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R352Q mutation of the *DHCR7* gene is common among Japanese Smith–Lemli–Opitz syndrome patients

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Abstract Smith–Lemli–Opitz syndrome (SLOS) is an autosomal recessive malformation syndrome characterized by microcephaly, syndactyly of toes, ambiguous genitalia, and mental retardation. The underlying *DHCR7* gene has been identified and a wide variety of distinct mutations were reported in USA and European SLOS patients. A significant difference has been suggested in the frequency of SLOS among different ethnic populations. Here, we report mutational analysis of seven Japanese SLOS patients. Five mutations, R352Q, R242H, G303R, X476Q, and S192F, were identified, and R352Q appeared most frequent, since nine out of the 13 mutations of Japanese origin were the same R352Q. These results suggest that R352Q is a predominant founder mutation in Japanese SLOS patients.

Keywords Smith–Lemli–Opitz syndrome · *DHCR7* · Mutation analysis · Founder mutation · Malformation syndrome

Introduction

Smith–Lemli–Opitz syndrome [SLOS; also known as RSH syndrome (MIM 270400)] is an autosomal recessive malformation syndrome characterized by a wide variety of congenital anomalies, including microcephaly, cleft palate, syndactyly of toes, polydactyly, visceral malformations, variable structural anomalies of the heart, ambiguous genitalia, and mental retardation. On the basis of severity of clinical symptoms, SLOS patients have been divided into two subgroups; a mild form, SLOS I, and a severe form, SLOS II. However, SLOS was found to be a disorder with a continuous spectrum of severity caused by a single biochemical defect, and the two forms represent two extremes of a single disorder.

Affected patients have elevated serum 7-dehydrocholesterol (7-DHC) levels and typically have reduced serum cholesterol levels (Cunniff et al. 1997; Tint et al. 1995). The 7-DHC reductase (*DHCR7*) gene, which

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encodes the enzyme responsible for converting 7-DHC to cholesterol, has been cloned (Moebius et al. 1998) and was found to be mutated in SLOS patients (Fitzky et al. 1998; Wassif et al. 1998; Waterham et al. 1998). The *DHCR7* gene spans 14 kb and consists of two 5' non-coding exons and seven coding exons (Fitzky et al. 1998). The *DHCR7* protein is localized in the membrane of the endoplasmic reticulum, and contains nine putative transmembrane domains, a large intracellular loop (the fourth cytoplasmic loop) and a highly conserved C-terminal domain (Fitzky et al. 1998; Moebius et al. 1998).

SLOS is estimated to be most common among European Caucasians, with an incidence of 1 in 20,000–30,000 births, and more than 80 different mutations have been reported, approximately 85% of which are missense mutations associated with reduced levels of *DHCR7* activity (Nowaczyk and Waye 2001). In contrast, it has an apparently lower incidence in Asian and African populations (Cunniff et al. 1997; Kelley 1997; Opitz 1994), and the genetic defects in these ethnic populations are poorly understood. We previously reported that SLOS might have a low frequency in the Japanese populations (Tsukahara et al. 1998). In this study, we searched for mutations in the *DHCR7* gene in seven unrelated Japanese patients, and found that the R352Q mutation most likely represents a Japanese founder mutation.

Materials and methods

Lymphoblastoid cell lines from seven patients (96003, 97021, 99010, 02003, 04002, 04004, and 04005) and their first-degree relatives were used for this study. All families were unrelated and non-consanguineous, and all the members were Japanese except for a Dutch father of

patient 97021. In all the index cases, the diagnosis was clinically made and biochemically confirmed (Table 1). Serum cholesterol and 7-DHC were measured by gas chromatography/mass spectrometry analysis (Table 2). Six patients were diagnosed as a mild form of SLOS I, and patient 99010 was diagnosed as a severe form of SLOS II, since he died at 5 months of age due to hepatic failure.

Total RNAs were extracted from the lymphoblastoid cell lines using a TRIZOL Reagent (Invitrogen). cDNA was synthesized from 10 µg of total RNA with 10 units of Moloney murine leukemia virus reverse transcriptase and 500 ng of random hexamer primers/µl. The *DHCR7* cDNA that includes the 1,425-bp ORF was PCR-amplified into three overlapping segments using the primer pairs (1F, ACTTTAGCCGTTGAGAAGGAT; 1R, CGATGCCCATCATGTAGTTGTA; 2F, GTCACCTTCCAGGTGCTTCT; 2R, ACAGGTCCTTCTGGTGGTTG; 3F, GAAGTGGTTTGACTTCAAGCTG; 3R, CAGAGCGTGATTAGGTACTGGA). The RT-PCR products were directly sequenced. The frequencies of the mutations in Japanese population were determined in 184 chromosomes of unrelated Japanese individuals.

