

DATA REPORT

Novel *DHCR7* mutation in a case of Smith–Lemli–Opitz syndrome showing 46,XY disorder of sex developmentMayuko Tamura^{1,2}, Tsuyoshi Isojima¹, Takeshi Kasama³, Ryo Mafune¹, Konomi Shimoda¹, Hiroki Yasudo¹, Hiroyuki Tanaka¹, Chie Takahashi¹, Akira Oka¹ and Sachiko Kitanaka¹

Smith–Lemli–Opitz syndrome is an autosomal recessive disease caused by mutations in 7-dehydrocholesterol reductase (*DHCR7*), which is rarely observed in Japan. We report a Japanese case with 46,XY disorder of sex development and Y-shaped 2–3 toe syndactyly. *DHCR7* gene analysis revealed compound heterozygous mutations including the novel mutation H442R. Early diagnosis led to starting cholesterol treatment at an early age.

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Smith–Lemli–Opitz syndrome (SLOS, OMIM #270400) is an autosomal recessive inheritance disease characterized by multiple anomaly, failure to thrive and intellectual disability.^{1–3} SLOS is caused by defective function of 7-dehydrocholesterol reductase (*DHCR7*), which converts 7-dehydrocholesterol (7-DHC) to cholesterol.⁴ Thus far, over 140 *DHCR7* mutations have been reported.^{5,6}

Because of defective cholesterol synthesis, serum cholesterol levels are reduced in patients, and 7-DHC and 8-dehydrocholesterol tend to accumulate. These abnormalities in cholesterol synthesis may cause various problems during the fetal period, including dysmorphic facial features, hypotonicity, microcephaly, Y-shaped 2–3 toe syndactyly, 46,XY disorder of sex development (DSD) and congenital heart diseases.³ Y-shaped 2–3 toe syndactyly, referring to incomplete cutaneous syndactyly of the second and third toes, is a unique SLOS symptom.² However, the other phenotypes show a wide spectrum of severity, ranging from mild to severe in nature. Clinical severity is thought to depend on the extent that the mutation impairs *DHCR7* function.⁵

Cholesterol treatment is beneficial for neurological development and neuropsychological symptom treatment, and is widely used in Europe.^{7,8} However, the reports of cholesterol treatment for SLOS in Japan are limited. We report a Japanese SLOS case with a novel mutation in *DHCR7* who was diagnosed early in life with 46,XY DSD and Y-shaped 2–3 toe syndactyly, and was treated with cholesterol.

The patient was born at 38 weeks of gestation to non-consanguineous Japanese parents. Birth weight, length and head circumference were 2,595 g (−0.92 s.d.), 45 cm (−1.7 s.d.) and 33 cm (−0.1 s.d.), respectively. Ambiguous genitalia, low set ears, a short nose and Y-shaped 2–3 toe syndactyly were detected at birth (Figure 1a and b). Examination of the patient's genitalia revealed a micropenis (stretch length: 1.5 cm), left lateral testicular hypoplasia (right and left diameters: 10 and 5 mm, respectively), hypospadias and a bifid scrotum (Figure 1c). Differential diagnosis of DSD was performed. Echography and magnetic resonance imaging failed to detect the presence of a uterus or ovaries. Blood examination revealed a relatively low total cholesterol (T. Chol)

level (46 mg/dl) (reference: 129–232), normal testosterone and slightly low anti-mullerian hormone level for males. Urine steroid profile for detection of defects in steroid hormone and testosterone synthesis was normal. G-banding analysis confirmed a normal 46,XY male karyotype. From these results, the patient was recorded in public office documents as male. The 46,XY DSD and the observation that his phenotype included syndactyly suggested that he had SLOS. We performed cholesterol analysis with high-performance liquid chromatography. This analysis revealed relative hypocholesterolemia and high 7-DHC and 8-dehydrocholesterol levels in the patient and normal levels (undetectable) in his parents (Table 1). T. Chol/7-DHC was significantly lower in the patient than in his parents. Therefore, he was biochemically diagnosed with SLOS. The patient also presented with allergies for milk and eggs, and eczema throughout his body. His eczema, considered to be atopic dermatitis, was treated with corticosteroid ointment.

