

ELECTRONIC LETTER

Smith-Lemli-Opitz syndrome: carrier frequency and spectrum of *DHCR7* mutations in Canada

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Smith-Lemli-Opitz syndrome (SLOS, OMIM 270400) is an autosomal recessive disorder of cholesterol biosynthesis resulting from deficient 3 β -hydroxysterol Δ^7 -reductase (*DHCR7*) activity.^{1,2} Patients with SLOS have a characteristic facial phenotype, various degrees of cleft palate and of syndactyly of toes 2 and 3, failure to thrive, behavioural problems, and mental retardation in addition to variable combinations of external and internal malformations.³⁻⁵ The spectrum of severity extends from prenatal death with holoprosencephaly or other lethal malformations to minimally physically affected patients with normal intelligence or minimal intellectual impairment. Most patients with SLOS have abnormally low levels of plasma cholesterol and all have raised levels of its immediate precursor, 7-dehydrocholesterol (7DHC).²

The *DHCR7* gene has been mapped to chromosome 11q13, spans approximately 14 kb, and encodes a protein of 475 amino acid residues.⁶ To date, 85 different *DHCR7* mutations have been identified in more than 200 SLOS patients.^{5,7,8} Missense mutations comprise over 85% of the known alleles, although the two most common alleles are a splice acceptor site mutation (IVS8-1G \rightarrow C) and a nonsense mutation (W151X).

SLOS is most common among people of European descent, with estimated incidences ranging from 1 in 10 000 to 1 in 60 000 depending on the diagnostic criteria and the reference population.⁵ Several groups have shown that the carrier rate for the most common mutation, IVS8-1G \rightarrow C, is approximately 1 in 100 for white populations in North America, and even higher in some European populations.⁹⁻¹² Haplotypes of *DHCR7* single nucleotide polymorphisms (SNPs) have been used to investigate the origin and distribution patterns of SLOS

mutations in different populations, showing both founder effects and recurrent mutations.¹³

In this report, we summarise our molecular diagnostic experiences with SLOS in Canada. *DHCR7* mutations and SNPs were identified in 30 unrelated SLOS patients and their families using a panel of allele specific assays and direct nucleotide sequencing. The carrier rate of SLOS in Canada was investigated by screening approximately 3000 anonymous subjects of various ethnic backgrounds for the five most common *DHCR7* mutations (IVS8-1G \rightarrow C, T93M, W151X, V326L, and R404C).

MATERIALS AND METHODS

Allele specific mutation assays

Allele specific assays were based on the amplification refractory mutation system (ARMS).^{11,14} Tests were designed to detect the five most common *DHCR7* mutant alleles, specifically IVS8-1G \rightarrow C (g.9447G \rightarrow C), T93M (c.278C \rightarrow T), W151X (c.453G \rightarrow A), V326L (c.976G \rightarrow T), and R404C (c.1210C \rightarrow T). Each ARMS assay uses four primers, two primers that amplify a region of the *HFE* gene and two primers that amplify a region of the *DHCR7* gene if the SLOS mutation is present (table 1). The assays were developed using control DNA samples of known *DHCR7* genotypes, generously provided by Dr F D Porter (NIH, Bethesda, Maryland). Sensitivity studies established that each ARMS assay could detect a single carrier in pools of at least 100 subjects.¹¹

Abbreviations: SLOS, Smith-Lemli-Opitz syndrome; SNP, single nucleotide polymorphism; ARMS, amplification refractory mutation system

Table 1 Primers used for allele specific ARMS assays

Mutation	Primers*	Size (bp)
IVS8-1G \rightarrow C	(g.9279-9301) 5'-CACAAACAGCCCTCAAGGTGGTG-3' (g.9468-9447) 5'-GTGGTACACCAAGTACAGTCCG-3'	190
T93M	(g.708-728) 5'-CAGTGTGACTGCCTGCATCCG-3' (g.938-917) 5'-TAGAGCTGGGCGGCTTCGTCA-3'	231
W151X	(g.3187-3208) 5'-CATACCACAGGGGCATCCTCAC-3' (g.3574-3553) 5'-CAGAGCAGGTGCGTGAGGTGCT-3'	388
V326L	(g.9279-9301) 5'-CACAAACAGCCCTCAAGGTGGTG-3' (g.9480-9460) 5'-CAGCTGCACGGGTGGTTCAGA-3'	202
R404C	(g.9675-9694) 5'-GGGCTTCTGGGCGTGCCCT-3' (g.9964-9942) 5'-TGGCAGAACACGCTCTTGACAGC-3'	286
Internal control	(g.3335-3357) 5'-GTGTGTGGAGCCTCAACATCCTG-3' (g.3763-3743) 5'-CACAAAGACCTCAGACTTCCAG-3'	429

* Accession numbers for the *DHCR7* and *HFE* genes are AF110060 and Z92910, respectively. The coordinates for each primer are given relative to the genomic DNA sequence, with position 1 being the first base of the translation initiation codon.

