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Clinical Effects of Cholesterol Supplementation in Six Patients With the Smith–Lemli–Opitz Syndrome (SLOS)

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We describe the clinical effects of cholesterol supplementation in 6 children with the RSH—"Smith–Lemli–Opitz" syndrome (SLOS). The children ranged in age from birth to 11 years at the onset of therapy, with pretreatment cholesterol levels ranging from 8 to 62 mg/dl. Clinical benefits of therapy were seen in all patients, irrespective of age at onset of treatment, or severity of cholesterol defect. Effects of treatment included improved growth, more rapid developmental progress, and a lessening of problem behaviors. Pubertal progression in older patients, a better tolerance of infection, improvement of gastrointestinal symptoms, and a diminution in photosensitivity and skin rashes were also noted. There were no adverse reactions to treatment with cholesterol. This preliminary study suggests that cholesterol supplementation may be of benefit to patients with the SLOS. Am. J. Med. Genet. 68:305–310, 1997.

KEY WORDS: cholesterol therapy; 7-dehydrocholesterol (7-DHC); Smith–Lemli–Opitz syndrome (SLOS)

INTRODUCTION

In 1993, a defect in cholesterol biosynthesis was first reported in Smith–Lemli–Opitz syndrome [Irons et al., 1993]. This defect in the enzyme 7-dehydrocholesterol-Δ^7-reductase results in markedly deficient plasma and tissue cholesterol levels, and accumulation of the cholesterol precursor, 7-dehydrocholesterol (7-DHC) [Tint et al., 1994]. Because cholesterol is an essential component of cell membranes and central nervous system myelin, and is the substrate for many biological compounds including bile acids and adrenal hormones, its deficiency has important biophysiological consequences. Many of the medical problems associated with SLOS, including severe growth failure, CNS dysgenesis, delayed myelinization, and ambiguous genitalia, can now be explained by the cholesterol deficiency, while problems such as hypersensitive skin and cataract formation probably result from the deposition of 7-DHC, or the substitution of 7-DHC in place of cholesterol [Irons et al., 1995a].

This paper summarizes our experience in developing a treatment protocol for SLOS patients, particularly the clinical effects of therapy in the 6 patients we have followed for up to 2 years. Because the cholesterol biosynthetic defect associated with SLOS causes anomalies of prenatal onset, and treatment was not started until late childhood in several of the patients, we did not expect to see many substantial effects of therapy. However, we have noted clinical consequences of therapy, even in older patients, and many of the changes seem beneficial.

EARLY THERAPEUTIC TRIALS

Since the initial description of the biochemical defect, we have been treating SLOS patients on an experimental research protocol, consisting of cholesterol replacement. The purpose of this treatment was to try to correct the biochemical defect found in SLOS patients, but also to help ameliorate some of the medical problems plaguing these children. The first attempts at therapy involved using high cholesterol foods, but were limited by severe gastrointestinal tract dysmotility and food allergies to egg and milk. However, improvement in plasma cholesterol levels was noted, as were some clinical effects including weight gain, a decrease in irritability, and improved muscle tone [Irons et al., 1994].

Because of severe feeding intolerance, one patient had been fed a special formula ("LOG") from age 6 months to 3 years. This formula, based on lamb's meat,

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contains 6–7 times the amount of cholesterol found in regular infant formulas. This child showed normal growth parameters for age while on the lamb-based formula. Her growth rate declined when changed to a more traditional low-cholesterol formula, and then increased again when begun on cholesterol supplementation at age 4 years (Fig. 1). LOG formula is an alternative therapy for infants with SLOS, and has led to improved growth and nutrition in at least one other SLOS patient (Nwokoro, personal communication). Its use is hampered by an unpleasant appearance and aroma, and difficulty in its preparation.

The early therapeutic attempts suggested that treatment with cholesterol and bile acids could alter the abnormalities in sterol and bile acid levels seen in SLOS, and could lead to some clinical improvement. However, GI tract dysfunction and food allergy limited the effectiveness of this trial. This prompted the development of a pure cholesterol medication for use in patients with SLOS.

