Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype

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Abstract

We have reviewed all known UK cases of Smith-Lemli-Opitz syndrome. Among 49 cases with proven 7-dehydrocholesterol reductase deficiency, half had been terminated or had died in infancy. The minimum incidence is 1 in 60 000. The frequent occurrence of hypospadias may account for 71% of recognised cases being male. Important common features which emerged include short thumbs, severe photosensitivity, aggressive behaviour, and atrioventricular septal defect. The typical facial appearance becomes less obvious with age and 20% of cases did not have 2/3 toe syndactyly. Biochemical measurements of serum 7-dehydrocholesterol did not correlate with clinical severity.

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Smith-Lemli-Opitz (SLO) syndrome is an autosomal recessive mental retardation/multiple congenital anomaly syndrome first described by Smith *et al*¹ in 1964. The incidence is estimated to be 1 in 20 000 to 1 in 50 000 with a carrier frequency of 0.014.^{2,3} Before 1993, diagnosis was based on characteristic clinical features, including mental retardation, failure to thrive, dysmorphic facies, cleft palate, congenital heart disease, hypospadias, and 2/3 toe syndactyly. There is a wide clinical spectrum, ranging from mild developmental delay and syndactyly to lethal multiorgan anomalies.^{2,4}

In 1993 and 1994, Irons *et al*⁵ and Tint *et al*⁶ showed that very low levels of cholesterol and markedly raised levels of the cholesterol precursor 7-dehydrocholesterol (7DHC) were present in subjects with both the severe and the milder forms of SLO. In 1995, Honda *et al*⁷ showed that SLO resulted from a primary defect in the enzyme 7-dehydrocholesterol reductase, which normally converts 7DHC to cholesterol. Hypocholesterolaemia and raised 7DHC are present in blood and other tissues. Cholesterol is vital for the normal structure of cell and mitochondrial membranes, development of the central nervous system, and myelination, and for normal synthesis and metabolism of steroids, bile acids, and vitamin D. It is postulated that the excess of 7DHC or deficiency of cholesterol or both results in abnormal plasma membrane and myelin formation. The protein product of the vertebrate early embryonic patterning gene, Sonic Hedgehog, undergoes autolysis and covalent

linkage with cholesterol, forming a cholesterol modified active product. Holoprosencephaly and other malformations present in SLO may be caused by incomplete or abnormal modification of the Sonic Hedgehog protein.⁸ Although a deficiency of the 7DHC reductase enzyme is suspected as the primary abnormality, little is currently known about the function and structure of the enzyme or its regulation. Work is presently under way to fully characterise and purify the enzyme. Positional cloning techniques are currently under way to identify putative genes in the 7q32.1 region, following the discovery of a de novo balanced translocation in a patient reported by Wallace *et al*⁹ and other patients with chromosome rearrangements in the 7q region.^{10,11} To date, the metabolic defect has not been associated with any gene in the 7q32 region.

It has been suggested that dietary intervention at an early age may improve the neurological and physical outcome in these children.^{4,12} Trials of high dose cholesterol and bile acid supplementation have been introduced to a small number of patients and preliminary reports suggest that the children are better behaved and there are some reports of acceleration of neurological progress. Conclusions on the value of therapy are dependent upon a clear delineation of the natural history of the disease.

With the advent of a biochemical marker for diagnosis, the "well defined clinical spectrum", born of "minimal diagnostic criteria",¹³ has become amenable to independent evaluation. We have reviewed all known SLO patients in the UK in order to establish the clinical spectrum.

Subjects and methods

Patients were ascertained through geneticists and paediatricians in the UK and through the UK SLO support group. Ethical approval for the study was obtained from the Newcastle and North Tyneside Health Authority Joint Ethics Committee. A total of 86 cases were initially identified as having "SLO". Thirty-seven cases were excluded from the study for a variety of reasons. One patient with a biochemically confirmed diagnosis refused to take part in the study. Six patients had had a clinical diagnosis but were found not to have SLO on biochemical testing. Two adults with a clinical diagnosis were not tested biochemically because of parental wishes. There were 28 cases who had died on whom we were unable to obtain tissue samples for biochemical confirmation; of these, 11 had features in keeping with the diagnosis of SLO.

