Sjögren-Larsson syndrome (SLS) is an inherited neurocutaneous disorder characterized by ichthyosis, mental retardation and spasticity. The disease is caused by mutations in the \textit{ALDH3A2} gene, which encodes for fatty aldehyde dehydrogenase (FALDH), an enzyme involved in the oxidation of aldehydes derived from metabolism of fatty alcohol and other lipids. The pathogenesis of the disease is poorly understood and therapeutic options are limited. We produced a FALDH\textsuperscript{-/-} gene targeting construct to generate a mouse model of SLS.

\textbf{RESULTS}

\textbf{Generation of knockout of ALDH3A2}

We constructed a gene targeting vector that replaced an exon of 4 and exon of 5 of the \textit{ALDH3A2} gene, encoding the catalytic cysteine (Cys 241) of FALDH, with the selectable neo gene (Figure 3, upper panel). The targeting construct was microinjected into pronuclei of C57B6/J blastocysts and selected for screening by PCR. Using primers in neo and intron 6 outside of the vector insert, a 4.2 kb product was found in 2 of 172 clones (Figure 3, lower panel). These clones were confirmed to have properly incorporated the mutant construct into the \textit{ALDH3A2} gene by detecting a 5.6 kb PCR product in the vector insert and in neo (data not shown). One of the ES clones was expanded, injected into blastocysts and implanted into pseudo-pregnant females.

\textbf{Biochemical features}

In wild-type mice, FALDH activity is highest in liver, with considerably less activity in intestine, brain and skin (Figure 4). \textit{ALDH3A2} \textit{-/-} mice showed undetectable (<0.05% of normal) FALDH activity in liver. Variable amounts of residual enzyme activity using octadecanal as substrate were seen in intestine (7 ± 20\% of wild-type), brain (9 ± 20\%) and skin (7 ± 30\% of wild-type). FALDH activity in cultured skin fibroblasts from \textit{ALDH3A2} \textit{-/-} mice was reduced to 15\% of normal (not shown). This residual activity probably reflects the presence of other aldehyde dehydrogenase enzymes that have overlapping substrate specificities with octadecanal. FALDH catalyzes the oxidation of fatty aldehyde derived from fatty alcohol metabolism by participating as a component of the fatty alcohol NAD(+) oxidoreductase enzyme complex. We measured fatty alcohol levels in tissues from \textit{FALDH}\textsuperscript{-/-} and wild-type mice. In all tissues examined, the predominant fatty alcohols detected were hexadecanol (16:0-DH) and octadecanol (18:0-DH). Fatty alcohol levels were increased in livers and brains of \textit{ALDH3A2} \textit{-/-} mice by 7 to 11-fold compared to wild-type animals. Fatty alcohols in the skin were only marginally elevated.

\textbf{Clinical phenotype}

When \textit{ALDH3A2} \textit{-/-} mice were mated, the number of littersmates with +/-, +/-, and -/- genotypes corresponded to the expected 1:2:1 ratio for an autosomal recessive trait, which indicated normal prenatal viability of the \textit{ALDH3A2} \textit{-/-} animals. The gene knockout mice survived and gained weight at a normal rate.

A small proportion of littersmates from homozygous crosses exhibited congenital ichthyosis with scaling (Figure 5A). The scales were seen on the posterior nuchal area with smaller scales on the back. These mice with ichthyosis, nevertheless, survived and seemed to grow normally.

As the animals aged to about 6 months, some of the homozygous knockout mice exhibited pruritis with excoriations and loss of ear pinna tissue (Figure 5C) or scratching of the skin and loss of whiskers on the snout (not shown). These behaviors were never observed on wild-type mice. In addition, all of some animals became sparse, suggesting underlying cutaneous disease. By one year of age, some animals exhibited obvious loss of fur. One animal displayed gait abnormalities which progressed to hindlimb paralysis and death within one year. The other \textit{-/-} mice have a more variable clinical phenotype compared to SLS patients. Only a minority of the mice exhibit congenital ichthyosis; other animals appear to develop cutaneous symptoms as they get older. The neurologic symptoms of spasticity and mental retardation may become apparent only after 1 year of age. This phenotypic variation in the knockout mice may arise from the presence of modifier genes in the mixed strain 129 genetic background of the animals. Efforts are ongoing to cross the \textit{ALDH3A2} \textit{-/-} mice with other pure genetic strains of mice and observe for modification of the incidence and onset of clinical symptoms. We also plan to obtain brain MRI studies on the knockout mice and examine the histologic changes in skin and brain.

\textbf{DISCUSSION}

The \textit{ALDH3A2} gene knockout mouse model should prove useful for understanding the biochemical abnormalities in human SLS, identifying the toxic lipid precursors that cause symptoms and developing new therapeutic approaches to the disease.

\textbf{ACKNOWLEDGMENTS}

This work was supported by NIH grant AR44552 and by the Nebraska Tobacco Settlement Fund.