ORIGINAL RESEARCH ARTICLE

Genetics in Medicine

A placebo-controlled trial of simvastatin therapy in Smith-Lemli-Opitz syndrome

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Background: Smith-Lemli-Opitz syndrome (SLOS) is a multiple malformation/cognitive impairment syndrome characterized by the accumulation of 7-dehydrocholesterol, a precursor sterol of cholesterol. Simvastatin, a 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor that crosses the blood–brain barrier, has been proposed for the treatment of SLOS based on in vitro and in vivo studies suggesting that simvastatin increases the expression of hypomorphic *DHCR7* alleles.

Methods: Safety and efficacy of simvastatin therapy in 23 patients with mild to typical SLOS were evaluated in a randomized, double-blind, placebo-controlled trial. The crossover trial consisted of two 12-month treatment phases separated by a 2-month washout period.

Results: No safety issues were identified in this study. Plasma dehydrocholesterol concentrations decreased significantly: $8.9 \pm 8.4\%$ on placebo to $6.1 \pm 5.5\%$ on simvastatin ($P < 0.005$); we observed a trend toward decreased cerebrospinal fluid dehydrocholesterol concentrations. A significant improvement ($P = 0.017$, paired *t*-test) was observed on the irritability subscale of the Aberrant Behavior Checklist–C when subjects were taking simvastatin.

Conclusion: This article reports what is, to our knowledge, the first randomized, placebo-controlled trial designed to test the safety and efficacy of simvastatin therapy in SLOS. Simvastatin seems to be relatively safe in patients with SLOS, improves the serum dehydrocholesterol–to–total sterol ratio, and significantly improves irritability symptoms in patients with mild to classic SLOS.

Genet Med advance online publication 11 August 2016

Key Words: Aberrant Behavior Checklist–Community; DHCR7; randomized, placebo-controlled trial; simvastatin; Smith-Lemli-Opitz syndrome

INTRODUCTION

Smith-Lemli-Opitz syndrome (SLOS; OMIM 270400) is an autosomal recessive, multiple malformation syndrome first described by Smith, Lemli, and Opitz in 1964 (ref. 1). Nearly 30 years passed before Irons et al.[2] determined the biochemical nature of the disorder. Biochemically, SLOS is characterized by the abnormal accumulation of 7-dehydrocholesterol (DHC). The gene 7-dehydrocholesterol reductase (*DHCR7*) encodes the enzyme that reduces 7-DHC to cholesterol. *DHCR7* was independently cloned by three groups in 1998, and mutations within the gene were shown to be the molecular basis of SLOS.[3–5] Impaired DHCR7 activity results in increased levels of 7-DHC and its isomer 8-dehydrocholesterol. Cholesterol levels are frequently low in SLOS patients but can be in the normal range. The incidence of SLOS has been estimated to be 1 in 25,000–60,000.[6–9]

The SLOS phenotype varies broadly. At the severe end of the phenotypic spectrum, SLOS is a lethal disorder due to multiple major congenital anomalies. By contrast, at the mild end of the phenotypic spectrum, SLOS patients manifest fewer and minor physical abnormalities in combination with lesser functional cognitive and fewer behavioral deficits. Congenital anomalies are variable but can include second- and third-toe syndactyly, microcephaly, neonatal or postnatal cataracts, micrognathia, cleft palate, postaxial polydactyly, cardiac defects, pyloric stenosis, and genital malformations.[10,11]

Currently, most SLOS patients are treated with dietary cholesterol supplementation. Although cholesterol therapy reduces serum 7-DHC concentrations to a degree, significant amounts of 7-DHC persist even after years of therapy.[12,13] Anecdotal case studies and case series support the idea that cholesterol supplementation benefits the overall well-being of SLOS patients;[10,14–18] however, the effects of dietary cholesterol supplementation on cognitive or behavioral aspects of this disorder have not been reported by others[19,20] or substantiated in a limited controlled trial.[21] The efficacy of dietary cholesterol supplementation is

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probably limited by the inability of dietary cholesterol to cross the blood–brain barrier.[22] Moreover, increased concentrations of 7-DHC or 7-DHC-derived oxysterol could have toxic effects.[23–26] Specialists have hypothesized that, in patients with mild to classic SLOS, many aspects of the abnormal behavioral and cognitive phenotype could be the result of altered sterol composition in the central nervous system. Thus, interventions that ameliorate the central nervous system biochemical disturbances in SLOS are critical to understanding the pathological processes that underlie this inborn error of cholesterol synthesis and to developing effective therapies to treat the neurological deficits.

