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Short Report

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The Exclusion of Sjögren-Larsson Syndrome in A High Risk Pregnancy Using Enzymatic Methods:

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Sjogren-Larsson Syndrome (SLS) is an inherited neurocutaneous disorder characterized by mental retardation, spasticity and ichthyosis. Since it was first described in 1957, over 200 patients have been reported in the literature (1).

The ichthyosis is usually evident at birth and brings the patient to medical attention. The onset of neurologic symptoms in the first or second year of life prompts the diagnosis of SLS. Mental retardation and spasticity become apperant with delay in achieving motor and verbal milestones. Most patients have an IQ less than 60 and show evidence of leukodystrophy with neuroimaging. Unlike other lipid disorders, SLS patients generally don't show neuroregression. Spastic dyplegia or tetraplegia impairs walking and use of the upper extremities. Additional clinical features include glistening white dots on the retina, seizures, short stature and speech defects.

SLS is associated with deficiency of fatty aldehyde dehydrogenase (FALDH) activity, which catalyzes the oxidation of long-chain aliphatic aldehydes to fatty acids. Affected patients have less than 10% of the normal mean FALDH activity in cultured skin fibroblasts, and obligate SLS heterozygotes show an enzyme activity reduced to about one-half of normal. The enzyme activity is also deficient in cultured keratinocytes, leucocytes and other tissues in affected patients. Since FALDH is involved in the oxidation of fatty alcohol to fatty acid, patients also show a reduction of fatty alcohol : NAD^+ oxidoreductase (FAO) activity. Enzymatic studies can distinguish SLS from other clinically related disorders, and permit carrier detection and prenatal diagnosis (2,3,4).

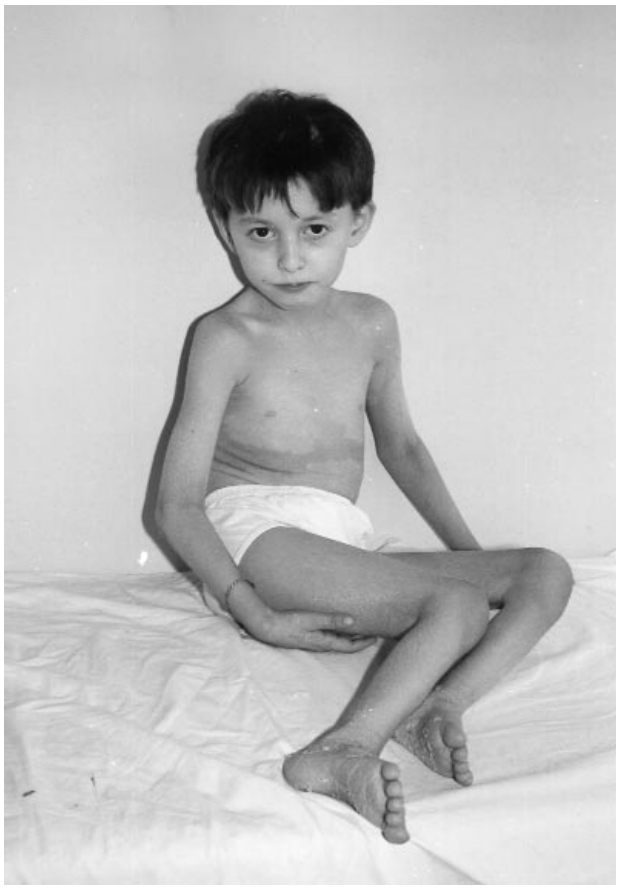
SLS is inherited as an autosomal recessive trait that maps to chromosome 17p 11.2 by linkage analysis. FALDH cDNA has recently been cloned and by mapping and mutation analysis it has been shown that it's the defective gene responsible from SLS (5).

We now report two siblings affected with SLS and results of prenatal diagnostic enzymatic studies predicting an unaffected fetus in a subsequent pregnancy.

The pregnant mother of two mentally retarded spastic dyplegic children was referred to our Medical Genetics Division for the possibility of prenatal diagnosis.

The younger child of the family, 8 3/12 years old boy (SB) was born to non-consanquineous parents both aged 31 years old. His birth weight was 2850 gr and he had a negative history for perinatal asphyxia. He was evaluated at the Pediatric Neurology Division at the age of 5 months for decreased muscle tone in the lower extremities. His neuromotor developmental milestones were delayed. At the age of 1.5-2 years, he started to speak with one or two words. He began to express his wishes at the age of 3 by sentences and gestures. He had a seizure at the age of six. At present he still can not stand up or walk. He reportedly had no collodion membrane but showed dry skin since birth with peeling on the scaly palms and soles.

