Genetic Homogeneity in Sjögren-Larsson Syndrome: Linkage to Chromosome 17p in Families of Different Non-Swedish Ethnic Origins

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Summary

Sjögren-Larsson syndrome (SLS) is a rare, autosomal recessive disorder that is characterized by congenital ichthyosis, mental retardation, and spastic diplegia or tetraplegia. Three United States families, three Egyptian families, and one Israeli Arab family were investigated for linkage of the SLS gene to a region of chromosome 17. Pairwise and multipoint linkage analysis with nine markers mapped the SLS gene to the same region of the genome as that reported in Swedish SLS pedigrees. Examination of recombinants by haplotype analysis showed that the gene lies in the region containing the markers D17S953, D17S805, D17S689, and D17S842. D17S805 is pericentromeric on 17p. Patients in two consanguineous Egyptian families were homozygous at the nine marker loci tested, and another patient from a third family was homozygous for eight of the nine, suggesting that within each of these families the region of chromosome 17 carrying the SLS gene is identical by descent. Linkage of the SLS gene to chromosome 17p in families of Arabic, mixed European, Native American, and Swedish descent provides evidence for a single SLS locus and should prove useful for diagnosis and carrier detection in worldwide cases.

Introduction

Sjögren-Larsson syndrome (SLS) is a rare, autosomal recessive disorder in which patients display a triad of clinical features—congenital ichthyosis, mental retardation, and spastic diplegia or tetraplegia (Sjögren and Larsson 1957). Other features associated with the disor-

der include glistening white dots on the retina, short stature, seizures, and speech defects. SLS patients also have a severe deficiency in the enzyme fatty aldehyde dehydrogenase (FALDH), which is a component of the fatty alcohol:NAD$^+$ oxidoreductase (FAO; EC 1.1.1.192) enzyme complex that catalyzes the oxidation of fatty alcohol to fatty acid. Measurement of dramatically reduced FALDH activity in cultured fibroblasts can be used for conclusive diagnosis of SLS (Rizzo et al. 1988).

SLS was first described in families from northern Sweden, where the largest number of affected patients has been identified (Sjögren and Larsson 1957). In most Swedish SLS families, the gene lineage could be traced back to a few common ancestors. The high prevalence of SLS in northern Sweden probably arose from founder effects and inbreeding (Jagell et al. 1981). However, additional non-Swedish SLS patients have been reported worldwide (Richards 1972; Theile 1974), and it is likely that most of these patients represent independent gene mutations. It is not known whether SLS exhibits locus heterogeneity.

Recently, a gene for SLS was localized to chromosome 17 by linkage analysis in 24 Swedish families (Pigg et al. 1994). Using haplotype and allele association analyses in patients with common lineage, the Swedish SLS gene was estimated to be within 600 kb of the microsatellite marker D17S805. However, because of founder effects in the Swedish study, it is probable that all or most of these patients have the same gene mutation inherited from a common ancestor. The significance of the chromosome 17 mapping of an SLS gene would be much greater if it can be shown that it is not restricted to Swedish pedigrees that are known to have common ancestors. In order to determine whether there is locus homogeneity in worldwide cases of SLS, we undertook a linkage study using pedigrees of non-Swedish ancestry.

