European Journal of Human Genetics (2000) 8, 299–306

www.nature.com/ejhg

ARTICLE

RNA-based mutation screening in German families with Sjögren-Larsson syndrome

C Kraus, C Braun-Quentin, WG Ballhausen and RA Pfeiffer

Institut für Humangenetik, Universität Erlangen-Nürnberg, Germany

Sjögren-Larsson syndrome (SLS) is a rare autosomal recessively inherited disorder characterised by mental retardation, spasticity and ichthyosis. SLS patients have a profound deficiency in fatty aldehyde dehydrogenase (FALDH) activity. The human cDNA of FALDH has been shown to map to the SLS locus on chromosome 17p11.2. Here we describe a method based on reverse transcriptase-polymerase chain reaction (RT-PCR) and protein truncation test to identify mutations in the *FALDH* gene in nine German SLS families. Using this detection system both disease-causing mutations were found in eight of the nine SLS families examined (17/18 chromosomes). Seven different mutations were identified: an exon 2 skipping due to exon 2 splice donor mutation; two different exon 3 splice donor mutations resulting in combined exon 2 and 3 skipping; a 906delT deletion in exon 6; a genomic deletion of about 6 kb including exon 9; a 1277T > G transversion resulting in a Leu426Ter nonsense mutation; and a 1297delGA deletion. Two of the mutations identified, the genomic exon 9 deletion and the 906delT in exon 6 affected five out of seven SLS patients from a small region of Northern Bavaria. Therefore these two mutations accounted for 71% (10/14 chromosomes) of Bavarian SLS alleles and so far have not been described in SLS families from other countries. Our findings do not support our 'historical' hypothesis, that a possible region clustering in Northern Bavaria could be due to the presence of Swedish soldiers during the 30 Years War (1618–1648), but suggest that two mutations causing SLS syndrome originated in Northern Bavaria. *European Journal of Human Genetics* (2000) **8**, 299–306.

Keywords: Sjögren-Larsson syndrome; fatty-aldehyde dehydrogenase (*FALDH*) gene; mutation screening; reverse-transcriptase polymerase chain reaction; protein truncation test; mRNA decay

Introduction

Sjögren-Larsson syndrome (SLS) is an autosomal recessive disorder which is characterised by the presence of ichthyosis, slowly progressive spastic diplegia or tetraplegia, and mental retardation.[1–3] The ichthyosis is usually congenital and first brings the patient to medical attention. Neurological findings, which show considerable variations, may not be evident within the first 3 years of life. Developmental retardation, particularly of motor and speech functions is mostly noted during infancy. Additional clinical abnormalities are seizures, glistening dots in the macular region of the retina, and short stature.

Although patients with these symptoms have been described previously, the clinical entity was delineated only in 1957 by Sjögren and Larsson.[1] Up to 1994 more than 200 patients had been reported from at least 23 countries[2,4] but mostly from Sweden.

Cultured fibroblasts of patients with SLS were shown to have deficient activity of fatty aldehyde dehydrogenase (FALDH),[5] an enzyme that is necessary for the oxidation of fatty alcohol to fatty acid as a component of the fatty alcohol: NAD^+ oxidoreductase (FAO) enzyme complex.[6] Therefore, affected patients with impaired fatty alcohol oxidation accumulate long-chain fatty alcohol in their tissues.[3,6] Although the pathogenesis of single abnormalities is yet unclear, fatty alcohol storage is thought to be responsible for the symptoms of the disease.[3] Prior to the

Correspondence: Dr rer. nat. Cornelia Kraus, Institut für Humangenetik der Universität, Schwabachanlage 10, D-91054 Erlangen, Germany. Tel: +49 9131 85 26587; Fax: +49 9131 209297; E-mail: cokraus@humgenet.uni-erlangen.de
Received 19 February 1999; revised 26 November 1999; accepted 29 November 1999

identification of the *SLS* gene, enzymatic activity testing was necessary for diagnosis of SLS patients, carrier detection[7] and prenatal diagnosis.[8]

The *SLS* gene was assigned to chromosome 17p11.2 by linkage analysis[9,10] and the *FALDH* gene was mapped to the same locus using yeast artificial chromosomes.[11] The gene consists of 10 exons spanning about 30.5 kb[12,13] and the coding region encompasses 1458 bp.

After identification of the first disease-causing mutations, suitable molecular screening methods were applied. Because most of the known mutations result in a premature translational stop codon,[11,14–18] we decided to use RT-PCR and a coupled *in vitro* transcription–translation assay as screening method. This paper will report on our findings in nine German families with Sjögren-Larsson syndrome.

Material and methods

Patients

The diagnosis of SLS in nine patients from Germany was concluded from the presence, though variable, of the three main symptoms, namely ichthyosis, spastic di- or tetraplegia and mental retardation. All but one have glistening macular dots. Two patients were sibs with minor findings in the male and severe abnormalities in the female. In contrast to her younger brother, the spastic tetraplegia and the mental retardation of the sister was more developed.

Preparation of DNA and RNA samples

Mutation analyses in the *FALDH* gene were carried out in nine unrelated SLS index patients and their parents, using genomic DNA and total RNA. Genomic DNA was extracted from 10 ml peripheral blood by the salting-out procedure. Total RNA was isolated from 10 ml EDTA-treated peripheral blood or from $2–3 \times 10^7$ Epstein-Barr virus immortalized B-lymphocytes using the commercial Tri™ reagent kit (Sigma, Deisenhofen, Germany).

Reverse transcription polymerase chain reaction

In order to analyse the whole *FALDH* coding region 2 μg of total RNA was reversely transcribed using the Superscript II RT (BRL, Eggenstein, Germany) according to the manufacturer's instructions.

