Carrier Detection for Sjögren–Larsson Syndrome

T. L. KELSON, D. A. CRAFT and W. B. RIZZO*
Departments of Human Genetics and Pediatrics, Medical College of Virginia, Virginia Commonwealth University, PO Box 259 MCV Station, Richmond, VA 23298, USA

Summary: Sjögren–Larsson syndrome (SLS) is an autosomal recessive disorder associated with reduced activity of the fatty alcohol:NAD⁺ oxidoreductase complex (FAO). Recent studies indicate that SLS patients are specifically deficient in the fatty aldehyde dehydrogenase (FALDH) component of FAO. To investigate the possibility of carrier detection for SLS, FAO and FALDH activities were measured in cultured skin fibroblasts from normal controls, obligate SLS heterozygotes, and SLS homozygotes using the 18-carbon substrates octadecanol and octadecanal. Three of 11 heterozygotes for SLS had FAO activities that were within the normal range; the other 8 SLS heterozygotes had FAO activities below normal. In contrast, fibroblast FALDH activity was more effective than FAO in discriminating SLS heterozygotes from normal controls. FALDH activity (nmol min⁻¹ (mg protein)⁻¹) in normal controls was 8.54 ± 1.16 (mean ± SD; range 6.95–10.77; n = 12) and in SLS heterozygotes was 5.12 ± 1.31 (range 3.28–6.96; n = 11), or 60 ± 15% of mean normal activity. One SLS heterozygote had an FALDH activity within the lower range of normal; this heterozygote had an FAO activity below normal. None of the SLS heterozygotes had an FAO or FALDH activity that was in the range of that measured in SLS homozygotes. These results indicate that measurement of FAO and FALDH activities in cultured skin fibroblasts using 18-carbon substrates is useful for SLS carrier detection.

Sjögren–Larsson syndrome (SLS; McKusick 270200) is an autosomal recessive disorder characterized with congenital ichthyosis, mental retardation, and spastic di- or tetraplegia (Sjögren and Larsson, 1957; Chaves-Carballo, 1987). SLS patients have been reported to have macular degeneration, glistening white dots in the optic fundus, speech defects, seizures, kyphosis of the thoracic spine, brain atrophy, and short stature (Chaves-Carballo, 1987; Jagell et al., 1981). Generalized hyperkeratosis is present at birth and is usually the first sign of SLS. Commonly, the diagnosis of SLS

*Correspondence
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is made in patients who present with congenital ichthyosis and show signs of spasticity and mental retardation before 3 years of age (Jagell and Heijbel, 1982).

Patients with SLS are deficient in fatty alcohol: NAD\textsuperscript{+} oxidoreductase (FAO), an enzyme that catalyses the oxidation of fatty alcohol to fatty acid (Rizzo et al. 1988, 1989; Judge et al. 1990). It has been postulated that FAO is an enzyme complex, one component functioning as a fatty alcohol dehydrogenase (FADH) necessary for the oxidation of fatty alcohol to fatty aldehyde, and another enzyme closely associated with FADH that functions as a fatty aldehyde dehydrogenase (FALDH) catalysing the oxidation of fatty aldehyde to fatty acid (Ichihara et al., 1986). Recent investigations have indicated that the primary biochemical defect in SLS patients is a deficiency of the FALDH component of FAO (Rizzo and Craft, 1991). In SLS fibroblasts, the extent of FALDH and FAO deficiency is distinctly more profound with 18-carbon substrates than with shorter-chain substrates.

The demonstration of partial FAO deficiency in some obligate SLS heterozygotes raises the possibility of using this test for SLS carrier detection. However, heterozygote detection for SLS is unreliable when FAO activity is measured in cultured fibroblasts using the 16-carbon substrate hexadecanol (Rizzo et al., 1989). The present study was undertaken in an effort to improve carrier detection by measuring FAO and FALDH activities using 18-carbon substrates. We investigated both crude homogenates and subcellular fractions in cultured skin fibroblasts from normal controls, obligate SLS heterozygotes, and affected homozygous patients.

**MATERIALS AND METHODS**

**Chemicals:** \[1-^{14}\text{C}\] Stearate (58 mCi/mmol) was obtained from ICN Radiochemicals, Irvine, CA, USA. \[1-^{14}\text{C}\] Octadecanol was synthesized from \[1-^{14}\text{C}\] stearate and purified by thin-layer chromatography as described by Rizzo et al. (1987). Thin-layer chromatography plates composed of silica gel G were obtained from Whatman Inc., Clifton, NJ, USA. Organic solvents, either reagent-grade or high-performance liquid-chromatography grade, were from J. T. Baker Inc., Phillipsburg, NJ, USA. Octadecanal was prepared from octadecanol according to the method of Ferrell and Yao (1972). Unless otherwise specified, all other biochemicals were purchased from Sigma Chemical Co., St. Louis, MO, USA.

**Cells:** Cultured skin fibroblasts were grown from skin punch biopsies taken from normal controls, parents of affected patients who are obligate heterozygotes for this autosomal recessive disorder, and affected homozygous patients. Cells were routinely grown at 37°C in an atmosphere of 5% CO\textsubscript{2}, 95% air in Dulbecco's MEM supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U/ml), and streptomycin (100 μg/ml). All experiments were performed on cells at passages 2 through 12.

**Enzyme assays:** Human skin fibroblasts were grown to confluence and harvested by trypsinization. The cells were pelleted by centrifugation at 1500g for 5 min and washed twice with phosphate-buffered saline. The final cellular pellet was resuspended...