Subjects and Methods

SLS Pedigrees

Seven pedigrees containing a total of 14 SLS patients were used in this study (fig. 1). All individuals gave
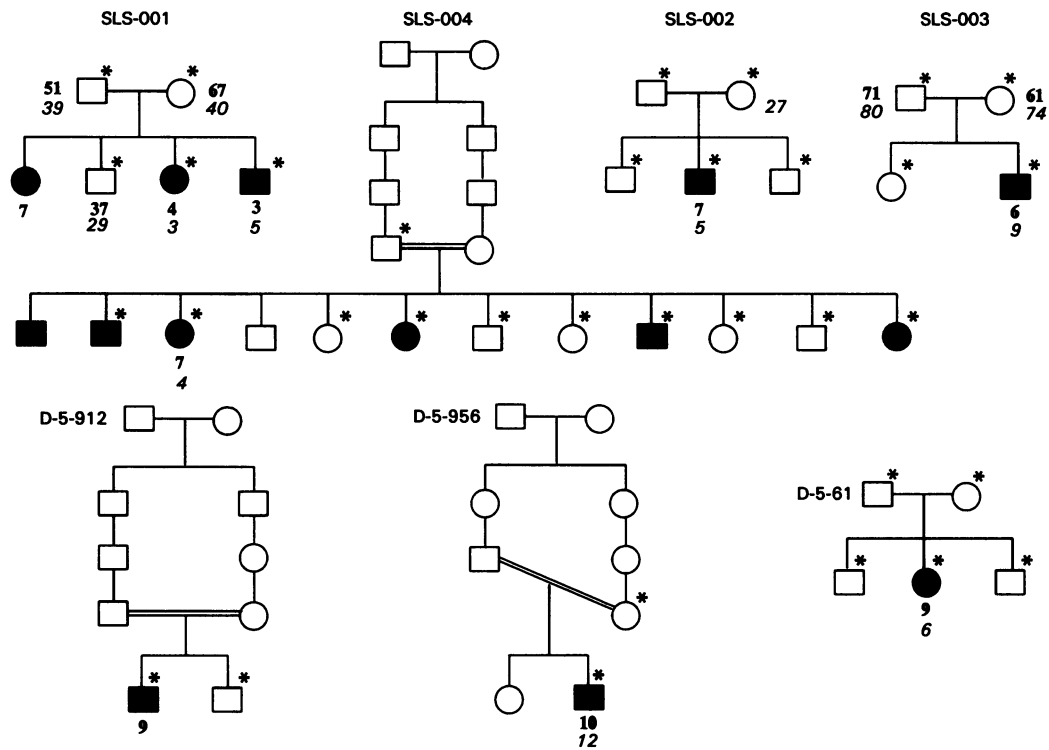
Figure 1   Family pedigrees of SLS patients. Affected individuals are denoted by solid squares (male) or circles (female). DNA from individuals marked with an asterisk (*) was used in the linkage studies. Numbers indicate FAO (upper bold) and FALDH (lower italicized) enzyme activity of cultured fibroblasts as a percentage of mean normal activity (normal FAO activity = 79 ± 13 pmol/min/mg protein [n = 15] and normal FALDH activity = 8640 ± 1080 pmol/min/mg protein [n = 12]).

informed consent to participate in an institutional review board–approved protocol. Three families were from the United States (SLS-001, SLS-002, and SLS-003), one Arab family was from Israel (SLS-004; Gomori et al. 1987), and three families were from Egypt (D-5-912, D-5-956, and D-5-61). The ancestors of the United States families were as follows: SLS-001—English; SLS-002—Irish/French (paternal) and French/Native American (maternal); SLS-003—English/German/Lithuanian (paternal) and Hungarian/Native American/French (maternal). Consanguinity was documented in two of the Egyptian families and the Israeli family and identified retrospectively in the third Egyptian family on the basis of homozygosity of marker typings in the SLS-affected patient.

The probands in each family had ichthyosis, mental retardation, and spasticity. The clinical diagnosis of SLS was further confirmed in each proband by measuring FAO and/or FALDH enzyme activity in cultured fibroblasts as described by Rizzo and Craft (1991). Figure 1 shows that all assayed SLS patients had reduced FAO and/or FALDH activity of <12% normal mean activity.

DNA Marker Analysis

The polymorphic markers D17S921, D17S953, D17S805, D17S959, D17S842, D17S783, D17S925,

D17S841, and D17S935 were amplified with oligonucleotide primer sequences given by Gyapay et al. (1994). Primers for the marker D17S689 were obtained from Gerken et al. (1995).

