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SHORT REPORT

# Smith-Lemli-Opitz syndrome: evidence of T93M as a common mutation of Δ7-sterol reductase in Italy and report of three novel mutations

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**The Smith-Lemli-Opitz syndrome (SLOS) is an autosomal recessive disorder of cholesterol biosynthesis characterised by facial dysmorphisms, mental retardation and multiple congenital anomalies. SLOS is caused by mutations of the human Δ7-sterol reductase (*DHCR7*) gene and, so far, 19 different mutations have been described. Among these, mutations impairing the activity of the C-terminus appear to be the most severe. Here we report the mutational analysis of the *DHCR7* gene in nine Italian SLOS patients. The T93M mutation, previously reported in one patient, results the most frequent one (7/18 alleles) in our survey. Furthermore, we identified three novel mutations, two missense mutations (N407Y and E448K), and a 33 bp deletion spanning part of exon 5 and the donor splice site of intron 5.**

**Keywords: Smith-Lemli-Opitz syndrome; Δ7-sterol reductase; DHCR7 mutations**

## Introduction

The Smith-Lemli-Opitz syndrome (SLOS, MIM 270400) is an autosomal recessive disorder of sterol metabolism that impairs morphogenesis.[1,2] SLOS patients are characterised by a wide spectrum of developmental abnormalities, including mental retardation, pre- and postnatal failure to thrive, severe craniofacial anomalies, limb malformations, incomplete development of male genitalia and variable structural anomalies of internal organs.[3] Mild and severe forms of SLOS have been described[4] (type 1 and type 2, respectively), but both types seem to represent a clinical and biochemical continuum of the same disorder.[2,3] High levels of serum 7-dehydrocholesterol (7-DHC) represent a biochemical marker of SLOS.[5] Molecular cloning, expression of human Δ7-sterol reductase cDNA (DHCR7) and mutational analysis of the *DHCR7* gene in SLOS patients have been recently reported.[6–9] Nineteen different mutations of *DHCR7*, mostly missense mutations, have been described in 19 SLOS patients to date. Although a clear-cut phenotype/genotype correlation cannot be established, homozygosity for mutations resulting in a severe disruption of *DHCR7*, eg those affecting the highly conserved carboxyl-terminal part of the protein, seems to cause the SLOS type II.

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Here we report the molecular characterisation of 9 unrelated Italian patients with SLOS type I.

## Methods

Nine unrelated patients from Central and Southern Italy, affected by SLOS type I, and their first-degree relatives were recruited in this study. In all the index cases, the diagnosis was clinically assessed and biochemically confirmed. Serum cholesterol and 7-DHC were measured by gas chromatography/mass spectrometry (GC/MS) analysis.[10] DNA samples were screened for mutations of the *DHCR7* gene by PCR/SSCP analysis[11] using oligonucleotide primers designed to amplify the coding region and the intron–exon boundaries of the gene.[8] Amplification of exon 9 was performed using two sets of primers.[8] Altered conformers, as seen by SSCP analysis, were sequenced by an automatic ABI PRISM 377 DNA sequencer (PE Applied Biosystems, Foster City, CA, USA).

As the 1342G > A (E448K) mutation abolishes a new Bsp1286 I site in exon 9, restriction enzyme analysis was carried out on relatives of patient 4 and on 100 unrelated control alleles. The same control group was tested for the occurrence of the 384–IVS5 + 4 del mutation using 2% agarose gel electrophoresis and ethidium bromide staining.

In order to clarify the effect of the 384–IVS5 + 4 del mutation on mRNA processing in patient 1, reverse transcriptase-PCR (RT-PCR) analysis was performed. The primers used were: 5'-CCTTCATCGTCTACTACTTC-3' sense primer, in exon 4, and 5'-CAGTTGTCGAAGATGATGGT-3' antisense primer in exon 6 (annealing temperature 60°C).

