Korean Journal of Pediatrics Vol. 51, No. 11, 2008
DOI : 10.3345/kjp.2008.51.11.1236
Case report

A case of Smith-Lemli-Opitz syndrome diagnosed by identification of mutations in the 7-dehydrocholesterol reductase (*DHCR7*) gene

Mee Rim Park, M.D., Jung Min Ko, M.D., Chong-Keun Cheon, M.D.
Gu-Hwan Kim, Ph.D.* and Han-Wook Yoo

Department of Pediatrics, Medical Genetics Clinic and Laboratory*,
Asan Medical Center, University of Ulsan, College of Medicine, Seoul, Korea

= Abstract =

Smith-Lemli-Opitz syndrome (SLOS) is a rare, autosomal recessive disease caused by an inborn error in cholesterol synthesis. Patients with this disease suffer from multiple malformations due to reduced activity of 7-dehydrocholesterol reductase (*DHCR7*), which increases 7-dehydrocholesterol (7DHC) and 8-dehydrocholesterol (8DHC) concentrations and decreases cholesterol concentration in body fluids and tissue. The SLOS phenotypic spectrum ranges from a mild disorder with behavioral and learning problems to a lethal disease characterized by multiple malformations. Here, we describe a newborn male with ambiguous genitalia who was diagnosed to have type II SLOS during the neonatal period. A clinical examination revealed low levels of unconjugated estriol in the maternal serum, and a variety of fetal ultrasound anomalies, including prenatal growth retardation. After birth, the infant was diagnosed to have congenital heart disease (Tetralogy of Fallot with severe pulmonary artery stenosis), cleft lip and palate, micrognathia, postaxial polydactyly, ambiguous genitalia, and cataracts. Clinical investigation revealed extremely low plasma cholesterol levels and the presence of mutation (homozygote of p.Arg352Gln) in the *DHCR7* gene. The patient underwent palliative heart surgery (to widen the pulmonary artery) and received intravenous lipid supplementation. Cholesterol levels increased slightly, but not to normal values. The patient died from cardiopulmonary failure and sepsis 72 days after birth. This report provides the first description of a Korean patient with SLOS confirmed by verification of *DHCR7* gene mutation and illustrates the need for early recognition and appropriate diagnosis of this disease.
(Korean J Pediatr 2008;51:1236-1240)

Key Words : Smith-Lemli-Opitz syndrome, Multiple malformations, 7-dehydrocholesterol reductase, Cholesterol

Introduction

Smith-Lemli-Opitz syndrome (SLOS; OMIM 270400) is an autosomal recessive disorder caused by mutations of the gene encoding 7-dehydrocholesterol reductase (*DHCR7*), which is located on chromosome 11q13[1]. Although this disease affects many ethnic groups, it appears to be more prevalent among Caucasians, with an estimated prevalence ranging from 1 in 20,000 to 1 in 60,000 individuals[2]. SLOS is characterized by faulty cholesterol biosynthesis, which can lead to mental retardation, growth retardation, facial abnormalities and various other congenital anomalies of the genitourinary and gastrointestinal tracts. The first report of this syndrome in 1964[1] was followed by descriptions of more than 700 patients who suffered from a broad range of symptoms, including isolated syndactyly of the toes, holoprosencephaly and the development of multiple visceral anomalies in utero[3]. Historically, clinicians have distinguished the relatively mild symptoms resulting from the classic SLOS (type I) from the more severe manifestations of this disease (type II).

The major malformations of SLOS stem from malfunctions in cholesterol biosynthesis[4]. Defects in the *DHCR7* gene, which catalyzes the final step in cholesterol biosynthesis, can block the synthesis of plasma cholesterol from 7DHC and lead to the accumulation of 7-dehydrocholesterol (7DHC) and its spontaneous isomer, 8-dehydrocholesterol (8DHC), in body tissues. Plasma cholesterol concentrations are often inversely correlated with the severity of the disease, while a minor relationship is observed between severity and 7DHC concentrations[5].