Table 2 Frequencies and characteristics of *DHCR7* mutations in Canadian SLOS patients

Mutation*	Sequence	Type†	Alleles (%)	Haplotypes‡ (No)	
				This study	Ref 13
IVS8-1G→C	g.9447G→C	0	24 (40.0)	A (14)	A (13); E (1)
R404C	c.1210C→T	4L	10 (16.7)	A (1); F (5)	B (1); D (1); F (3)
T93M	c.278C→T	TM	5 (8.3)	J (4)	A (1); E (2)
W151X	c.453G→A	0	3 (5.0)		C (2); F (2); G (3)
E448K	c.1342G→A	CT	3 (3.0)	A (1)	
R242C	c.724C→T	TM	2 (3.3)	A (1)	
V326L	c.976G→T	TM	2 (3.3)	A (1)	A (5)
P109L	c.326T→C	TM	2 (3.3)	F (1)	
L157P	c.470T→C	TM	1 (1.7)		
W248R*	c.742T→C	TM	1 (1.7)		
Y280C*	c.839A→G	TM	1 (1.7)	A (1)	
T289I	c.866C→T	TM	1 (1.7)	A (1)	A (1)
Y318N	c.952T→A	TM	1 (1.7)	F (1)	
R352W	c.1054C→T	TM	1 (1.7)	A (1)	
C380Y	c.1139G→A	4L	1 (1.7)		
Y408H	c.1222T→C	4L/TM	1 (1.7)	A (1)	
Unknown			1 (1.7)		

*Mutations that have not been previously reported.

†Mutations are classified as 0 (null), TM (missense mutation of transmembrane domain), 4L (missense mutation of 4th cytoplasmic loop), or CT (missense mutations of C-terminal domain).¹⁸

‡*DHCR7* haplotypes are as defined by Witsch-Baumgartner *et al.*¹³ The J haplotype has not been previously reported; positions c.189 (G), c.207 (C), c.231 (T), c.438 (C), c.969 (G), c.1158 (C), c.1272 (C), c.1350 (C).

For each ARMS assay, PCR was conducted in a total volume of 20 µl containing 150–250 ng genomic DNA, 50 mmol/l KCl, 10 mmol/l Tris-HCl, 0.2 mmol/l each dNTP, 0.147 mmol/l forward control primer, 0.133 mmol/l reverse control primer, 0.140 mmol/l forward and reverse ARMS primers, and 2.5 U AmpliTaq Gold® polymerase (Applied Biosystems, Foster City, CA). Reactions were run in either the GeneAmp® 2400, 9600, or 9700 thermocyclers (Applied Biosystems, Foster City, CA) under the following cycling parameters: eight minutes denaturation at 94°C followed by repeated cycles of 94°C denaturation for 30 seconds, 60°C annealing for 30 seconds, and 72°C extension for 30 seconds, concluding with a seven minute final extension at 72°C. The IVS8-1G→C and T93M PCR assays were run for 32 cycles, and 40 cycles were used for the W151X, V326L, and R404C assays. The PCR products were analysed by electrophoresis on 8% non-denaturing polyacrylamide gels and visualised by ethidium bromide staining and UV fluorescence.

Sequencing strategy

For comprehensive nucleotide sequencing, the *DHCR7* exons and exon/intron boundaries were amplified as four fragments using the following primer sets: exons 3–4, forward primer 5'-GTG GAT GCA ACA GGG AAA GGTG-3', reverse primer 5'-GTG CCA GAC AAA TGG AAG GAC TAC-3'; exons 5–6, forward primer 5'-ATT CTC ATG TTC CTG CTA TGC GTC-3', reverse primer 5'-GCC ATC TCC AGC TGC ATT CTT GC-3'; exons 7–8, forward primer 5'-TCT GCT GAG CAG CAG CTG AATG-3', reverse primer 5'-TCT GCC CTA CTT TGC AGA TGT TAG-3'; exon 9, forward primer 5'-CAC AAC AGC CCT CAA GGT TGG TG-3', reverse primer 5'-TGG CAG AAC ACG CTC TTG ACA GC-3'. The PCR products were sequenced using internal primers, the BigDye® Terminator Cycle Sequencing kit, and the ABI PRISM® 310 automated fluorescent sequencer (Applied Biosystems, Foster City, CA). The entire coding region of the *DHCR7* gene can be covered in eight sequencing reactions. This approach is capable of detecting every known *DHCR7* mutation, plus the eight SNPs that have been used to define *DHCR7* haplotypes.¹³

Carrier screening

DNA samples from almost 3000 people were screened for the five most common *DHCR7* mutant alleles using the ARMS assays described above. The samples were from anonymous

subjects belonging to various ethnic groups, the majority of whom were white, African and Caribbean Blacks, and south east Asians. Smaller numbers of east Indians, Middle Easterners, and North American Aboriginals were also included. Samples were screened in pools as previously described.¹¹ Briefly, pools of 30 samples were screened using the five ARMS assays and the individual constituents of each positive pool were then screened to identify the positive samples.

