Case Report

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A case of Smith-Lemli-Opitz syndrome confirmed by molecular analysis: Review of mutation spectrum of the *DHCR7* gene in Korea

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Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive disorder caused by 7-dehydrocholesterol reductase deficiency. The characteristic clinical features are syndactyly of the second and third toes, facial dysmorphism, multiple malformations, and intellectual disability. Few cases of SLOS have been reported in Korea. We observed a male patient with SLOS who presented with typical facial features, undescended testes, microcephaly, bilateral syndactyly of the second and third toes, and cardiac defects, including patent ductus arteriosus and atrial septal defect. Mutation analysis of the *DHCR7* gene identified compound heterozygous mutations of c.907G>A (p.Gly303Arg) and c.1055G>A (p.Arg352Gln). In a review of the literature, c.1054C>T (p.Arg352Trp) was the most common mutation reported in Far East Asian countries. This report describes the clinical features, biochemical data, molecular characteristics, and clinical outcome of a Korean patient with SLOS.

Key words: Smith-Lemli-Opitz syndrome, 7-Dehydrocholesterol reductase, *DHCR7*.

Introduction

Smith-Lemli-Opitz syndrome (SLOS) is a rare autosomal recessive disorder with multiple congenital anomalies. It is caused by an inborn error of cholesterol biosynthesis, which results from the deficiency of 7-dehydrocholesterol (7-DHC) reductase enzyme that converts 7-DHC to cholesterol [1-3]. The estimated incidence of SLOS ranged from 1 in 20,000 to 1 in 60,000 in the Caucasian population, and was less common in the Asian population [3]. SLOS is characterized by growth retardation, intellectual disability, and multiple malformations. The most common clinical features are facial dysmorphism (narrow forehead, epicanthal folds, ptosis, short mandible, short nose, anteverted nares, and low-set ears), syndactyly of the second and third toes, microcephaly, hypospadias, cleft palate, and postaxial polydactyly [1,3,4].

Although the diagnosis of SLOS is based on elevated serum 7-DHC and low serum cholesterol levels, the serum cholesterol level can be normal in mild cases [5]. Therefore, mutation analysis of the *DHCR7* gene, which encodes 7-DHC reductase, is useful for confirmatory diagnosis [6].

To date, few cases of SLOS have been reported in Korea [7-12]. We recently encountered a case of SLOS where a male patient presented with typical facial dysmorphism, undescended testes, microcephaly, syndactyly, and cardiac defects, and this was

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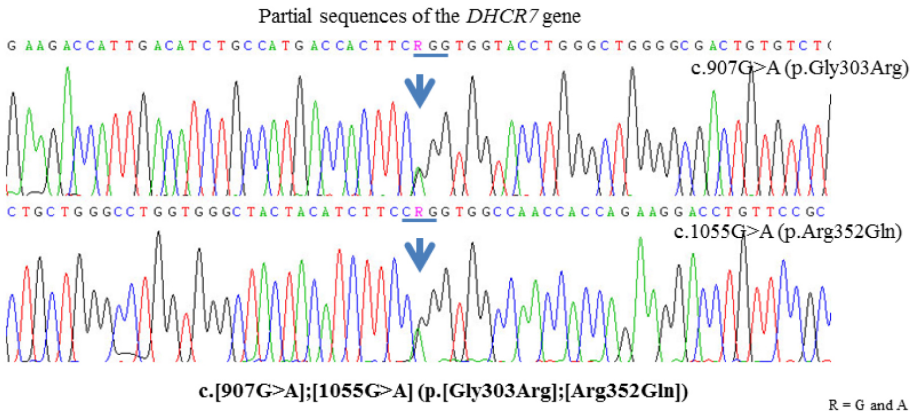

Fig. 1. Direct sequencing of the *DHCR7* gene. Compound heterozygous mutations of c.907G>A (p.Gly303Arg) and c.1055G>A (p.Arg352Gln) were identified, as indicated in blue.

confirmed by molecular analysis of the *DHCR7* gene. Here, we describe the clinical features, biochemical data, and molecular characteristics of this case, with a review of the literature.

Case

The patient was born at 40 weeks of gestation, by cesarean section because of fetal distress and placental abruption. The patient weighed 2,700 g (−1.55 standard deviation score [SDS]) at birth and was the first child born to nonconsanguineous parents. As the triple marker test was abnormal at 16 weeks of gestation, amniocentesis was carried out, and the karyotype was 46,XY.