The use of 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitors as an adjunctive therapy to dietary cholesterol supplementation was considered soon after the biochemical defect was determined,[27] and several case reports with divergent outcomes were published. For example, Starck et al.[18] reported adverse events in two severely affected patients with SLOS treated with simvastatin, and Jira et al.[12,28] reported that simvastatin therapy reduced serum and cerebrospinal fluid (CSF) concentrations of 7-DHC and, paradoxically, increased plasma and CSF cholesterol concentrations in two SLOS patients. Although the studies were confounded by concurrent dietary cholesterol supplementation, an increase in serum cholesterol in SLOS patients treated with simvastatin was also reported in two other case reports.[29,30]

Expression of *DHCR7* is regulated by SREBP2, which, when activated by low levels of cholesterol in the endoplasmic reticulum, increases the transcription of most genes of the cholesterol synthetic pathway. Having shown that *DHCR7* expression is increased in SLOS fibroblasts treated with simvastatin,[31] we hypothesized that the paradoxical increase in serum cholesterol could be the result of increased expression of a *DHCR7* allele with residual enzymatic function, and we demonstrated that many *DHCR7* alleles encode an enzyme with residual activity.[31] Furthermore, both in vitro experiments with human fibroblasts[31] and in vivo experiments using hypomorphic *Dhcr7*[T93M/delta] mice[32] support the hypothesis that increased expression of *DHCR7* alleles with residual enzymatic activity can significantly improve plasma and tissue sterol concentrations. Because residual DHCR7 activity varies among patients with SLOS, this hypothesis could explain the paradoxical increase in cholesterol in some patients and the adverse reactions observed in others.

Subsequent to the initiation of our trial, additional reports describing the use of simvastatin in SLOS were published, although none were placebo-controlled trials. Chan et al.[33] reported a decrease in plasma 7-DHC but no increase in the cholesterol level in three subjects treated with up to 0.4 mg/kg/day of simvastatin and a high-cholesterol diet. Szabó et al.[30] reported decreased 7-DHC and increased cholesterol levels in a single subject after 2 years of 0.2 mg/kg/day simvastatin and dietary cholesterol supplementation (150–250 mg/kg/day). They also reported improved behavior and gross motor function. Roullet et al.[34] observed no change in the dehydrocholesterol (7-DHC + 8-DCH)–to–cholesterol ratio in 9 subjects with SLOS treated with an average simvastatin dose of 0.23 mg/kg/day. Hass et al.[35] published a retrolective study of simvastatin treatment (1 mg/kg/day) of 15 subjects with SLOS. They found a decrease in the dehydrocholesterol-to-cholesterol ratio but no positive effects on behavior. Six of their subjects taking this dose of simvastatin had significant adverse effects (sleep disturbance, increased serum transaminase concentrations, autoagression) that resulted in the reduction or discontinuation of simvastatin therapy. None of these studies included a control group, none evaluated CSF sterols, and none included prespecified clinical outcome measures. In this article we report the findings of a placebo-controlled crossover trial of simvastatin therapy in SLOS, in which we demonstrate a significant decrease in the plasma dehydrocholesterol-to-total sterol ratio, a trend toward decreased CSF 7-DHC concentrations, and a significant decrease in irritability.

MATERIALS AND METHODS

Human subjects research protections

This study was approved by the National Institute of Child Health and Human Development Institutional Review Board. Neurocognitive testing performed at the Kennedy Krieger Institute in Baltimore, Maryland, was also approved by the Johns Hopkins Institutional Review Board. This study was registered on ClinicalTrials.gov (NCT00064792). Informed consent was obtained from parents or guardians. Because of the cognitive impairment associated with this disorder, patient assent was not feasible. The study was monitored by the National Institute of Child Health and Human Development Data Safety Monitoring Committee.

Subjects

The study was limited to individuals 4 to 18 years of age at enrollment with a biochemical diagnosis of SLOS (based on an increased level of 7-DHC). For both safety reasons and because our hypothesis assumes the presence of a *DHCR7* allele with residual enzymatic function, enrollment was limited to subjects with clinically mild to typical SLOS, with an SLOS severity score[10] ≤30, a diagnostic DHC-to-cholesterol ratio ≤1.0, and demonstration in the subjects' fibroblasts of residual cholesterol synthesis ≥10% of that observed in control lines.[31] Subjects were excluded if they had acute liver disease or serum transaminase concentrations more than three times normal, a history of myopathy or serum creatine phosphokinase (CPK) more than three times normal, or a weight less than 10 kg. The accrual limit was 25 subjects, with the goal of having at least 18 subjects complete the study.

Simvastatin, placebo, and cholesterol preparations

Simvastatin was obtained from Xenos Bioresources and compounded by the National Institutes of Health Clinical Center Pharmacy Development Service at a concentration of 5 mg/ml in ORA-Plus 0.75 ml and cherry flavor 0.25 ml. The Pharmacy Development Service also prepared a placebo that was indistinguishable from the active product in appearance and taste. The

use of simvastatin in this research study was covered by IND 67,321. Simvastatin was administered as a single daily dose of 0.5 mg/kg for the first 6 weeks of the trial and 1.0 mg/kg/day for the remaining 46 weeks of the trial. The maximum dose was 40 mg/day. The dose selected for this study was based on that used by Jira et al.[12] Dose adjustments for weight were made at 6, 14, and 20 months. The placebo dose matched the corresponding simvastatin volume. All subjects were maintained on dietary cholesterol supplementation (150 mg/kg/day) using a cholesterol suspension (150 mg/ml) in ORA-Plus prepared by the Pharmacy Development Service.