PCR amplification of the whole *FALDH* coding region was carried out using the T7 promotor sequence-modified sense primer FALDHT7 5'GGATCCTAATACGACTCACTATAGGAACAGACCACCATGGAGCTCGAAGTCCGGCGG3' in combination with the antisense oligonucleotide FALDH1487 5'AGGCACTAGGAGGTTGAACAGG3'. Primer sequences were designed using the GenBank file L47162 deposited by De Laurenzi *et al*[11]

We amplified 1 μl of the cDNA in a 25 μl total reaction volume using the Expand™ Long Template PCR System (puffer system 3) according to the manufacturer's instructions (Boehringer Mannheim GmbH, Mannheim, Germany).

PCR conditions were 94°C for 2 min, followed by 15 cycles at 94°C for 10 s, 66°C for 30 s, 68°C for 2 min, 25 cycles at 94°C for 10 s, 65°C for 30 s, 68°C for 2 min and or 20 s extension, and final extension at 68°C for 7 min.

Protein truncation test (PTT)

Screening for translational stop mutations in the coding region of the *FALDH* gene was carried out by the protein truncation test (PTT).[19] Approximately, 100 ng of PCR amplified products of reversely transcribed mRNA was directly used in a 12.5 μl volume coupled TNT T7 reticulocyte system *in vitro* transcription/translation reaction (Promega, Heidelberg, Germany) substituted with *in vitro* translation grade (^{35}S)-L-methionine (ICN, Meckenheim, Germany). The synthesized polypeptide chains were separated by a 14% SDS-polyacrylamide gel at 200 V for 2 h at 10°C. Fluorography was carried out after treating the gel with 1 M sodium salicylate for 30 min followed by vacuum drying of the gel and exposure to an X-omat X-ray film (Kodak, Wiesbaden, Germany) for 24 h at –80°C.

cDNA cloning and sequencing

PCR amplified products were purified with Jetsorb (Genomed, Bad Oeyenhausen, Germany) and subsequently cloned in the T-vector pCR2.1™ (Invitrogen, Heidelberg, Germany). Recombinants were isolated and subsequently subjected to enzymatic dideoxy-sequencing reactions using a commercial sequencing kit (Biozym, Hess. Oldendorf, Germany) and dye-modified M13 universal primers. Sequencing products were analysed with the help of a computer assisted automated LiCor-electrophoresis apparatus (MWG, Ebersberg, Germany).

Genomic DNA sequence analysis

DNA amplification of exons 2, 3, 5, 6, 9 and 10 were performed in a 25 μl total reaction volume containing 2.5 μl of 10 × buffer, 2 μl 2.5 mM dNTPs 10 pmol of each primer, 1.5 mM $MgCl_2$ and 0.6 U Taq polymerase (PAN Script, PAN Systems, Nürnberg, Germany) and subjected to 35 PCR cycles (30 s at 94°C, 30 s at 55–65°C, 30 s at 72°C). Primer sequences for the amplification of FALDH exons 2, 3, 6 and 10 were already published.[18] For PCR amplification of genomic FALDH exons 5 and 9 we used the following primer sequences:

FALDH5MS: 5'MS-TATATAGCTGTTCTGGATGTTTTCC3'
FALDH5MR: 5'MR-GGGGATGCTGCCTGCGAATAG3'
FALDH9MS: 5'MS-AGCTTGCATCATCTACAGTGAAGC3'
FALDH9MR: 5'MR-GCTAGAATATGCATCTGGCAGCC3'

Primer sequences were designed using the GenBank files U75286–U75296 deposited by Rogers *et al*.[13] Each sense primer was modified at its 5' end by a universal M13 sequence (MS: 5'TGTAAAACGACGGCCAGT3') and each antisense

primer was preceded at the 5' end by a universal M13 reverse sequence (MR: 5'CAGGAAACAGCTATGACC3') allowing sequence analysis as described above.

Multiplex-PCR

DNA amplification encompassing the genomic regions of exons 8 to 10 and exons 9 to 10, respectively, was performed in one single reaction containing two different 5' primers (FALDH8MS: 5'MS-TCACTGACCTGGACACCTTTGG3'; FALDH9MS: 5'MS-AGCTTGCATCATCTACAGTGAAGC3') and one common 3' primer (FALDH1487: 5'AGGCACTAG-GAGGTTGAACAGG3'). 500 ng of genomic DNA was amplified in a 25 µl total reaction volume using the Expand™ Long Template PCR System (Boehringer Mannheim GmbH).

PCR conditions were 94°C for 2 min, followed by 15 cycles at 94°C for 10 s, 65°C for 30 s, 68°C for 3 min, 25 cycles at 94°C for 10 s, 63°C for 30 s, 68°C for 3 min and 20 s extension, and final extension at 68°C for 7 min.

Puromycin treatment

For inhibition of nonsense mediated RNA decay lymphoblastoid cells were grown for 12 h in the presence of 200 µg/ml puromycin (Sigma, Deisenhofen, Germany) and then harvested in PBS buffer.

Results

Three different exon deletions identified by RT-PCR analyses

For initial RT-PCR analysis leukocytes from our patients and their healthy parents were collected to extract total RNA, which was reversely transcribed into complementary DNA with the help of random primers. RT-PCR based amplification of the 1458 bp *FALDH* coding region was carried out and PCR products were analysed in a 1.2% agarose gel. Figure 1 depicts the results of the RT-PCR analysis in five of our nine Sjögren-Larsson families.