## Results

Cholesterol and 7-DHC serum levels of SLOS patients are shown in Table 1. For all nine patients additional conformers were observed at the SSCP analysis. DNA sequencing revealed six different mutations: four missense mutations (T93M, R352W, N407Y, E448K), a nonsense mutation (W151X) and a deletion (384–IVS5 + 4 del) (Table 1). Three are novel mutations: N407Y – found in heterozygosity in patient 2, E448K – present in homozygosity in patient 4 (without known parental consanguinity), and the 33 bp deletion 384–IVS5 + 4 del) – found in heterozygosity in patient 1. Mutation 1342 G > A (E448K) destroys a Bsp1286 I site, altering the wild type restriction pattern of exon 9 (Figure 1a). The 33 bp deletion is easily recognized in patient 1 and her relatives by a 2% agarose gel electrophoresis of exon 5 amplicons and ethidium bromide staining (Figure 1b). RT-PCR, using primers designed in exon 4 and exon 6 in order to clarify the possible transcriptional effect of the deletion, failed to show amplification of any aberrant cDNA (data not shown). Three previously described mutations were also found in this survey (T93M, R352W, W151X).[8] Heterozygosity for the T93M mutation was found in five patients, whereas a single patient, without known

**Table 1**

| *Patients (age at diagnosis)* | *Serum CH (μmol/L)* | *Serum 7-DHC (μmol/L)* | *Exon* | *Nucleotide change* | *Effect on coding sequence* |
|---|---|---|---|---|---|
| 1 | 556 | 444 | 4 | pat278C>T | T93M |
| (4y 6m) | | | 5 | mat384-IVS5+4 del | altered RNA processing[b] |
| 2 | 934 | 234 | 4 | pat278C>T | T93M |
| (13y) | | | 9 | mat1219A>T | N407Y[b] |
| 3 | 1373 | 1261 | 4 | pat278C>T | T93M |
| (2y) | | | 6 | mat452G>A | W151X |
| 4 | 178 | 426 | 9 | pat1342G>A | E448K[b] |
| (1m) | | | 9 | mat1342G>A | E448K[b] |
| 5 | 879 | 390 | 4 | pat278C>T | T93M |
| (5m) | | | 4 | mat278C>T | T93M |
| 6 | 509 | 278 | 9 | mat1054C>T | R352W |
| (4y 9m) | | | | | |
| 7 | 1397 | 364 | 4 | mat278C>T | T93M |
| (1y 2m) | | | | | |
| 8 | 853 | 247 | 9 | mat1054C>T | R352W |
| (9m) | | | | | |
| 9 | 310[a] | 130[a] | 4 | mat278C>T | T93M |
| (4y) | | | | | |

CH (cholesterol) normal range: 1–3 y: 1150–4700 μmol/L; 4–6 y: 2800–4800 μmol/L; 10–14y (female): 3360–5280 μmol/L[13]
7-DHC (7-dehydrocholesterol) normal range: 0.13–0.39 μmol/L[3]
[a]non-esterified sterols
[b]newly described mutation
Allele origin: pat, paternal; mat, maternal

parental consanguinity, was found to be homozygous for this mutation. The R352W mutation was present in heterozygosity in two patients, whereas the W151X mutation was detected in a single allele.

The sequence analysis of the altered conformers, observed in all the patients, revealed the presence of polymorphic variants including 189A > G, 231C > T and 1272T > C, previously described.[8]

## Discussion

This is the first molecular characterisation of Italian SLOS patients. We describe three novel mutations (Table 1). Among these, an A to T transversion (1219A > T) causes an asparagine to tyrosine change (N407Y). This mutation alters the last cytosolic loop nearby the ninth putative trans-membrane domain. In the same region, very close to asparagine 407, two other mutations, R404C and G410S, have been described previously.[8] These three mutations were localised into an amino acid stretch, highly conserved in some reductases of different species and in the C-termini of human and chicken lamin-B receptor.[12] Similarly to the effect produced by the other two close mutations, N407Y mutation might impair the DHCR7 expression.

A G to A transition at position 1342 causes a glutamic acid to lysine change (E448K). This mutation produces an inversion of the polar charge of an amino acid residue at the C-terminus of the protein. The causative role of this mutation is further supported by the segregation analysis in the pedigree of patient 4 (Figure 1a).

The 33 bp deletion (384–IVS5 + 4 del) spans the 3' end of exon 5 and the first 4 bp of intron 5 (Figure 1b). The causative role of the 384–IVS5 + 4 del mutation is supported by the effect observed when the splicing signals of the IVS5 are removed, as no aberrant product is obtained by RT-PCR using primers including the deletion. This suggests that mRNA arising from the allele carrying this mutation might be rapidly degraded.