Genomic DNA was prepared by standard procedures from either blood cells or buccal mucosa (Richards et al. 1993). PCR was performed in a 10-μl volume containing ~100 ng purified genomic DNA or 1 μl buccal DNA preparation, 200 nM of each oligonucleotide primer, 200 μM each of dATP/dTTP/dGTP, 25 μM dCTP, 1 μCi [α-^{32}P]dCTP (3,000 Ci/mmol), 0.5 U *Taq* polymerase (Boehringer Mannheim), and supplied reaction buffer containing 10 mM Tris-HCl, 50 mM KCl, and 1.5 mM MgCl$_2$. Thirty-five cycles of amplification consisting of 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min 30 s were performed followed by a final extension of 10 min at 72°C. The PCR products were separated on a 6% denaturing polyacrylamide gel and visualized by autoradiography.

Linkage Analysis

Two-point linkage analyses were performed using the computer program LINKAGE (subprogram MLINK) on a VAX workstation. SLS was modeled as a rare, autosomal recessive disorder (disease allele frequency = .001) with complete penetrance. Marker allele frequencies

Table I

Pairwise LOD Scores between Chromosome 17 Markers and the SLS Locus

LOCUS	RECOMBINATION FRACTION AT θ =						Z_{max} (θ)
	.001	.05	.10	.20	.30	.40	
D17S921	1.43	2.74	2.63	2.04	1.27	.46	2.74 (.05)
D17S953	3.77	3.35	2.92	2.09	1.26	.45	3.78 (0)
D17S805	3.17	2.87	2.55	1.89	1.17	.43	3.18 (0)
D17S689	3.83	3.43	3.03	2.19	1.32	.47	3.83 (0)
D17S842	1.60	1.45	1.30	.95	.57	.19	1.60 (0)
D17S783	.95	2.25	2.14	1.59	.94	.32	2.25 (.05)
D17S925	.65	1.97	1.89	1.45	.89	.32	1.97 (.06)
D17S841	−1.53	.04	.20	.23	.15	.05	.24 (.16)
D17S935	−4.32	.37	.86	.95	.66	.25	.98 (.16)

were taken from the Genome Data Base on-line database when available or were calculated from the data. Multipoint linkage analysis for all nine markers with SLS was performed on a DEC Alpha workstation using the program MAPMAKER/HOMOZ (Kruglyak et al. 1995). This program is designed to calculate multipoint LOD scores for recessive traits in nuclear families, including those with inbreeding. The marker order and distance for multipoint analysis were taken from published data (Gyapay et al. 1994; Pigg et al. 1994) or estimated from observed recombination events in our family data and was as follows: 17pter–D17S921–6 cM–D17S953–6 cM–D17S805–0.5 cM–D17S689–0.5 cM–D17S842–1 cM–D17S783–1 cM–D17S925–2 cM–D17S841–1 cM–D17S935–17qter. The exact position of the centromere on this map is uncertain, but it lies in the direction of the q arm from D17S783 (see Discussion).

The Israeli family (SLS-004) was treated as an outbred family for all analyses because of the significant computer time required to analyze an inbred family with such a large sibship. The HOMOG program (Smith 1963; Ott 1992) was used to test for heterogeneity of the recombination fraction across families.

Results

Linkage Analysis

Family members were initially genotyped with panels of microsatellite DNA markers on several chromosomes, but the results did not show any evidence of linkage to the SLS gene. Subsequent analysis with markers in the region of chromosome 17 recently reported to harbor the Swedish SLS gene (Pigg et al. 1994) gave significant evidence for linkage to the SLS gene. D17S959 was uninformative in several families and was not used for further analysis. Table 1 gives the maximum LOD scores and corresponding recombination fraction between each of

the nine markers and the SLS locus in the seven families. Complete cosegregation with the SLS gene and LOD scores >3.0 were found with D17S953, D17S805, and D17S689. Three other markers (D17S935, D17S841, and D17S842) gave positive LOD scores even though each marker was uninformative in several families.

The relative position of D17S689 was unknown, so multipoint linkage was performed three times, with D17S689 placed between D17S953 and D17S805, between D17S805 and D17S842, and between D17S842 and D17S783. In each case the results were essentially identical and yielded a maximum LOD >9.0 at the location of the marker D17S842. A plateau occurred with nearly identical LOD scores in the interval between D17S842 and D17S953 (fig. 2). These results implicate an interval of ~7–10 cM in length as harboring the SLS locus. Likelihoods of 1,000–10,000 times lower were found adjacent to this region on chromosome 17.