At physical examination (S.B) his weight was 17 kg (below 3rd percentile), height 115 cm (below 3rd percentile), OFC 52 cm (25-50 percentile). He had a big tongue, prominent ears and high arched palate. His teeth



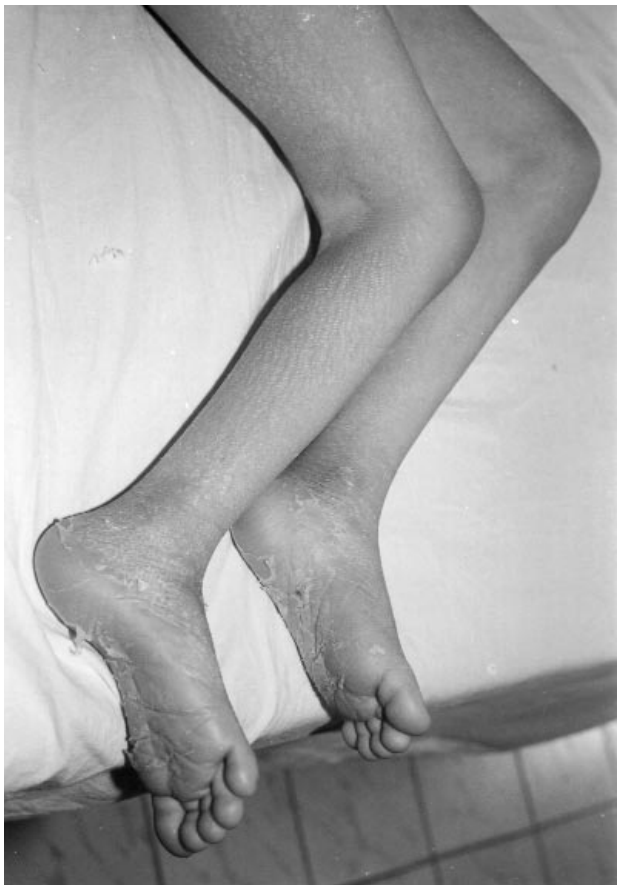


Figure 1 a : Clinical photograph of SB at 8 3/12 years

b : Note scaly, ichthyotic skin changes at the lower extremities

were widely spaced with caries. He had scaly skin accentuated at the hands and feet with decreased sweating (Figure 1 a-b). He was mildly mentally retarded. Neurological examination revealed generalized hypertonia, and bilateral pyramidal signs at the lower extremities. Extrapyramidal and cerebellar examination were normal. There were no sensory deficits. Ophthalmologic examination revealed glistening white dots at the nasal side of the right eye and pigmentary changes at the nasal side of the left eye on the retina.

His 14 years old sister (N.B) was born at the 8th month of gestation with a birth weight of 3000 gr complicated by perinatal asphyxia. She was referred to Neurology Division for developmental delay, and carried a diagnosis of "cerebral palsy". She still can't walk or speak and is severely mentally retarded. She had febrile seizures three times before the age of 4. She also had dry and scaly skin since birth. Neurologic examination revealed generalized hypertonia and bilateral pyramidal signs. Other systemic findings were normal.

Amniocyte cultures were grown from amniotic fluid cells obtained at 20 weeks gestation. Cells were grown in minimal essential medium containing 10 per cent fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml) under an atmosphere of 95 percent air, 5 percent CO_2 . Cultured chorionic villus cells were grown from chorionic villus biopsy obtained at 20 weeks' gestation. Control amniocytes and chorionic villus cells were obtained from women undergoing amniocentesis or chorionic villus sampling (CVS) for advanced maternal age. Fetal skin fibroblast cultures were established and grown using standard methods as described in Rizzo et al (6).

FAO was assayed using [^{14}C] octadecanol as a substrate as described in Rizzo et al (7). FAO was also assayed using [^{3}H]dihydrophytol (specific activity 30 Ci/mmol) as a substrate at a sub-saturating concentration (40 nm). FALDH activity was measured fluorometrically

Table 1. Enzyme activities measured in fibroblasts of SLS patients.