Received : 12 June 2008, Revised : 20 August 2008,
Accepted : 10 September 2008
Address for correspondence : Han-Wook Yoo, M.D., Ph.D.
Department of Pediatrics, Asan Medical Center, Pungnap-2 Dong, Songpa-Gu, Seoul 138-736, Korea
Tel : +82.2-3010-3374, Fax : +82.2-473-3725
E-mail : hwyoo@amc.seoul.kr

SLOS is diagnosed on the basis of elevated 7DHC levels, which are typically measured in plasma or tissue samples. *DHCR7* mutation analysis can be performed to confirm the diagnosis.

Measurement of unconjugated estriol (uE3) in maternal serum or urine levels is a novel, non-invasive method that can be used to screen for SLOS during pregnancy. Prenatal SLOS can be diagnosed by measuring 7DHC levels in amniotic fluid or chorionic villus samples (CVS), or via direct mutation analysis of DNA isolated from amniocytes or CVS[6].

Here, we describe a pregnancy characterized by low levels of maternal uE3 and abnormal ultrasound findings. A female infant with the 46, XY phenotype was subsequently born and diagnosed with type II SLOS during the neonatal period. The diagnosis was verified by mutation analysis of the *DHCR7* gene, with mutations identified in both alleles. This is the first description of a Korean patient with SLOS who was diagnosed using *DHCR7* mutation analysis.

Case report

A 36 year-old woman (gravida 3, para 1) presented at 30 weeks of gestation with an abnormal antenatal ultrasound evaluation and a screen-positive triple marker test for trisomy 18. An antenatal ultrasound scan of the fetus indicated a possible heart defect (i.e., pulmonary artery atresia with a ventricular septal defect), cleft lip, polydactyly and female genitalia. The maternal serum alphaferoprotein (AFP) level was 0.61 multiples-of-median (MoM), the human chorionic gonadotropin (hCG) level was 0.29 MoM, and the unconjugated estriol (uE3) level was 0.17 MoM. Amniocentesis demonstrated a normal karyotype of 46, XY on chromosome analysis and fluorescence in situ hybridization (FISH) analysis did not detect defects in the TUPLE1 gene. The mother had previously given birth to one healthy son, a half-brother of the fetus, and there was no evidence of consanguinity. A follow-up ultrasound revealed intrauterine growth retardation and similar physical findings as before. The patient was delivered by cesarean section at 38^{+1} weeks of gestation with a weight of 1894 g, a length of 39 cm, and an occipitofrontal head circumference of 29.3 cm; all three dimensions were lower than the third percentile. Apgar scores were seven and eight at 1 and 5 minutes, respectively.

The infant had a shallow orbit at birth, with a flat supraorbital ridge, cleft lip, soft cleft palate, micrognathia, wide-spaced nipples, ambiguous genitalia with undescended testes, club foot, postaxial polydactyly of the toes and skin tags on both of the fifth fingers. The patient had cyanosis with a cardiac murmur. Echocardiography demonstrated tetratology of Fallot with severe pulmonary artery hypoplasia. After 1 hour, the patient required oxygen, as well as prostaglandin E1 (alprostadil) and diuretics to maintain cardiac function.

On the second day after birth, cholesterol level was 12 mg/dL and intravenous lipid supplementation was started. Cholesterol levels increased, but fluctuated between 35 mg/dL and 75 mg/dL. Mutational analysis of the *DHCR7* gene was performed, but laboratory equipment was not available to measure the levels of 7-dehydrocholesterol and 8-dehydrocholesterol.

The patients blood counts and thyroid function remained normal, and there was no evidence of adrenal insufficiency. An ultrasound scan of the patients brain revealed no abnormalities and images of the abdomen revealed testes, as well as normal adrenal glands and kidneys.

Additional clinical problems were detected during hospitalization, including cataracts, cholestatic liver disease of unknown causes, recurrent clinical sepsis (i.e., without detectible bacteria or viruses) and feeding intolerance.

The patient had abdominal distention for one week after birth due to chyloascites, and subsequently fasted for 2 weeks. As he continued to feed poorly, a nasogastric tube was inserted. The patient continued to experience several episodes of fasting due to sepsis and cardiopulmonary instability.