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Table 1 The main clinical features in 49 SLO subjects in this series and comparison with previous studies

Clinical features	No of affected*	Percentage	Percentages of previous studies
General			
Males	35	71	64†, 79‡
Mental retardation	23/25	92	100†, 100§
Blonde hair	17/26	65	—
Photosensitivity	13/24	54	—
Abnormal sleep pattern	16/23	70	—
Face			
Microcephaly	32/40	80	63‡
Ptosis	26/44	59	32‡
Cataracts	6	12	18†, 22§, 53‡
Anteverted nares	34	69	84‡
Low set ears	23	47	—
Cleft palate (hard or soft)	18	37	52†, 68‡
Tongue abnormalities	6	12	63‡
Micrognathia	33	67	100‡
Skeletal abnormalities			
Postaxial polydactyly	24	49	43†, 52§, 95‡
Toe syndactyly	43/48	90	89†, 99†, 100§
Short/proximally placed thumb	24	49	63‡
Other abnormalities			
Heart defect	18	37	36§, 38†, 84‡
Genital anomaly	32/35	91	100‡
Renal anomaly	14	29	13†, 40§, 47‡
Lung anomaly	12	24	33§, 56‡
Gastrointestinal abnormality	15	31	22§, 25†, 68‡
Brain structural anomaly	6/28	21	16§, 26‡

*Unless otherwise stated, the number of subjects with each abnormality is from a total of 49 cases.

†Cunniff *et al.*,⁴ 80 patients from the Kennedy Krieger Institute, Baltimore.

‡Curry *et al.*,¹⁰ 19 severely affected patients from the USA.

§Lin *et al.*,²⁸ 59 patients from the Kennedy Krieger Institute, Baltimore.

Forty-nine cases from 43 sibships were included in the study. Most patients had an initial clinical diagnosis which was confirmed biochemically following availability of the 7DHC assay. Where the patient had died, retrospective biochemical confirmation of clinical diagnosis was obtained from tissue samples. However, if no tissue samples were available, parents were tested for raised 7DHC, indicating carrier status (three cases).¹⁴ Prenatal diagnosis was performed on amniotic fluid in one fetus. Plasma samples were collected and kept frozen at -20°C until analysis. Serum cholesterol and 7DHC measurements were performed using gas chromatography-mass spectrometry (GC-MS) of plasma sterols following solvent extraction.⁶ It is important that serum cholesterol measurements are made by stable isotope dilution by GC-MS, as standard cholesterol methods report serum cholesterol as the sum of cholesterol + 7DHC + 8DHC. We have banked DNA and obtained

Figure 1 Frequency of severity scores for 49 SLO subjects. Malformations of the brain, oral cavity, heart, kidney, gastrointestinal tract, liver, lung, bowel, genitals, eyes, and skeleton were scored as 0=normal, 1=minor malformation, and 2=major malformation. These were added to obtain a score of between 0 and 20.

lymphocyte cell lines from 20 patients and 14 sets of parents.

Twenty-four patients were alive, 20 were dead (including one stillbirth), and five fetuses were terminated. All patients' medical and genetic notes were reviewed and all living patients were examined personally by one of the authors (AR). Seven of the cases in this series have been previously reported; four by Seller *et al.*^{15, 16} (two unrelated fetuses and two sibs who died early in the neonatal period) and three by McGaughan *et al.*¹⁷ (a family of two sibs and prenatal diagnosis in a third terminated fetus).

All cases were assigned a severity score, initially devised by Bialer *et al.*¹⁸ to quantify degree of severity and recently modified by Kelley to remove the bias for early death (Kelley, personal communication). Malformations of the brain, oral cavity, heart, kidney, liver, lung, bowel, genitals, eyes, and skeleton were scored as 0=normal, 1=minor malformation, and 2=major malformation. These were added to obtain a score of between 0 and 20.

Parents of the 24 living patients were given behavioural and sleep questionnaires to complete. The behavioural questionnaire asked detailed questions on developmental assessment (vision and hearing, manual dexterity, mobility, feeding, continence) and behaviour (feeding, dressing, communication, education, sleep, social behaviour, activities, movements, interests, self injury, aggression, pain awareness, emotional state). The sleep questionnaire asked for details on present and past sleeping habits and behaviours and treatment of sleep disturbances.

Results

In some instances, data were not available or were not applicable, for example, the presence of ptosis cannot be determined in fetuses and feeding data were not available in those infants who died in the early neonatal period. We have given numbers of patients with each abnormality for each section, and where the denominator is not the total 49 patients, this is specified. The main clinical features are summarised in table 1.