Using this method we were able to identify truncated RT-PCR products of reduced size in the index patients P1, P2, P3, P4 and P5 (Figure 1: Lanes 1, 6, 8, 10). Since in all four cases no full length *FALDH* product was observed, we speculated that they could be homozygous for the particular deletion.

RT-PCR analysis of the *FALDH* gene of the parents revealed that both parents of patients P2, P3 and P4 were heterozygous for the deletions (Figure 1: lanes 4, 5, 7, 9). However, only one parent of patient P1 showed a shortened RT-PCR product (Figure 1: lane 3), whereas in the second parent (Figure 1: lane 2) only a *FALDH*-transcript of normal size was identified. Blood samples of parents of patient P5 were not available.

To characterise the missing sequences in *FALDH* transcripts of reduced size, aberrant cDNA products of the five patients shown in Figure 1 were cloned into the T-vector pCR2.1. Results of the sequence analysis of cloned fragments are summarised in Figure 2. Sequence data derived from patients P1, P2 and P3 revealed that the RT-PCR fragments of

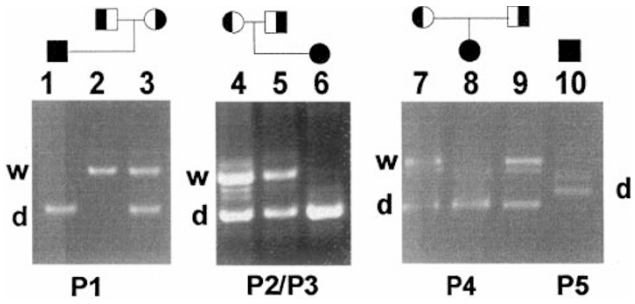

Figure 1 Three different exon deletions identified by RT-PCR analyses. RT-PCR analysis of the *FALDH* gene of four SLS patients and their healthy parents. RT-PCR based amplification of the 1458 bp *FALDH* coding region was carried out and PCR products were analysed in a 1.2% agarose gel. Full length *FALDH* PCR products of 1458 bp (w) and RT PCR products of reduced size (d) were indicated. RT-PCR products loaded from left to right are derived from: Lane 1: SLS patient P1; Lane 2: father of SLS patient P1; Lane 3: mother of SLS patient P1; Lane 4: mother of SLS patient P2/P3; Lane 5: father of SLS patient P2/P3; Lane 6: SLS patient P2/P3. Lane 7: mother of SLS patient P4; Lane 8: SLS patient P4; Lane 9: father of SLS patient P4; Lane 10: SLS patient P5. Parents of P5 were not available.

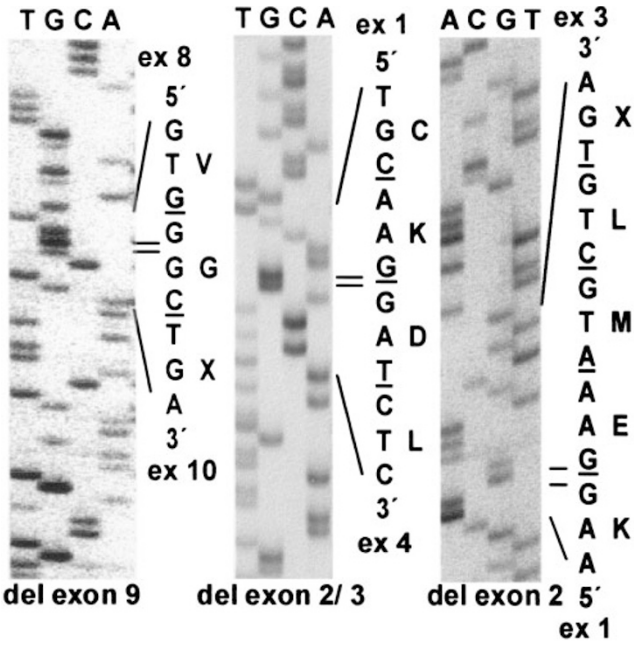

Figure 2 Identification of three different FALDH exon deletions by cDNA sequencing. RT-PCR products of reduced size of patients P1, P2/P3, P4 and P5 were cloned into the T-vector pCR2.1 and individual alleles were subsequently sequenced. An aberrant 8 to 10 connection in patient P1 and P2/P3 (del exon 9), an exon 1 to 4 connection in patient P4 (del exon 2/3) and an exon 1 to 3 connection in patient P5 (del exon 2) was observed. Nucleotide sequences surrounding the aberrant exon connections including deduced amino acids (one letter code) are given on the right hand side.

reduced size were the result of exon 9 skipping (Figure 2: del exon 9), which leads to an out-of-frame reading of exon 10. The probands with this deletion also have a polymorphic nucleotide exchange 1446A > T in exon 10 on the same allele. The combination of both mutation and polymorphism creates a premature stop codon after three altered amino acids in the last exon. Since PCR amplification with primers specific for genomic sequences of exon 9 failed to yield a product in patients P2 and P3, respectively, we postulated that exon 9 skipping detected at the RNA level could be due to a genomic deletion including exon 9, which has also been described by Sillen *et al*.[18] This genomic deletion was further analysed in more detail (see below).

Sequence analysis of recombinant clones derived from patient P4 (Figure 1: lane 6) revealed that the shortened transcript was due to in-frame skipping of exon 2 and 3 (Figure 2: del exon 2/3). Interestingly, this deletion resulted from two different splice donor mutations affecting exon 3. The maternal allele carried a G-deletion of the canonical GT-splice donor dinucleotide, whereas the GT-splice donor sequence of exon 3 of the paternal allele was altered by a G to C transversion resulting in an abnormal CT site (data not shown).