The T93M mutation, previously reported in heterozygosity in a single patient,[8] turned out to be the most

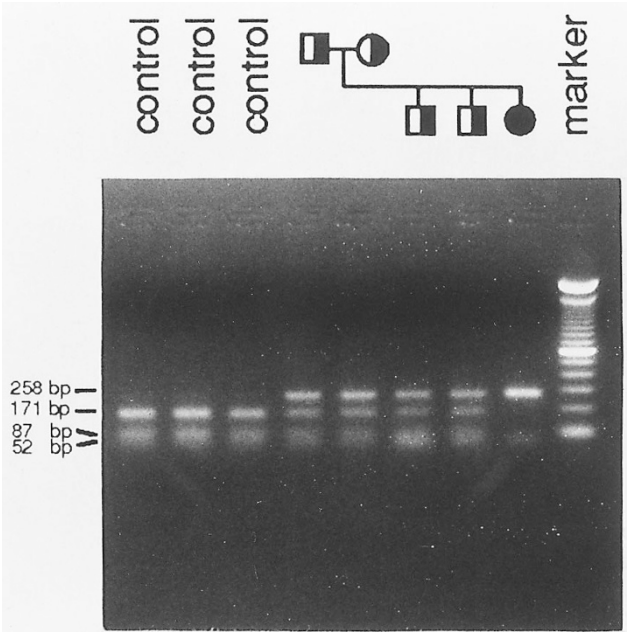


**Figure 1a** *Agarose gel of BSP1286 I restriction analysis of PCR products of the distal part of exon 9 from patient 4, her relatives and normal healthy controls. Digestion of wild type samples yields a 171 bp, a 87 bp and a 52 bp fragment; if G > A mutation at 1342 site occurs, Bsp1286 I fails to cut and yields only a 258 bp and a 52 bp fragment. Heterozygotes present all four bands. The marker is a 100-bp ladder*

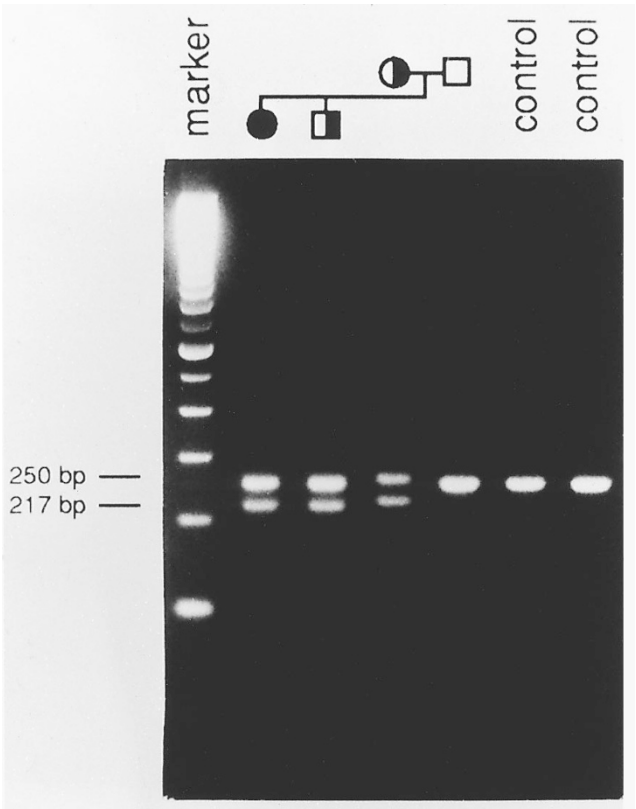


**Figure 1b** *Analysis of PCR products of exon 5 by 2% agarose gel electrophoresis and ethidium bromide staining from patient 1, her relatives and normal controls. The 384–IVS5 + 4 del is easily recognisable, as a normal allele (250 bp) and a smaller product (217 bp) are observed in individuals bearing this mutation. The marker is a 100-bp ladder*

common mutation among Italian SLOS patients (seven out of 18 alleles analysed).

In the present study, the search for mutations of the *DHCR7* gene in SLOS patients focused on coding exons, including the splice junctions. In five patients, the molecular characterisation was complete, whereas only a single mutant allele was identified in four cases. Failure to find the second mutation in three patients could be caused by mutations in other regions of the gene, as discussed elsewhere.[8]

Including the mutations here reported, a total of 22 different mutations of the *DHCR7* gene in SLOS patients is known to date. Although most *DHCR7* mutations rarely recur, the presence of the T93M mutation in some 39% of the Italian SLOS alleles suggests an important role of this mutation, at least in Italy.

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