INCIDENCE

Fifteen subjects (10 male, five female) were known to have been born or terminated in the UK in 1995 and 1996. The male birth rate in the UK is approximately 300 000 births per year. Therefore, the minimum incidence is 1/60 000 males per year and the minimum birth prevalence is 1/67 000 males per year. Assuming females and males are affected in equal numbers (but that more males are diagnosed because of the presence of genital abnormalities), this is equivalent to a minimum carrier frequency of 1 in 122 (0.8%). The parents of one child were distantly related.

NATURAL HISTORY

The 49 cases in this study were from 43 sibships. There were 35 males and 14 females (71% males). There were 113 known conceptions in these 43 sibships: 90 births (39

Figure 2 (A) An 18 month old male with blonde hair, downward slanting palpebral fissures, bilateral ptosis, and large right ear. (B) A 16 month old female with blonde hair, left sided ptosis, a small nose with anteverted nares, and low set ears. (All photographs reproduced with permission.)

probands), 16 miscarriages, and seven terminated fetuses. Subsequent to the probands' births, there were nine affected SLO cases from 28 conceptions (28%); six were liveborn and three were terminated. Three of these subsequently affected cases (one liveborn, two terminated fetuses) were not included in the study because of insufficient clinical detail.

For 44 pregnancies, a maternal obstetric history was available. Of these, 16 (36%) were complicated by pregnancy induced hypertension, oligohydramnios, or abnormal scans. Polyhydramnios was present in only one pregnancy. Five pregnancies were complicated by oligohydramnios; all had either hypoplastic kidneys or bilateral renal agenesis. The

Figure 3 This boy has the typical "SLO face". He has microcephaly, a narrow bitemporal diameter, downward slanting palpebral fissures, ptosis, a short nose with anteverted nares, micrognathia, and low set ears.

antenatal ultrasound showed intrauterine growth retardation in seven patients and congenital abnormalities in seven patients (one pregnancy had both features). Based on information from 30 mothers, half reported reduced fetal movements. Nuchal oedema was present in two infants at birth and was present at necropsy in another fetus at 22 weeks gestation.¹⁵ Of the 41 patients for whom gestation was known, seven (17%) were born prematurely, before 37 weeks' gestation. In 39 patients, delivery data were available; nine were born by caesarean section (23%) and 11 patients (28%) were a breech presentation.

Five fetuses were terminated between 17 and 23 weeks' gestation, owing to congenital abnormalities detected on antenatal ultrasound (4) or because of confirmation of SLO by amniocentesis following a previously affected sib (1). One infant was stillborn following intrauterine fetal death at 30 weeks' gestation. There were 19 postnatal deaths; seven (37%) occurred within the first day of life, 12 (63%) within the first month, and 18 (95%) within the first year. The oldest death was at 14 years. Death was the result of congenital heart disease or multiple congenital abnormalities including lung and renal hypoplasia in the majority of cases. Twenty-four patients were alive, with ages ranging between 4 months and 38 years at the time of examination; the majority were aged less than 12 years. There were five adults, aged 22 to 38 years.

The majority of patients were diagnosed as having SLO at necropsy or within the first month of life. All subjects were scored to rate their severity of congenital anomalies (range 2-15, mean=6.51, SD 2.84) (fig 1). The majority of patients had one or more major structural congenital abnormality in addition to facial dysmorphism and developmental delay. There were 11 patients without major organ malformations but with one or more minor malformations. For example, one male patient had bilateral 2/3 toe syndactyly, right talipes equinovagum, mild dysmorphic features, mild learning difficulties, feeding difficulties, and ptosis (fig 2A). Another male infant had congenital dislocation of the hip, bifid uvula, right undescended testis, and facial dysmorphism. One female had mild developmental delay, bilateral 2/3 toe syndactyly, short thumbs, mild dysmorphic features, and failure to thrive, but did not require nasogastric tube feeding (fig 2B).

Assessment of severity of mental retardation in those patients living at least six months (25 patients) was based upon developmental milestones, medical records, school performance reports, formal developmental assessment, and physical examination. Two were in the normal range, although they were aged only 8 months and 10 months at the time of assessment. Twenty-three of 25 (92%) had mental retardation; four (16%) had mild, seven (28%) had moderate, and 12 (48%) had severe mental retardation.