To reveal the genetic background of his disease, we performed *DHCR7* gene analysis of the patient (age: 2 months) and his parents. We obtained written informed consent from the parents for DNA analysis and approval from the ethics committee of the University of Tokyo. The analysis revealed two heterozygous missense mutations, c.907G>A, p.Gly303Arg (G303R) and c.1325A>G, p.His442Arg (H442R) (Figure 2a). G303R, located on exon 8, had been reported in a Japanese SLOS case.⁹ However, H442R represented a novel missense mutation located on exon 9 and was not found in a single-nucleotide polymorphism database of 934 Japanese people nor in the single-nucleotide polymorphism database of the 1,000 Genome Project.¹⁰ *In silico* functional prediction using PolyPhen 2¹¹ and Mutation Taster¹² indicated that the H442R substitution was 'probably damaging' and 'disease causing', respectively. Genetic analysis of the parents revealed that his father and mother carried G303R and H442R, respectively, in a heterozygous manner (Figure 2a).

Although oral cholesterol is widely used in Europe, cholesterol powder is not an approved medical drug in Japan. However, for our patient, we obtained approval from the ethics committee of Tokyo University and informed consent from his parents to begin

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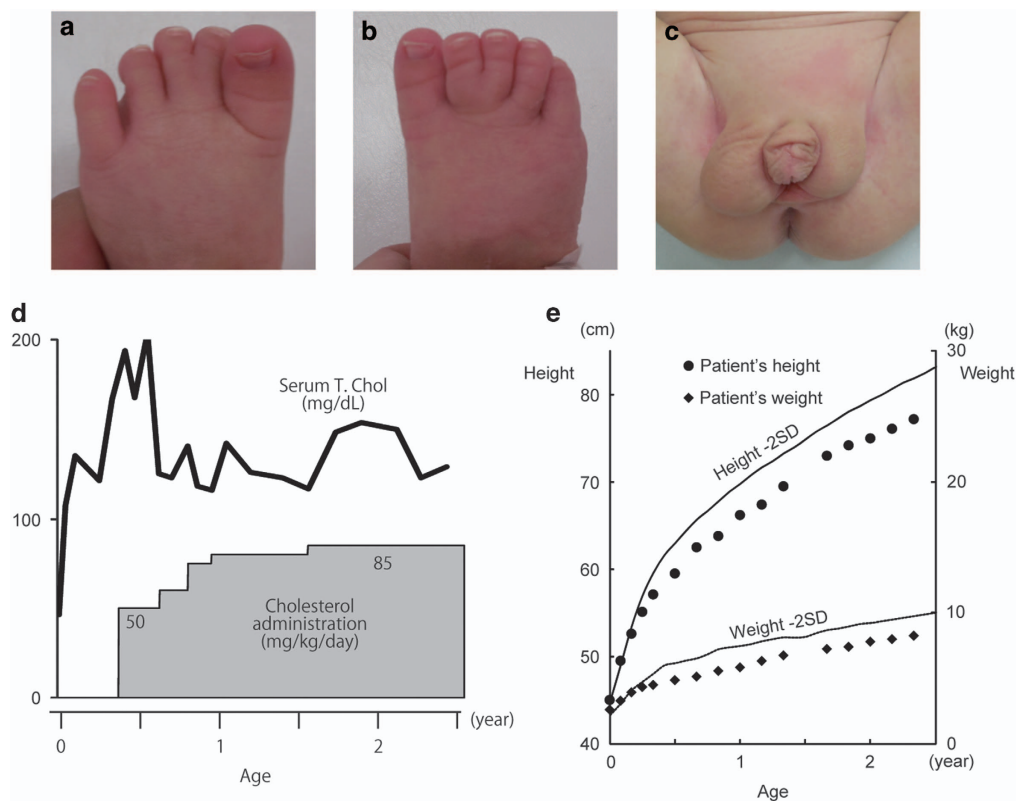


Figure 1. Clinical appearance of the patient. (a, b) Bilateral Y-shaped 2–3 toe syndactyly. (c) Ambiguous genitalia, including micropenis, left lateral testicular hypoplasia, hypospadias and a bifid scrotum. (d) Chart depicting the cholesterol dose and serum T. Chol level. (e) Growth curve showing development after treatment initiation. T. Chol, total cholesterol.