Trial design

The study was designed as a randomized, double-blind, placebo-controlled, crossover trial consisting of two 12-month treatment and placebo arms separated by a 2-month washout period. Randomization was performed by the Pharmacy Development Service in blocks of four. Neither the participants nor the evaluating physicians knew the assignments. Study participants were clinically evaluated, and behavioral assessments were obtained, at baseline and 6, 12, 20, and 26 months during the study. Behavioral assessment was also obtained at 14 months. Anthropomorphic measurements were obtained at each admission. The Side Effects Profile for Exceptional Children modified with additional questions related to SLOS and simvastatin (**Supplementary Information** online) was used to screen participants for adverse effects. Serum/plasma was collected for sterol, biochemical, or safety testing at 0, 0.5, 1, 3, 9, 14, 14.5, 15, 17, 23, and 26 months. CSF was collected at 0, 12, and 26 months. Parents and guardians could elect to enroll the study participants in an open-label extension after completing the crossover trial. Data regarding adverse events observed during the open-label extension were also collected.

Laboratory testing

Clinical laboratory testing was performed by the National Institutes of Health Clinical Center Department of Laboratory Medicine. Sterol concentrations were measured by the Kennedy Krieger Institute Biochemical Genetics Laboratory. Residual cholesterol synthesis in subject fibroblasts was determined as previously reported.[31]

Statistical evaluation

The primary outcome measure for this study was the plasma DHC–to–total sterol ratio. Secondary outcome measures were a change in the severity of the irritability subscale of the Aberrant Behavior Checklist–Community (ABC-C)[36] completed by the parent or primary caretaker and the assessment of overall behavioral plus cognitive improvement using the Clinician's Global Impression–Improvement scale (CGI-I)[37] completed by the behavioral clinician (ET) at each follow-up visit.

The study was powered based on the primary outcome measure. Prior data on plasma DHC–to–total sterol ratios from our natural history study gave a standard deviation of 0.13 for the between-subject variability. Serial data from four subjects gave

a correlation of 0.95 supporting feasibility of a crossover design and yielded an expected same-subject SD of approximately 0.04. A power calculation indicated that, in a crossover design, we could detect a difference of 0.03 between treatment and control means at $P = 0.05$, with 80% power in 18 subjects. Statistical analysis was performed using Prism 6 (GraphPad Software, La Jolla, CA). Two sided paired t-tests were used when data were available from both phases; otherwise, unpaired t-tests were used. An F-test was performed to compare variances. A P value less than 0.05 was considered significant, and values between 0.05 and 0.10 were considered to indicate a trend. No adjustment was made for multiple comparisons of the prespecified primary and secondary outcome measures. Data are expressed as mean ± SD unless otherwise noted.

RESULTS

Study subjects

Of 23 subjects with SLOS initially enrolled in this study, 22 were randomized and 18 completed the study (**Figure 1**). Demographics, SLOS severity, *DHCR7* genotype, SLOS severity score, residual cholesterol synthesis, and baseline sterol values for all participants are provided in **Table 1**. Of the 22 randomized subjects, 13 (59%) were male and 9 were female. Age at enrollment ranged from 4.0 to 17.5 years, with a mean and median of 8.2 and 7.3 years, respectively. The SLOS severity score ranged from 6 to 28, with a mean and median of 13.2 and 11, respectively. Fibroblast residual fractional cholesterol synthesis ranged from 0.11 to 0.76, with a mean and median of 0.37 and 0.30, respectively. Baseline DHC-to-sterol ratio for plasma and CSF were 9.1 ± 7.4% and 6.7 ± 4.0%, respectively.
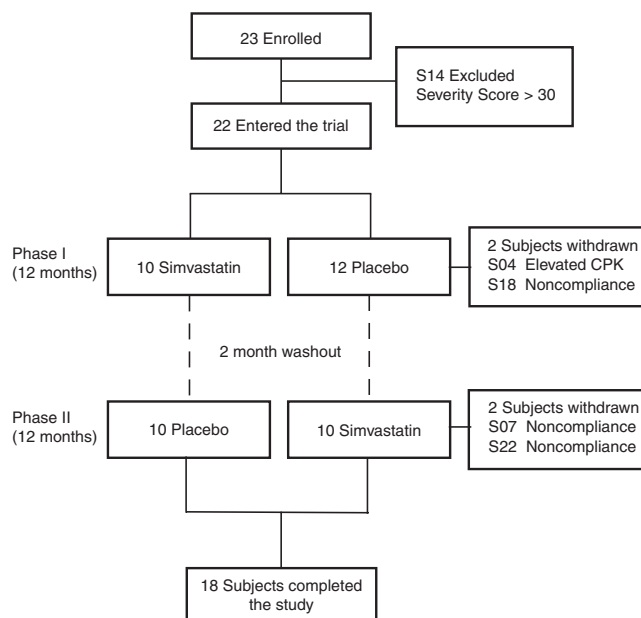
Figure 1 Study design. Subjects were randomized to either placebo or simvastatin in the first phase of this crossover trial. After 12 months in phase 1 and a 2-month washout period, subjects were switched to the alternative therapy for 12 months. One subject was excluded and four subjects were withdrawn.