There was no evidence of heterogeneity in the recombination fraction for either of the two highly informative marker loci tested (D17S805 and D17S953). χ^2 values for testing the hypothesis of linkage and heterogeneity versus linkage with homogeneity were 0.000, and α, the proportion of linked families, was estimated to be 1.0 (θ = 0).

Haplotype Analysis

The most probable haplotypes were constructed for each individual on the basis of previously published reports of marker order: pter–D17S921–D17S953–D17S805–[D17S783/D17S842/D17S925]–[D17S841/D17S935]–qter (Gyapay et al. 1994; Pigg et al. 1994). D17S689 is tightly linked to D17S805 and D17S783 (Gerken et al. 1995). Recombination in several families provided information that allowed us to narrow the location of the SLS gene and refine the published marker order.

Two recombinations were observed in family SLS-003—one occurred between D17S841 and D17S935,
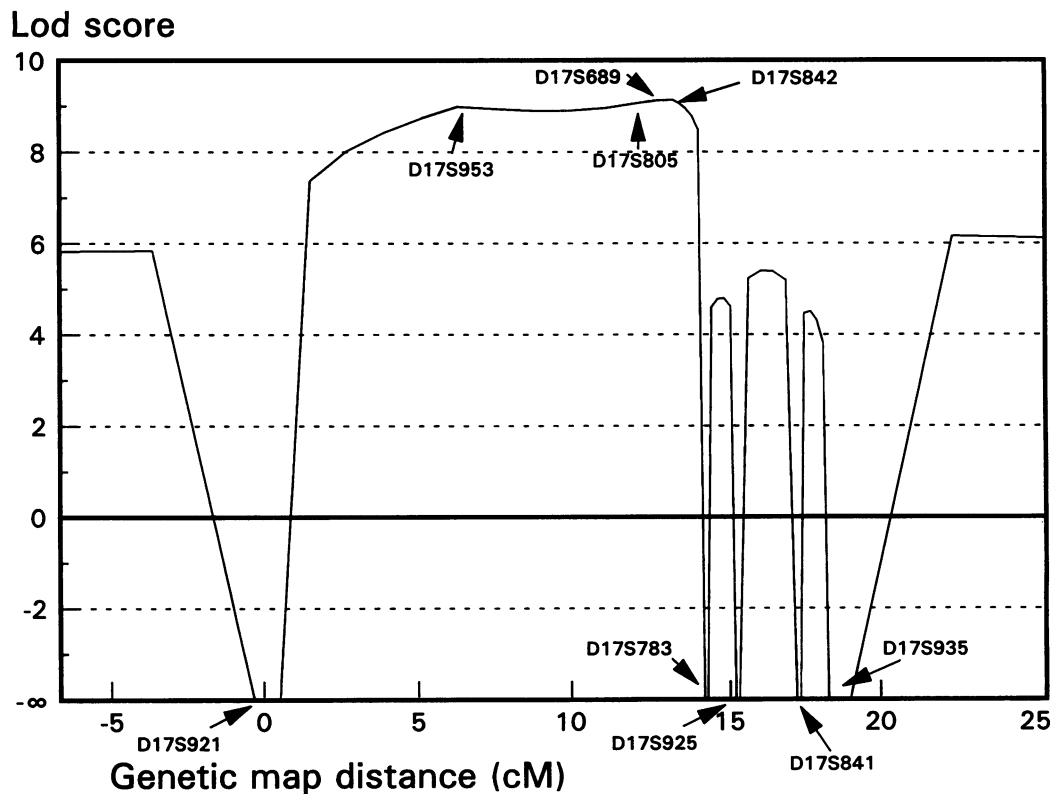
Figure 2   Multipoint LOD scores for SLS versus chromosome 17 markers.

placing the SLS gene in the direction of pter from D17S935, while the other occurred between D17S921 and D17S953, placing the SLS gene in the direction of qter from D17S921. These haplotypes also supported the marker order pter–D17S921–D17S953–qter and pter–D17S841–D17S935–qter.