There were 11 sibs from five families. Three sets of sibs are alive and two sibships of two and three sibs (two sibs and one terminated fetus)

Figure 4 The same male photographed at 3 months, 4 years, 8 years, and 26 years. The typical face of childhood becomes less obvious with time.

all died within the first month of life. The phenotype was broadly concordant within sibships; their severity scores were (3,5), (9,9), (5,7), (8,6,8), and (6,3).

The majority of patients had severe feeding difficulties in infancy with poor suck, lack of interest in feeds, and vomiting. All but six patients required nasogastric tube feeding for between 4 months and 8 years. The vomiting and feeding difficulties tended to improve as the children grew older, though gastrostomy/fundoplication was required in eight patients. Medical treatment for gastro-oesophageal reflux was generally ineffective.

PHYSICAL FINDINGS

Growth

Growth data were available for all 24 living patients. Average birth weight was 2700 g at 38 weeks' gestation. Failure to thrive was seen in 21/24 (88%), who were below the 3rd centile for height/weight parameters. The majority were born with heights and weights within the normal range but gradually fell across and then

below the centile charts by 6 months of age. Adult height ranged between 150 and 160 cm for four males and was 152 cm in the only adult female (all <3rd centile).

Facial features

Fig 3 shows the typical facial appearance in late childhood while fig 4 shows the evolution of facial appearance with age. Microcephaly with bitemporal narrowing was present in 32/40 (80%). In the eight patients whose head circumference was $\geq$ 3rd centile, the majority were less than or equal to the 10th centile and only one patient had a head circumference $>$ 50th centile.

Ptosis was present in 26/44 patients (59%) and in three it was unilateral. The palpebral fissures were either upward or downward slanting in 13/49 patients (27%). Six of 49 (12%) patients had cataracts (two unilateral, four bilateral) and 10 patients had hypertelorism. Vision was generally reported as normal, although eight had strabismus. There were no reports of coloboma or retinal abnormalities.

The nose was typically small with a broad, flat nasal bridge and the nares were anteverted in 34 patients (69%), although this feature became less obvious with age. The ears, usually of normal size and shape, were low set in 23 patients (47%) and were posteriorly rotated in 16 patients (33%). The cheeks often had a full appearance. Micrognathia was present in 33 (67%) patients and became less obvious with age.

Mouth shape was usually normal, but a few patients had large or small mouths. Seven patients had dental abnormalities consisting of either large central front teeth or crowded teeth as a result of a small mouth or palate (table 2). Six patients had hypoplastic or grooved tongues or both (bifid tongue in one patient). Thirty-seven (76%) had a palatal abnormality; 18 (37%) had either a cleft of the soft or hard palate and the remainder had either a cleft uvula or high arched palate. In addition to this, 18 (37%) patients had broad alveolar margins, which, like the palate, was typically rugose in appearance (fig 5).

Cardiac defects

Eighteen patients (37%) had congenital heart disease. Atrioventricular septal defect was present in six patients (12%) and was associated with coarctation in two cases. Two other cases had isolated coarctation and two had left ventricular hypoplasia. Three infants had patent ductus arteriosus (not associated with prematurity) and there was one of each of the following: atrial septal defect, right ventricular hypoplasia, aortic stenosis, complex disease, and unknown type.

Skeletal abnormalities

Skeletal abnormalities were a frequent finding, including shortened limbs in 10 patients and a broad range of mild skeletal abnormalities, listed in table 3. Postaxial polydactyly was present in 26 patients (53%) and was seen twice as frequently in the upper limbs compared to

Table 2 Range of oral abnormalities in 49 SLO subjects

	No of affected
Tongue	
Hypoplastic	4
Adherent to floor of the mouth	1
Bifid	1
Longitudinal grooves	3
Teeth	
Crowded	3
Large or widely spaced	4
Palate abnormalities	
Cleft of the hard palate	7
Cleft of the soft palate	11
Cleft uvula	5
High arched palate	14

Figure 5 Cleft of the soft palate, showing the typical rugosity of the alveolar ridges and palate.

the lower limbs. Soft tissue syndactyly was seen in 43/48 patients (90%). The “classic” 2/3 toe syndactyly was seen in 39/48 (81%) patients (three unilateral, 36 bilateral) and, while present in isolation in the majority, it was also seen in combination with various patterns of toe syndactyly. In only two patients was mild 3/4 hand syndactyly present.