Results

Five mutations were detected in seven SLOS patients, and their parents were found to be heterozygous carriers for the mutation (Table 2). Patients 96003 and 02003 had a homozygous G → A transition at position 1,055 in exon 9, resulting in an Arg to Gln substitution at the 352nd residue (R352Q). Patient 97021 had the same heterozygous R352Q mutation and a heterozygous 725G → A substitution, which results in an Arg to His

Table 1 Clinical symptoms of SLOS patients

	96003	97021	99010	02003	04002	04004	04005
Microcephaly	+	+	+	+	+	+	+
Prenatal growth retardation	–	+	+	–	+	–	+
Characteristic facial appearance	+	+	+	+	+	+	+
Cleft palate	+	–	–	–	+	+	–
Syndactyly of the 2nd and 3rd toes	+	+	+	+	+	+	+
Sexual ambiguity	–	–	+	–	–	–	+
Pyrolic stenosis	–	+	–	–	–	–	–

Table 2 Plasma sterol concentrations and mutations in SLOS patients (ND not determined)

Patient	Sex	Age in years	Cholesterol (mg/dl)	7-DHC (mg/dl)	Mutation	Effect on protein
96003	M	19	48	15.4	1,055G → A/1,055G → A	R352Q/R352Q
97021	M	5	107	35.0	1,055G → A/725G → A	R352Q/R242H
99010	M	1 day	34	ND	1,055G → A/907G → A	R352Q/G303R
02003	M	10 day	54	9.7	1,055G → A/1,055G → A	R352Q/R352Q
04002	F	14	79	11.9	1,055G → A/1,426T → C	R352Q/X476Q
04004	F	3 day	64	28.4	1,055G → A/907G → A	R352Q/G303R
04005	M	10	55	13.6	1,055G → A/575C → T	R352Q/S192F

at the 242nd residue (R242H). The R242H allele was derived from his Dutch father. Patients 99010 and 04004 were a compound heterozygote for the R352Q mutation and a 907G → A substitution, which results in a Gly to Arg at the 303rd residue (G303R). Patient 04002 was a compound heterozygote for the R352Q and a 1,426T → C substitution, which results in the disruption of a termination codon (X476Q). Patient 04005 had the R352Q mutation and a 575C → T substitution, which results in a Ser to Phe at 192nd residue (S192F). The S192F, G303R, and X476Q mutations were not detected in 184 chromosomes of normal Japanese individuals. We identified single nucleotide polymorphisms, 189G/A, 207T/C, 438C/T, 1,158C/T, 1,272T/C (Fitzky et al. 1998), and the nine alleles with the R352Q mutation showed a conserved haplotype of 189G-207C-438C-1,158C-1,272C.

Discussion

More than 80 different mutations have been reported in SLOS patients from the USA and Europe (Nowaczyk et al. 2001; Witsch-Baumgartner et al. 2005). Three mutations account for over 50% of those observed: IVS8-1G → C, T93M, and V326L (Yu et al. 2000a). Among them, IVS8-1G → C was most common in European SLOS patients (Nowaczyk et al. 2001; Witsch-Baumgartner et al. 2001; Yu et al. 2000b). In contrast, this mutation has not been detected in any of 95 Chinese and 103 Japanese individuals (Yu et al. 2000b) and not in 475 individuals in Asian (Nowaczyk et al. 2001). These data suggest that the different mutations may distribute in Asian populations.

We have identified five mutations in seven SLOS patients. The R242H, R352Q, and S192F mutations have been reported in non-Japanese populations (Krakowiak et al. 2000; Witsch-Baumgartner et al. 2000, 2005), whereas G303R and X476Q were previously undescribed mutations. The S192F, R242H, G303R and R352Q mutations are located within the fourth, the fifth, the seventh, and the eighth transmembrane domains, all of which represent a highly conserved sterol-sensing domain (Bae et al. 1999; Krakowiak et al. 2000). The R242H and R352Q both likely affect DHCR7 protein stability, since functional analysis, by expression of similar mutations (R242C and R352W) in a human cell line, demonstrated that the mutations resulted in reduced protein expression (<3%) (Fitzky et al. 1998; Witsch-Baumgartner et al. 2000). The S192F mutation has recently been reported in a European patient with mild severity (Witsch-Baumgartner et al. 2005). The G303R newly identified in two patients 99010 and 04004 is likely pathogenic because it was heterozygous in the patients and their parents, but absent in 184 control chromosomes, and the 303rd residue is conserved in the orthologues of cattle, mouse, rat, and zebrafish. Patient 99010 was diagnosed as a severe form of SLOS II, whereas patient 04004 showed a mild form of SLOS I.

We, therefore, assumed that the G303R mutation, if combined with unknown genetic or environmental factors, could be associated with severe clinical phenotypes. The X476Q mutation we identified is also likely pathogenic, since this substitution results in a 51-amino-acids addition to the C-terminal end of DHCR7, and is absent in 184 control chromosomes.

Out of five mutations, R352Q is the most common in Japanese SLOS patients, since nine out of the 13 mutations were the same R352Q mutation, and R242H allele of patient 97027 was derived from his Dutch father. Furthermore, the nine alleles with the R352Q mutation showed a conserved haplotype in the *DHCR7* locus. The presence of the R352Q mutation and the conserved haplotype in each of the unrelated patients suggests that the spread of this mutation by founder effect in Japan would play an important role for Japanese SLOS patients.

In summary, we identified the common R352Q mutation in seven unrelated Japanese patients, suggesting that this is the founder mutation in Japanese SLOS patients. Continued screening for the *DHCR7* gene may provide a better understanding of the genetic background of Japanese SLOS patients.

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