Another recombination event was observed in an affected child (II:3) of pedigree SLS-004 and placed the SLS gene in the region containing the markers D17S953, D17S805, D17S689 and D17S842 (fig. 3). Furthermore, this recombination indicated that the order of markers in the cluster can be refined to [pter–D17S842–D17S783/D17S925–qter] and that D17S689 is closer to D17S805 and D17S842 than to D17S783. Placement of D17S925 was deduced to be closest to 17qter on the basis of a recombination on the paternal chromosome of an unaffected child in family SLS-002, which separated D17S925 from D17S842/D17S783 (data not shown).

Analysis of the Egyptian pedigrees revealed no shared haplotypes, thus providing no evidence of common ancestry among the families. However, the affected individual from Egyptian family D-5-912 was homozygous at all nine marker loci tested, while the proband from Egyptian family D-5-956 had a different haplotype that was homozygous for all loci except

D17S921. This is consistent with the inheritance of both disease-carrying chromosomes from a common ancestor in each of these consanguineous families. Although consanguinity had not been reported by the family members in Egyptian pedigree D-5-61 it was indicated by homozygosity at all nine marker loci tested in the SLS patient but not in her two unaffected siblings. Further investigation into this complex family revealed that the parents shared a single common ancestor and had an inbreeding coefficient (F) of 1/32. Affected children from the Israeli consanguineous family (SLS-004) showed homozygosity at only two informative markers (D17S805 and D17S689), probably resulting from recombination of the common chromosome in a previous generation. This suggests, and is consistent with the data of Pigg et al. (1994), that D17S805 is very close to the SLS gene.

Allele Association

Comparison of allele frequencies at the D17S805 locus between SLS-carrying ($n = 11$) and non-SLS-carrying ($n = 13$) chromosomes over all our pedigrees showed no significant allele association ($\chi^2 = 3.5$, df $= 3$; not significant). Furthermore, allele association was not observed when the data were stratified into two groups on the basis of geographical origin of the families (United States vs. Middle East).
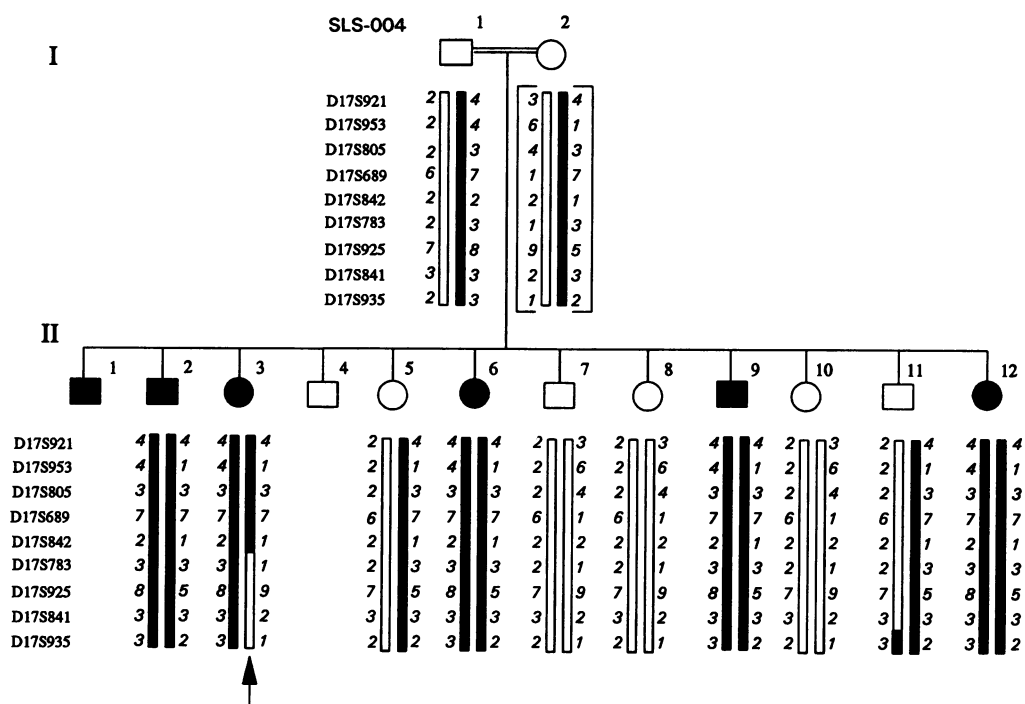
Figure 3   Haplotype analysis of pedigree SLS-004. DNA was not available for individuals I:2, II:1, or II:4; the haplotype for I:2 was deduced. The paternal chromosome is depicted on the left for each child, and the solid chromosome carries the mutated SLS gene. Marker loci are listed in order toward 17qter, except the position of D17S689 relative to D17S805 and D17S842, which is unknown. SLS-affected individual II:3 displays a recombination in the maternal chromosome (arrow).