The shortened transcript identified in the fifth patient P5 (Figure 1: lane 10) was due to exon 2 skipping creating a premature stop codon after three altered amino acids in the third exon (Figure 2: del exon 2). Sequence analysis of genomic DNA of patient P5 revealed that the patient was heterozygous for an exon 2 splice donor mutation. The GT-splice donor sequence of exon 2 of one allele was altered by a T to C transition resulting in an abnormal GC site (data not shown).

Characterising a genomic exon 9 deletion by multiplex-PCR analysis

To further characterise the presumed genomic deletion of exon 9 described above, a multiplex PCR was established as shown in Figure 3. Two different 5' primers specific for exon 8 and exon 9, respectively, were combined with an antisense primer located in exon 10. We theoretically expected amplification of a 4 kb and a 11 kb fragment in healthy controls, but only the smaller fragment of 4 kb was amplified (Figure 3: lane 4 and 8). With this set of primers we obtained an additional fragment of 4.4 kb in patients P1, P2 and P3 (Figure 3: lanes 1 and 7) and in their healthy parents (Figure 3: lanes 3, 5, 6). We concluded that this 4.4 kb fragment represents the mutated allele resulting from a 6 kb genomic deletion including exon 9.

This genomic analysis provided the additional information in patient P1 (Figure 3, lane 1) that he is heterozygous for the exon 9 deletion in contrast to the initial results obtained by RT-PCR analysis (Figure 1, lane 1), which suggested expression of a single truncated *FALDH* transcript.

In order to map precisely the deletion breakpoints, the 4.4 kb junction fragments of patients P1, P2 and P3, respectively, and corresponding wild type fragments were cloned and sequenced. The forward primer to characterise the 5' breakpoint of the region spanning the deletion was 5' GAT ACC CTG TGG CAT CAT TGA C 3' located in intron 8 (IVS8 + 1259 to 1280). The reverse primer 5' GTT CAT CTC TGC TTA CTG GAC C 3' located in the alternative spliced exon 9*[13] was used to delineate the 3' breakpoint. This led to the identification of the putative 5' and 3' breakpoints of the deletion as delineated in Figure 4. Comparison of the junction fragment with the corresponding wild type sequence revealed 8 bp (5' AGGAGAAT 3') at the deletion junction that was identical with two 8 bp sequences in intron 8 (IVS8 + 1644 to 1651) and intron 9 (197–204 bp downstream of exon 9*), respectively.

Therefore, we postulate that the 5' breakpoint is located in between base pairs 1644–1651 upstream of exon 8 in intron 8 and the 3' breakpoint is located in between base pairs 197–204 downstream of the alternative spliced exon 9* in intron 9. This deletion removes about 6 kb and causes complete loss of exon 9. The presence of identical 8 bp

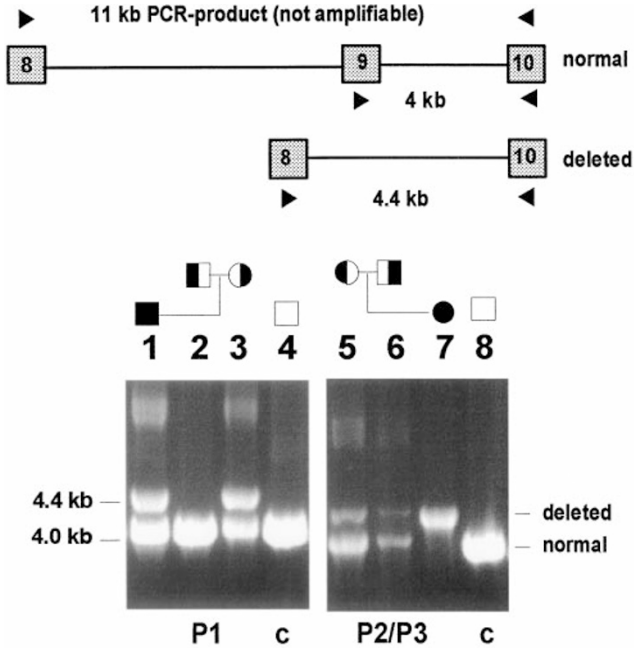

Figure 3 Multiplex PCR analysis of a genomic FALDH exon 9 deletion. DNA amplification encompassing the genomic regions of exons 8 to 10 and exons 9 to 10, respectively, was performed in one single reaction containing two different 5' primers specific for exon 8 and 9, and one 3' primer specific for exon 10. Expected sizes of PCR products are given in the upper scheme. The lower part documents the results of multiplex analyses of this particular genomic region in families of index patients P1 (Lanes 1–3) and P2/P3 (Lanes 5–7). PCR products loaded from left to right are derived from: Lane 1: SLS patient P1; Lane 2: father of SLS patient P1; Lane 3: mother of SLS patient P1; Lane 4: healthy control; Lane 5: mother of SLS patient P2; Lane 6: father of SLS patient P2; Lane 7: SLS patient P2; Lane 8: healthy control.

