ARTICLE

Mutational Spectrum of Smith–Lemli–Opitz Syndrome

HANS R. WATERHAM* AND RAOUL C.M. HENNEKAM**

Smith–Lemli–Opitz syndrome (SLOS; OMIM #270400) is an autosomal recessive malformation syndrome characterized by a large spectrum of morphogenic and congenital anomalies. SLOS is caused by mutations in the *DHCR7* gene, which encodes 7-dehydrocholesterol reductase, the enzyme that catalyzes the final step in cholesterol biosynthesis. We report on 154 currently known mutations in *DHCR7* identified in patients affected with SLOS and discuss their coding consequences. These 154 mutations include 130 missense, 8 nonsense, 8 deletions, 2 insertions, 1 indel, and 5 splice site mutations. Using information available from published case reports and from patients identified in our clinical diagnostic laboratory, we analyzed correlations between genotype, clinical presentation and 7-dehydrocholesterol level. © 2012 Wiley Periodicals, Inc.

KEY WORDS: Smith–Lemli–Opitz syndrome; SLOS; 7-dehydrocholesterol (7DHC); cholesterol; inborn error of metabolism; DHCR7; severity score; review

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INTRODUCTION

Smith–Lemli–Opitz syndrome (SLOS, OMIM #270400) is an autosomal recessive malformation syndrome characterized by prenatal and postnatal growth retardation, characteristic facial appearance, genital abnormalities, 2–3 toe syndactyly and intellectual disabilities [Smith et al., 1964]. SLOS presents with malformations of many organ systems [Kelley and Hennekam, 2000]. The incidence of SLOS ranges from 1:15,000 to 1:60,000 with a higher incidence observed in some East-European countries, presumably secondary to founder effects [Kelley and Hennekam, 2000]. SLOS is the most common disorder of the cholesterol biosynthesis pathway known to date [Waterham, 2006] with at least 450 published patients. SLOS is caused by a defective functioning of the enzyme 7-dehydrocholesterol reductase (DHCR7; E.C. 1.3.1.21; OMIM #602858), which catalyzes the reduction of the C7–C8 double bond of 7-dehydrocholesterol (cholesta-5,7-dien-3β-ol) to produce cholesterol (cholest-5-en-3β-ol), which is generally regarded as the predominant final step in cholesterol biosynthesis [Tint et al., 1994]. As a consequence of the DHCR7 deficiency, low cholesterol levels and elevated 7-dehydrocholesterol (7DHC) levels are found in plasma, cells, and tissues of the vast majority of SLOS patients.

DHCR7 is encoded by the *DHCR7* gene, which is located at chomosome 11q13.2-q13.5, spans ~14 kb and contains nine exons with the translation initiation codon located in exon 3 (Fig. 1a). The gene produces two main *DHCR7* mRNAs (~2.9 and ~2.3 kb), which vary in length of the 3′ noncoding regions encoded by exon 9. The gene is ubiquitously expressed both in adult and fetal tissues with highest levels in the adrenal gland (adult), liver, and brain.

The *DHCR7* open reading frame of 1,425 bp codes for a polypeptide of 475 amino acids with a calculated molecular weight of 54.5 kDa. There are nine putative trans-membrane helices and a sterol sensing domain (Fig. 1b), which is found in different proteins involved in

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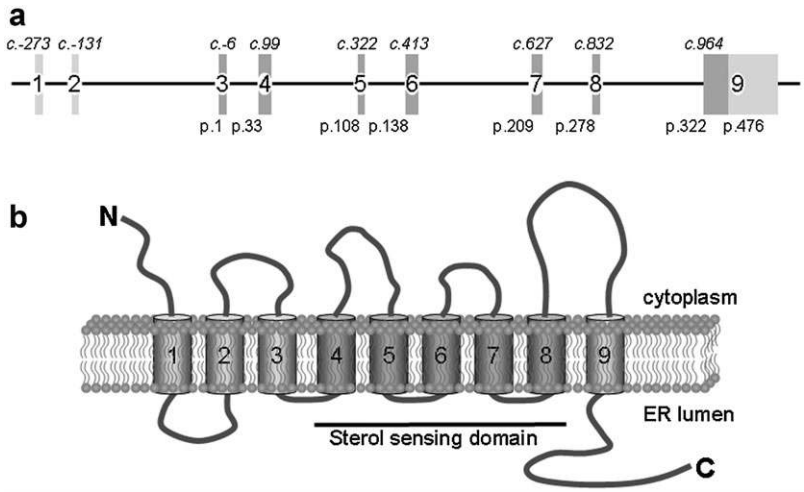

Figure 1. **a**: DHCR7 gene structure. Numbers of first nucleotide (c.) and first encoded amino acid (p.) of each coding exon (exons 3–9) are given. **b**: Predicted structure and membrane topology of DHCR7 protein. Localization of the nine membrane-associated helices (MAH): MAH 1: p.W37-p.F57, MAH 2: p.Y100-p.C118, MAH 3: p.A150-p.F174, MAH 4: p.W177-p.F202, MAH 5: p.K236-p.A257, MAH 6: p.V266-p.W286, MAH 7: p.L306-p.V326, MAH 8: p.Q331-p.F351, MAH 9: p.L412-p.I434.

cholesterol biosynthesis, transport, and homeostasis or cholesterol-dependent signaling pathways [Chang et al., 2006]. The DHCR7 protein shows structural similarities to other sterol reductases and is localized in the endoplasmic reticulum membrane. The catalytic and NADPH binding sites are most probably contained in the large cytosolic loop 8–9 that follows the sterol sensing domain (Fig. 1b).

Here we present the comprehensive list of currently known mutations in *DHCR7* identified in affected SLOS patients and discuss their coding consequences. We also analyze the correlations between genotype, 7DHC levels and clinical presentation.

DHCR7 MUTATIONS IN SLOS

Table IA shows the current list of 154 different SLOS-causing mutations and their predicted consequences. Mutations are extracted from reports published since the recognition of the genetic defect in 1998 [Fitzky et al., 1998; Wassif et al., 1998; Waterham et al., 1998] supplemented with unpublished mutations identified in our clinical DNA diagnostic unit at the Academic Medical Center in Amsterdam. The majority of mutations were identified through sequence analysis of the coding exons and flanking intronic sequences of *DHCR7*, which identifies at least 95% of possible mutations in affected patients. In addition to the disease-causing mutations, several polymorphic variants have been found in *DHCR7*, some of which are more common than others (Table IB).

The 154 different mutations include 130 missense, 8 nonsense, 8 deletions, 2 insertions, 1 indel, and 5 splice site mutations. Mutations are distributed widely along the gene, with many mutations found in single or only a few patients, whereas other mutations are found frequently only in selected populations due to founder effects. Several mutations are generally more common including p.T93M, p.R404C, p.W151X, p.V326L, and c.964-1G>C. By far the most prevalent mutation in Caucasians is the severe c.964-1G>C splice site mutation (allele frequency of ~30%), which leads to aberrant splicing of the *DHCR7* mRNA at a cryptic splice acceptor site located 5′ of the mutated splice site resulting in the partial retention of 134-bp intron sequence, and produces no functional protein. The localization of the various mutations in the protein according to the topology model (Fig. 1b) is depicted in Table IA.

The 154 different mutations include 130 missense, 8 nonsense, 8 deletions, 2 insertions, 1 indel, and 5 splice site mutations. Mutations are distributed widely along the gene, with many mutations found in single or only a few patients, whereas other mutations are found frequently only in selected populations due to founder effects.

GENOTYPE–PHENOTYPE CORRELATIONS

To analyze genotype–phenotype correlations, we performed a literature search through PubMed using the search terms Smith–Lemli–Opitz syndrome, SLOS, 7DHC, and *DHCR7*. All publications were examined to determine whether complete data on individual SLOS patients were provided. Only publications in which patients were reported with either the 7DHC plasma level or sufficient clinical information of each individual case to derive a clinical severity score were included in our evaluation. Reference lists of all useful publications were hand searched for additional publications. We used the clinical severity score originally described by Bialer et al. [1987] and adapted by Kelley and Hennekam [2000], which allows scoring of 10 embryologically separate organ systems equally (Table II). To use this score, clinical information must be available for at least five organ systems. The sum was normalized to 100 to allow comparison of patients in whom