Discussion

We present localization of the gene causing SLS in families of Egyptian, Arabic, mixed European, and Native American origins to the pericentromeric region of chromosome 17 and linkage to the markers D17S953, D17S805, D17S689, and D17S842. This is consistent with gene-linkage mapping in Swedish kindreds reported by Pigg et al. (1994), who first placed the gene on chromosome 17, in the region flanked by D17S805 and the closely linked group of markers D17S783/ D17S959/ D17S842/D17S925. Strong allelic association between locus D17S805 and SLS in the Swedish pedigrees, supported by historical records, indicates a founder effect in this population (Pigg et al. 1994) and that a single mutation in the SLS gene affects this population. Common ancestors were not expected among our seven pedigrees, and no allele association of D17S805 to SLS was evident in the present study. Our results from families of diverse non-Swedish ethnicity, all but certain to represent independent genetic defects, provide evidence that SLS is a genetically homogeneous disease.

The SLS gene is most likely to be located at 17p11.2 (fig. 4) rather than on 17q11 as previously suggested (Pigg et al. 1994). Pigg et al. (1994) calculated that the SLS gene was located within 600 kb of D17S805. This marker has been placed on the two most proximal YACs
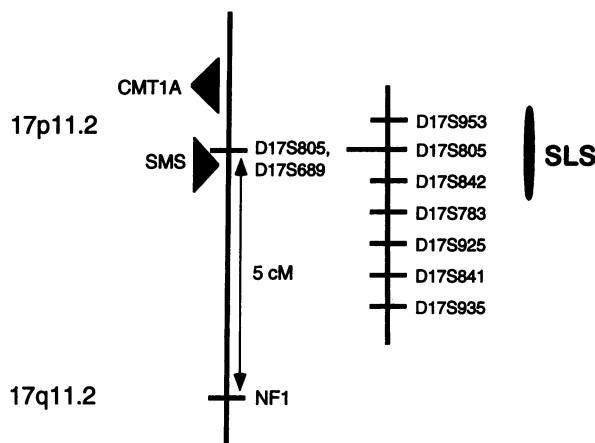

Figure 4   Map of the SLS locus on chromosome 17. The SLS gene was placed within the marker grouping D17S953, D17S805, D17S689, and D17S842 in this study. This is shown relative to the CMT1A duplication and the SMS deletion regions on 17p11.2 (Chevillard et al. 1993). D17S805 is the common marker in these studies. D17S689 has been placed on 17p ~3 cM from the centromere and 5 cM from the NF1 locus on 17q11.2 (Gerken et al. 1995). The marker order comes from published maps (Gyapay et al. 1994) and recombination events observed in this study; Gerken et al. (1995) reported colocalization of D17S689 and D17S805. This map does not depict marker to marker distances from D17S953 to D17S935.

of a contig spanning the Charcot-Marie-Tooth type 1A (CMT1A) duplication and the distal part of four micro-deletions in Smith-Magenis syndrome (SMS) on 17p11.2-p12 (Chevillard et al. 1993). Furthermore, a recent high-resolution genetic map of chromosome 17p placed D17S689 on 17p ~3 cM from the centromere and 5 cM from the NF1 locus on 17q11.2 (Gerken et al. 1995). Pairwise linkage analysis showed that D17S689 was tightly linked to D17S805 (Z_{max} = 12.6; θ = .001) and D17S783 (Z_{max} = 12.6; θ = .001). Our study also showed close linkage between D17S689 and the SLS locus (Z_{max} = 3.83; θ = 0). Taken together, our linkage results (table 1) and those reported by Pigg et al. (1994) are consistent with the assignment of the SLS gene to chromosome 17p11.2.