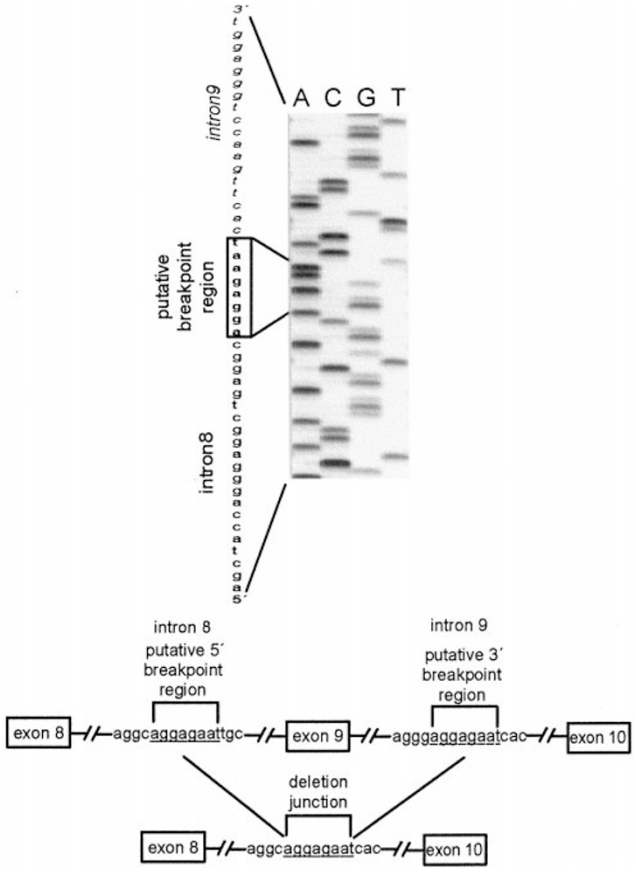

Figure 4 Characterisation of the genomic breakpoint. Upper part: Sequence data of the deletion junction and portions of the joining introns derived from the 4.4 kb junction fragment. Sequences 5' to the breakpoint region were derived from intron 8 and that in 3' to the junction from intron 9. Lower part: Nucleotide sequences from the 4.4 kb junction fragment and the normal fragments from intron 8 and intron 9 containing the 5' and 3' breakpoint regions. An 8bp region of homology at the deletion junction and in the vicinity of breakpoints is underlined. The putative breakpoint regions are indicated by brackets.

(AGGAGAAT) sequences at both breakpoints, with one being part of the deleted sequence, suggests a common slippage mispairing mechanism for this deletion.[20]

Premature termination codons induce nonsense-mediated RNA decay

Since only one allele of patient P1 appeared to be affected by the genomic 6 kb deletion including exon 9 (Figure 3: lane 1), we considered a second mutation which could probably lead to RNA instability. Meanwhile, this particular patient had already been analysed in Sweden and was found to carry a deletion of one out of four thymidins at nucleotide position 903–906 in exon 6, designated nt906delT in addition to the genomic exon 9 deletion.[18] This deletion nt906delT created a frameshift that introduced a stop signal 12 codons downstream from Ala301.

To demonstrate that this particular mutation leads to RNA instability due to a process designated 'nonsense-mediated mRNA decay', lymphoblastoid cells were treated with the translation inhibitor puromycin[21] and total RNA was isolated. Upon cDNA synthesis RT-PCR analyses were carried out as shown in Figure 5. In contrast to the initial RT-PCR analysis (Figure 1), a full-length and a shortened *FALDH*-transcript were identified in patients P1 and P5 (Figure 5, lanes 2 and 8). In addition, a *FALDH* transcript of normal size was demonstrated for the first time in patients P6 and P7 (Figure 5, lanes 5 and 6).

Subsequently, RT-PCR products were subjected to a coupled *in vitro* transcription/translation reaction and S35-methionine labelled proteins were size-fractionated by polyacrylamide gel electrophoresis.[19] Autoradiographic signals were obtained, which are depicted in Figure 6. As expected, patient P1 (Figure 6: lane 2) presented a truncated polypeptide chain of 45 kD resulting from the exon 9 deletion and an additional small polypeptide chain of 32 kD due to the frameshift mutation in exon 6. Since the father of P1 (Figure 6; lane 1) is heterozygous for the latter mutation, an identical 32 kD polypeptide chain and a 54 kD polypeptide chain corresponding to the wild type *FALDH* were observed. This PTT also allowed the identification of identical truncated FALDH proteins in patients P6 and P7 (Figure 6; lanes 5 and 6), respectively. Since the pattern of their *in vitro* translated polypeptides was similar to the low molecular

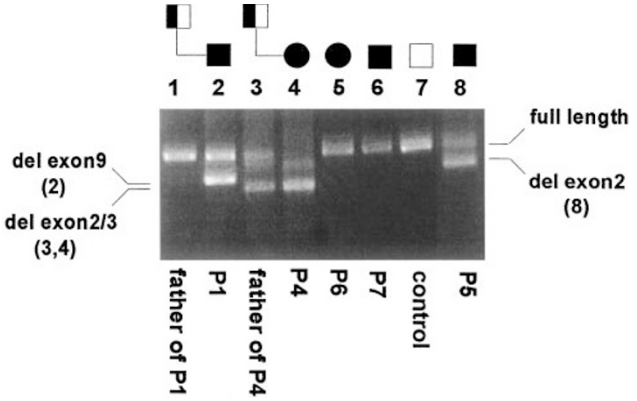

Figure 5 RT-PCR analysis of the *FALDH* gene after puromycin treatment. RT-PCR analysis of the *FALDH* gene of five SLS patients, healthy parents and one normal control person. RT-PCR based amplification of the 1458 bp *FALDH* coding region was carried out on whole RNA derived lymphoblastoid cell lines after puromycin treatment. RT-PCR products were separated through a 1.2% agarose gel. The deletions identified are indicated on the margins with an assignment to the sample lanes given in brackets. RT-PCR products loaded from left to right are derived from: Lane 1: father of SLS patient P1; Lane 2: SLS patient P1; Lane 3: father of SLS patient P4; Lane 4: SLS patient P4; Lanes 5–6: SLS patients P6 and P7; Lane 7: normal control; Lane 8: SLS patient P5.