Table 1. HPLC cholesterol analysis of the patient and his parents

	T. Chol (mg/dl)	7-DHC (mg/dl)	8-DHC (mg/dl)	T. Chol/7-DHC
Patient	79.8	8.0	2.0	9.975
Father	232.7	0.0	0.0	∞
Mother	248.2	0.0	0.0	∞

Abbreviations: 7-DHC, 7-dehydrocholesterol; 8-DHC, 8-dehydrocholesterol; HPLC, high-performance liquid chromatography; T. Chol, total cholesterol. Cholesterol analysis by HPLC of the patient and his parents.

cholesterol treatment at 3 months of age. There are several forms of cholesterol treatment, including cholesterol suspension, yolk oils and egg yolk. Here, we selected cholesterol suspension because of the patient's food allergies. After 3 months of treatment, the T. Chol level increased to the normal range, and the 7-DHC level reduced from 8.0 to 3.8 mg/dl. The cholesterol dose was gradually increased from 50 to 85 mg/kg/day at 2 years (Figure 1d). His height gain followed approximately the -3 s.d. line of the normal growth curve (Figure 1e). Although many SLOS cases exhibit microcephaly, his head circumference also remained at -3 s.d., which is not small compared to his height. At 2 years, his motor and language development, and his social development had reached that normally expected at 1 and 1.5 years of age, respectively. Eczema is often seen in SLOS patients. This patient also had refractory eczema, which was ameliorated after cholesterol treatment together with topical corticosteroid ointment.

We identified a novel mutation H442R of *DHCR7* in a patient with a mild SLOS phenotype. This substitution was not recorded in the single-nucleotide polymorphism database. The H442 residue is highly conserved in mouse, chicken and zebrafish (Figure 2b);

in silico analysis shows that mutations of this residue are predicted to damage protein function. *DHCR7* is composed of 9 exons, and the predicted structure of the protein contains 9 membrane-associated helices (MAH).¹³ H442 is located in exon 9 at the C-terminus (between membrane-associated helices 9 and the 3'untranslated region), the area where other missense mutations have been reported (Figure 2c). The phenotype of a patient with one mutation in the C-terminus region is reportedly mild.¹³ Considering the mild phenotype of our patient, we suspected that *DHCR7* with the H442R mutation exhibited residual enzyme activity. The other mutation, G303R, has been reported in several Japanese cases and is located in exon 8, encoding loop 6–7 within the sterol sensing domain.¹⁴ Symptoms of cases possessing G303R as a compound heterozygote usually present with mild-to-severe phenotypes.⁹ Therefore, we believe that the compound heterozygous mutation caused SLOS.

SLOS incidence is estimated as 1:20,000–1:60,000 in Europe, but it is lower in other areas and is extremely rare in Japan.² Genital anomalies are seen in more than 70% of SLOS patients.² Although the mechanism of genital anomalies in SLOS has not been elucidated, it is suggested that it might be caused by the lack of substrate to produce adrenal and testicular steroids owing to low-cholesterol synthesis.¹⁵ Because the phenotype occurs before birth, cholesterol treatment after birth is unlikely to be beneficial for the genital anomalies. However, early diagnosis and treatment of SLOS is important because cholesterol treatment appears to improve physical and neurological development.^{7,8} In our case, we diagnosed SLOS by differential diagnosis of 46,XY DSD and subsequently confirmed our suspicions by cholesterol analysis and *DHCR7* gene analysis (at 2 months). This enabled treatment at the early age of 3 months. Even though SLOS is a very rare syndrome, it should be considered in a differential diagnosis of 46,XY DSD.