Although the current human gene map contains no likely candidate for the SLS gene, the presence of a stomach aldehyde dehydrogenase gene (ALDH3) on 17p11.2 may be of significance (Hiraoka et al. 1995). Biochemical studies of SLS patients have identified a deficiency in a related enzyme activity, FALDH, which catalyzes the oxidation of fatty aldehyde to fatty acid. Its activity was profoundly reduced (<10% normal) in the cultured fibroblasts of seven unrelated SLS patients from six countries of origin, including Sweden (Rizzo and Craft 1991). Similar deficiency of FALDH activity has been observed in 20 additional unrelated SLS patients (W. B. Rizzo, unpublished data). Parents of SLS patients, who are predicted to be obligate SLS heterozygotes, were found to have an intermediate FALDH activity, reduced to about one-half of mean normal activity (Rizzo and Craft 1991; Kelson et al. 1992). This is consistent with carriers producing an equal proportion of nonfunctioning mutant enzyme and normal enzyme. The dramatic reduction in FALDH activity is characteristic of SLS and not of cutaneous or neurological disorders in general (Rizzo 1993) and can be used for heterozygote detection (Kelson et al. 1992) and prenatal diagnosis of the disorder (Rizzo et al. 1994). Taken together, the enzymatic data strongly point to FALDH as the likely candidate gene for SLS.

Aldehyde dehydrogenases comprise a complex family of enzymes that have been classified on the basis of intracellular localization, substrate specificity, and amino acid sequence similarities (Lindahl 1992). Seven nonallelic gene sequences for human aldehyde dehydrogenases have been reported and localized to six different chromosomal regions (Yoshida 1992; Kurys et al. 1993; Hsu et al. 1994a, 1994b). A recent report localized the ALDH3 gene to 17p11.2 by FISH (Hiraoka et al. 1995). This isozyme is not a likely SLS candidate, because it is expressed predominantly in the stomach and lung and not in fetal tissues or fibroblasts (Santisteban et al. 1985). Moreover, ALDH3 and FALDH have different substrate specificities (Santiseban et al. 1985; T. L. Kel-

son and W. B. Rizzo, unpublished data). However, the FALDH gene may be part of a cluster of aldehyde dehydrogenase genes on chromosomal 17p11.2.

Regardless of the nature of the SLS gene, the common biochemical deficiency of FALDH and our linkage results from seven unrelated SLS families combined with the findings of Pigg et al. (1994) provide strong evidence for a single SLS locus on chromosome 17. The linkage of the SLS gene to several microsatellite markers on chromosome 17 should prove useful for the diagnosis of SLS when enzymatic studies are not available. In addition, DNA-based linkage analysis will be invaluable for carrier detection.

Acknowledgments

References

Chevillard C, Le Paslier D, Passage E, Ougen P, Billault A, Boyer S, Mazan S (1993) Relationship between Charcot-Marie-Tooth 1A and Smith-Magenis regions: snU3 may be a candidate gene for the Smith-Magenis syndrome. Hum Mol Genet 2:1235–1243

Gerken SC, Albertson H, Elsner T, Ballard L, Holik P, Lawrence E, Moore M, et al (1995) A strategy for constructing high-resolution genetic maps of the human genome: a genetic map of chromosome 17p, ordered with meiotic breakpoint-mapping panels. Am J Hum Genet 56:484–499

Gomori JM, Leibovici V, Zlotogorski A, Wirguin I, Haham-Zadeh S (1987) Computed tomography in Sjögren-Larsson syndrome. Neuroradiology 29:557–559

Gyapay G, Morissette J, Vignal A, Dib C, Fizames C, Millasseau P, Marc S, et al (1994) The 1993–1994 Généthon human genetic linkage map. Nat Genet 7:246–339