Forty days after birth, the patient required intubation and ventilatory support to counteract the worsening symptoms of heart disease (e.g., tachypnea, chest retraction and desaturation). On the forty-first day after birth, the patient underwent cardiac surgery to widen the right ventricular outflow tract. After surgery, the patient could not be weaned from the ventilator and echocardiography revealed fluctuation of the pulmonary artery flow. With progressive cardiopulmonary decompensation, the patient died 72 days after birth.

The clinical diagnosis of SLOS was confirmed after death. Mutational analysis of the *DHCR7* gene revealed that the patient was a homozygote for *DHCR7* mutation: [c.(1055G>A)+(1055G>A)] and [p.(Arg352Gln)+(Arg352Gln)]. Autopsy was not performed. The SLOS severity score was determined to be 10 using a scale to weigh embryologically separated organs that was initially introduced by Bialer et al.[7] and was recently modified by Kelley and Hennekam[8].

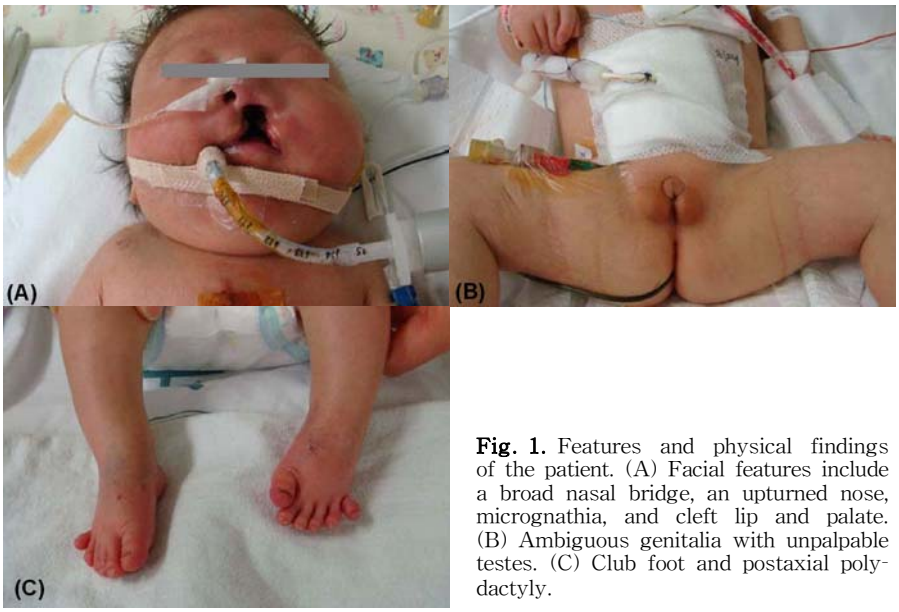

Fig. 1. Features and physical findings of the patient. (A) Facial features include a broad nasal bridge, an upturned nose, micrognathia, and cleft lip and palate. (B) Ambiguous genitalia with unpalpable testes. (C) Club foot and postaxial polydactyly.

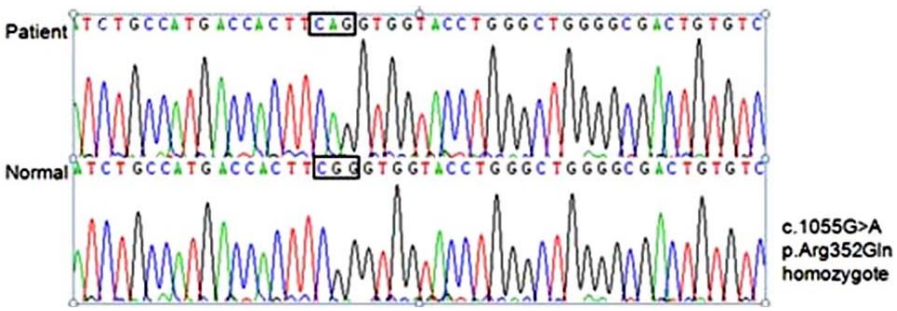

Fig. 2. Partial genomic DNA sequence of the *DHCR7* gene. The patient was homozygote for Arg352Gln mutation caused by a G to A base substitution at the 1055 nucleotide of cDNA of the *DHCR7* gene.