TABLE I. Variants Identified in the Human *DHCR7* Gene

Mutation	Type	Exon	Coding effect	Position in DHCR7 protein[a]	Refs. (first reported)
A: Pathogenic mutations causing SLOS					
Del exon 3–4	Deletion	3+3i+4	—	—	Weaver et al. [2010]
c.1A>G	Missense	3	Translation initiation defect	—	Witsch-Baumgartner et al. [2005], Scalco et al. [2005]
c.3G>A	Missense	3	Translation initiation defect	—	Waterham and Wanders [2000]
c.60delTinsAA	Indel	3	Frame shift	—	Waterham, this report
c.89G>C	Missense	3	p.G30A	N terminus	Blahakova et al. [2007]
c.98_194del	Deletion	3	p.W33SfsX4	—	Wassif et al. [1998]
c.98+2_+6delTAAGG	Splice defect	3i	?	—	Waterham [this report]
c.99-4G>A	Splice defect	3i	?	—	Waterham [this report]
c.111G>A	Nonsense	4	p.W37X	—	Yu et al. [2000]
c.149C>A	Missense	4	p.A50D	MAH 1	Witsch-Baumgartner et al. [2005]
c.149C>T[b]	Missense	4	p.A50V	MAH1	Quélin et al. [2012]
c.151C>T	Missense	4	p.P51S	MAH 1	Fitzky et al. [1998]
c.152C>A	Missense	4	p.P51H	MAH 1	Anstey et al. [2005]
c.176G>T	Missense	4	p.M59R	Loop 1–2	Waterham and Wanders [2000]
c.185A>T	Missense	4	p.D62V	Loop 1–2	Waterham and Wanders [2000]
c.203T>C	Missense	4	p.L68P	Loop 1–2	Ciara et al. [2004]
c.208G>T[b]	Missense	4	p.G70C	Loop 1–2	Quélin et al. [2012]
c.278C>T	Missense	4	p.T93M	Loop 1-2	Fitzky et al. [1998]
c.292C>T	Nonsense	4	p.Q98X	–	Correa-Cerro et al. [2005], Cardoso et al. [2005]
c.296T>C	Missense	4	p.L99P	Loop 1–2	Fitzky et al. [1998]
c.321G>C	Missense	4	p.Q107H	MAH 2	Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.326T>C	Missense	5	p.L109P	MAH 2	Waterham and Wanders [2000], Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.338T>C	Missense	5	p.S113C	MAH2	Waye et al. [2005]
c.355delC	Deletion	5	p.H119IfsX8	—	Witsch-Baumgartner et al. [2005]
c.356del13nt	Deletion	5	p.His119ProFsX23	—	Quélin et al. [2012]
c.356A>T	Missense	5	p.H119L	MAH 2	Waterham et al. [1998]
c.385_412+5del	Deletion	5+5i	?	—	De Brasi et al. [1999]
c.400G>T	Missense	5	p.V134L	Loop 2–3	Waterham [this report]
c.412+3A>T	Splice defect	5i	?	—	Koo et al. [2010]
c.413G>T	Missense	6	p.G138V	Loop 2–3	Waye et al. [2005]
c.433A>C	Missense	6	p.I145L	Loop 2–3	Waye et al. [2005]
c.438C>G	Missense	6	p.N146K	Loop 2–3	Jezela-Stanek et al. [2010]
c.440G>A	Missense	6	p.G147D	Loop 2–3	Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.443T>G	Missense	6	p.L148R	Loop 2–3	Yu et al. [2000]
c.445C>T	Nonsense	6	p.Q149X	—	Waterham and Wanders [2000]
c.452G>A	Nonsense	6	p.W151X	—	Fitzky et al. [1998]
c.453G>A	Nonsense	6	p.W151X	—	Witsch-Baumgartner et al. [2000]
c.461C>T	Missense	6	p.T154M	MAH 3	Waterham and Wanders [2000], Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.461C>G	Missense	6	p.T154R	MAH 3	Witsch-Baumgartner et al. [2001b]

(*Continued*)

TABLE I. (*Continued*)

Mutation	Type	Exon	Coding effect	Position in DHCR7 protein[a]	Refs. (first reported)
c.470T>C	Missense	6	p.L157P	MAH 3	Fitzky et al. [1998]
c.474G>T	Missense	6	p.W158C	MAH 3	Waye et al. [2007]
c.502T>A	Missense	6	p.F168I	MAH 3	Yu et al. [2000]
c.506C>T	Missense	6	p.S169L	MAH 3	Waterham and Wanders [2000], Witsch-Baumgartner et al. [2000], Yu et al. [2000]
c.521T>C	Missense	6	p.F174S	MAH 3	Cardoso et al. [2005]
c.523G>C	Missense	6	p.D175H	Loop 3–4	Yu et al. [2000]
c.529T>C	Missense	6	p.W177R	MAH 4	Krakowiak et al. [2000]
c.532A>T	Missense	6	p.I178F	MAH 4	Nowaczyk et al. [2001]
c.533T>A	Missense	6	p.I178N	MAH 4	Witsch-Baumgartner et al. [2001b]
c.536C>T	Missense	6	p.P179L	MAH 4	Yu et al. [2000]
c.545G>T	Missense	6	p.W182L	MAH 4	Waterham and Wanders [2000]
c.546G>C	Missense	6	p.W182C	MAH 4	Witsch-Baumgartner et al. [2000]
c.548G>A	Missense	6	p.C183Y	MAH 4	Waterham and Wanders [2000]
c.575C>T	Missense	6	p.S192F	MAH 4	Witsch-Baumgartner et al. [2005]
c.577A>C	Missense	6	p.T193P	MAH 4	Waterham [this report]
c.592A>G	Missense	6	p.K198E	MAH 4	Waterham and Wanders [2000]
c.628A>T	Nonsense	7	p.K210X	—	Quélin et al. [2012]
c.651C>A	Nonsense	7	p.Y217X	—	Waterham and Wanders [2000]
c.655T>G	Missense	7	p.Y219D	Loop 4–5	Jezela-Stanek et al. [2008]
c.670G>A	Missense	7	p.E224K	Loop 4–5	Witsch-Baumgartner et al. [2005]
c.679C>T	Missense	7	p.P227S	Loop 4–5	Ko et al. [2010]
c.679C>A	Missense	7	p.P227T	Loop 4–5	Waterham [this report]
c.682C>T	Missense	7	p.R228W	Loop 4–5	Witsch-Baumgartner et al. [2005]
c.682insC	Insertion	7	p.Frame shift	—	Wassif et al. [1998]
c.704T>C	Missense	7	p.F235S	Loop 4–5	Waye et al. [2005]
c.720_735del	Deletion	7	Frame shift	—	Fitzky et al. [1998]
c.724C>T	Missense	7	p.R242C	MAH 5	Neklason et al. [1999]
c.725G>A	Missense	7	p.R242H	MAH 5	Waterham and Wanders [2000], Krakowiak et al. [2000]
c.728C>G	Missense	7	p.P243R	MAH 5	Yu et al. [2000]
c.730G>A	Missense	7	p.G244R	MAH 5	Waterham et al. [1998]
c.740C>T	Missense	7	p.A247V	MAH 5	Fitzky et al. [1998]
c.742T>C	Missense	7	p.W248R	MAH 5	Waye et al. [2002]
c.744G>T	Missense	7	p.W248C	MAH 5	Waterham et al. [1998]
c.744G>C	Missense	7	p.W248C	MAH 5	Jezela-Stanek et al. [2010]
c.752T>A	Missense	7	p.I251N	MAH 5	Romano et al. [2005]
c.753C>G	Missense	7	p.I251M	MAH 5	Waterham and Wanders [2000]
c.755A>G	Missense	7	p.N252S	MAH 5	Waterham and Wanders [2000]
c.760T>G	Missense	7	p.S254A	MAH 5	Goldenberg et al. [2003]
c.762insT	Insertion	7	Frame shift	—	Wassif et al. [1998]
c.765C>A	Missense	7	p.F255L	MAH 5	Waterham and Wanders [2000]
c.808A>G	Missense	7	p.M270V	MAH 6	Waterham and Wanders [2000]
c.818T>G	Missense	7	p.V273G	MAH 6	Witsch-Baumgartner et al. [2005]
c.822C>A	Missense	7	p.N274K	MAH 6	Goldenberg et al. [2003]
c.839A>G	Missense	8	p.Y280C	MAH 6	Waye et al. [2002]
c.841G>A	Missense	8	p.V281M	MAH 6	Witsch-Baumgartner et al. [2000]

(*Continued*)

TABLE I. (*Continued*)

Mutation	Type	Exon	Coding effect	Position in DHCR7 protein[a]	Refs. (first reported)
c.852C>A	Missense	8	p.F284L	MAH 6	Waterham and Wanders [2000], Yu et al. [2000]
c.861C>A	Missense	8	p.N287K	Loop 6–7	Yu et al. [2000]
c.862G>A	Missense	8	p.E288K	Loop 6–7	Witsch-Baumgartner et al. [2001b]
c.866C>T	Missense	8	p.T289I	Loop 6–7	Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.890T>C	Missense	8	p.I297T	Loop 6–7	Waye et al. [2005]
c.896A>G	Missense	8	p.H299R	Loop 6–7	Waterham [this report]
c.902A>G	Missense	8	p.H301R	Loop 6–7	Cardoso et al. [2005]
c.906C>G	Missense	8	p.F302L	Loop 6–7	Waterham and Wanders [2000], Yu et al. [2000]
c.907G>A	Missense	8	p.G303R	Loop 6–7	Matsumoto et al. [2005]
c.920G>A	Missense	8	p.G307D	MAH 7	Witsch-Baumgartner et al. [2001b]
c.925G>A	Missense	8	p.G309S	MAH 7	Witsch-Baumgartner et al. [2001b]
c.931T>G	Missense	8	p.C311G	MAH 7	Witsch-Baumgartner et al. [2000]
c.932G>A	Missense	8	p.C311Y	MAH 7	Witsch-Baumgartner et al. [2000]
c.950T>G	Missense	8	p.L317R	MAH 7	Scalco et al. [2005]
c.952T>A	Missense	8	p.Y318N	MAH 7	Krakowiak et al. [2000]
c.956C>T	Missense	8	p.T319M	MAH 7	Waterham and Wanders [2000]
c.957G>A	Missense	8	p.T319A	MAH 7	Witsch-Baumgartner et al. [2001b]
c.964-1G>C	Splice defect	8i	p.G322KfsX136	—	Fitzky et al. [1998], Waterham et al. [1998], Wassif et al. [1998]
c.964-1G>T	Splice defect	8i	?	—	Waterham and Wanders [2000]
c.970T>C	Missense	9	p.Y324H	MAH 7	Witsch-Baumgartner et al. [2000], Yu et al. [2000]
c.976G>T	Missense	9	p.V326L	MAH 7	Fitzky et al. [1998]
c.986C>T	Missense	9	p.P329L	Loop 7–8	Patrono et al. [2000]
c.1022T>C	Missense	9	p.L341P	MAH 8	Krakowiak et al. [2000]
c.1030G>C	Missense	9	p.G344R	MAH 8	Waye et al. [2005]
c.1039G>A	Missense	9	p.G347S	MAH 8	Witsch-Baumgartner et al. [2001a]
c.1054C>T	Missense	9	p.R352W	Loop 8–9	Fitzky et al. [1998]
c.1055G>A	Missense	9	p.R352Q	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1055G>T	Missense	9	pR352L	Loop 8–9	Al-Owain et al. [2012]
c.1057Gdel	Deletion	9	Frame shift	—	Waterham and Wanders [2000]
c.1058T>C	Missense	9	p.V353A	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1063A>G	Missense	9	p.N355D	Loop 8–9	Waterham and Wanders [2000]
c.1068-1070del	Deletion	9	p. H356del	Loop 8–9	Evans et al. [2001]
c.1079T>C	Missense	9	p.L360P	Loop 8–9	Ciara et al. [2004]
c.1084C>T	Missense	9	p.R362C	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1097G>T	Missense	9	p.G366V	Loop 8–9	Waterham [this report]
c.1127_1128delA	Nonsense	9	p.K376RfsX37	—	Chae et al. [2007]
c.1138T>A	Missense	9	p.C380S	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1138T>C	Missense	9	p.C380R	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1139G>A	Missense	9	p.C380Y	Loop 8–9	Waterham and Wanders [2000], Witsch-Baumgartner et al. [2000]
c.1139G>C	Missense	9	p.C380S	Loop 8–9	Fitzky et al. [1998]
c.1187T>A	Missense	9	p.V396E	Loop 8–9	Waterham [this report]
c.1190C>T	Missense	9	p.S397L	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1210C>T	Missense	9	p.R404C	Loop 8–9	Fitzky et al. [1998]