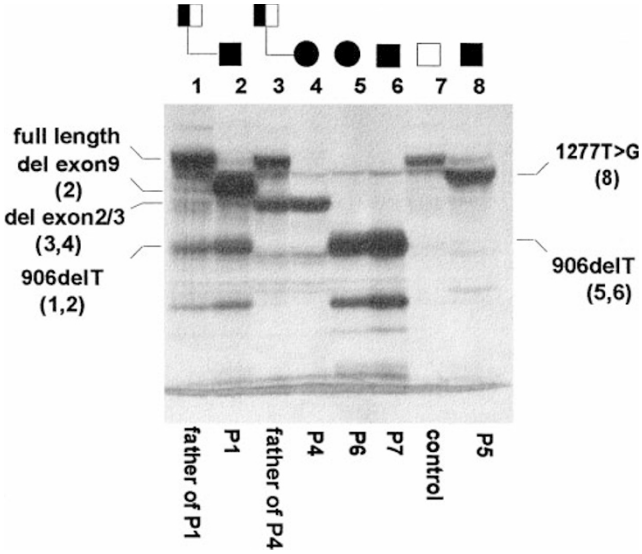

Figure 6 Protein truncation test after puromycin treatment. Reverse transcriptase-polymerase chain reaction based protein truncation test of the whole coding region of the *FALDH* gene. Autoradiographic signals of (^{35}S)-L–methionine labeled *in vitro* synthesised proteins, that were separated through a 14% SDS-polyacrylamide gel are shown after 24h of exposure to an x-ray film. The mutations identified in patients are indicated on the margins. The numbers in brackets given below the mutations refer to the lanes loaded. Protein samples are derived from: Lane 1: father of SLS patient P1; Lane 2: SLS patient P1; Lane 3: father of SLS patient P4; Lane 4: SLS patient P4; Lane 5: SLS patient P6; Lane 6: SLS patient P7; Lane 7: control; Lane 8: SLS patient.

weight bands of patient P1 and his father (Figure 6, lanes 1 and 2), we expected the same 906delT alteration, which was confirmed by cDNA sequencing. Genomic sequencing of patients P6 and P7 and their parents showed that both patients were homozygous for this particular mutation (data not shown). Interestingly, cDNA sequencing revealed that this 906delT mutation was always associated with two polymorphism 733G > A (exon 5) and 901G > C (exon 6) already described.[18] These two polymorphisms were neither identified in other SLS patients nor in healthy controls (data not shown). In patient P5 a 47 kD polypeptide chain was identified by PTT (Figure 6, lane 8). Cloning and sequencing of the full length RT-PCR product of P5 revealed a TTA → TGA transversion at nucleotide position 1277, which resulted in an Leu426Ter stop mutation in exon 9. In this patient the exon 2 skipping initially shown by RT-PCR resulted in a 4.9 kD polypeptide chain. This latter fragment was not identifiable by the PTT.

With the help of the PTT and subsequent cDNA sequencing in patient P8 the identical 906delT mutation as in patients P1, P6 and P7 was identified on one FALDH allele. On the second FALDH allele a deletion of GA at nucleotide position 1297–1298 in exon 9 was identified (data not shown). This deletion in exon 9, designated 1297delGA, was already described by other groups.[15,16,18] In patient P9 a wild type *FALDH* and a truncated polypeptide were identified by the PTT (data not shown). Cloning and sequencing of the cDNA revealed the identical 1297delGA deletion as described in patient P8 on one FALDH allele. On the other allele a TAT → AAT transversion was identified at nucleotide position 835, which resulted in a Tyr279Asn missense mutation in exon 6. Comparison of the amino acid sequence of human *FALDH* to rat microsomal aldehyde dehydrogenase (*rmsALDH*), human stomach *ALDH3* (*hALDH3*), and human *ALDH7* (*hALDH7*), revealed that Tyr279 is conserved in *rmsALDH* and *hALDH3*, but not in *hALDH7*.[11] To exclude that Tyr279Asn is a polymorphism, 35 control persons were analysed. None of them showed this alteration in exon 6. Therefore, we postulate that *Tyr279Asn* is a missense mutation inactivating the second FALDH allele of patient P9.

Discussion

The present paper describes our efforts to identify mutations in the *FALDH* gene of nine German families in which at least one child suffered from SLS. Seven of them are living in a limited region in Northern Bavaria, one in Southern Bavaria and one in Northern Germany. To our knowledge neither the parents are consanguineous nor the families are related to each other.

An initial screening revealed that the most common point mutation *C943T*[14,17] was not identified in any of our analysed patients. For further screening of the *FALDH* gene we used RT-PCR analysis, RT-PCR based PTT, cDNA sequencing, genomic amplification and DNA sequencing. With the help of these combined methods we were able to identify eight different mutations and three different polymorphisms listed in Table 1 and Table 2. Both disease causing mutations were uncovered in the 9 SLS families examined (Table 3).

This is the first paper using RT-PCR and PTT to screen for mutations in the *FALDH* gene. Using this approach the molecular analysis of our patients uncovered two different types of mutational mechanisms.

First, RT-PCR analyses carried out on RNA isolated from untreated peripheral blood cells revealed different exon deletions in SLS patients and their healthy parents (Figure 1). The second type of mutation consisted of one or two base pair deletions and a single base pair exchange within different exons which resulted in premature stop codons and were identified by truncated polypeptide chains in the PTT (Figure 6). In the course of our experiments we realised, that in these instances RT-PCR products were never obtained when RNA was extracted from peripheral blood cells. This observation suggested that these mutated mRNA molecules with premature stops might undergo nonsense-mediated RNA decay.