Genital anomalies

Of the 35 males in our series, 32 (91%) had genital abnormalities. Eight had phenotypic sex reversal (23%) and 11 (31%) had ambiguous genitalia. Of the 13 remaining males, four had hypospadias and cryptorchidism, seven had hypospadias, and two had cryptorchidism. All females had apparently normal genitalia. Two adult males had delayed puberty; one of these males had had prophylactic bilateral orchidopexy as a child.

Skin and appendages

A total of 15/24 (63%) patients had marked photosensitivity; one child has been shown to have an ultraviolet A light photosensitivity (Dr A Anstey, personal communication). All are reported to have marked dermal erythematous reactions on exposure to sunlight. Eczema was reported in four patients. Of the 26 patients whose hair colour was recorded, 17 (65%) had blonde hair, eight had brown hair, and one had red hair. The fair colouring was often in contrast to hair colouring in first degree relatives.

Table 3 Skeletal abnormalities in 49 SLO subjects

	No of affected	Percentage
General		
Dislocated/subluxed hips	8/44	18
Symmetrical limb shortening	6	12
Upper limb shortening	4	8
Hand abnormalities		
Short/proximally placed thumbs	24	49
Postaxial polydactyly	24	49
Brachydactyly of 1 or more fingers	10	20
Ulna deviation of fingers	7	14
Clinodactyly	4	8
Overriding index finger	3	6
Foot abnormalities		
Soft tissue syndactyly	43/48	90
Postaxial polydactyly	14	29
Talipes calcaneovalgus	13	27
Short/broad toes	8	16
Overriding toes	3	6
Varus deformity	2	4

Miscellaneous features

Fourteen (29%) patients had structural renal abnormalities, consisting of bilateral renal agenesis/hypoplastic kidneys (6), single kidney (3), cystic kidneys (2), lack of fetal lobulations (1), and renal tract dilatation (2). In two patients with renal hypoplasia, the ureters and bladder were also hypoplastic. Functional gastrointestinal problems were frequently seen. Constipation was a major problem in 11 patients, while three had chronic diarrhoea. One patient with intractable diarrhoea had marked improvement in symptoms once cholesterol therapy was implemented. Structural anomalies were present in 14 (29%) patients, and several had more than one affected organ. Defects observed were malrotation (4), pyloric stenosis (6), and Hirschsprung's disease (4). Liver abnormalities were infrequent, with cholestatic liver disease being present in three patients and only one patient having an atretic gall bladder. Ten patients were reported as having frequent lower respiratory tract infections; these were often the result of aspiration of feeds and tended to improve as vomiting decreased or following gastrostomy insertion. Twelve patients had hypoplastic/incomplete lobulation of the lungs. Six patients had excess skin folds/nuchal oedema over the nape of the neck and skin webbing was noted in three patients (groin, neck and axilla, and elbow).

Brain structure

Twenty-eight patients had either brain imaging or postmortem examinations. Apart from microcephaly, malformations were present in six patients (21%). These consisted of myelin maturation delay (2), lissencephaly and agenesis of the corpus callosum (1), hypoplasia of the corpus callosum (1), choroidal cyst (1), and a hypoplastic cerebellum (1). Of note, holoprosencephaly was not reported in any patient. Seizures were seen transiently in only two infants.

BEHAVIOURAL PHENOTYPE

A pattern of behavioural characteristics emerged from questionnaires completed by the parents of 23 living children and adults aged 6 months and older. A striking feature was the abnormal sleeping pattern, seen in 16 (70%) patients. As infants, the majority were sleepy and floppy. In the first few years of life, children frequently slept as little as two to three hours a night, without catch up sleep during the day. The majority were difficult to settle and would wake during the night and in the early hours of the morning. Sedatives were ineffective in all but one case. Most children gradually required more sleep as they aged, sleeping for seven to eight hours a night by the age of 5–6 years, although one boy was 9 years old before his sleeping pattern normalised. Common behavioural characteristics included self injurious behaviour in eight (35%) and aggressive behaviour in 12 (52%) (fig 6). Ritualistic behaviours were seen in 12 (52%); these commonly included playing the same audio or video cassette repeatedly, obsessions about

Figure 6 This 25 year old female has blonde hair, microcephaly, bilateral ptosis, a small nose with anteverted nares, and micrognathia. The abrasion on her left cheek was caused by self injurious behaviour.

placement of objects, or repeated actions, for example, spinning self or objects repeatedly. Many of the children were described as happy and loving to the extent of being over affectionate with strangers. In four cases, parents reported that their children's feet were excessively sensitive to tactile stimulus.