(*Continued*)

TABLE I. (*Continued*)

Mutation	Type	Exon	Coding effect	Position in DHCR7 protein[a]	Refs. (first reported)
c.1210C>A	Missense	9	p.R404S	Loop 8–9	Witsch-Baumgartner et al. [2000], Yu et al. [2000]
c.1213C>T	Missense	9	p.H405Y	Loop 8–9	Waye et al. [2005]
c.1219A>T	Missense	9	p.N407Y	Loop 8–9	De Brasi et al. [1999]
c.1222T>C	Missense	9	p.Y408H	Loop 8–9	Witsch-Baumgartner et al. [2000], Krakowiak et al. [2000]
c.1228G>A	Missense	9	p.G410S	Loop 8–9	Fitzky et al. [1998]
c.1228G>C	Missense	9	p.G410R	Loop 8–9	Witsch-Baumgartner et al. [2000]
c.1277A>C	Missense	9	p.H426P	MAH 9	Waye et al. [2005]
c.1289A>G	Missense	9	p.Y432C	MAH 9	Witsch-Baumgartner et al. [2005]
c.1327C>T	Missense	9	p.R443C	C terminus	Waterham and Wanders [2000], Witsch-Baumgartner et al. [2000]
c.1328G>A	Missense	9	p.R443H	C terminus	Witsch-Baumgartner et al. [2001b]
c.1331G>A	Missense	9	p.C444Y	C terminus	Krakowiak et al. [2000]
c.1336C>T	Missense	9	p.R446W	C terminus	Waterham [this report]
c.1337G>A	Missense	9	p.R446Q	C terminus	Witsch-Baumgartner et al. [2000]
c.1342G>C	Missense	9	p.E448Q	C terminus	Witsch-Baumgartner et al. [2000]
c.1342G>A	Missense	9	p.E448K	C terminus	De Brasi et al. [1999]
c.1349G>T	Missense	9	p.R450L	C terminus	Neklason et al. [1999]
c.1351T>C	Missense	9	p.C451R	C terminus	Waterham [this report]
c.1370G>T	Missense	9	p.G366V	C terminus	Szabó et al. [2010]
c.1384T>C	Missense	9	p.Y462H	C terminus	Yu et al. [2000]
c.1396G>A	Missense	9	p.V466M	C terminus	Waterham [this report]
c.1397T>C	Missense	9	p.V466A	C terminus	Scalco et al. [2005]
c.1400C>T	Missense	9	p.P467L	C terminus	Witsch-Baumgartner et al. [2001b]
c.1406G>C	Missense	9	p.R469P	C terminus	Yu et al. [2000]
c.1409T>A	Missense	9	p.L470Q	C terminus	Ginat et al. [2004]
c.1423T>C	Missense	9	p.F475S	C terminus	Witsch-Baumgartner et al. [2005]
c.1426T>A	Missense	9	p.X476Q	C terminus	Matsumoto et al. [2005]

Nucleotide change	Exon	Effect on coding sequence
B: Nonpathogenic mutations		
−223T>C	1	Noncoding
−23T>C	2	Noncoding
c.139C>T	4	p.L47L
c.189A>G	4	p.Q63Q
c.207C>T	4	p.T69T
c.231C>T	4	p.T77T
c.285A>G	4	p.K95K
c.438C>T	6	p.N146N
c.969G>T	9	p.L323L
c.1158C>T	9	p.D386D
c.1272T>C	9	p.G424G
c.1341C>T	9	p.D447D
c.1350C>G	9	p.R450R

[a]Localization according to topology model of Figure 1b. MAH = membrane-associated helix; for numbering of MAHs see Figure 1b.

TABLE II. Severity Score for Anatomical Abnormalities in Smith–Lemli–Opitz Syndrome [Kelley and Hennekam, 2000]*

Organ	Score	Criteria
Brain	1	Seizures; qualitative MRI abnormality
	2	Major CNS malformations; gyral defects
Oral	1	Bifid uvula or submucous cleft
	2	cleft hard palate or median cleft lip
Acral	0	Non-Y shaped minimal toe syndactyly
	1	Y shaped 2–3 toe syndactyly; club foot; upper or lower limb polydactyly; other syndactyly
	2	Any two of the above
Eye	2	Cataract; frank microphthalmia
Heart	0	Functional defects Single chamber or vessel defect
	2	Complex cardiac malformation
Kidney	0	Functional defects
	1	Simple cystic kidney disease
	2	Renal agenesis; clinically important cystic disease
Liver	0	Induced hepatic abnormality
	1	Simple structural abnormality
	2	Progressive liver disease
Lung	0	Functional defects
	1	Abnormal lobation; underdevelopment
	2	Pulmonary cysts; other major malformations
Bowel	0	Functional defects
	1	Pyloric stenosis
	2	Hirschsprung disease
Genitalia	1	Simple hypospadias
	2	Ambiguous or female genitalia in 46,XY; frank genital malformation in 46,XX

*Overall severity can only be determined if at least five organ systems can be scored. The sum of the scores should be normalized to 100.

different numbers of organ systems were scored.

In total we collected 251 patients from 33 publications (Table III). The patients originated from 23 countries. Fifteen patients (6.0%) had two nonsense mutations, 77 patients (30.7%) had two missense mutations, and 159 (63.3%) had a combination of one missense and one nonsense mutation. The positions of the different mutations in the DHCR7 protein are indicated also in Table III. The 13 most common *7DHCR* mutations constitute 67% of all mutations, indicating a large number of infrequently or even uniquely reported mutations. In our cohort, the c.964-1G>C mutation is the most commonly reported mutation and occurs in almost all countries, but several other mutations showed regionally marked differences in incidence because of founder effects.

To determine if there is a correlation between the 7DHC levels and the clinical severity of patients, we compared the plasma 7DHC levels to the severity score in the 165 patients in whom both values were available (Fig. 2). Severity score and 7DHC levels correlated with one another, but this was less pronounced in patients who had a combination of a nonsense mutation and missense mutation (Fig. 2a; $r = 0.22$) compared to patients who had two missense mutations (Fig. 2b; $r = 0.36$). The number of patients with two nonsense mutations in whom both severity score and 7DHC levels was known ($n = 4$) was too small for analysis. Their severity scores varied between 50 and 78, and their 7DHC level ranged from 290 and 670 μmol/L. We performed a Student's *t*-test to evaluate whether either the severity scores or the 7DHC levels between the groups of patients with two missense mutations and a combined missense–nonsense mutation were different, and the differences were not significant (two tailed $P > 0.1$ for both).

To determine if there is a correlation between the 7DHC levels and the clinical severity of patients, we compared the plasma 7DHC levels to the severity score in the 165 patients in whom both values were available (Fig. 2). Severity score and 7DHC levels correlated with one another, but this was less pronounced in patients who had a combination of a nonsense mutation and missense mutation (Fig. 2a; r = 0.22) compared to patients who had two missense mutations (Fig. 2b; r = 0.36).

To evaluate the correlation between genotype, clinical severity, and 7DHC levels, we grouped the severity scores and when known, 7DHC levels from 207 published patients for whom severity scores were available (see Table III) according to the position of the mutations in the DHCR7 protein (Table IV).

Based on data from 55 patients, it was previously reported that patients with either two null alleles or two mutations in loop 8–9 generally have the highest 7DHC to total sterol ratios and

TABLE III. Genotypes and Phenotypic Characteristics of 251 Published Patients With Smith–Lemli–Opitz Syndrome*

