0.81) were found, there were no significant correlations for cholesterol, 7-DHC, and 8-DHC levels with scores from either the ADOS or the GARS (−0.24 to 0.32). A one-way ANOVA yielded no differences between the three ADOS categories in terms of measured cholesterol levels or baseline 7-DHC and 8-DHC levels (see Table V).

DISCUSSION

Results from the present evaluation confirm our hypothesis that most children with SLOS demonstrate behavior typically seen in individuals diagnosed with ASDs. More specifically, based on scores from the ADOS, considered the "gold standard" for autism assessment, 12 out of 14 children with SLOS in the present study, roughly 86%, scored in the Autism or ASD categories (57% in the Autism category, 29% in the ASD category), while only 2 patients (14%) scored in the non-spectrum category. Parent report

TABLE IV. Cholesterol Levels, Baseline 7-DHC and 8-DHC Levels, and Length of Supplementation for Each Participant

Patient	Baseline cholesterol (M = 83.4) (SD = 28.4)	Cholesterol with supplementation (M = 133.3) (SD = 41.4)	Cholesterol on date of evaluation (M = 116.6) (SD = 37.6)	Baseline 7-DHC (M = 6.1) (SD = 4.3)	Baseline 8-DHC (M = 5.6) (SD = 3.7)
14	90.8	131.5	98.7	11.5	10.3
63	86.0	118.0	95.0	3.8	4.6
28	105.0	128.7	120.7	3.3	5.3
03	60.3	123.2	123.2	10.0	11.4
55	131.0	158.1	131.0	2.0	2.3
02	44.3	101.6	75.6	11.2	9.6
20	39.2	57.7	53.2	12.4	11.4
41	121.1	149.9	149.4	0.6	1.5
77	132.5	N/A	137.7	0.2	0.5
06	75.5	181.3	152.7	9.4	4.5
64	82.4	127.2	84.3	4.9	5.3
33	94.8	160.4	161.5	4.8	2.6
21	101.6	215.2	177.6	2.3	2.5
62	53.4	80.0	71.8	9.0	6.6

of autistic behaviors, using clinical interview and completion of the DSM-IV Checklist, identified a slightly lower prevalence of ASD (10 out of 14, 71%), while a behavior checklist, the GARS, yielded scores in the autism range for only 3 out of 14 subjects (21%).

The high rate of agreement between the ADOS and the DSM-IV Checklist suggests that the GARS, by itself, under reports autistic behaviors, at least in the present study. Lecavalier [2005] found similar results in his study of 284 children with ASDs. He reported a low sensitivity score for the GARS, perhaps due to an emphasis on repetitive/restricted behaviors, rather than on social and communication deficits. Our results, in combination with results from the studies by South et al. [2002] and Lecavalier [2005] suggest that future investigation of autistic symptoms in known genetic disorders should rely on direct observation and/or parent interview rather than completion of a behavior questionnaire, even a behavior questionnaire specific to autism.

Our results support previous prevalence rates of traditional autism (i.e., DSM-IV-TR Autistic Disorder) as presented by Tierney et al. [2001], even though different methods were used (i.e., we used the direct observation ADOS while Tierney used the parent report ADI-R). Agreement between the ADOS and the ADI-R was 57% versus 53%, and between the DSM-IV Checklist and ADI-R was 50% versus 53%. Strikingly, we also found that an additional 30% of individuals had enough symptoms to be considered on the autism spectrum, by ADOS scoring. In contrast to the findings from Tierney et al. [2001] children in the present study beginning cholesterol supplementation prior to age 5, which constituted the majority (11 out of 14) were more, rather than less, likely to have behaviors consistent with ASDs, though the number of subjects not supplemented prior to age 5 (n = 3, Subjects 14, 63, and 64) was too small to draw any reliable conclusions. We do not suggest that cholesterol supplementation increases the risk of autism in children with SLOS, as this does not seem biochemically plausible. Our findings likely reflect better identification of children with SLOS over time, resulting in only the mildest cases being identified later than age 5 (Patients 14, 63, and 64 did have relatively low SLOS severity scores compared to the other subjects). Also in support of these findings, our previous study on developmental progress in children with SLOS found that baseline levels of cholesterol, not age that supplementation began, best predicted developmental outcomes [Sikora et al., 2004].

Results from the present evaluation suggest that blood cholesterol levels are not related to the severity of autistic symptoms as measured by the ADOS, at least in our sample of children with SLOS. This finding is somewhat surprising, given the very strong relationship between cholesterol levels and developmental outcome [Sikora et al., 2004]. However,

TABLE V. ANOVA Results Comparing Differences in Cholesterol, 7-DHC and 8-DHC Levels Across the ADOS Categories of Autism, Autism Spectrum, And Non-Spectrum

Sterol	AD	ASD	Non-S	F(2, 12)	*P*-value
Baseline cholesterol	M = 84.6, SD = 36.9	M = 80.0, SD = 21.5	M = 86.6, SD = 6.0	0.041	0.960
Supplemental cholesterol	M = 138.0, SD = 50.0	M = 127.0, SD = 41.8	M = 129.3, SD = 3.0	0.085	0.919
Cholesterol during evaluation	M = 126.2, SD = 42.2	M = 110.1, SD = 34.7	M = 91.5, SD = 10.2	0.734	0.502
Baseline 7-DHC	M = 5.4, SD = 5.0	M = 6.4, SD = 3.3	M = 8.2, SD = 4.7	0.303	0.754
Baseline 8-DHC	M = 5.2, SD = 4.7	M = 5.2, SD = 1.0	M = 7.8, M = 3.5	0.368	0.700