	FALDH (pmol/min/mg protein	FAO (Octadecanol pmol/min per mg)
Patient 1 (S.B)	366	4.1
Patient 2 (N.B)	468	3.6
SLS Patients	654±412 (n: 37)	5.8±2.7 (n: 61)
Normal Controls	8540±1158 (n: 12)	75.1±13.4 (n: 14)
Range	6950-10722	61-107

Table 2. Enzyme activities measured in amniocytes of at-risk pregnancy (B. Family) for SLS.

	FAO	
	Octodecanol	Dihydrophytol cpm/min/mgprotein
Family B		
At-risk fetus	NT	13777
Family 1*		
At-risk fetus	45 (n: 1)	NT
Family 2*		
At-risk fetus	4 (n: 1)	645 (n: 1)
N. Control	46.9±13.2 (n: 16)	33055±12908 (n: 6)
Range	25.5-77.6	19208-50910

NT: Not tested
*: (4)
Fetus 1* unaffected
Fetus 2* affected

as described by Rizzo and Craft (2), except that 0.1 per cent Triton X - 100 was included in the assay buffer. The protein concentration of cell homogenates was assayed according to Lowry et al (8).

FAO activity in cultured skin fibroblasts of S.B. was 5.5 percent of the mean normal activity and FALDH activity was 4.3 % percent of the mean normal activity. FAO and FALDH activities of N.B. were respectively 4.8 % and 7.7 % percent of normal. Both results confirmed the diagnosis of SLS (Table 1).

Amniocentesis and chorionic villi sampling were performed at the 20th week of the mother's 3rd pregnancy. Both FAO activities in cultured amniocytes and chorionic villi cells and FALDH activity in cultured chorionic villi were within normal limits as with controls (Table 2-3).

Based on these results, an unaffected fetus was predicted. The pregnancy was continued and a female unaffected infant was subsequently born. Physical examination at the age of 6 months showed no evidence of skin or neurological disease in this infant.

This is the first reported case of SLS from Turkey which has been enzymatically confirmed and is also the first attempt at prenatal diagnosis for SLS. Since initially reported by Sjogren and Larsson in 1957 in Sweden, about 200 cases have been reported with approximately 50 of them enzymatically analysed (1).

SLS has been reported worldwide without any ethnic or racial prediction but the true incidence of SLS outside of Sweden is unknown. Swedish patients comprise the largest single identified group. The overall prevalence in Sweden is 0.4 per 100.000. The majority of patients in Sweden come from the county of Vasterbotten and the prevalence of the disease is found to be 8.3 per 100.000 in that area (9).

Prenatal diagnosis of SLS was first established by electron microscopic evaluation of a fetal skin biopsy at 23 weeks of gestation (10). An affected fetus can be missed if the skin biopsy is performed before 23 weeks of gestation (11). Deficient FAO and FALDH activity has been observed in cultured chorionic villi cells and amniocytes from affected fetuses (4). The enzymatic approach is preferable since obtaining a skin biopsy is much more riskier than obtaining fetal cells by amniocentesis or chorion villus sampling. Presently, CVS is the best choice for early prenatal diagnosis but cultured chorionic villi cells is obligatory, since the results are

Table 3. Enzyme activities measured in cultured chorionic villus cells from family B's pregnancy at-risk for SLS.

	FAO		
	Octadecanol	Dihydrophytol	FALDH
		cpm/min/mgprotein	pmol/min per mg
Family B	31.3	26617	2888
At-risk fetus			
Family 3*	4 (n:1)	827 (n: 1)	84 (n:1)
At-risk fetus			
Family 4*	74 (n: 1)	22359 (n: 1)	NT
At-risk fetus			
Normal Controls	81±25 (n: 19)	24206±12527 (n: 8)	1031±219 (n: 8)
Range	41-119	11.274-53129	730-1358

NT: Not tested

*: (4)

Fetus 3* affected

Fetus 4* unaffected

ambigious with uncultured cells.

The human FALDH cDNA has recently been cloned and several SLS patients were found to carry distinct mutations, including deletions, an insertion and a point mutation. (5). The best way to proceed for future prenataldiagnostic testing will be DNA based when mutation analysis is completed in our family.

We conclude that with a high rate of consanguineous matings in Turkey (12), all cases with spastic dyplegia/quadriplegia should be carefully evaluated for ichthyotic skin changes, eye findings and other symptoms prompting the diagnosis of SLS, since there is a high risk

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