The mechanisms leading to reduced mRNA levels are unclear, as mRNA processing, transport, stability and/or translation could be involved.[22] Our own data give strong

Table 1 *FALDH* mutations identified in nine German SLS patients (*n*/18 number of FALDH chromosomes with the mutation in the sample of 18 FALDH chromosomes)

Exon	*Genomic DNA change*	*cDNA change*	*Consequence*	*Identified by*	*n/18*	*Reference*
2	IVS2+2T>C	del exon 2	out of frame deletion of exon 2; termination	RT-PCR	1/18	this study
3	IVS3+1delG	del exons 2+3	in frame deletion of exons 2+3	RT–PCR	1/18	this study
3	IVS3+1G>C	del exon 2+3	in frame deletion of exon 2+3	RT-PCR	1/18	this study
6	835T>A	835T>A	Tyr279Asn	sequencing	1/18	this study
6	906delT	906delT	frameshift; termination	PTT	6/18	18
9	6kb genomic deletion	del exon 9	out of frame deletion of exon 9, termination	RT-PCR	5/18	18
9	1277T>G	1277T>G	Leu426Ter, termination	PTT	1/18	this study
9	1297delGA	1297delGA	frameshift, termination	PTT	2/18	15, 16, 18

Note: numbering of the nucleotides (nt) and amino acids is in accord with the GenBank file L47162 starting with the A of the initiator ATG as nt number 1.

Table 2 Sequence polymorphisms/variations identified in nine German SLS patients

Exon	*Genomic DNA change*	*cDNA change*	*Consequence*	*Identified by*	*Frequence of heterozygosity*	*Reference*
5	733G>A	733G>A	Asp245Asn	sequencing	only on SLS alleles in combination with 906delT	18
6	901G>C	901G>C	Ala301Pro	sequencing	only on SLS alleles in combination with 906delT	18
10	1446A>T	1446A>T	Ala482Ala	sequencing	0.385	18

Note: numbering of the nucleotides (nt) and amino acids is in accord with the GenBank file L47162 starting with the A of the initiator ATG as nt number 1.

Table 3 SLS patients and identified SLS alleles

Patient	*Allele 1*	*Allele 2*
P1	[del exon 9; 1446A>T]	[733G>A; 901G>C; 906delT]
P2	[del exon 9; 1446A>T]	[del exon 9; 1446A>T]
P3	[del exon 9; 1446A>T]	[del exon 9; 1446A>T]
P4	IVS3+1delG	IVS3+1G>C
P5	1277T>G	IVS2+2T>C
P6	[733G>A; 901G>C; 906delT]	[733G>A; 901G>C; 906delT]
P7	[733G>A; 901G>C; 906delT]	[733G>A; 901G>C; 906delT]
P8	1297delGA	[733G>A; 901G>C; 906delT]
P9	1297delGA	835T>A

Note: complex SLS alleles are given in brackets; numbering of the nucleotides (nt) and amino acids is in accord with the GenBank file L47162 starting with the A of the initiator ATG as nt number 1.

support to the theory that cytoplasmatic ribosomes are involved in the pathway of nonsense-mediated mRNA decay since application of the translation inhibitor puromycin increased the steady-state level of the mutated mRNA (Figure 5 and Figure 6).

Nonsense-mediated mRNA is a mechanism evolved by cells to get rid of aberrant transcripts and proteins. Through rapid degradation of transcripts containing premature stop codons the synthesis of incomplete and potentially deleterious proteins is prevented. However, it is unknown to what extent the remaining altered *FALDH* transcripts in patients P1, P2, P3, P4 and P5 are translated in functional proteins and whether they have a dominant negative or a moderating effect.

It is noteworthy that the different mutations were not related to specific clinical manifestations of the disease. All patients were typically affected.

As the first German patients with SLS had been noted in a limited region of Northern Bavaria[23] the question was raised whether a possible regional clustering could be due to the presence of Swedish soldiers during the 30 Years War (1618–1648) in this area with special reference to a camp near Nürnberg.[24] If this hypothesis were true we would expect the same genetic alteration not only in all our patients, but the mutation should also be the same as in the Swedish patients. However, on the other hand it was shown that not all Swedish SLS patients shared the same genotype. Particularly, one of the most common Swedish mutations, 943 C > T, which replaces a highly conserved proline 315 with serine was not identified in our families. The second common SLS mutation, 1297delGA, which, together with the 943 C > T mutation, is reported to account for 48% of the SLS alleles[14] was, however, identified in two of our analysed families. On the other hand, the two most common mutations in the Northern Bavarian SLS patients are the chromosomal exon 9 deletion and the 906delT in exon 6. The latter mutation is always associated with a 901G > C polymorphism in the same exon and a 733G > A polymorphism in exon 5. Both alterations have not shown in Swedish patients. Moreover, two of the seven Northern Bavarian patients are homozygous for the exon 9 deletion. Two of them are homozygous for the exon 6 mutation and one further patient is compound heterozygous for both mutations. Therefore these two mutations together account for 71% (10/14) of the Northern Bavarian SLS alleles analysed. Interestingly, these two mutation have only been described in German SLS patients so far. However, as the exon 6 mutation is always associated with 901 G gt; C in exon 6 and with 733G > A in exon 5 of the same allele, a common origin might be possible. Analyses of all SLS patients, their parents and 60 normal controls revealed that both polymorphisms are only present in patients and their parents who carry the exon 6 mutation. Thus our findings did not support the 'historical' hypothesis

but rather suggest that two new mutations causing SLS syndrome originated in Northern Bavaria.