Hiraoka LR, Hsu L, Hsieh C-L (1995) Assignment of ALDH3 to human chromosome 17p11.2 and ALDH5 to human chromosome 9p13. Genomics 25:323–325

Hsu LC, Chang W-C, Hiraoka L, Hsieh C-L (1994a) Molecular cloning, genomic organization, and chromosomal localization of an additional human aldehyde dehydrogenase gene, ALDH6. Genomics 24:333–341

Hsu LC, Chang W-C, Yoshida A (1994b) Cloning of a cDNA encoding human ALDH7, a new member of the aldehyde dehydrogenase family. Gene 151:285–289

Jagell S, Gustavson K-H, Holmgren G (1981) Sjögren-Larsson syndrome in Sweden: a clinical, genetic and epidemiological study. Clin Genet 19:233–256

Kelson TL, Craft DA, Rizzo WB (1992) Carrier detection for Sjögren-Larsson syndrome. J Inher Metab Dis 15:105–111

Kruglyak L, Daly MJ, Lander ES (1995) Rapid multipoint linkage analysis of recessive traits in nuclear families, including homozygosity mapping. Am J Hum Genet 56:519–527

Kurys G, Shah PC, Kikonyogo, A, Reed D, Ambroziak W, Pietruszko R (1993) Human aldehyde dehydrogenase:

cDNA cloning and primary structure of the enzyme that catalyzes dehydrogenation of 4-aminobutyraldehyde. Eur J Biochem 218:311–320

Lindahl R (1992) Aldehyde dehydrogenases and their role in carcinogenesis. Crit Rev Biochem Mol Biol 27:283–335

Ott J (1992) The HOMOG programs. Columbia University

Pigg M, Jagell S, Sillén A, Weissenbach J, Gustavson K-H, Wadelius C (1994) The Sjögren-Larsson syndrome gene is close to D17S805 as determined by linkage analysis and allelic association. Nat Genet 8:361–364

Richards B, Skoletsky J, Shuber AP, Balfour R, Stern TC, Dorkin HL, Parad RB, et al (1993) Multiplex PCR amplification from the CFTR gene using DNA prepared from buccal brushes/swabs. Hum Mol Genet 2:159–163

Richards BW (1972) Sjögren-Larsson Syndrome. In: Winken PJ, Bruyn GW (eds) Handbook of clinical neurology, vol 13. North Holland, Amsterdam, pp 468–482

Rizzo WB (1993) Sjögren-Larsson syndrome. Sem Dermatol 12:210–218

Rizzo WB, Craft DA (1991) Sjögren-Larsson syndrome: deficient activity of the fatty aldehyde dehydrogenase component of fatty alcohol:NAD$^+$ oxidoreductase in cultured fibroblasts. J Clin Invest 88:1643–1648

Rizzo WB, Craft DA, Kelson TL, Bonnefont J-P, Saudubray J-M, Schulman JD, Black SH, et al (1994) Prenatal diagnosis of Sjögren-Larsson syndrome in the first and second trimester using enzymatic methods. Prenat Diagn 14:577–581

Rizzo WB, Dammann AL, Craft DA (1988) Sjögren-Larsson syndrome: impaired fatty alcohol oxidation in cultured fibroblasts due to deficient fatty alcohol:nicotinamide adenine dinucleotide oxidoreductase activity. J Clin Invest 81:738–744

Santisteban I, Povey S, West LF, Parrington JM, Hopkinson DA (1985) Chromosome assignment, biochemical and immunological studies on a human aldehyde dehydrogenase, ALDH3. Ann Hum Genet 49:87–100

Sjögren T, Larsson T (1957) Oligophrenia in combination with congenital ichthyosis and spastic disorders. Acta Psychiatr Neurol Scand Suppl 32:1–113

Smith CAB (1963) Testing for heterogeneity of recombination fraction values in human genetics. Ann Hum Genet 27:175–182

Theile U (1974) Sjögren-Larsson syndrome: oligophrenia-ichthyosis-di/tetraplegia. Humangenetik, 22:91–118

Yoshida A (1992) Molecular genetics of human aldehyde dehydrogenase. Pharmocogenetics 2:139–147