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
Netherlands	M	c.356A>T	Missense	Loop 2–3	42	595	Waterham et al. [1998] (case 1)
		c.730G>A	Missense	MAH 5			
Netherlands	M	c.964-1G>C	Splice defect	—	71	?	Waterham et al. [1998] (case 2)
		c.964-1G>C	Splice defect	—			
Netherlands	M	c.744G>T	Missense	MAH 5	33	559	Waterham et al. [1998] (case 3)
		c.964-1G>C	Splice defect	—			
Germany	F	c.452G>A	Nonsense	—	?	406	Fitzky et al. [1998] (SLO1)
		c.976G>T	Missense	MAH 7			
Germany	F	c.452G>A	Nonsense	—	?	1,124	Fitzky et al. [1998] (SLO2)
		c.976G>T	Missense	MAH 7			
Germany	M	c.452G>A	Nonsense	—	?	304	Fitzky et al. [1998] (SLO3a)
		c.1130G>C	Missense	MAH 8			
Germany	M	c.452G>A	Nonsense	—	?	304	Fitzky et al. [1998] (SLO3b)
		c.1130G>C	Missense	MAH 8			
Germany	F	c.278C>T	Missense	Loop 1–2	?	260	Fitzky et al. [1998] (SLO4)
		c.720_735del	Frame shift	—			
Germany	M	c.296T>C	Missense	Loop 1–2	?	174	Fitzky et al. [1998] (SLO5)
		c.964-1G>C	Splice defect	—			
Germany	M	c.151C>T	Missense	MAH 1	?	262	Fitzky et al. [1998] (SLO6)
		c.964-1G>C	Splice defect	—			
Germany	M	c.452G>A	Nonsense	—	?	282	Fitzky et al. [1998] (SLO7)
		c.470T>C	Missense	MAH 3			
Germany	F	c.740C>T	Missense	MAH 5	?	231	Fitzky et al. [1998] (SLO8)
		c.1210C>T	Missense	Loop 8–9			
Germany	F	c.296T>C	Missense	Loop 1–2	?	231	Fitzky et al. [1998] (SLO9)
		c.1228G>A	Missense	Loop 8–9			
Germany	M	c.976G>T	Missense	MAH 7	?	422	Fitzky et al. [1998] (SLO10)
		c.1054C>T	Missense	Loop 8–9			
Italy	F	c.278C>T	Missense	Loop 1–2	?	444	De Brasi et al. [1999] (pat 1)
		c.384_412+4del	Frame shift	—			
Italy	?	c.278C>T	Missense	Loop 1–2	?	234	De Brasi et al. [1999] (pat 2)
		c.1219A>T	Missense	Loop 8–9			
Italy	?	c.278C>T	Missense	Loop 1–2	?	1,261	De Brasi et al. [1999] (pat 3)
		c.452G>A	Nonsense	—			
Italy	F	c.1342G>A	Missense	MAH 9	?	426	De Brasi et al. [1999] (pat 4)
		c.1342G>A	Missense	MAH 9			
Italy	?	c.278C>T	Missense	Loop 1–2	?	390	De Brasi et al. [1999] (pat 5)
		c.278C>T	Missense	Loop 1–2			
USA	?	c.964-1G>C	Splice defect	—	17	177	Neklason et al. [1999] (pat b)
		c.278C>T	Missense	Loop 1–2			
Italy	?	c.964-1G>C	Splice defect	—	?	352	Patrono et al. [2000]
		c.986C>T	Missense	Loop 7–8			
Austria	F	c.452G>A	Nonsense	—	75	?	Löffler et al. [2000] (pat 1)
		c.452G>A	Nonsense	—			
Austria	M	c.452G>A	Nonsense	—	80	?	Löffler et al. [2000] (pat 2)
		c.452G>A	Nonsense	—			
USA	M	c.964-1G>C	Splice defect	—	11	62	Krakowiak et al. [2000] (pat 1)
		c.278C>T	Missense	Loop 1–2			
USA	M	c.452G>A	Missense	—	22	140	Krakowiak et al. [2000] (pat 2)
		c.278C>T	Missense	Loop 1–2			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
USA	M	c.964-1G>C	Splice defect	—	17	432	Krakowiak et al. [2000] (pat 3)
		c.278C>T	Missense	Loop 1–2			
USA	F	c.964-1G>C	Splice defect	—	33	650	Krakowiak et al. [2000] (pat 4)
		c.278C>T	Missense	Loop 1–2			
USA	F	c.964-1G>C	Splice defect	—	56	280	Krakowiak et al. [2000] (pat 5)
		c.326T>C	Missense	MAH 2			
USA	F	c.964-1G>C	Splice defect	—	11	900	Krakowiak et al. [2000] (pat 6)
		c.461C>T	Missense	MAH 3			
USA	F	c.321G>C	Missense	MAH 2	17	310	Krakowiak et al. [2000] (pat 7)
		c.1331G>A	Missense	C terminus			
USA	F	c.529T>C	Missense	Loop 3–4	17	940	Krakowiak et al. [2000] (pat 8)
		c.724C>T	Missense	MAH 5			
USA	M	c.964-1G>C	Splice defect	—	11	320	Krakowiak et al. [2000] (pat 9)
		c.740C>T	Missense	MAH 5			
USA	F	c.964-1G>C	Splice defect	—	25	212	Krakowiak et al. [2000] (pat10)
		c.1222T>C	Missense	Loop 8–9			
USA	F	c.964-1G>C	Splice defect	—	45	503	Krakowiak et al. [2000] (pat11)
		c.1342G>A	Missense	MAH 9			
USA	F	c.964-1G>C	Splice defect	—	17	700	Krakowiak et al. [2000] (pat12)
		c.952T>A	Missense	MAH 7			
USA	F	c.964-1G>C	Splice defect	—	31	830	Krakowiak et al. [2000] (pat13)
		c.1022T>C	Missense	MAH 8			
USA	M	c.964-1G>C	Splice defect	—	6	890	Krakowiak et al. [2000] (pat14)
		c.866C>T	Missense	Loop 6–7			
USA	M	c.964-1G>C	Splice defect	—	11	900	Krakowiak et al. [2000] (pat15)
		c.866C>T	Missense	Loop 6–7			
USA	F	c.725G>A	Missense	MAH 5	55	83	Krakowiak et al. [2000] (pat16)
		c.440G>A	Missense	Loop 2-3			
USA	F	c.278C>T	Missense	Loop 1–2	74	676	Yu et al. [2000] (pat 2)
		c.964-1G>C	Splice defect	—			
USA	F	c.1406G>C	Missense	C terminus	63	338	Yu et al. [2000] (pat 3)
		c.1342G>A	Missense	MAH 9			
USA	F	c.278C>T	Missense	Loop 1–2	26	494	Yu et al. [2000] (pat 7)
		c.964-1G>C	Splice defect	—			
USA	F	c.964-1G>C	Splice defect	—	32	286	Yu et al. [2000] (pat 12)
		c.502T>A	Missense	MAH 3			
USA	M	c.443T>G	Missense	Loop 2-3	60	624	Yu et al. [2000] (pat 24)
		c.536C>T	Missense	MAH 4			
USA	F	c.970T>C	Missense	MAH 7	21	286	Yu et al. [2000] (pat 28)
		c.964-1G>C	Splice defect	—			
USA	M	c.278C>T	Missense	Loop 1–2	15	411	Yu et al. [2000] (pat 33)
		c.964-1G>C	Splice defect	—			
USA	F	c.1384T>C	Missense	C terminus	5	494	Yu et al. [2000] (pat 43)
		c.964-1G>C	Splice defect	—			
USA	F	c.976G>T	Missense	MAH 7	45	390	Yu et al. [2000] (pat 52)
		c.964-1G>T	Frame shift	—			
USA	F	c.728C>G	Missense	MAH 5	37	403	Yu et al. [2000] (pat 55)
		c.976G>T	Missense	MAH 7			
USA	F	c.1210C>A	Missense	Loop 8–9	32	286	Yu et al. [2000] (pat 63)
		c.964-1G>C	Splice defect	—			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
USA	F	c.1054C>T	Missense	Loop 8–9	32	1,040	Yu et al. [2000] (pat 66)
		c.964-1G>C	Splice defect	—			
USA	F	c.1054C>T	Missense	Loop 8–9	32	702	Yu et al. [2000] (pat 67)
		c.964-1G>C	Splice defect	—			
USA	M	c.976G>T	Missense	MAH 7	40	361	Yu et al. [.2000] (pat 70)
		c.964-1G>C	Splice defect	—			
USA	F	c.278C>T	Missense	Loop 1–2	33	712	Yu et al. [2000] (pat 71)
		c.964-1G>C	Splice defect	—			
USA	F	c.278C>T	Missense	Loop 1–2	16	182	Yu et al. [2000] (pat 75)
		c.976G>T	Missense	MAH 7			
USA	F	c.278C>T	Missense	Loop 1–2	26	551	Yu et al. [2000] (pat 88)
		c.964-1G>C	Splice defect	—			
USA	M	c.1054C>T	Missense	Loop 8–9	15	317	Yu et al. [2000] (pat 90)
		c.964-1G>C	Splice defect	—			
USA	M	c.523G>C	Missense	Loop 3–4	45	320	Yu et al. [2000] (pat 93)
		c.964-1G>C	Splice defect	—			
USA	F	c.506C>T	Missense	MAH 3	32	60	Yu et al. [2000] (pat 109)
		c.964-1G>C	Splice defect	—			
USA	M	c.906C>G	Missense	Loop 6–7	20	299	Yu et al. [2000] (pat 113)
		c.964-1G>C	Splice defect	—			
USA	F	c.744G>T	Missense	MAH 5	21	125	Yu et al. [2000] (pat 117)
		c.976G>T	Missense	MAH 7			
USA	M	c.1342G>A	Missense	MAH 9	20	127	Yu et al. [2000] (pat 330-3)
		c.151C>T	Missense	MAH 1			
USA	F	c.151C>T	Missense	MAH 1	79	390	Yu et al. [2000] (pat 1100-3)
		c.964-1G>C	Splice defect	—			
Sweden	F	c.861C>A	Missense	Loop 6–7	42	962	Yu et al. [2000] (pat Nr1)
		c.964-1G>C	Splice defect	—			
Sweden	M	c.861C>A	Missense	Loop 6–7	35	1,014	Yu et al. [2000] (pat Nr2)
		c.964-1G>C	Splice defect	—			
Sweden	M	c.296T>C	Missense	Loop 1–2	50	364	Yu et al. [2000] (pat Nr3)
		c.964-1G>C	Splice defect	—			
Sweden	M	c.861C>A	Missense	Loop 6–7	35	159	Yu et al. [2000] (pat Nr4)
		c.728C>G	Missense	MAH 5			
Netherlands	F	c.964-1G>C	Splice defect	—	58	430	Jira et al. [2001] (pat 1)
		c.964-1G>C	Splice defect	—			
Netherlands	M	c.964-1G>C	Splice defect	—	40	300	Jira et al. [2001] (pat 3)
		c.529A>G	Missense	MAH 4			
Germany	M	c.964-1G>C	Splice defect	—	75	750	Jira et al. [2001] (pat 5)
		c.326T>C	Missense	MAH 2			
Netherlands	M	c.964-1G>C	Splice defect	—	20	410	Jira et al. [2001] (pat 6)
		c.765C>A	Missense	MAH 5			
Netherlands	F	c.964-1G>C	Splice defect	—	8	362	Jira et al. [2001] (pat 7)
		c.461C>T	Missense	MAH 3			
Netherlands	M	c.964-1G>C	Splice defect	—	25	154	Jira et al. [2001] (pat 8)
		c.461C>T	Missense	MAH 3			
Germany	M	c.964-1G>C	Splice defect	—	8	239	Jira et al. [2001] (pat 9)
		c.545G>T	Missense	MAH 4			
Germany	M	c.964-1G>C	Splice defect	—	8	181	Jira et al. [2001] (pat 10)
		c.548G>A	Missense	MAH 4			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