RESULTS AND DISCUSSION

We have used allele specific assays and direct nucleotide sequencing to identify the *DHCR7* mutations in 30 unrelated SLOS patients and their families. There were 22 different genotypes involving 16 different mutations, two of which have not been previously reported (W248R and Y280C).¹⁵ There was only one patient for whom only one mutation was identified. It is possible that this patient has a yet to be identified mutation in another region of the *DHCR7* gene, such as the introns or 5' untranslated regions.

The IVS8-1G→C mutation was by far the most common *DHCR7* mutant allele, as has been observed for SLOS patients from elsewhere in North America and Western Europe. The IVS8-1G→C allele was represented in two-thirds of the unrelated probands tested and had an observed frequency of 0.40 (table 2). The next two most common mutations observed in the Canadian patients were R404C (0.17) and T93M (0.08), mutations that have previously been identified within Acadians (French-Canadians originating in Nova Scotia and related to Louisiana Cajuns) and Italian SLOS patients, respectively. All of our families with R404C were of French-Canadian background and the mutation was predominantly associated with haplotype F (table 2). Several of the families with the T93M allele were of Mediterranean descent (Italian, Greek). This mutation was associated with a single haplotype that has not previously been reported (designated J).

We have taken two approaches to determine the incidence of SLOS in Canada. First, we are conducting a national surveillance programme in which paediatricians, who are members of the Canadian Paediatric Society, and related caregivers are asked to identify potential SLOS cases. To date, the programme has identified 20 confirmed cases of SLOS. Based on census data and current birth rates, the overall incidence of SLOS is 1 in 29 000 in the Canadian population, and

Table 3 Carrier frequencies of the five most common *DHCR7* mutations determined by screening 2865 anonymous DNA samples of known ethnic backgrounds

Mutation	White (n=1557)	Black (n=504)	Asian (n=475)	Others* (n=329)
IVS8-1G→C	17	4	0	0
T93M	0	0	0	0
W151X	2	0	0	0
V326L	0	0	0	0
R404C	0	0	0	0
Total	19	4	0	0
Carrier frequency (95% CI)	1.22% (0.50-2.32%)	0.79% (0.56-1.03%)		

*East Indian (215), Middle Eastern (94), North American Aboriginal (20).

somewhat greater (1 in 22 000) in the sector of the population that is of European descent.¹⁶⁻¹⁷ This corresponds to a predicted carrier rate of approximately 1 in 74 for Canadians of European descent.

We have also approached the incidence issue by investigating the population carrier frequencies for the most common SLOS mutations (table 3).¹¹ We identified 17 carriers of IVS8-1G→C and two carriers of W151X in our white population sample (n=1557), but failed to detect any carriers of T93M, V326L, or R404C. This is markedly different from the mutation distribution of our SLOS patients, for which IVS8-1G→C comprised only 40% of the mutant alleles. This discrepancy may in part be because of differences between our white population sample and the patient group. For example, French-Canadians are over-represented in the patient sample relative to our population sample, perhaps accounting for the failure to detect R404C in the population sample.

Rather than representing 40% of the *DHCR7* mutations in the white population, the IVS8-1G→C mutation may actually account for the majority of all *DHCR7* alleles. Surveys of SLOS patients have shown a deficiency of the observed versus expected number of null/null homozygotes and a corresponding excess of null/missense compound heterozygotes.¹⁹ Based on these observations, Kelly and Herman¹⁹ have suggested that null mutations such as IVS8-1G→C and W151X may account for up to 80% of the *DHCR7* mutant alleles.¹⁹ Applying the 80% factor to our empirical data for IVS8-1G→C and W151X carriers among whites (19/1557 or 1 in 82), the carrier rate for all *DHCR7* mutations would be 1 in 66. This, in turn, corresponds to a birth incidence of approximately 1 in 17 000. Ascertainment bias may account for this discrepancy between the predicted and observed incidence of SLOS, in that homozygosity for null mutations may result in fetal death and resultant misdiagnosis in a significant proportion of affected pregnancies. Moreover, mild SLOS cases may remain undiagnosed.

To date, most studies of SLOS have dealt with whites of North American and European origins. We have screened subjects of different ethnic groups for the five most common *DHCR7* mutations found among SLOS patients. Four carriers of the IVS8-1G→C mutation were found in our black population sample, corresponding to an estimated carrier frequency of 0.79%. Subsequent haplotype analysis of these four subjects showed that the IVS8-1G→C mutation is found on haplotype A. This is the most common white haplotype and is also the haplotype associated with the IVS8-1G→C mutation in white populations. Haplotype A is not a common *DHCR7* haplotype among African blacks (our unpublished data), suggesting that population admixture is the most likely explanation for the observation of IVS8-1G→C carriers in our black population sample. The extent to which SLOS occurs in other population groups and the underlying spectra of *DHCR7* mutations remains to be determined.

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