Acknowledgements

The expert technical assistance of Mrs A Vogler, Mrs B Schenker and Mrs K Thoma is gratefully acknowledged. The authors thank Dr D Abeck, Dr KH Grimm, Dr M Greiwe and Dr L Neumann for referring SLS patients included in this study.

References

1 Sjögren T, Larsson T: Oligophrenia in combination with congenital ichthyosis and spastic disorders. *Acta Psychiatr Neuro Scand* 1957; **32**: 1–113.
2 Jagell S, Gustavson KH, Holmgren G: Sjögren-Larsson syndrome in Sweden. A clinical, genetic and epidemiological study. *Clin Genet* 1981; **19**: 233–256.
3 Rizzo WB: Sjögren-Larsson syndrome. *Sem Dermat* 1993; **12**: 210–218.
4 Theile U: Sjögren-Larsson syndrome: oligophrenia-ichthyosis-di/tetraplegia. *Humangenetik* 1974; **22**: 91–118.
5 Rizzo WB, Craft DA: Sjögren-Larsson syndrome: Deficient activity of the fatty aldehyde dehydrogenase component of fatty alcohol: NAD^+ oxidoreductase in cultured fibroblasts. *J Clin Invest* 1991; **88**: 1643–1648.
6 Rizzo WB, Dammann AL, Craft DA *et al*: Sjögren-Larsson syndrome: Inherited defect in the fatty alcohol cycle. *J Pediatr* 1989; **115**: 228–234.
7 Kelson TL, Craft DA, Rizzo WB: Carrier detection for Sjögren-Larsson syndrome. *J Inherit Metab Dis* 1992; **15**: 105–111.
8 Rizzo WB, Craft DA, Kelson TL *et al*: Prenatal diagnosis of Sjögren-Larsson syndrome using enzymatic methods. *Prenat Diagn* 1994; **14**: 577–581.
9 Pigg M, Jagell S, Sillen A, Weissenbach J, Gustavson KH, Wadelius C: The Sjögren-Larsson syndrome gene is close to D17S805 as determined by linkage analysis and allelic association. *Nat Genet* 1994; **8**: 361–364.
10 Rogers GR, Markova NG, De Laurenzi V, Rizzo WB, Compton JG: Genetic homogeneity in Sjögren-Larsson syndrome: Linkage to chromosome 17p in families of different non-Swedish ethnic origin. *Am J Hum Genet* 1995; **57**: 1123–1129.
11 De Laurenzi V, Rogers GR, Hamrock DJ *et al*: Sjögren-Larsson syndrome is caused by mutations in the fatty aldehyde dehydrogenase gene. *Nat Genet* 1996; **12**: 52–57.
12 Chang C, Yoshida A: Human fatty aldehyde dehydrogenase gene (*ALDH10*): Organisation and tissue-dependent expression. *Genomics* 1997; **40**: 80–85.
13 Rogers GR, Markova NG, De Laurenzi V, Rizzo WB, Compton JG: Genomic organization and expression of the human fatty aldehyde dehydrogenase gene (*FALDH*). *Genomics* 1997; **39**: 127–135.
14 De Laurenzi V, Rogers GR, Tarcsa E *et al*: Sjögren-Larsson syndrome is caused by a common mutation in Northern European and Swedish patients. *J Inv Derm* 1997; **109**: 79–83.
15 Rizzo WB, Carney G, De Laurenzi V: A common deletion mutation in European patients with Sjögren-Larsson syndrome. *Biochem Molec Med* 1997; **62**: 178–181.
16 Tsukamoto N, Chang C, Yoshida A: Mutations associated with Sjögren-Larsson syndrome. *Ann Hum Genet* 1997; **61**: 235–242.
17 Sillen A, Jagell S, Wadelius C: A missense mutation in the *FALDH* gene identified in Sjögren-Larsson syndrome patients originating from the northern part of Sweden. *Hum Genet* 1997; **100**: 201–203.
18 Sillen A, Anton-Lambrecht I, Braun-Quentin C *et al*: Spectrum of mutations and sequence variants in the *FALDH* gene in patients with Sjögren-Larsson syndrome. *Hum Mut* 1998; **12**: 377–384.
19 Kraus C, Günther K, Vogler A, Hohenberger W, Pfeiffer RA, Ballhausen WG: Rapid RT-PCR based protein truncation test in the screening for 5' located mutations of the *APC* gene. *Mol Cell Probes* 1998; **12**: 143–147.
20 Cooper DN, Krawczak M: *Human Gene Mutation*. Bios Scientific: Eynsham, UK, 1993.
21 Andreutti-Zaugg C, Scott RJ, Iggo R: Inhibition of nonsense-mediated messenger RNA decay in clinical samples facilitates detection of human *MSH2* mutations with an *in vivo* fusion protein assay and conventional techniques. *Cancer Res* 1997; **57**: 3288–3293.
22 Maquat LE: When cells stop making sense: Effect of nonsense codons on RNA metabolism in vertebrate cells. *RNA* 1995; **1**: 453–465.
23 Jäger B: Das Sjögren-Larsson Syndrom – ein Bericht über fünf Patienten aus dem nordbayerischen Raum. Dissertation. Institut für Humangenetik: Erlangen.
24 Braun-Quentin C, Bathke KD, Pfeiffer RA: Das Sjögren-Larsson Syndrom in Deutschland. *Deutsches Ärzteblatt* 1996; **93**: 1039–1043.