Germany	F	c.964-1G>C	Splice defect	—	8	57	Jira et al. [2001] (pat 11)
		c.548G>A	Missense	MAH 4			
Scottish	M	c.278C>T	Missense	Loop 1–2	8	749	Jira et al. [2001] (pat 12)
		c.725G>A	Missense	MAH 5			
Canada	M	c.964-1G>C	Splice defect	—	61[b]	1,326	Nowaczyk et al. [2001a]
		c.1138T>C	Missense	Loop 8–9			
Canada	M	c.964-1G>C	Splice defect	—	88[c]	?	Nowaczyk et al. [2001b] (pat 1)
		c.964-1G>C	Splice defect	—			
Canada	M	c.964-1G>C	Splice defect	—	61	?	Nowaczyk et al. [2001b] (pat 2)
		c.964-1G>C	Splice defect	—			
Canada	M	c.964-1G>C	Splice defect	—	80	?	Nowaczyk et al. [2001b] (pat 3)
		c.964-1G>C	Splice defect	—			
Lebanon	M	c.1400C>T	Missense	C terminus	16	52	Nezarati et al. [2002] (pat 1)
		c.1400C>T	Missense	C terminus			
Lebanon	M	c.1400C>T	Missense	C terminus	16	64	Nezarati et al. [2002] (pat 2)
		c.1400C>T	Missense	C terminus			
Italy	M	c.278C>T	Missense	Loop 1–2	56	707	Patrono et al. [2002] (pat 1)
		c.453G>A	Nonsense	MAH 3			
Italy	F	c.964-1G>C	Splice defect	—	52	412	Patrono et al. [2002] (pat 2)
		c.1337G>A	Missense	C terminus			
Italy	F	c.278C>T	Missense	Loop 1–2	29	211	Patrono et al. [2002] (pat 3)
		c.730G>A	Missense	MAH 5			
Italy	M	c.964-1G>C	Splice defect	—	22	430	Patrono et al. [2002] (pat 4)
		c.986C>T	Missense	Loop 7–8			
Italy	F	c.988G>A	Missense	Loop 7–8	26	80	Patrono et al. [2002] (pat 5)
		c.1087C>T	Missense	Loop 8–9			
Italy	F	c.964-1G>C	Splice defect	—	32	120	Patrono et al. [2002] (pat 7)
		c.278C>T	Missense	Loop 1–2			
Canada	F	c.964-1G>C	Splice defect	—	14	200	Prasad et al. [2002]
		c.839A>G	Missense	MAH 6			
USA	F	c.852C>A	Missense	MAH 6	21	226	Mueller et al. [2003]
		c.976C>T	Missense	MAH 7			
Netherlands	M	c.964-1G>C	Splice defect	—	14	8.6	Langius et al. [2003] (pat 1)
		c.3G>A	Missense	N terminus			
Netherlands	M	c.964-1G>C	Splice defect	—	7	2.6	Langius et al. [2003] (pat 2)
		c.3G>A	Missense	N terminus			
Netherlands	F	c.1342G>A	Missense	MAH 9	14	5	Langius et al. [2003] (pat 3)
		c.3G>A	Missense	N terminus			
Poland	M	c.452G>A	Nonsense	—	55	?	Ciara et al. [2004] (pat 1)
		c.452G>A	Nonsense	—			
Poland	M	c.964-1G>C	Splice defect	—	60	642	Ciara et al. [2004] (pat 2)
		c.964-1G>C	Splice defect	—			
Poland	M	c.964-1G>C	Splice defect	—	57	169	Ciara et al. [2004] (pat 3)
		c.976C>T	Missense	MAH 7			
Poland	F	c.452G>A	Nonsense	—	20	52	Ciara et al. [2004] (pat 4)
		c.925G>A	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	66	?	Ciara et al. [2004] (pat 5)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	62	374	Ciara et al. [2004] (pat 6)
		c.976C>T	Missense	MAH 7			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
Poland	F	c.452G>A	Nonsense	—	60	?	Ciara et al. [2004] (pat 7)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	60	?	Ciara et al. [2004] (pat 8)
		c.976C>T	Missense	MAH 7			
Poland	F	c.452G>A	Nonsense	—	56	?	Ciara et al. [2004] (pat 9)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	55	494	Ciara et al. [2004] (pat 10)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	50	702	Ciara et al. [2004] (pat 11)
		c.976C>T	Missense	MAH 7			
Poland	F	c.452G>A	Nonsense	—	40	?	Ciara et al. [2004] (pat 12)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	38	114	Ciara et al. [2004] (pat 13)
		c.976C>T	Missense	MAH 7			
Poland	M	c.453G>A	Nonsense	MAH 3	30	135	Ciara et al. [2004] (pat 14)
		c.976C>T	Missense	MAH 7			
Poland	M	c.452G>A	Nonsense	—	30	?	Ciara et al. [2004] (pat 15)
		c.976C>T	Missense	MAH 7			
Poland	F	c.452G>A	Nonsense	—	60	712	Ciara et al. [2004] (pat 16)
		c.1079T>G	Missense	Loop 8–9			
Poland	F	c.452G>A	Nonsense	—	50	?	Ciara et al. [2004] (pat 17)
		c.1190C>T	Missense	Loop 8–9			
Poland	M	c.452G>A	Nonsense	—	22	380	Ciara et al. [2004] (pat 18)
		c.1054C>T	Missense	Loop 8–9			
Poland	M	c.452G>A	Nonsense	—	30	62	Ciara et al. [2004] (pat 19)
		c.1054C>T	Missense	Loop 8–9			
Poland	M	c.452G>A	Nonsense	—	40	247	Ciara et al. [2004] (pat 20)
		c.1054C>T	Missense	Loop 8–9			
Poland	M	c.964-1G>C	Splice defect	—	30	260	Ciara et al. [2004] (pat 21)
		c.1054C>T	Missense	Loop 8–9			
Poland	M	c.452G>A	Nonsense	—	22	601	Ciara et al. [2004] (pat 22)
		c.808A>G	Missense	MAH 6			
Poland	M	c.452G>A	Nonsense	—	22	127	Ciara et al. [2004] (pat 23)
		c.533T>A	Missense	MAH 4			
Poland	M	c.452G>A	Nonsense	—	60	221	Ciara et al. [2004] (pat 24)
		c.470T>C	Missense	MAH 3			
Poland	F	c.452G>A	Nonsense	—	15	845	Ciara et al. [2004] (pat 25)
		c.278C>T	Missense	Loop 1–2			
Poland	M	c.976G>T	Missense	MAH 7	91	?	Ciara et al. [2004] (pat 26)
		c.1079T>G	Missense	Loop 8–9			
Poland	M	c.470T>C	Missense	MAH 3	72	767	Ciara et al. [2004] (pat 27)
		c.470T>C	Missense	MAH 3			
Poland	M	c.470T>C	Missense	MAH 3	16	572	Ciara et al. [2004] (pat 28)
		c.470T>C	Missense	MAH 3			
Poland	M	c.470T>C	Missense	MAH 3	35	268	Ciara et al. [2004] (pat 29)
		c.976G>T	Missense	MAH 7			
Poland	F	c.976G>T	Missense	MAH 7	22	562	Ciara et al. [2004] (pat 30)
		c.976G>T	Missense	MAH 7			
Poland	F	c.976G>T	Missense	MAH 7	16	75	Ciara et al. [2004] (pat 31)
		c.976G>T	Missense	MAH 7			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
Poland	M	c.1054C>T	Missense	Loop 8–9	45	759	Ciara et al. [2004] (pat 32)
		c.1337G>A	Missense	C terminus			
Poland	F	c.976G>T	Missense	MAH 7	25	99	Ciara et al. [2004] (pat 33)
		c.1328G>A	Missense	C terminus			
Poland	M	c.151C>T	Missense	MAH 1	5	73	Ciara et al. [2004] (pat 34)
		c.1337G>A	Missense	C terminus			
Poland	M	c.151C>T	Missense	MAH 1	5	39	Ciara et al. [2004] (pat 35)
		c.1337G>A	Missense	C terminus			
Poland	M	c.976G>T	Missense	MAH 7	15	265	Ciara et al. [2004] (pat 36)
		c.203T>C	Missense	Loop 1–2			
Poland	M	c.976G>T	Missense	MAH 7	10	509	Ciara et al. [2004] (pat 37)
		c.203T>C	Missense	Loop 1–2			
Cuba	M	c.278C>T	Missense	Loop 1–2	17	596	Nowaczyk et al. [2004] (pat 1)
		c.841G>A	Missense	MAH 6			
Cuba	M	c.278C>T	Missense	Loop 1–2	17	302	Nowaczyk et al. [2004] (pat 2)
		c.278C>T	Missense	Loop 1–2			
Cuba	M	c.278C>T	Missense	Loop 1–2	11	219	Nowaczyk et al. [2004] (pat 3)
		c.906C>G	Missense	Loop 6–7			
Cuba	F	c.278C>T	Missense	Loop 1–2	10	283	Nowaczyk et al. [2004] (pat 4)
		c.700G>T	Missense	Loop 4–5			
Cuba	F	c.278C>T	Missense	Loop 1–2	22	276	Nowaczyk et al. [2004] (pat 5)
		c.964-1G>C	Splice defect	—			
Cuba	F	c.278C>T	Missense	Loop 1–2	17	311	Nowaczyk et al. [2004] (pat 6)
		c.964-1G>C	Splice defect	—			
Cuba	F	c.278C>T	Missense	Loop 1–2	17	454	Nowaczyk et al. [2004] (pat 7)
		c.964-1G>C	Splice defect	—			
Cuba	F	c.278C>T	Missense	Loop 1–2	19	367	Nowaczyk et al. [2004] (pat 8)
		c.964-1G>C	Splice defect	—			
Cuba	M	c.278C>T	Missense	Loop 1–2	28	599	Nowaczyk et al. [2004] (pat 9)
		c.964-1G>C	Splice defect	—			
Cuba	M	c.278C>T	Missense	Loop 1–2	11	427	Nowaczyk et al. [2004] (pat10)
		c.964-1G>C	Splice defect	—			
Italy	F	c.278C>T	Missense	Loop 1–2	17	134	Nowaczyk et al. [2004] (pat 11)
		c.278C>T	Missense	Loop 1–2			
Portugal	M	c.278C>T	Missense	Loop 1–2	20	158	Nowaczyk et al. [2004] (pat 12)
		c.964-1G>C	Splice defect	—			
Greece	M	c.278C>T	Missense	Loop 1–2	20	375	Nowaczyk et al. [2004] (pat 13)
		c.964-1G>C	Splice defect	—			
Portugal	F	c.278C>T	Missense	Loop 1–2	11	462	Nowaczyk et al. [2004] (pat 14)
		c.964-1G>C	Splice defect	—			
Irish-Ukrainian	M	c.278C>T	Missense	Loop 1–2	17	558	Nowaczyk et al. [2004] (pat 15)
		c.964-1G>C	Splice defect	—			
UK	F	c.964-1G>C	Splice defect	—	?	361	Anstey et al. [2005] (pat 1)
		c.152C>A	Missense	MAH 1			
UK	M	c.964-1G>C	Splice defect	—	?	297	Anstey et al. [2005] (pat 2)
		c.1349G>T	Missense	C terminus			
UK	M	c.1342G>A	Missense	MAH 9	?	431	Anstey et al. [2005] (pat 3)
		c.1342G>A	Missense	MAH 9			
UK	M	c.964-1G>C	Splice defect	—	?	706	Anstey et al. [2005] (pat 4)
		c.296T>C	Missense	Loop 1–2			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
UK	M	c.964-1G>C	Splice defect	—	?	183	Anstey et al. [2005] (pat 2)
		c.278C>T	Missense	Loop 1–2			