TREATMENT PROTOCOL (JANUARY 1994–PRESENT)

Six patients with clinically suspected and biochemically confirmed SLOS were started on an experimental treatment protocol approved by the New England Medical Center Human Investigation Review Board, after obtaining informed consent from the parents. The patient group consisted of 4 girls, ranging in age from birth to 11 years at onset of therapy, and 2 boys, age 2 and 8 years at onset (Table I). Patients 2 and 3 are second cousins and patients 5 and 6 are sibs.

Since January 1994, an FDA-approved pure cholesterol suspension has been used. In the first 4 patients the cholesterol was initially used in combination with two bile acids given to enhance cholesterol absorption, ursodeoxycholic acid (Actigall®), and chenodeoxycholic acid (Chenix®). In addition to facilitating cholesterol absorption, Chenix® also suppresses the rate-controlling enzyme of cholesterol biosynthesis (3-hydroxy-3-methylglutaryl CoA or HMG CoA reductase), and it was hoped that its use would lead to a decrease in 7-DHC biosynthesis.

The suspension of pure cholesterol dissolved in soybean oil (200 mg/ml) was administered either orally or per gastrostomy tube and was well tolerated without side effects. The pure cholesterol was initially administered at a dose of 40–60 mg/kg/day, and gradually increased to the current dose of 100–125 mg/kg/day. Actigall®, given at a dose of 15 mg/kg/day, is a bile acid which has been used extensively in pediatric patients with liver diseases, and was also well tolerated. Chenix®, used at a dose of 7 mg/kg/day, was associated with some mild GI side effects in two patients.

All patients showed increases in plasma cholesterol on therapy and in the percent of total sterols consisting of cholesterol. The combination of cholesterol plus the two bile acids Actigall® and Chenix® seemed to be more effective then cholesterol plus Actigall alone in raising serum cholesterol levels. Unfortunately, late in 1994, the Chenix® became unavailable, due to manufacturing issues. The patients were then continued on cholesterol plus Actigall® alone. The cholesterol levels started to drop in two patients on this regimen, and information became available that Actigall® may actually decrease cholesterol absorption from the GI tract [Wang et al., 1995]. The Actigall® was then discontinued, and since 1995, patients have been treated with cholesterol alone [M. Irons et al., presented at NIH-sponsored SLOS conference, Bethesda, 9/95].

METHODS

To study the clinical consequences of cholesterol and bile acid therapy the patients were evaluated at