several possible explanations for the finding exist. First, the ADOS may simply not be sensitive enough to detect differences in symptom severity. Second, a clinical diagnosis of Autistic Disorder is independent of developmental level (i.e., children with autism may function at any developmental level, from mental retardation to intellectually gifted). Therefore, it certainly may be possible that while blood cholesterol level is related to developmental level in SLOS, it may not be related to autism severity. Third, blood cholesterol levels do not reflect cholesterol levels in the brain, as previous animal studies suggest that CNS cholesterol is synthesized on site rather than imported from the blood [Bjorkhem et al., 2001]. It is therefore possible that blood cholesterol levels are not a valid equivalent for brain cholesterol levels. Finally, there may be some other aspect of SLOS, rather than cholesterol levels, that is more closely related to autism severity. For example, cholesterol metabolism, both in the body as well as the brain, is critical for the production of steroids. All steroid hormones have cholesterol as precursor. In addition, it has been well documented that the brain is capable of producing its own steroids, called neurosteroids [Shackleton et al., 2002; Marcos et al., 2004]. Increasing evidence suggests that these neurosteroids play a vital role in many neuronal processes, including neuroendocrine functions and behavior, as well as neurotransmitter functioning [Mellon and Griffin, 2002]. Indeed, recently, neurosteroid deficiency has been documented in idiopathic autism [Strous et al., 2004]. Neurosteroid production in individuals with SLOS has been evaluated in a preliminary study and found to be atypical [Marcos et al., 2004], though neurosteroids were evaluated systemically, not in the CNS. Therefore, it may be atypical neurosteroid production, rather than cholesterol deficiency, that results in autistic behavior in individuals with SLOS. Clearly, further evaluation of the reasons for the high rate of autism in SLOS is warranted, including the use of more specific measures of symptom severity, such as the Aberrant Behavior Checklist, and investigation of brain cholesterol and steroid metabolism.

Interestingly, more of our subjects scored in the Autism category on the ADOS in the area of Communication (12 out of 14) as opposed to Social Interaction (8 out of 14). It is unclear whether this difference is simply an artifact of the small sample size, or whether it represents a true difference in symptom presentation for children with SLOS displaying autistic behavior versus autism in non-SLOS individuals. Communication deficits measured by the ADOS not only include repetitive language and conversational skills, but also speech abnormalities and gesture use. The role of communication deficits in the overall behavior phenotype of SLOS is worthy of further investigation, as it has been well documented that atypical and aggressive behavior increases in individuals with poor communication skills.

Recently, SLOS has been described as 1 of 15 different known genetic conditions associated with autistic behavior [Cohen et al., 2005], and experience with parents of children participating in our study suggests that the SLOS community is increasingly aware of the link between autism and SLOS. Results from the present study suggest that SLOS has the strongest association with autism of any genetic condition. Indeed, the prevalence of autism in individuals with SLOS is higher than in other genetic conditions previously considered to have high rates of autism, such as Fragile X syndrome, where the rate of autism is 15–33% or tuberous sclerosis where the rate of autistic behaviors is 25–60% [Cohen et al., 2005].

The high rate of autism in SLOS has implications for determining the pathogenesis of idiopathic autism, which is currently unknown. The striking relationship we found between autism and a defect in cholesterol metabolism suggests that cholesterol metabolism is not only linked to the pathogenesis of autism in SLOS, but also to autism in general. SLOS, therefore, is an excellent model of autism for future study.

The important role of cholesterol metabolism in both brain development and brain functioning is well established. Cholesterol is an essential structural component of the plasma membrane of every cell in the body and in the CNS [Dietschy and Turley, 2004]. In addition, cholesterol was recently identified as the signal for synaptogenesis and is critical in glial cells and neuron proliferation. Cholesterol is synthesized locally in all regions of the CNS. In the brain, 24S-hydroxycholesterol (24S) is associated with brain cholesterol turnover. Recent work with patients suffering from Alzheimer's disease has found higher levels of 24S in their plasma when compared to healthy control subjects, suggesting a link between cholesterol turnover and brain pathology [Lutjohann et al., 2000; Kolsch et al., 2003]. Cholesterol metabolism has yet to be systematically studied in individuals with idiopathic autism.

Finally, the high rate of ASD in the present study suggests that cholesterol supplementation in its present form does not, by itself, prevent the development of autistic symptoms in individuals with SLOS. All subjects had received continuous cholesterol supplementation for a minimum of 2 years, and some for more than 7 years at the time of testing. However, providing autism-specific interventions for children with SLOS may allow for improvement in social and communicative behaviors, as suggested by the outcome of one child participating in our study. His ADOS scores improved in all domains following participation in an autism program through his local school district, while cholesterol supplementation since shortly after

birth did little to improve his symptoms. Certainly, combining dietary intervention with autism-specific approaches, especially when the dietary intervention is augmented by the use of statins, may prove to be the most effective intervention yet for individuals with SLOS [Jira et al., 2000]. Some statins cross the blood-brain barrier and may beneficially affect cholesterol metabolism and/or turnover in CNS tissue that is inaccessible to dietary cholesterol.

Several limitations of the present study are worthy of discussion. The study design was not randomized and did not include a group of children with SLOS who had not received cholesterol supplementation. A second limitation was the small sample size, although the total number of subjects is similar to the sample size for several multi-center studies on SLOS. This primarily reflects the rarity of the condition, and the difficulties of carrying out research in rare disorders. However, the lack of significant findings in the relationship between cholesterol levels and autism may be due to the small sample size rather than the absence of a true relationship. In addition, as discussed above, some of the assessment instruments chosen may not detect subtle, yet possibly meaningful, differences in autism severity. Fourth, no true measurement of brain cholesterol metabolism is available for the subjects, although indirect measurement of brain cholesterol metabolism, using plasma 24S, is planned for the future.

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