LABORATORY DATA

Forty-seven patients had a karyotype performed. All were reported as normal, with no abnormalities at 7q32. One patient had sexing determined by Y probe identification on histological tissue.

In three cases, the clinical diagnosis of SLO was confirmed after their parents were shown to have raised 7DHC levels, compatible with parental heterozygote levels. For the remaining 46 cases, all had raised 7DHC, with the exception of one case, who had markedly raised 8DHC (the 8-dehydro isomer of 7DHC), without any detectable 7DHC. This patient had a severe, but typical phenotype. For 19 patients, both pretreatment serum cholesterol and 7DHC values were available. Pretreatment serum cholesterol ranged from 0.2 to 3.1 mmol/l (mean=1.71, SD 0.84) and pretreatment 7DHC ranged from 143 to 991 μ mol/l (mean=397, SD 275). Correlation of these values against each other and severity scores were tested by first order regression analysis. No significant correlation was found between these

Figure 7 Very weak correlation between 7DHC values and severity score in 19 patients.

variables: 7DHC *v* cholesterol ($r^2=0.29$), 7DHC *v* severity ($r^2=0.16$), and cholesterol *v* severity ($r^2=0.33$). Fig 1 shows the frequency distribution of clinical severity while fig 7 shows the very weak correlation of severity scores with 7DHC levels.

DIETARY INTERVENTION

Fifteen patients were on cholesterol supplementation. While there has been no dramatic improvement in developmental ability of any of these patients, anecdotal reports of improvement in alertness, interest in the environment, and decreased aggression have been reported. Physical changes included improvement in eczema and photosensitivity and resolution of chronic diarrhoea. One child was thought to be more irritable on treatment and the cholesterol was ceased temporarily. This has subsequently been restarted without adverse effect. Apart from this one report, no other complications of the diet were seen.

Discussion

Based on our figures from male patients born and diagnosed with SLO in 1995 and 1996 in the UK, we suggest a minimum incidence of 1/60 000 and a carrier frequency of at least 1/122. Continuing difficulty in diagnosis means this is undoubtedly an underestimate. A single example of parental consanguinity is in keeping with the hypothesis that SLO is a relatively common disorder. For counselling purposes, a carrier frequency of 1-2% is appropriate. These figures are in keeping with the estimates of prevalence of 1/20 000-1/50 000 in the northern European population and the American population respectively, and a carrier frequency of 1-2%.^{2,3,19} There appears to be a wide variation in carrier frequency in different ethnic groups, with a higher frequency in northern Europeans and a low frequency in Asian and African races.³ Whether this will prove to be because of some selective advantage or other factors is unknown. The true birth incidence could be determined if the entire newborn population for a given location was screened. This is now theoretically possible; Zimmerman *et al*²⁰ have recently published a method of screening dried filter paper blood specimens for raised 7DHC and low cholesterol.

With the advent of a biochemical marker, the already wide clinical spectrum has expanded even further. None of the features in SLO are pathognomonic or obligatory for diagnosis. While the majority of cases are diagnosed in the newborn period because of multiple congenital abnormalities and facial dysmorphism, there were 11 patients in this series without major structural abnormalities, but with mild dysmorphic features and developmental delay. Patients representing the mild end of the spectrum require a high level of suspicion for diagnosis. Three patients in our series were diagnosed after the clinician sent serum for analysis to "exclude SLO"; they were only mildly dysmorphic, but had at least two minor anomalies consistent with the diagnosis of SLO. This suggests that more patients with

developmental delay, dysmorphic features, and compatible minor congenital abnormalities should be considered for biochemical testing of the cholesterol pathway, even in the absence of more severe physical abnormalities. Recently, a second defect in the conversion of lanosterol to cholesterol, desmosterolosis, was described.²¹ This and other aberrations of the cholesterol biosynthetic pathway can be detected using GC-MS.

The "SLO face" is easily recognised in the majority of patients, although may be subtle. We feel that the striking features are microcephaly with narrow bitemporal diameter, ptosis, a short nose with anteverted nares, palatal abnormality (ridged, high arched, cleft uvula, or cleft palate), and broad alveolar ridges. As patients age, the face becomes less typical, and so should not be relied on for diagnosis.