Table 1. Subject demographics, genotype, and baseline sterol levels

Subject identifier	NIH SLOS subject identifier	Gender	Age at enrollment	DHCR7 genotype	SLOS severity score	Fibroblast residual cholesterol synthesis	Baseline plasma sterols				Baseline CSF sterols			
							Cholesterol (µg/ml)	7-DHC (µg/ml)	8-DHC (mcg/ml)	DHC-to-total sterols (%)	Cholesterol (mcg/ml)	7-DHC (mcg/ml)	8-DHC (mcg/ml)	DHC-to-total sterols (%)
S01[a]	SLOS-025	F	10.0	R242C/R450L	11	0.19	1,168	40	20	4.9	NSO	NSO	NSO	NSO
S02	SLOS-055	F	4.4	C380Y/c.964-1 G>C	11	0.61	1,287	22	28	3.7	1.94	0.035	0.083	5.7
S03	SLOS-004	M	8.8	c.964-1 G>C/T93M	22	0.21	649	95	85	21.7	1.64	0.065	0.140	11.1
S04[b]	SLOS-026	M	13.3	R242C/R450L	11	0.24	783	33	44	9.0	2.02	0.028	0.084	5.3
S05	SLOS-012	M	8.9	c.964-1 G>C/T289I	11	0.47	1,061	28	33	5.4	1.66	0.024	0.077	5.7
S06	SLOS-013	M	10.6	c.964-1 G>C/T289I	6	0.49	1,102	20	29	4.3	2.68	0.043	0.132	6.1
S07[c]	SLOS-001	M	7.1	c.964-1 G>C/T93M	11	0.31	858	93	66	15.6	1.69	0.088	0.155	12.6
S08	SLOS-002	M	9.8	W151X/T93M	28	0.19	788	71	63	14.5	2.00	0.081	0.151	10.4
S09	SLOS-024	F	7.6	T154M/R443H	22	0.51	1,085	59	54	9.4	1.98	0.029	0.080	5.2
S10	SLOS-018	F	12.0	R242C/W177R	17	0.48	819	40	42	9.1	1.94	0.033	0.073	5.2
S11	SLOS-033	M	5.6	c.964-1 G>C/A247V	6	0.49	1,008	63	62	11.0	1.73	0.041	0.079	6.5
S12	SLOS-005	F	17.5	c.964-1 G>C/R352W	17	0.11	849	139	98	21.8	1.22	0.086	0.101	13.3
S13	SLOS-062	F	10.3	P51S/W151X	6	0.27	942	17	28	4.6	1.66	0.020	0.047	3.9
S14[b]	SLOS-034	M	5.7	c.964-1 G>C/V326L	33	0.33								
S15	SLOS-060	M	4.8	T289I/c.964-1 G>C	6	0.35	1,495	29	49	5.0	1.90	0.036	0.102	6.8
S16	SLOS-063	F	5.4	T93M/R443C	22	0.21	738	168	121	28.1	0.96	0.057	0.094	13.6
S17	SLOS-061	M	6.3	S169L/c.964-1 G>C	11	0.20	1,082	2	8	0.9	2.99	0.003	0.028	1.0
S18	SLOS-064	M	4.0	c.964-1 G>C/V466M	11	0.76	467	4.2	3.7	1.7	1.22	0.010	0.038	3.8
S19	SLOS-066	M	4.1	M1V/Q98X	17	0.25	1,620	0.8	2.6	0.2	2.18	0.000	0.006	0.3
S20	SLOS-073	M	6.9	M1V/R446Q	6	0.37	1,311	0.4	1.4	0.1	3.66	0.002	0.009	0.3
S21	SLOS-068	F	4.1	c.964-1 G>C/Y462H	11	0.66	1,053	45	35	7.1	1.85	0.049	0.074	6.2
S22	SLOS-070	M	13.3	W151X/V466M	22	0.29	776	71	68	15.2	1.81	0.097	0.165	12.6
S23	SLOS-056	F	6.1	S192F/Y462H	6	0.51	962	34	29	6.1	1.58	0.026	0.063	5.3

[a]Shaded rows indicate phase 1 placebo and phase 2 simvastatin. [b]Excluded (severity score >30).
CSF, cerebrospinal fluid; DHC, dehydrocholesterol; F, female; M, male; NIH, National Institutes of Health; NSO, no sample obtained; SLOS, Smith-Lemli-Opitz syndrome.

The plasma and CSF DHC-to-sterol ratios were highly correlated ($r^2 = 0.83$; $P < 0.0001$).

Subjects were randomized in blocks of four, with 10 and 12 subjects starting on simvastatin and placebo, respectively, for the first 12-month phase. Simvastatin therapy was initiated at 0.5 mg/kg/day and increased to 1.0 mg/kg/day at 6 weeks. The placebo was increased by a corresponding volume. Two subjects on placebo were withdrawn during the first phase. Subject S04 reported symptoms consistent with muscular pain and had increasing CPK concentrations, and thus was withdrawn at 1 month. Subject S18 was withdrawn at 6 months because of noncompliance. Twenty subjects, equally distributed between the simvastatin and placebo groups, were crossed over to the alternate study arm after a 2-month washout period. Two subjects (S07 and S22) were withdrawn at 14 and 20 months into the second phase for noncompliance. Although both of these subjects were on simvastatin, there were no indications that noncompliance was the result of adverse drug effects. A total of 18 subjects completed both phases of the trial. There were

no significant differences in gender, age, SLOS severity score, fibroblast residual cholesterol synthesis, or baseline sterol ratios between subjects who were initially randomized to either simvastatin or placebo or the 4 subjects withdrawn from the study (**Supplementary Table S1** online). Because we did not observe an effect of phase order, the data were analyzed as simvastatin treatment versus placebo.