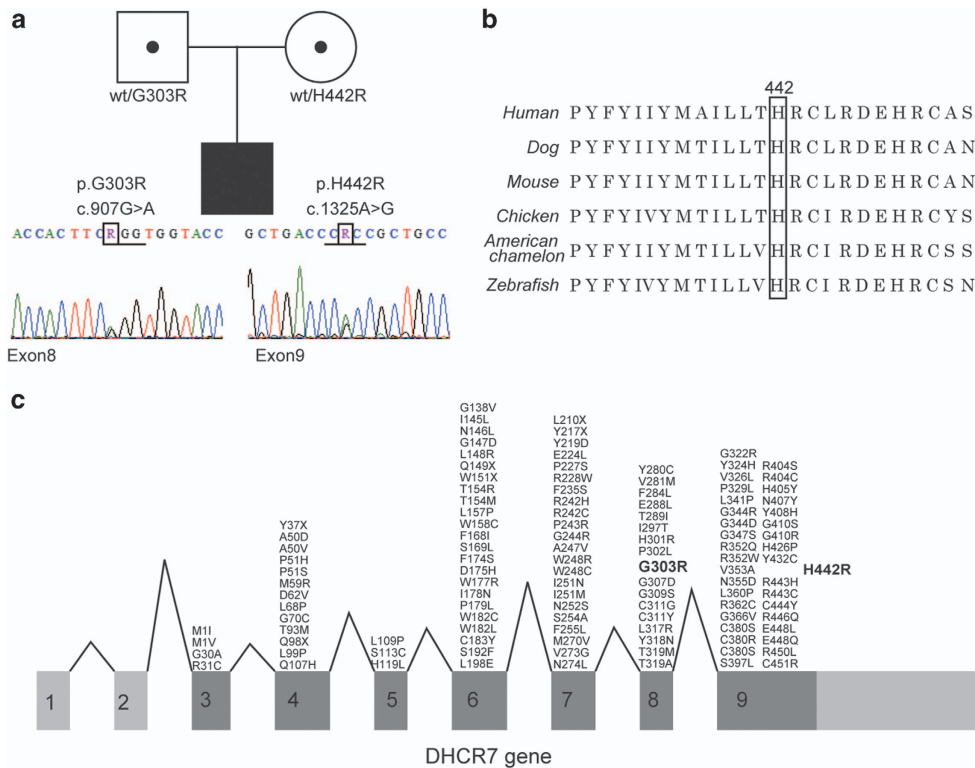


Figure 2. *DHCR7* gene analysis. (a) The patient exhibited two non-synonymous substitutions. G303R, inherited from his father, has been reported previously in Japanese cases; H442R, inherited from his mother, has not been reported previously nor deposited in an established SNP database. Genetic tests showed that both parents were carriers. (b) Homologs of *DHCR7* at the H442 residue, which is highly conserved among different species. (c) *DHCR7* gene structure. Boxes indicate exons and lines indicate introns. H442R was located in a region where other missense and nonsense mutations exist. SNP, single-nucleotide polymorphism.

In SLOS, cholesterol levels vary. Calculating T. Chol/7-DHC allows differentiation between affected (0.1–10) and healthy individuals (>10,000).¹⁶ In our case, the T. Chol level was relatively low, and T. Chol/7-DHC was definitively low compared with that of healthy controls. Consequently, this important ratio was very useful for diagnosing SLOS. The 7-DHC level, which is reported to correlate with severity, was 8.0 mg/dl before treatment. Other reported Japanese cases showed higher 7-DHC levels (9.7–35 mg/dl).⁹ Considering clinical, genetic and laboratory data, this case appeared to be mild compared with other reported cases. Although cholesterol analysis by high-performance liquid chromatography led to the definitive diagnosis of SLOS, it can be conducted only in research institutes. We consider genetic analysis to be more applicable for diagnosis.

Dietary cholesterol treatment is broadly used as a treatment for SLOS worldwide and results in an increase in the serum cholesterol level and decrease in 7-DHC and 8-dehydrocholesterol levels by feedback inhibition.⁷ Elias *et al.*¹⁷ reported that neuropsychological symptoms were ameliorated rapidly with cholesterol treatment. Although our patient had shown postnatal growth failure before treatment, he has maintained growth after cholesterol treatment and has not shown a decline in development in either microcephaly or other neurological symptoms. However, the extent to which cholesterol treatment has contributed to his growth and neurological development is unclear because the natural course of SLOS varies and he was classified to have only mild severity.

In summary, we report a Japanese SLOS case with a novel *DHCR7* mutation, who presented with 46,XY DSD, Y-shaped 2–3 toe syndactyly and a low-T. Chol/7-DHC ratio, and who was treated with cholesterol. SLOS should be considered as a potential

differential diagnosis of 46,XY DSD because early diagnosis leads to early treatment.

ACCESSION CODES

Nucleotide sequence data reported are available in the DDBJ/EMBL/GenBank databases under the accession numbers: LC201749, LC202815 and LC202816.

HGV DATABASE

The relevant data from this Data Report are hosted at the Human Genome Variation Database at <http://dx.doi.org/10.6084/m9.figshare.hgv.1348>.

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COMPETING INTERESTS

The authors declare no conflict of interest.

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