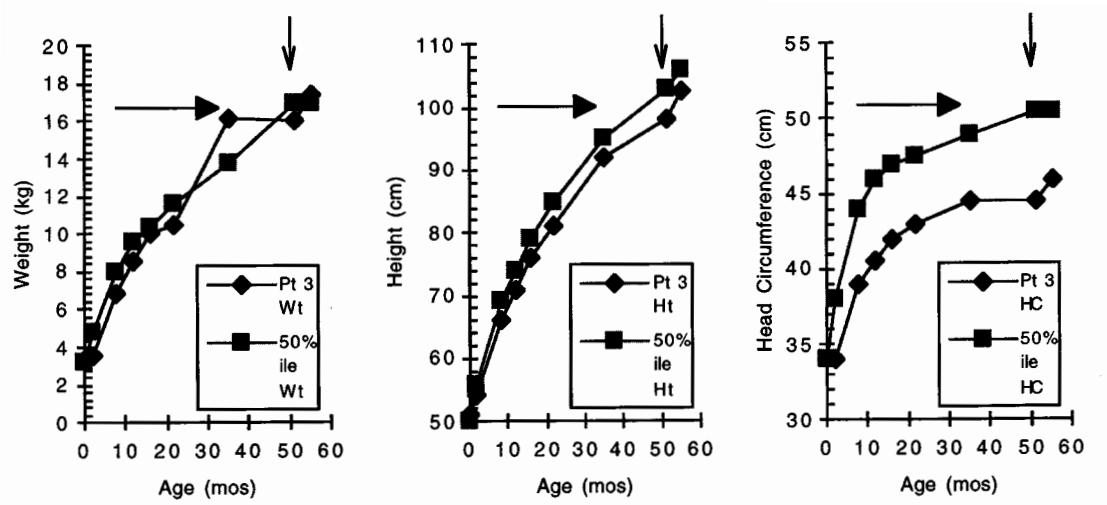

Fig. 1. Growth parameters of patient 3. These graphs illustrate that growth velocity was normal in this SLOS patient while receiving a high-cholesterol diet between age 6 months and 3 years, slowed on regular (low cholesterol) formula between age of 3 and 4 years, and then improved again once the cholesterol suspension was begun (horizontal arrow denotes high-cholesterol lamb-based formula, vertical arrow denotes cholesterol and bile acid therapy).

TABLE I. Patients on SLOS Therapy

Pt.	Sex	Age[a]	Plasma cholesterol mg/dl[a]
1	F	23 mo	8
2	F	11 yr	20
3	F	4 yr	62
4	M	8 yr	44
5	M	2 yr	33
6	F	Birth	29

[a] Pre-treatment.

monthly clinic visits. Interval history and physical examination were documented to assess effects of therapy on development, behavior, pubertal status, skin findings, cataracts, intercurrent illness, and GI symptoms. Table II lists the clinical and laboratory parameters which were evaluated during this study.

RESULTS

Evidence of clinical benefits of therapy have been seen, even in older patients. None of the patients developed side effects from the cholesterol suspension.

Growth and Nutrition

Patients have shown improved rates of growth on the cholesterol supplementation. An acceleration in weight, height, and head circumference was noted, even in older patients. For example, the head circumference of Pt. 2 was 48 cm from age 6 years to age 11 years, grew 1 cm in the first 4 months of therapy, and 2 cm since starting therapy. Serum levels of fat-soluble vitamins were measured and were normal in all patients tested [Irons et al., 1995b]. Lipoprotein levels have been added to the protocol only recently.

Neuropsychological Course

Behavior changes on therapy occurred fairly rapidly, within days to weeks of starting therapy. Parents, teachers, and clinicians noted a decrease in irritability, hyperactivity, and self-injurious behaviors, and an increased attention span. Improvement in muscle tone has occurred, but following a lag time of about 6 months on therapy. Improved rate of developmental progress was seen, also with a lag time of about 6 months following the onset of cholesterol. For example, Pt. 2 was wheelchair-bound before therapy, and is now able to walk with a walker, and Pt. 3, formerly in a wheelchair, is now walking independently and running.

Improvement in language and cognitive skills has also been noted, particularly as decreased irritability and improved attention span allow for a more beneficial outcome from early intervention and school programs. Pt. 2, nonverbal for the first 11 years of her life, can now indicate "yes" and "no," and make choices with a computer switch. Pt. 3, also nonverbal at the onset of therapy, now consistently uses 30 signs to communicate.

Of particular interest is a sib pair; the older brother, Pt. 5, started cholesterol therapy at age 2 years, and

TABLE II. Variables to Be Monitored on Therapy

Source	Test	Frequency of testing
Blood	Cholesterol	Monthly (q 3 mo once stable)
	7-DHC	Monthly (q 3 mo once stable)
	CBC, platelets	Every 3 months
	SGOT/SGPT	Every 3 months
	BUN/Creatinine	Every 3 months
	Cortisol	Yearly (if abnl initially)
	Sex hormones[a]	Yearly
	Fat-soluble vitamins	Yearly (if abnl initially)
	Lipoproteins	Yearly
Urine	Bile acids	Every 6 months
Stool	Bile acids	Every 6 months
Clinical	Wt, ht, OFC	Every month
	Interval history	Every month
	Infection	
	Rashes	
	GI	
	Psychomotor development	
	Cataracts	Every 6 months
	Head MRI	At entry and every 2 years
	IQ/behavior	Yearly (formal testing)[b]

[a] Testosterone in males, estradiol in females.