Chorionic villus biopsy and amniocentesis sterol analysis have proven to be reliable for prenatal diagnosis and is useful in those families at high risk of having an affected child.²²⁻²⁴ Features seen on scan include intrauterine growth retardation, renal abnormalities, congenital heart disease, polydactyly, nuchal oedema, decreased fetal movements, and oligohydramnios.²⁴⁻²⁶ Recently, prenatal diagnosis of SLO based on suggestive abnormalities seen on antenatal scan has been reported.²⁶ Sterol analysis could be performed at the same time as chorionic villus biopsy or amniocentesis in those pregnancies affected by compatible congenital abnormalities. Similarly, low maternal serum oestriol values have been associated with SLO affected pregnancies.²⁴⁻²⁷ The non-specific nature of the structural anomalies in SLO detectable by anomaly scanning raises the question of routine measurement of 7DHC in amniotic fluid collected for chromosome analysis on the basis of an abnormal scan. Given that the number of cases detected could be of the order of 1/1000 diagnostic tests, routine adoption is not justified without formal evaluation.

Eighteen (37%) patients had congenital heart disease; a disproportionate increase in atrioventricular septal defect was seen, a finding noted in previous reports.¹⁸⁻²⁸⁻²⁹ We did not, however, find a significant increase in atrial septal defect or anomalous pulmonary venous drainage. None of our patients, nor any reported SLO patients, have had conotruncal anomalies. The atrioventricular septal defect excess may reflect alterations in cell morphology and loss of cellular attachments, resulting in defective migration of cells during development of the endocardial cushions.³⁻³⁰

Marked photosensitivity, with a striking erythematous reaction on brief exposure to sunlight, was seen in 63% of patients. Sunburn is a major problem and children are generally kept indoors as burning may occur within a few minutes of exposure to sunlight or through the car window. The photosensitivity has been postulated to be the result of the accumulation of 7DHC in fibroblasts or abnormal cell membrane structure caused by deficient cholesterol.³¹

Limb abnormalities were commonly seen, though it is of interest that only 81% had the "hallmark" 2/3 toe syndactyly. No patient was reported to have holoprosencephaly, a malformation which has been reported in SLO and has been implicated to be, in part, the result of the effect of the Sonic Hedgehog gene.⁸ Given the severity of the neurological impairment in SLO, the rarity of epilepsy is worthy of note. Cunniff *et al*⁸ described two patients from their series of 80 who died of cholestatic liver disease. The addition of a further three patients in this series with cholestatic liver disease makes it likely that this is an uncommon, but serious manifestation of the SLO phenotype.

In reviewing the biochemistry of 19 patients, there was limited correlation between pretreatment values of serum cholesterol, 7DHC, and severity of congenital abnormalities. While other groups also found little correlation between 7DHC and clinical severity, Cunniff *et al*⁸ and Tint *et al*²⁹ found an inverse correlation between clinical severity and cholesterol level. The poor correlation between 7DHC, cholesterol, and clinical severity taken together with the distribution of severity scores in our series points to the presence of one or more important modifier genes in the population.

8DHC is an isomer of 7DHC; neither are normally detected in controls, but are present in SLO homozygotes. There was one patient in our series with raised 8DHC, without any detectable 7DHC. This case had a severe, but otherwise typical phenotype. The absence of 7DHC may be artefactual, as 7DHC may be more prone to photodegradation or auto-oxidation compared to 8DHC and cholesterol. Alternatively, it is possible that this case may have had a different inborn error of cholesterol metabolism.

A high cholesterol diet can increase serum cholesterol and reduce 7DHC in SLO patients. It has been suggested that it may improve developmental outcome. Given the wide clinical spectrum, small numbers, and placebo effect on development and perceptions of behaviour, it will be difficult to prove an effect without fully randomised trials. For this reason, we have made no specific comment on the effect of dietary intervention and must await results of continuing studies. While there has been no dramatic improvement in developmental ability in any of the patients seen in our series, anecdotal reports of improvement in behaviour and alertness were noted by some parents. Any improvement in behaviour such as aggressive outbursts and sleep patterns is worthwhile, making these children and adults more manageable for their care givers. Importantly, very few adverse effects of the diet have been reported. Recent reports by Elias *et al*³¹ and Nwokoro and Mullvihill³² have noted improvements in growth parameters although improvements in behaviour are still largely subjective.

In summary, we have found a wide spectrum of abnormalities in patients with SLO. Since the advent of a biochemical marker, the clinical features are being refined and some patients have been diagnosed with minimal features. We

believe clinicians should have a low threshold for biochemical testing in patients who are dysmorphic and have developmental delay.

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