Safety and adverse events

Twenty-seven adverse events occurred in 15 subjects during the controlled part of the trial (**Supplementary Table S2** online). Seven additional adverse events were noted during the open-label extension. Five adverse events were classified as serious. Only one serious adverse event, a headache after lumbar puncture requiring extended hospitalization, occurred while taking simvastatin. No serious adverse events were considered to be drug related. Thirteen (48%) of the adverse events occurred while taking simvastatin, and 10 of the 27 (37%) had an infectious etiology. Eight episodes among seven subjects involved
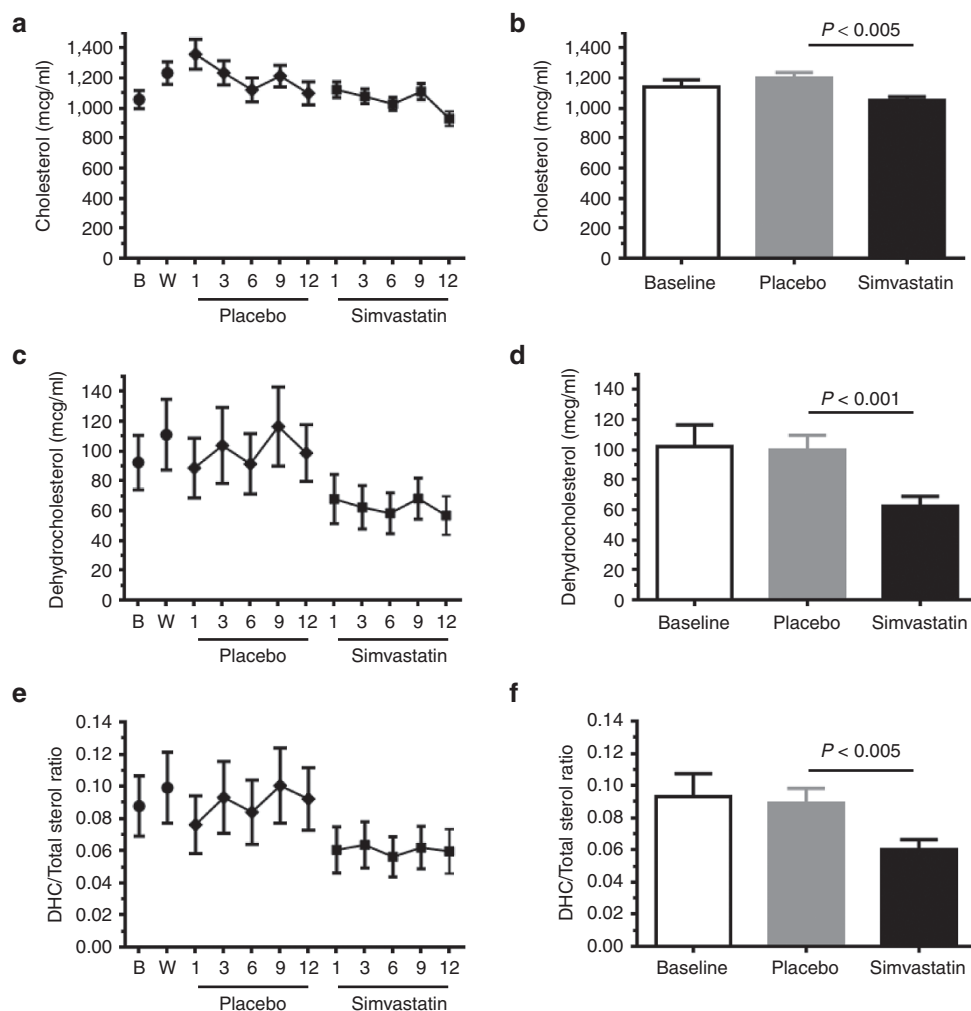
Figure 2 Plasma sterol concentrations. Cholesterol and dehydrocholesterol (DHC; 7-DHC + 8-DHC) levels were measured at baseline (B); washout (W; 14 months); and 1, 3, 6, 9, and 12 months in both the placebo and simvastatin treatment phases. Plasma cholesterol (**a, b**) and DHC (**c, d**) concentrations decreased significantly during the simvastatin phase compared with the placebo phase. The plasma DHC–to–total sterol ratio (**e, f**), which was the primary outcome measure of this study, also decreased significantly. Data are expressed as mean ± SEM.