Portugal	M	c.902A>G	Missense	Loop 6–7	38	47	Cardoso et al. [2005] (pat 1)
		c.545G>T	Missense	MAH 4			
Portugal	F	c.964-1G>C	Splice defect	—	11	153	Cardoso et al. [2005] (pat 2)
		c.521T>C	Missense	MAH 3			
Portugal	M	c.964-1G>C	Splice defect	—	44	246	Cardoso et al. [2005] (pat 3)
		c.278C>T	Missense	Loop 1–2			
Portugal	F	c.292C>T	Nonsense	Loop 1–2	17	1,135	Cardoso et al. [2005] (pat 4)
		c.278C>T	Missense	Loop 1–2			
Portugal	F	c.822C>A	Missense	MAH 6	33	247	Cardoso et al. [2005] (pat 5)
		c.278C>T	Missense	Loop 1–2			
Portugal	F	c.278C>T	Missense	Loop 1–2	19	148	Cardoso et al. [2005] (pat 6)
		c.278C>T	Missense	Loop 1–2			
Portugal	M	c.964-1G>C	Splice defect	—	44	347	Cardoso et al. [2005] (pat 7)
		c.902A>G	Missense	Loop 6–7			
USA	M	c.964-1G>C	Splice defect	—	78	290	Correa-Cerro et al. [2005] (pat 1)
		c.452G>A	Nonsense	—			
USA	F	c.461C>G	Missense	MAH 3	67	210	Correa-Cerro et al. [2005] (pat 2)
		c.452G>A	Nonsense	—			
USA	M	c.1054C>T	Missense	Loop 8–9	30	60	Correa-Cerro et al. [2005] (pat 3)
		c.452G>A	Nonsense	—			
Poland	M	c.470T>C	Missense	MAH 3	60	220	Correa-Cerro et al. [2005] (pat 4)
		c.452G>A	Nonsense	—			
Poland	M	c.1054C>T	Missense	Loop 8–9	20	380	Correa-Cerro et al. [2005] (pat 5)
		c.452G>A	Nonsense	—			
Poland	F	c.925G>A	Missense	MAH 7	20	60	Correa-Cerro et al. [2005] (pat 6)
		c.452G>A	Nonsense	—			
Poland	M	c.976G>T	Missense	MAH 7	30	130	Correa-Cerro et al. [2005] (pat 7)
		c.452G>A	Nonsense	—			
Poland	M	c.808A>G	Missense	MAH 6	22	600	Correa-Cerro et al. [2005] (pat 8)
		c.452G>A	Nonsense	—			
Poland	M	c.1054C>T	Missense	Loop 8–9	40	250	Correa-Cerro et al. [2005] (pat 9)
		c.452G>A	Nonsense	—			
Poland	M	c.976G>T	Missense	MAH 7	55	490	Correa-Cerro et al. [2005] (pat 10)
		c.452G>A	Nonsense	—			
USA	M	c.1349G>T	Missense	C terminus	6	140	Correa-Cerro et al. [2005] (pat 11)
		c.292C>T	Missense	Loop 1–2			
Japan	M	c.1055G>A	Missense	Loop 8–9	?	400	Matsumoto et al. [2005] (pat 1)
		c.1055G>A	Missense	Loop 8–9			
Japan	M	c.1055G>A	Missense	Loop 8–9	?	910	Matsumoto et al. [2005] (pat 2)
		c.725G>A	Missense	MAH 5			
Japan	M	c.1055G>A	Missense	Loop 8–9	?	252	Matsumoto et al. [2005] (pat 4)
		c.1055G>A	Missense	Loop 8–9			
Japan	F	c.1055G>A	Missense	Loop 8–9	?	309	Matsumoto et al. [2005] (pat 5)
		c.1426T>C	Missense	C terminus			
Japan	F	c.1055G>A	Missense	Loop 8–9	?	738	Matsumoto et al. [2005] (pat 6)
		c.907G>A	Missense	Loop 6–7			
Japan	M	c.1055G>A	Missense	Loop 8–9	?	354	Matsumoto et al. [2005] (pat 7)
		c.575C>T	Missense	MAH 4			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
USA	?	c.203T>C	Missense	Loop 1–2	17	?	Waye et al. [2005] (pat 1)
		c.1210C>T	Missense	Loop 8–9			
USA	?	c.433A>C	Missense	Loop 2-3	17	?	Waye et al. [2005] (pat 4)
		c.1222T>C	Missense	Loop 8–9			
USA	?	c.704T>C	Missense	Loop 4–5	28	?	Waye et al. [2005] (pat 5)
		c.964-1G>C	Splice defect	—			
USA	?	c.890T>C	Missense	Loop 6–7	11	?	Waye et al. [2005] (pat 6)
		c.964-1G>C	Splice defect	—			
USA	?	c.1030G>C	Missense	MAH 8	39	?	Waye et al. [2005] (pat 7)
		c.724C>T	Missense	MAH 5			
USA	?	c.1277A>C	Missense	MAH 9	11	?	Waye et al. [2005] (pat 9)
		c.724C>T	Missense	MAH 5			
Israel	?	c.1A>G	Missense	N terminus	9	7.3	Witsch-Baumgartner et al. [2005] (pat 1)
		c.964-1G>C	Splice defect	—			
Austria	?	c.149C>A	Missense	MAH 1	25	7.8	Witsch-Baumgartner et al. [2005] (pat 2)
		c.964-1G>C	Splice defect	—			
Poland	?	c.203T>C	Missense	Loop 1–2	11	510	Witsch-Baumgartner et al. [2005] (pat 3)
		c.976G>T	Missense	MAH 7			
Poland	M	c.203T>C	Missense	Loop 1–2	10	265	Witsch-Baumgartner et al. [2005] (pat 4)
		c.976G>T	Missense	MAH 7			
Germany	?	c.533T>A	Missense	MAH 4	30	9.6	Witsch-Baumgartner et al. [2005] (pat 8)
		c.725G>A	Missense	MAH 5			
Germany	?	c.533T>A	Missense	MAH 4	4	5.2	Witsch-Baumgartner et al. [2005] (pat 9)
		c.452G>A	Nonsense	—			
Spain	?	c.545G>T	Missense	MAH 4	22	304	Witsch-Baumgartner et al. [2005] (pat 10)
		c.670G>A	Missense	Loop 4–5			
Spain	?	c.278C>T	Missense	Loop 1–2	20	317	Witsch-Baumgartner et al. [2005] (pat11)
		c.670G>A	Missense	Loop 4–5			
Ireland	?	c.575C>T	Missense	MAH 4	15	?	Witsch-Baumgartner et al. [2005] (pat 12)
		c.278C>T	Missense	Loop 1–2			
Turkey	?	c.818T>G	Missense	MAH 6	1	702	Witsch-Baumgartner et al. [2005] (pat 13)
		c.1289A>G	Missense	MAH 9			
UK	?	c.1039G>A	Missense	MAH 8	33	6.8	Witsch-Baumgartner et al. [2005] (pat 14)
		c.452G>A	Nonsense	—			
Poland	?	c.203T>C	Missense	Loop 1–2	66	710	Witsch-Baumgartner et al. [2005] (pat 15)
		c.452G>A	Nonsense	—			
Germany	?	c.1289A>G	Missense	MAH 9	2	341	Witsch-Baumgartner et al. [2005] (pat 17)
		c.964-1G>C	Splice defect	—			
Italy	?	c.1423T>C	Missense	C terminus	10	364	Witsch-Baumgartner et al. [2005] (pat 18)
		c.964-1G>C	Splice defect	—			
Korea	F	c.1054C>T	Missense	Loop 8–9	25	176	Chae et al. [2007]
		c.1127delA	Frame shift	—			
Canada	F	c.964-1G>C	Splice defect	—	64	?	Waye et al. [2007]
		c.411A>G	Missense	Loop 2-3			
Poland	M	c.461C>T	Missense	MAH 3	42	188	Jezela-Stanek et al. [2008] (pat 5)
		c.655T>G	Missense	Loop 4–5			
Poland	F	c.326T>C	Missense	MAH 2	?	341	Jezela-Stanek et al. [2010] (pat 1)
		c.452G>A	Nonsense	—			
Poland	M	c.461C>T	Missense	MAH 3	?	189	Jezela-Stanek et al. [2010] (pat 2)
		c.655T>G	Nonsense	Loop 4–5			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
Poland	F	c.1054C>T	Missense	Loop 8–9	?	328	Jezela-Stanek et al. [2010] (pat 3)
		c.452G>A	Nonsense	—			
Poland	M	c.1055G>A	Missense	Loop 8–9	?	621	Jezela-Stanek et al. [2010] (pat 4)
		c.452G>A	Nonsense	—			
Poland	F	c.976G>T	Missense	MAH 7	?	419	Jezela-Stanek et al. [2010] (pat 5)
		c.1342G>A	Nonsense	MAH 9			
Poland	M	c.976G>T	Missense	MAH 7	?	442	Jezela-Stanek et al. [2010] (pat 9)
		c.452G>A	Nonsense	—			
Poland	M	c.433A>C	Missense	Loop 2-3	?	159	Jezela-Stanek et al. [2010] (pat 10)
		c.452G>A	Nonsense	—			
Poland	M	c.470T>C	Missense	MAH 3	?	161	Jezela-Stanek et al. [2010] (pat 11)
		c.452G>A	Nonsense	—			
Poland	M	c.326T>C	Missense	MAH 2	?	1,160	Jezela-Stanek et al. [2010] (pat 12)
		c.964-1G>C	Splice defect	—			
Poland	F	c.976G>T	Missense	MAH 7	?	289	Jezela-Stanek et al. [2010] (pat 14)
		c.452G>A	Nonsense	—			
Poland	F	c.452G>A	Nonsense	—	?	536	Jezela-Stanek et al. [2010] (pat 15)
		c.452G>A	Nonsense	—			
Poland	M	c.452G>A	Nonsense	—	?	187	Jezela-Stanek et al. [2010] (pat 16)
		c.452G>A	Nonsense	—			
Poland	M	c.1054C>T	Missense	Loop 8–9	?	523	Jezela-Stanek et al. [2010] (pat 17)
		c.452G>A	Nonsense	—			
Poland	M	c.452G>A	Nonsense	—	?	143	Jezela-Stanek et al. [2010] (pat 19)
		c.452G>A	Nonsense	—			
Poland	M	c.744G>C	Missense	MAH 5	?	96	Jezela-Stanek et al. [2010] (pat 10)
		c.452G>A	Nonsense	—			
Poland	M	c.452G>A	Nonsense	—	?	705	Jezela-Stanek et al. [2010] (pat 21)
		c.452G>A	Nonsense	—			
Poland	M	c.744G>C	Missense	MAH 5	?	114	Jezela-Stanek et al. [2010] (pat 22)
		c.452G>A	Nonsense	—			
Poland	F	c.326T>C	Missense	MAH 2	?	429	Jezela-Stanek et al. [2010] (pat 24)
		c.326T>C	Missense	MAH 2			
Korea	M	c.679C>T	Missense	Loop 4–5	55	567	Ko et al. [2010]
		c.907G>A	Missense	Loop 6–7			
USA	F	c.964-1G>C	Splice defect	—	50	670	Koo et al. [2010]
		c.412+3A>T	Frame shift	—			
Hungary	M	c.964-1G>C	Splice defect	—	50	260	Szabó et al. [2010]
		c.1370G>T	Missense	C terminus			
USA	M	c.964-1G>C	Splice defect	—	50[c]	?	Weaver et al. [2010]
		c.del exon 3–4	Frame shift	—			
USA	M	c.740C>T	Missense	MAH 5	6	128	Tierney et al. [2010] (pat 1)
		c.964-1G>C	Splice defect	—			
USA	F	c.278C>T	Missense	Loop 1–2	22	410	Tierney et al. [2010] (pat 2)
		c.1327C>T	Missense	C terminus			
USA	F	c.151C>T	Missense	MAH 1	6	26	Tierney et al. [2010] (pat 3)
		c.452G>A	Nonsense				
USA	F	c.964-1G>C	Splice defect	—	28	273	Tierney et al. [2010] (pat 4)
		c.278C>T	Missense	Loop 1–2			
USA	F	c.964-1G>C	Splice defect	—	17	135	Tierney et al. [2010] (pat 5)
		c.952T>A	Missense	MAH 7			