[b] The patients were evaluated by a licensed clinical psychologist at New England Medical Center (ADH), who performed formal neuropsychological testing to determine the child's developmental/intellectual quotient (DQ/IQ). This testing utilized the Bayley Scale of Infant Development, the Vineland Adaptive Behavior Scale, the Stanford-Binet Intelligence Scale 4th edition, the Stanford-Binet Intelligence Scale LM, the Peabody Picture Vocabulary Test—Revised, and the McCarthy Scales of Children's Abilities, as appropriate. These tests are well standardized and reproducible neuropsychological tests. In older patients, rate of developmental progress is compared to that seen before therapy. Behavior was scored objectively using the Aberrant Behavior Checklist, and the Reiss Scales for Children's Dual Diagnosis. These neuropsychological tests are designed to assess behavior problems in children with mental retardation and are standardized tests.

the younger sister, Pt. 6, at birth. The parents feel, and formal testing confirms, that the younger sib is showing a faster rate of development than the brother, although both had comparable pretreatment cholesterol levels (Fig. 2).

Endocrine Function

Cortisol levels were normal in all patients. Results of adrenal stimulation testing, performed in Pts. 1, 2, and 4, have also been normal. Of interest is that rapid pubertal advancement was noted in the 2 older patients once cholesterol was available as a substrate for sex hormone production. Pt. 2 advanced from Tanner I to Tanner III over a 6-month period (physical changes which normally occur over a 2-year period), once started on cholesterol. Pt. 4 has developed pubic hair, but no further pubertal changes have occurred so far.

Dermatological Problems

Severe photosensitivity improved significantly in Pts. 2, 3, and 4, for whom it was a problem pretreat-