gastrointestinal symptoms (gastroenteritis, diarrhea, vomiting, gas), with three of these (38%) occurring when taking simvastatin. Increased aggression and self-injurious behavior was reported clinically in one subject during the open-label extension phase. Safety laboratory testing was performed at 0, 0.5, 1, 3, 6, 9, 12, 14, 14.5, 15, 17, 20, 23, and 26 months. Values remained within normal ranges, and there were no significant changes in serum transaminase, alkaline phosphatase, or total bilirubin concentrations (**Supplementary Figure S1** online) between the placebo and simvastatin phases. Serum CPK concentrations remained within the normal range and did not significantly differ between the placebo and simvastatin phases. The trend toward increased serum aspartate aminotransferase and CPK concentrations during the simvastatin phase was not considered clinically significant. Subject S04 was noted to have increasing CPK concentrations after initiation of the study. CPK concentrations in subject S04 increased from 199 U/l (normal range, 52–386 U/l) at baseline to 220 and 347 U/l at 2 and 4 weeks, respectively. Although the concentrations were still within the normal range, because the subject reported thigh pain and exhibited signs of weakness, fatigue, and worsening behavior, the investigational product was discontinued and the subject was withdrawn from the study. CPK concentrations were followed and returned to baseline after the treatment was stopped. After completion of the study, it was determined that the subject had been assigned to the placebo. No abnormalities in serum electrolytes, creatinine, glucose, inorganic phosphate, magnesium, or ionized calcium were observed. Hemoglobin and hematocrit, as well as white blood cell and platelet counts, were normal. These data suggest that patients with mild to typical SLOS are not at increased risk of liver or muscle toxicity when treated with simvastatin.

Anthropomorphic parameters

Weight and height were measured at each admission. Since subjects were not seen at 14 months, the values obtained at the 12-month visit were used as a baseline for phase 2. Only two subjects lost weight during the trial. Subjects S12 and S16 each lost 1.2 kg during the placebo phase. Mean monthly weight gain during the placebo and simvastatin treatment periods were 0.20 ± 0.16 and 0.21 ± 0.13 kg, respectively (**Supplementary**

Figure S2A online; $n = 18$; $P = 0.76$, paired t-test). Complete data sets for height were available for 15 subjects. For two subjects, the monthly height gain could only be determined for either the simvastatin or placebo phase. Mean monthly height gain during the placebo and simvastatin treatment periods were 0.49 ± 0.23 and 0.42 ± 0.20 cm, respectively (**Supplementary Figure S2B** online; $n = 16$; $P = 0.42$, unpaired t-test). Our data do not support a significant change in growth parameters in subjects with mild to typical SLOS treated with simvastatin.

Plasma and CSF sterol biochemistry

Cholesterol and cholesterol precursor levels were measured at 0 (baseline); 1, 3, 6, 9, 12, and 14 months (washout); and 15, 17, 20, 23, and 26 months. Plasma sterol data are presented in **Figure 2** and **Supplementary Table S3** online. No significant differences were noted between plasma cholesterol concentrations ($P = 0.07$), DHC concentrations ($P = 0.54$), or the DHC–to–total sterol ratio ($P = 0.69$) at baseline (time 0) or 14 months after a 2-month washout. Because changes in the plasma sterol concentrations were evident by 1 month of treatment (**Figure 2a,c,e**), data from all time points in each phase were combined. In contrast to previous case reports, our data do not support a paradoxical increase in plasma cholesterol concentrations with simvastatin therapy when subjects are evaluated either as a group (**Figure 2b**) or as individuals. When the subjects were evaluated as a group, we observed a significant decrease ($P < 0.005$, paired t-test) in mean plasma cholesterol concentrations while taking simvastatin (105 ± 16 mg/dl) compared with placebo (120 ± 31 mg/dl). On an individual basis (**Supplementary Table S3** online), a trend toward increased plasma cholesterol was only observed in one subject (S06; $P = 0.05$). By contrast, a significant decrease in plasma cholesterol was observed in seven subjects (S04, S10, S12, S14, S15, S16, and S18). Plasma DHC concentrations were decreased in subjects with SLOS treated with simvastatin compared with placebo (**Figure 2c,d**). Mean DHC concentrations decreased from 106 ± 91 μg/ml on placebo to 66 ± 58 μg/ml on simvastatin ($P < 0.001$, paired t-test).

The primary outcome measure of this study was the plasma DHC–to–total sterol ratio. We observed a decrease in the plasma DHC–to–total sterol ratio in subjects treated with simvastatin. The mean fraction of DHC decreased from 8.9 ± 8.4%
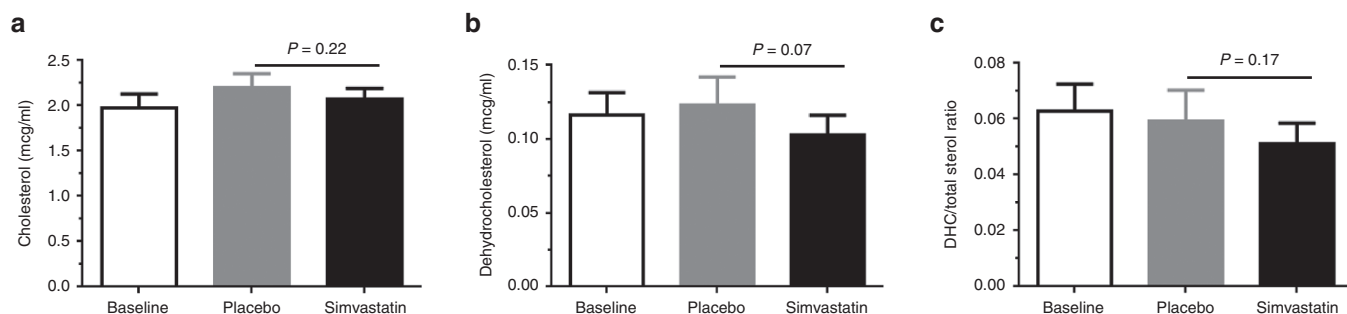
Figure 3 Cerebrospinal fluid (CSF) sterol concentrations. Neither cholesterol (**a**) nor the DHC–to–total sterol ratio (**b**) were decreased in CSF from subjects when they were treated with simvastatin compared with when they were treated with placebo. A trend toward decreased DHC (**c**) levels was observed during simvastatin therapy compared with placebo. Data are expressed as mean ± SEM.