(*Continued*)

TABLE III. (*Continued*)

Country	Gender	Mutation	Type	Position in DHCR7 protein[a]	Severity score	7DHC (μmol/L)	Refs.
USA	M	c.1139G>A	Missense	MAH 9	11	59	Tierney et al. [2010] (pat 6)
		c.1342G>A	Missense	MAH 9			
USA	M	c.964-1G>C	Splice defect	—	6	52	Tierney et al. [2010] (pat 7)
		c.866C>T	Missense	Loop 6–7			
USA	F	c.724C>T	Missense	MAH 5	17	81	Tierney et al. [2010] (pat 9)
		c.529T>C	Missense	Loop 3–4			
USA	F	c.964-1G>C	Splice defect	—	22	142	Tierney et al. [2010] (pat 10)
		c.1054C>T	Missense	Loop 8–9			
Saudi Arabia	F	c.861C>A	Missense	Loop 6–7	35	259	Al-Owain et al. [2012] (pat 1)
		c.861C>A	Missense	Loop 6–7			
Saudi Arabia	M	c.1055G>A	Missense	Loop 8–9	75	430	Al-Owain et al. [2012] (pat 2)
		c.1055G>A	Missense	Loop 8–9			
Saudi Arabia	M	c.1055G>A	Missense	Loop 8–9	75	660	Al-Owain et al. [2012] (pat 3)
		c.1055G>A	Missense	Loop 8–9			
Saudi Arabia	F	c.1055G>A	Missense	Loop 8–9	80	885	Al-Owain et al. [2012] (pat 4)
		c.1055G>A	Missense	Loop 8–9			
Sudan	M	c.1055G>T	Missense	Loop 8–9	45	105	Al-Owain et al. [2012] (pat 5)
		c.1055G>T	Missense	Loop 8–9			
France	F	c.356del13nt	Frame shift	—	63	?	Quélin et al. [2012] (pat 1)
		c.906C>G	Missense	Loop 6–7			
France	M	c.1228G>A	Missense	Loop 8–9	75	299	Quélin et al. [2012] (pat 2)
		c.964-1G>C	Splice defect	—			
France	M	c.438C>G	Missense	Loop 2-3	50	?	Quélin et al. [2012] (pat 3)
		c.964-1G>C	Splice defect	—			
France	F	c.506C>T	Missense	MAH 3	25	?	Quélin et al. [2012] (pat 4)
		c.964-1G>C	Splice defect	—			
France	F	c.149C>T	Missense	MAH 1	56[c]	?	Quélin et al. [2012] (pat 5)
		c.628A>T	Missense	Loop 4–5			
France	M	c.964-1G>C	Splice defect	—	63	?	Quélin et al. [2012] (pat 6)
		c.964-1G>C	Splice defect	—			
France	M	c.964-1G>C	Splice defect	—	56	?	Quélin et al. [2012] (pat 7)
		c.964-1G>C	Splice defect	—			
France	F	c.964-1G>C	Splice defect	—	56	?	Quélin et al. [2012] (pat 8)
		c.452G>A	Nonsense	—			
France	M	c.964-1G>C	Splice defect	—	44	?	Quélin et al. [2012] (pat 9)
		c.682C>T	Missense	Loop 4–5			
France	F	c.964-1G>C	Splice defect	—	50	?	Quélin et al. [2012] (pat 10)
		c.964-1G>C	Splice defect	—			

*Patients have only been tabulated if both mutations of the individual patient were available and either the 7DHC plasma levels or sufficient data to determine the clinical severity score were available.

[a]Localization according to topology model of Figure 1b. MAH = membrane-associated helix; for numbering of MAHs see Figure 1b.