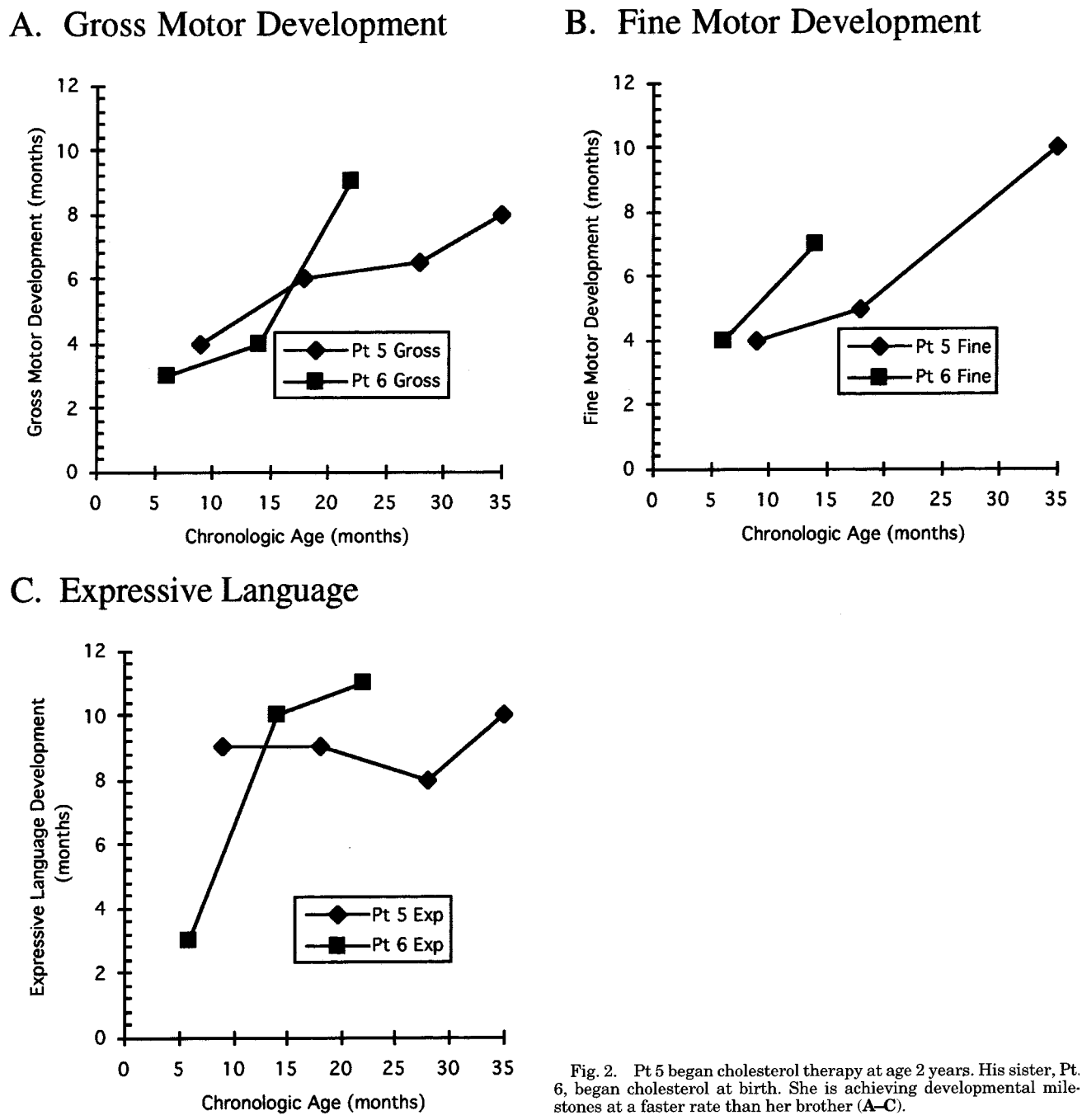

Fig. 2. Pt 5 began cholesterol therapy at age 2 years. His sister, Pt. 6, began cholesterol at birth. She is achieving developmental milestones at a faster rate than her brother (**A–C**).

ment. Improvement in skin rashes was seen in these same patients.

Ophthalmological Problems

Two of the 6 patients had cataracts. A cataract from Pt. 1 was extracted and proved to be composed of 7-DHC, demonstrating that the enzyme defect in SLOS causes storage disease-like manifestations. The cataract of Pt. 4 is stable, during the 4 years before onset of therapy, and in the 2 years since therapy began.

Infectious Diseases

There appears to be a diminution in the number and severity of intercurrent infections while on therapy. Prior to therapy, multiple ear infections necessitating placement of PE tubes, and frequent pneumonias, some requiring hospitalization, were seen in Pts. 1–4. Since the institution of cholesterol therapy, the number of courses of antibiotics prescribed, the number of hospitalizations required, and the clinical severity of intercurrent illnesses all are diminished. For example, prior to treatment, Pt. 2 had 4–6 episodes of otitis per year, necessitating placement of PE tubes several times. She required 2 hospitalizations for pneumonia, and one for staphylococcal sepsis. Since being on cholesterol therapy, she has only had 5 bouts of otitis over a 2-year period, and no hospitalizations for infection. Pt. 3 had difficulty handling upper respiratory infection and required hospitalization at age 2 for meningitis. She had 2–5 episodes of otitis per year, resulting in conductive hearing loss by age 2, but on therapy has been infection-free since April 1995 and now has normal hearing. Hearing loss also improved in Pt. 1, perhaps secondary to reduced episodes of otitis.

Gastrointestinal Problems

Gastrointestinal problems including tolerance of G tube feeding, dysmotility with severe constipation, and recurrent GI bleeds are clinically improved on therapy in Pts. 1–4. Pts. 5 and 6 are able to tolerate oral feedings, and did not have as severe GI symptoms pretreatment.

DISCUSSION

We report preliminary observations of a treatment protocol which has been evolving over time. The study patients are difficult to compare because they are few in number, have different degrees of severity of their biochemical defects, and were started on treatment at different ages. If the older patients are used as their own controls (i.e., when the clinical course is compared pre- and posttreatment), a number of clinical characteristics seem to be affected by therapy.