on placebo to $6.1 \pm 5.5\%$ on simvastatin ($P < 0.005$, paired t-test). On an individual basis, the mean fraction of DHC decreased in 17 of 18 subjects (**Supplementary Table S3** online). The decrease was significant ($P < 0.05$) in 12 subjects, and 2 other subjects demonstrated a trend toward significance ($P < 0.10$).

CSF sterols were evaluated as an exploratory outcome measure (**Figure 3**, **Supplementary Table S3** online). We observed a trend ($P = 0.07$, paired t-test) toward decreased CSF DHC concentrations when subjects were treated with simvastatin compared with placebo. No significant difference was observed for either the CSF cholesterol level or the DHC–to–total sterol ratio in paired samples.

Clinical outcome measures

In addition to determining whether simvastatin had an effect on sterol levels, we investigated whether treatment would provide a clinical benefit by improving behavior. Our secondary outcome measures were the irritability subscale of the ABC-C and the CGI-I completed by the behavioral clinician at each follow-up visit. No significant differences were observed with the CGI-I. For the ABC-C, paired data were available for both baseline (0 month) and washout (14 months) for 13 subjects. We did not observe any significant difference ($P = 0.89$, paired t-test) in the ABC-C irritability subscale between these two control time points (**Figure 4a**). However, paired comparison of the 14 subjects for whom we had data from both the placebo and simvastatin treatment arms demonstrated a significant decrease (clinical improvement; $P = 0.017$) on the irritability subscale of the ABC-C–(**Figure 4b**).

DISCUSSION

This study reports the first randomized, placebo-controlled trial of simvastatin therapy in SLOS. A crossover design was used. Although crossover trials have limitations, this design facilitates the conduct of controlled trials in rare disorders, where the number of participants is limited and clinical heterogeneity is great. Our study design necessarily had several limitations. Because dietary cholesterol therapy has become the standard of care in SLOS, participants were maintained on 150 mg/kg/day of cholesterol supplementation for the duration of the study. Thus, an evaluation of simvastatin therapy independent of dietary cholesterol supplementation was precluded. Only subjects with a mild to classic SLOS phenotype were included; severely affected subjects were excluded because of safety concerns. Although apparently random, missing data may have introduced an unrecognized bias. Because of the rarity of SLOS, the necessarily small sample size in this study limited the number of outcome measures that could be evaluated. Also, the lack of generally accepted outcome measures for the characterization of abnormal behavior in SLOS necessitated the selection of clinical outcome measures without significant prior data available to validate their use.

Although difficult to conduct in rare disorders, controlled trials are essential for understanding both the safety and potential efficacy of experimental therapies. Repurposing existing drugs can expedite the development of therapies for rare and neglected diseases. However, off-label use and case reports are not substitutes for controlled trials, and the conflicting literature regarding the safety and efficacy of simvastatin therapy in SLOS highlights the limitations of case reports and uncontrolled studies.

The underlying hypothesis for this study was that treatment with simvastatin would increase DHCR7 activity by increasing the expression of *DHCR7* alleles with residual enzymatic function. Increased DHCR7 activity would then decrease DHC levels relative to cholesterol and thus result in a lower DHC–to–total sterol ratio. This mechanism could also potentially explain the paradoxical increases in cholesterol that have previously been reported.[12,28] This potential mechanism was supported by preclinical in vitro and in vivo studies.[31,32] We further hypothesized that the behavioral and cognitive aspects of SLOS result from both developmental and functional defects due to altered neuronal cholesterol composition. Although brain developmental defects are irreversible, functional defects due to altered sterol composition or neurosteroid metabolism should be amendable to therapeutic interventions that correct the biochemical defect.