At birth, the patient displayed transient tachypnea and weak crying; this improved spontaneously after several days. Low-set and large ears, bilateral syndactyly of the second and third toes, a grade 2 systolic murmur at the left sternal border, developmental dysplasia of both hips, and ambiguous genitalia with hypospadias were noted. Patent ductus arteriosus (diameter, 1.5 mm) and a secundum atrial septal defect (ASD; diameter, 6 mm) were identified by echocardiography. Pelvic ultrasonography revealed the right testis in the peritoneum and left testis in the inguinal canal, without a uterus or ovaries. Auditory evoked potential measurements showed a sensorineural hearing defect in both ears (70 dB discrepancy in the right, 100 dB discrepancy in the left). At the age of 3 months, the patient was hospitalized because of pneumonia and pulmonary edema. Upon follow-up echocardiography, an almost common atrium, due to a large ASD (diameter, >8 mm), and a dilated right ventricle were observed. The patient was treated with intravenous antibiotics and diuretics.

The patient was referred to our institute for evaluation of multiple congenital anomalies at 1.6 years of age, when his height, weight, and head circumference were 77.6 cm (−1.81 SDS), 6.3 kg (−5.57 SDS), and 40.5 cm (−4.50 SDS), respectively. He manifested bilateral Y-shaped partial syndactyly of the second and third toes, micropenis, bilateral undescended testes, and facial dysmorphism such as bilateral epicanthic folds, large ears, and strabismus. The serum cholesterol level was 17 mg/dL (normal range, 45-182 mg/dL). Subsequently, he underwent ASD closure surgery using fresh autologous pericardium for a large ASD (diameter, 17.1 mm). Direct sequencing of the *DHCR7* gene, using genomic DNA isolated from peripheral blood leukocytes, identified compound heterozygous mutations of c.907G>A (p.Gly303Arg) and c.1055G>A (p.Arg352Gln) (Fig. 1), which were already reported to be pathogenic [13]. Mutation analysis of the patient's parents was not carried out.

Discussion

This study described a case of SLOS, which was confirmed by molecular analysis of the *DHCR7* gene. Two missense mutations in our case, p.Gly303Arg and p.Arg352Gln, were previously reported to be pathogenic in Japanese SLOS patients [13]. SLOS was first described in 1964 by David Smith, Luc Lemli, and John Opitz, as distinctive facial appearance, microcephaly, broad alveolar ridges, hypospadias, severe feeding disorder, and developmental delay [2]. Tint et al. [14] reported that SLOS patients had an increased plasma concentration of 7-DHC, suggesting a deficiency in the 7-DHC reductase the final step of the cholesterol biosynthesis pathway. In 1998, it was discovered that defects in the *DHCR7* gene located on chromosome 11q13.4 cause SLOS [6].

The *DHCR7* gene contains 9 exons and 8 introns within

Table 1. Previously reported patients with Smith-Lemli-Opitz syndrome in Korea

Case	Sex	Facial dysmorphism	Syndactyly of the 2nd and 3rd toes	Genitourinary anomaly	Heart defect	Other findings	Cholesterol (mg/dL)	7-DHC[a] (μg/mL)	Mutation
Present case	Male	Microcephaly, epicanthic folds, large ears, strabismus	Bilateral	Micropenis, undescended testes, hypospadias	Atrial septal defect	Developmental dysplasia of hip, sensorineural hearing defect	17	-	p.Gly303Arg and p.Arg352Gln
Ko et al., 2010 [7]	Male	Microcephaly micrognathia, small nose, anteverted nares, cleft palate, ptosis	Bilateral	Ambiguous genitalia	Patent ductus arteriosus, atrial septal defect	Hypertrophic pyloric stenosis, neonatal cholestasis, failure to thrive, generalized tonic seizure, hypotonia	21	567	p.Pro227Ser and p.Gly303Arg
Lee et al., 2010 [8]	Female	Hypertelorism, long cilia, low-set ears, short nasal root with anteverted nares, cleft palate, bifid uvula	Bilateral	-	-	Failure to thrive, feeding intolerance, irritability, sleep disorder	64	3,493	p.Gly303Arg and p.Arg352Trp
Park et al., 2008 [9]	Male	Flat supraorbital ridge, cleft lip and palate, micrognathia	-	Ambiguous genitalia, undescended testes	Tetralogy of Fallot with severe pulmonary atresia	Club foot, postaxial polydactyly of toes, cholestasis, cataract, feeding intolerance	12	-	p.Arg352Gln homozygote
Chae et al., 2007 [10]	Female	Cleft palate, ptosis, short nasal root	Bilateral	-	-	Failure to thrive, developmental delay, feeding intolerance, hypotonia	47-63	176	p.Lys376Argfs*37 and p.Arg352Trp
Ko, 2014 [11]	Female	Microcephaly, short nasal root with anteverted nares, bifid uvula	Bilateral	-	-	Failure to thrive	107	-	p.Arg352Trp homozygote
Ko, 2014 [11]	Male	Microcephaly, cleft palate, short nasal root with anteverted nares, micrognathia	-	-	-	Developmental delay, feeding intolerance, failure to thrive	119	-	p.Arg352Trp homozygote
Jeong et al., 2014 [12]	Female	Microcephaly, ptosis, epicanthic folds, cleft palate, micrognathia	Bilateral	-	-	Feeding intolerance, ventriculomegaly	64	3,493	p.Gly303Arg and p.Arg352Trp

[a]7-DHC, 7-dehydrocholesterol.