The children show a more rapid growth rate on cholesterol, including growth in head circumference. This is despite the fact that cholesterol given enterally is not thought to cross the blood–brain barrier. It is possible that the blood–brain barrier may be more permeable to cholesterol in the face of severe cholesterol deficiency. Reduced photosensitivity and incidence of skin rashes were noted on treatment, the mechanism of which is unclear, but may relate to abnormal skin cell membranes caused by deficient cholesterol and 7-DHC accumulation in the epidermis. The improvement in GI symptoms and tolerance of infection seen may be secondary to improved nutritional status. Fat-soluble vitamins were normal in all patients tested, suggesting that vitamin deficiency does not cause clinical symptoms in SLOS patients.

None of the patients had symptomatic heart or renal disease. Pt. 2 had successful surgical repair of an asymptomatic atrial septal defect in 1995. Although the cardiovascular team had initially elected not to operate, this decision was reversed based on the team's perception of significant clinical improvement in this patient on the cholesterol therapy.

Developmentally, a more rapid rate of progress has surprised teachers and therapists, as well as parents. The 11-year-old girl who learned to walk with a walker and manipulate switches, and her 4-year-old cousin who learned to walk independently and use 30 signs since therapy have commenced, are 2 examples of such progress. Without blinded placebo testing, it is difficult to prove that these changes are in fact the result of therapy, and might not have occurred otherwise. However, these children have been followed by the same clinician, teachers, and therapists for many years both pre- and posttreatment, and the same improvement has been noted by all, even by those educational professionals unaware that the child was on a research protocol.

Behavior changes have been interesting to observe, as they appear to occur fairly rapidly after starting cholesterol therapy. The most obvious change is a decrease in irritability, and an increase in alertness. A decrease in self-injurious behaviors is also noted. Again, these changes were observed by therapists unaware of the onset of cholesterol treatment, as well as by parents and many different clinicians involved in the childrens' care. The rapidity with which these behavior changes are manifested has suggested a possible "sterol effect"—i.e., cholesterol is now available as a substrate for sterol production, which then affects behavior, rather than the changes being an effect of the cholesterol on central nervous system structure.

Pubertal changes were rapid in 2 older patients and are easily explained by the cholesterol now being available to serve as substrate for sex hormone production. Improved nutrition may also play a role in the pubertal advancement.

Although these preliminary observations are gratifying, a great deal of work remains to be done before the cholesterol defect in SLOS is fully understood, and it can be proven that cholesterol therapy is truly beneficial in this disease. Continued study of these patients over time, and study of a larger cohort of patients will be necessary, so that children of comparable age and degree of biochemical defect can be identified, and meaningful statistical analysis accomplished. Because the effects of the cholesterol deficiency begin prenatally, treatment, even if started immediately after birth as in Pt. 6, is unlikely to result in a normal outcome. Animal studies in pregnant rats fed an inhibitor of the enzyme defective in SLOS, and supplemented with cholesterol, suggest that there may be some benefit to prenatal

therapy [Barbu et al., 1988]. This possibility is an area for future research.

Better understanding of cholesterol absorption through the GI tract, and its passage across the blood–brain barrier, will be important in helping to design more effective treatment strategies. A greater understanding of the enzyme defect may allow more specific and appropriate treatment to be designed for the different levels of severity seen in SLOS. Whether bile acids in addition to cholesterol are helpful, and which bile acids are most effective, are further questions which collaboration with ongoing laboratory research efforts [Xu et al., 1995] will help to answer. The roles of lipoproteins and lipoprotein receptors in SLOS is another area for future study.

In conclusion, SLOS patients treated with cholesterol have shown improvement in rate of growth, behavior, rate of developmental progress, and in dermatologic, gastrointestinal and infectious disease, which suggest that treatment with cholesterol has a beneficial effect. No patient has experienced any harmful consequences of treatment. Further study is ongoing to attempt to develop an effective treatment protocol for SLOS patients. It is hoped that these preliminary observations of cholesterol therapy in SLOS will lead to a greater understanding of the biochemistry involved, but more importantly, will lead to an improved quality of life for these patients and their families.

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