Consistent with our first hypothesis, we observed a significant decrease (**Figure 2**) in the plasma DHC–to–total sterol ratio. This decrease was the result of a greater decrease in DHC relative to
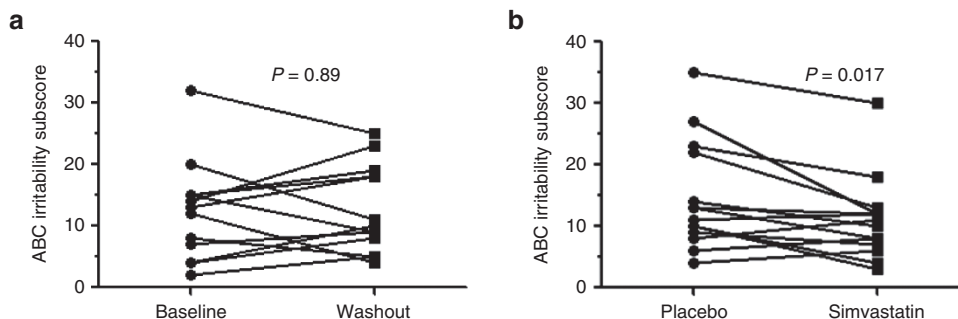
Figure 4 Simvastatin therapy reduces irritability in subjects with Smith-Lemli-Opitz syndrome. (**a**) Comparison of the Aberrant Behavior Checklist-Community (ABC-C) Irritability subscores from baseline (0 months) and washout (14 months). No significant differences were noted. (**b**) Paired evaluation of data from the 14 subjects for whom we had data from both the placebo and simvastatin treatment arms demonstrated a significant improvement in the ABC-C irritability subscale ($P = 0.017$, paired t-test).

that of cholesterol. This is consistent with the data reported by Haas et al.[35] in their retrospective study. Our data do not support a concomitant increase in plasma cholesterol concentrations. For CSF sterol concentrations, which were an exploratory outcome measure in this trial, we observed a trend toward decreased DHC concentrations in CSF (**Figure 3**), although this was not reflected in the CSF DHC–to–total sterol ratio. CSF sterol concentrations were measured under the assumption that they might reflect synaptic cholesterol levels, but the validity of this assumption is not known. Nonetheless, this result is consistent with our observation of a disproportionate decrease in plasma 7-DHC concentrations with simvastatin therapy. It is plausible that a decrease in 7-DHC levels could have a beneficial effect on central nervous system function in SLOS. In vitro and in vivo preclinical studies supported the idea that 7-DHC or 7-DHC metabolites are toxic. 7-DHC alters the physiochemical properties, protein composition, and signaling functions of cellular membranes (reviewed in ref. 25). More recently, Francis et al.[26] showed that 7-DHC disrupts Wnt/β-catenin signaling, and Xu et al.[23,24] showed that 7-DHC-derived oxysterols, in particular 3β,5α-dihydroxycholest-7-en-6-one, accumulate in SLOS and probably have toxic effects. Thus reduction of 7-DHC or 7-DHC metabolite levels could ameliorate abnormal SLOS behaviors.

In this study we also evaluated the potential of simvastatin to alter specific aspects of the SLOS behavioral phenotype. Our secondary outcome measures were the CGI-I and ABC-C irritability scores. Although we observed no significant effect on the CGI-I, we did observe significant improvement in the ABC-C irritability score (**Figure 4**). This article therefore represents the first controlled study to demonstrate improved behavior in subjects with SLOS in response to a therapeutic intervention. The CGI-I is an assessment that seeks to ascertain physicians' impressions of global improvement in both cognition and behavior. The ABC-C was developed as an outcome measure for pharmacological trials including people with developmental disabilities. The ABC-C is completed by a parent or guardian. The 15-item irritability subscale includes questions about aggression, self-injury, tantrums, agitation, and unstable mood on a scale of 0 to 45. Although we cannot directly demonstrate improved synaptic sterol composition, we were able to demonstrate changes in plasma and CSF 7-DHC concentrations consistent with the hypothesis that therapies correcting CNS sterol composition might improve behavior in SLOS and thus have a clinical benefit. Alternatively, the reduction in 7-DHC levels could have led to a reduction in 7-DHC-derived metabolites, with adverse biological effects. The small size of this study precludes the statistical evaluation of multiple neurocognitive outcome measures, and thus the evidence for clinical improvement is limited. However, the fact that we observed a significant improvement on the irritability subscale of the ABC-C provides a proof of principle for the continued development of drugs that can increase DHCR7 activity and decrease 7-DHC levels in individuals with SLOS. DHCR7 activity could be enhanced by drugs that either increase *DHCR7* expression or stabilize the mutant DHCR7 protein with residual enzymatic activity.

Although increased aggression and self-injurious behavior were reported in one subject during the open-label extension phase of the study, we did not observe any consistent, treatment-limiting adverse effects or laboratory abnormalities in subjects with SLOS treated with simvastatin, nor did we observe sleep disturbances, increased aggression, or self-injurious behavior during the controlled part of the study. We also observe no significant alteration in growth. This study supports the hypothesis that simvastatin therapy can be safely used in patients with mild to classic SLOS. However, caution is still warranted with regard to the long-term safety of simvastatin in these patients. We are aware of one subject (S23) who developed cataracts while taking off-label simvastatin therapy after this trial ended. Postnatal development of cataracts in SLOS is rare after infancy.

In summary, this study represents the first controlled trial of simvastatin therapy in SLOS and the first controlled trial demonstrating the potential of drug therapy to modulate sterol composition and to improve behavior in SLOS. We have established that treatment with simvastatin is relatively safe, can decrease DHC levels, and can improve at least one aspect of the behavioral phenotype. These data support continued efforts to identify and rigorously evaluate potential therapies that may have clinically meaningful benefits for patients with SLOS.

SUPPLEMENTARY MATERIAL
Supplementary material is linked to the online version of the paper at http://www.nature.com/gim

DISCLOSURE
The authors declare no conflict of interest.

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