14,100 base pairs. The 7-DHC reductase is encoded by exons 3-9 [15]. Thus far, 165 mutations in the *DHCR7* gene have been reported in the Human Gene Mutation Database (http://www.hgmd.org/), and the majority are missense mutations. Among these, the splice-site mutation c.832-1G>C (IVS8-1G>C) is the most common (allele frequency: ~30%) in Caucasians [16,17]. In contrast, there have been reports of the c.832-1G>C mutation in Korean patients with SLOS [7-12]. Thus far, 8 cases of SLOS have been reported in Korea, including the present case [7-12]. The clinical and molecular findings of previously reported cases of Korean patients with SLOS are summarized in Table 1. All cases were confirmed by mutation analysis of the *DHCR7* gene. Among these cases, the c.1054C>T (p.Arg352Trp) mutation was the most common (43.75%, 7/16), followed by c.907G>A (p.Gly303Arg) (25.00%, 4/16), c.1055G>A (p.Arg352Gln) (18.75%, 3/16), c.679C>T (p.Pro227Ser) (6.25%, 1/16), and c.1128delA (p.Leu376Argfs*37) (6.3%, 1/16) [7-12]. The c.1055G>A (p.Arg352Gln) mutation is the most common mutation in Japanese patients [13], whereas the p.Arg352Gln mutation has been reported in a few Caucasian patients [16]. Overall, there is a difference in the mutation spectrum of the *DHCR7* gene according to ethnicity, suggesting a founder effect.

Although it is difficult to establish a correlation between genotype and phenotype, some genotypes are known to cause a severe phenotype. Witsch-Baumgartner et al. [15] grouped mutations into 4 classes: nonsense and splice-site mutations resulting in putative null alleles, missense mutations in the transmembrane domain (TM), mutations in the fourth cytoplasmic loop (4L), and mutations in the carboxy-terminal endoplasmic reticulum domain (CT). They reported that patients with null and 4L mutations had severe clinical phenotypes, while patients with TM and CT mutations had mild clinical phenotypes. Both the c.907G>A (p.Gly303Arg) and c.1055G>A (p.Arg352Gln) mutations in our case are located in the TM region, indicating a mild clinical phenotype.

Common clinical findings in SLOS were second and third toe syndactyly, developmental delay, microcephaly, and postnatal growth retardation; each finding was observed in at least 80% of SLOS patients. Approximately 50-60% of SLOS patients had genital anomalies and congenital heart defects, including the case reported here [3,18]. The serum concentration of cholesterol is helpful for the diagnosis of SLOS, because most affected infants have hypocholesterolemia (<40 mg/dL). However, the serum cholesterol value can be normal in approximately 10% of SLOS patients [5]. Therefore, high serum concentration of 7-DHC is an important clue for the diagnosis of SLOS [3], and mutation analysis of the *DHCR7* gene is helpful for confirmatory diagnosis.

Although therapeutic options for SLOS are limited, cholesterol supplementation might improve clinical symptoms such as irritability, hyperactivity, sleep disorders, and growth retardation. Supplementation of dietary cholesterol not only raises blood cholesterol to normal levels, but also often prevents the accumulation of 7-DHC via feedback inhibition. The estimated daily cholesterol requirement during infancy is 30-40 mg · kg^{-1} · day^{-1}, which decreases to 10 mg · kg^{-1} · day^{-1} in adults; experimental treatment protocols included 30 mg · kg^{-1} · day^{-1} of egg yolk or 150-300 mg · kg^{-1} · day^{-1} of cholesterol suspension [3,19].

Low maternal concentration of unconjugated estriol could suggest the possibility of SLOS [18]. Fetal ultrasound findings of intrauterine growth retardation; major malformation of the brain, heart, kidneys, or limbs; and ambiguous genitalia may be helpful for prenatal diagnosis of SLOS. When SLOS is suspected, the elevated 7-DHC and/or decreased cholesterol level in the amniotic fluid is a helpful biomarker for diagnosis. In addition, molecular sequencing of the *DHCR7* gene, using chorionic villus tissue or amniocytes, can be considered for prenatal diagnosis [1,5,20].

In conclusion, we have reported the case of a patient with SLOS who carried heterozygous mutations of c.907G>A (p.Gly303Arg) and c.1055G>A (p.Arg352Gln) in the *DHCR7* gene, and described the mutation spectrum of *DHCR7* in Korea via a review of available literature. As the clinical features and biochemical findings of SLOS are variable, molecular analysis of the *DHCR7* gene is useful for confirmation of its diagnosis.

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