[b]Adrenal insufficiency.

a more severe phenotype. In contrast, patients with either two mutations in a trans-membrane domain or with one mutation in a trans-membrane domain and a second in the C-terminal domain tend to have the lowest DHC ratios and a mild to moderate phenotype [Witsch-Baumgartner et al., 2000]. Our more detailed evaluation on a larger cohort in Table IV indeed confirms that the most severe phenotypes are observed in patients with two null mutations or with two mutations in loop 8–9. Patients with one or two mutations in Loop 1–2, one mutation in the N terminus, and to a lesser extent, one mutation in the C terminus have milder phenotypes. Most other genotypes result in more

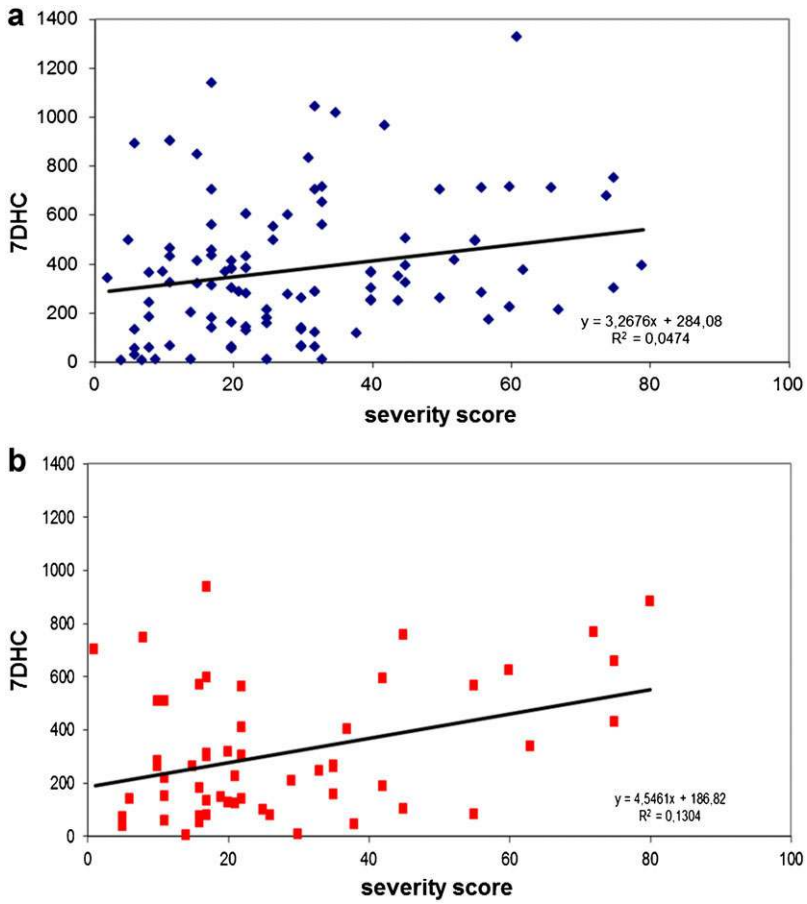

Figure 2. Correlations between clinical severity scores and plasma 7DHC levels in patients with a missense and a nonsense mutation (**a**) and patients with two missense mutations (**b**). Correlations were r = 0.217 and r = 0.361, respectively.

moderate phenotypes. As follows from the weak correlation between clinical severity and 7DHC (Fig. 1), the correlation between genotype and 7DHC levels is rather weak and often shows wide ranges within the same group.

Our more detailed evaluation on a larger cohort in Table IV indeed confirms that the most severe phenotypes are observed in patients with two null mutations or with two mutations in loop 8–9. Patients with one or two mutations in loop 1–2, one mutation in the N terminus, and to a lesser extent, one mutation in the C terminus have milder phenotypes. Most other genotypes result in more moderate phenotypes.

DISCUSSION

Historically SLOS has been subdivided into a classic (type I) and severe (type II). The introduction of the severity score that evaluated 10 different organ systems in an equal way and the finding that both types are due to mutations in the *DHCR7* gene have made clear that these two types form a continuum and are not distinct disorders [Bialer et al., 1987; Kelley and Hennekam, 2000]. In accordance to this continuum the severity scores in the analyzed 165 patients do not show a bimodal distribution (Fig. 2).

We found a weak correlation between the plasma 7DHC levels and the severity score, irrespective of the nature of the mutation (Fig. 2), which is in line with previous observations.

Indeed, genetically confirmed SLOS patients have been reported with a very mild phenotype and, accordingly, low severity scores ranging from 1 to 12, but with plasma 7DHC levels that vary widely from 2.6 to 900 μmol/L (mean 288 μmol/L) [Nowaczyk et al., 1998; Krakowiak et al., 2000; Yu et al., 2000; Jira et al., 2001; Prasad et al., 2002; Langius et al., 2003; Ciara et al., 2004; Cardoso et al., 2005; Correa-Cerro et al., 2005; Witsch-Baumgartner et al., 2005; Jezela-Stanek et al., 2008].

Although there appears to be a correlation between certain genotypes and clinical severity, patients with the same *DHCR7* genotype can have marked differences in severity score and plasma 7DHC levels (Tables III and IV). Thus, we concur with earlier conclusions that SLOS is a mendelian disorder with significant influence from other genetic, epigenetic, and environmental factors [Kelley and Hennekam, 2000; Ciara et al., 2004; Anstey et al., 2005; Jezela-Stanek et al., 2008; DeBarber et al., 2011]. As previously reported, the maternal–fetal cholesterol transfer during pregnancy is dependent on maternal diet, maternal apolipoprotein levels and probably several other factors, and thus will have a significant impact

Although there appears to be a correlation between certain genotypes and clinical severity, patients with the same DHCR7 genotype can have marked differences in severity score and plasma 7DHC levels.

TABLE IV. Clinical Severity and 7DHC Levels of 207 Published Patients With Smith–Lemli–Opitz Syndrome Based on Location of Mutations*

Location in DHCR7		Severity score					7DHC (μmol/L)				
				Multiple cases					Multiple cases		
Mutation 1	Mutation 2	No. patients	Single case	Average	Median	Range	No. patients	Single case	Average	Median	Range
—	—	16		61	61	50–88	4		508	536	290–670
N terminus	—	3		10	9	7–14	3		6	7	3–9
N terminus	MAH 9	1	14				1	5			
MAH 1	—	3		37	25	6–79	3		141	26	8–390
MAH 1	Loop 4–5	1	56								
MAH 1	MAH 9	1	20				1	127			
MAH 1	C terminus	2		5	5	5–5	2		56	56	39–73
Loop 1–2	—	26		27	21	11–74	26		417	419	62–845
Loop 1–2	Loop 1–2	4		18	17	17–19	4		430	225	148–1,135
Loop 1–2	MAH 3	1	56				1	707			
Loop 1–2	MAH 4	1	15								
Loop 1–2	Loop 4–5	2		15	15	10–20	2		300	300	283–317
Loop 1–2	MAH 5	2		19	19	8–29	2		480	480	211–749
Loop 1–2	MAH 6	2		25	25	17–33	2		275	275	247–302
Loop 1–2	Loop 6–7	1	11				1	219			
Loop 1–2	MAH 7	5		12	11	10–16	5		346	265	182–510
Loop 1–2	Loop 8–9	1	17								
Loop 1–2	C terminus	2		14	14	6–22	2		275	275	140–410
MAH 2	—	2		66	66	56–75	2		515	515	280–750
MAH 2	C terminus	1	17				1	310			
Loop 2–3	—	1	64								
Loop 2–3	MAH 4	1	60				1	624			
Loop 2–3	MAH 5	2		49	49	42–55	2		339	339	83–595
Loop 2–3	Loop 8–9	2		34	34	17–50					
MAH 3	—	10		29	33	8–67	9		285	220	60–900
MAH 3	MAH 3	2		44	44	16–72	2		670	670	572–767
MAH 3	Loop 4–5	1	42				1	188			
MAH 3	MAH 7	2		33	33	30–35	2		202	202	135–268
Loop 3–4	—	1	45				1	320			
Loop 3–4	MAH 5	2		17	17	17–17	2		511	511	81–940
MAH 4	—	6		15	8	4–40	6		152	154	5–300
MAH 4	Loop 4–5	1	22				1	304			
MAH 4	MAH 5	1	30				1	10			
MAH 4	Loop 6–7	1	38				1	47			
Loop 4–5	—	2		36	36	28–44					
Loop 4–5	Loop 6–7	1	55				1	567			
MAH 5	—	4		18	16	6–33	4		354	365	128–559
MAH 5	Loop 6–7	1	35				1	159			
MAH 5	MAH 7	2		29	29	21–37	2		264	264	125–403
MAH 5	MAH 8	1	39								
MAH 5	MAH 9	1	11								
Loop 6–7	—	8		22	16	6–44	7		638	890	52–1,014
Loop 6–7	Loop 6–7	1	35				1	259			
Loop 6–7	Loop 8–9	1	75				1	299			
MAH 6	—	3		19	22	14–22	3		467	600	200–601
MAH 6	MAH 7	1	21				1	226			

(*Continued*)

TABLE IV. (*Continued*)

Location in DHCR7		Severity score					7DHC (μmol/L)				
				Multiple cases					Multiple cases		
Mutation 1	Mutation 2	No. patients	Single case	Average	Median	Range	No. patients	Single case	Average	Median	Range
MAH 6	MAH 9	1	1					702			
MAH 7	—	20		42	43	17–66	14		318	324	60–702
MAH 7	MAH 7	2		19	19	16–22	2		319	319	75–562
MAH 7	Loop 8–9	1	91								
MAH 7	C terminus	1	25				1	99			
Loop 7–8	—	1	22				1	430			
Loop 7–8	Loop 8–9	1	26				1	80			
MAH 8	—	2		32	32	31–33	1	830			
Loop 8–9	—	11		30	30	20–61	11		445	286	60–1,040
Loop 8–9	Loop 8–9	4		68	75	40–80	4		520	545	105–885
Loop 8–9	C terminus	1	45				1	759			
MAH 9	—	8		35	35	2–60	7		358	341	62–712
MAH 9	MAH 9	1	11				1	59			
MAH 9	C terminus	1	63				1	338			
C terminus	—	4		29	30	5–52	4		383	388	260–494
C terminus	C terminus	2		16	16	16–16	2		58	58	52–64

*Localization according to topology model of Figure 1b. MAH = membrane-associated helix; for numbering of MAHs see Figure 1b. Of the patients listed in Table III only patients with reported severity scores have been included.

on the phenotype [Witsch-Baumgartner et al., 2004]. Although SLOS is caused only by mutations in the *DHCR7* gene, the SLOS phenotype appears to be greatly influenced by variants in many as yet unidentified genes [Hennekam and Biesecker, 2012]. Circumstantial evidence, such as the altered auxotrophy of cell lines to produce cholesterol by epigenetic influences [Seth et al., 2006] and the role of a changed methylation of ATP-binding cassette A1 (*ABCA1*) on cholesterol transport [Guay et al., 2012] indicate the significant influence of epigenetics on lipid metabolism and vice versa [Houten and Argmann, 2011]. We anticipate that such epigenetic influences on the phenotype of SLOS will also be identified in future investigations.

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