Discussion

Defects in the enzymes responsible for post-squalene cholesterol biosynthesis have recently emerged as important causes of congenital dysmorphology syndrome. Identification of SLOS led to the discovery that similar dysmorphology syndromes [e.g., desmosterolosis, lathosterolosis, CHILD syndrome and CDPX2, a form of chondrodysplasia punctata] are caused by inborn errors in cholesterol biosynthesis. SLOS was first delineated in 1964 by Smith, Lemli and Opitz[1].

In 1993, SLOS was found to stem from a metabolic defect in cholesterol biosynthesis[9] that results from an underlying deficiency in 7-dehydrocholesterol reductase (i.e., an enzyme that catalyses the final step in cholesterol biogenesis). Subsequently, several groups have identified *DHCR7* mutations in SLO patients[10].

SLOS was originally described as a syndrome characterized by mental retardation and multiple congenital anomalies. Clinical reports have described patients with characteristic facial phenotypes, various degrees of cleft palate and syndactyly of toes two and three, as well as various combinations of external and internal malformations. In addition, failure to thrive, behavioral problems, and mental retardation are hallmarks of the disorder. The symptoms of SLOS range from severe problems such as prenatal death, holoprosencephaly or other lethal malformations, to minor problems such as minimal physical abnormalities accompanied by normal intelligence or minimal intellectual impairment.

Suspected cases of SLOS are typically confirmed by the presence of elevated 7DHC levels in plasma or tissue sam-

ples. Although UV spectroscopy can detect 7DHC, it is best assayed via gas chromatography/mass spectroscopy (GC/MS). As standard laboratory cholesterol tests cannot distinguish between cholesterol and 7DHC, cholesterol values may appear to fall within normal ranges due to the presence of DHC; thus, normal cholesterol levels cannot rule out the possibility of SLOS. Prior to the biochemical elucidation of SLOS, diagnoses were divided into SLOS Type I (i.e., the mild form of the disease) and Type II (i.e., the severe form of the disease). Tint et al.[11] demonstrated that patients with both type I and type II SLO have similar metabolic defects; thus, differences in clinical severity appear to be caused by the degree that the enzymatic pathway is blocked. Hence, patients with type II SLO typically have lower cholesterol levels and higher 7 DHC levels than do patients with less severe phenotypes. Cunniff et al.[12] concluded that the best predictor of clinical severity is the cholesterol level, and that severity correlates inversely with cholesterol levels and not with 7DHC levels.

DHCR7 mutation analysis can be used to confirm a diagnosis of SLOS or can provide assistance in cases where biochemical testing is equivocal. The *DHCR7* gene, which was cloned in 1998, is encoded on human chromosome 11q12-13 by nine exons that span 14,100 bp of genomic DNA. More than 130 different *DHCR7* mutations have been identified in patients with SLOS. About 86% of these are missense mutations associated with reduced levels of *DHCR7*[13]. Approximately 30% of mutations in SLO are splice acceptor mutations (i.e., IVS8-1G>C). Homozygous null mutations often result in severe phenotypes and prenatal or perinatal death. The patient described in our report had a homozygous mutation of p.Arg352Gln (c.1055G>A), which is already reported[14].

The correlation between genotype and phenotype among patients with SLOS is relatively poor, and patients with the same genotype can have mild to severe phenotypes[15]. Thus, it is likely that factors other than genotype can significantly influence phenotype. One such factor appears to be the maternal ApoE protein. Witsch-Baumgartner et al.[16] reported that the maternal apo $\varepsilon 2$ mutation predisposes patients with SLOS to a more severe phenotype.

Some of the malformations associated with SLOS are consistent with impaired function of Sonic Hedgehog (SHH), which plays an important role in the formation of the central nervous system, facial structures and limbs[17, 18]. SHH is typically modified by cholesterol, secreted from a signaling cell, and binds to a receptor called Patched protein (PTCH). PTCH regulates transmembrane signaling by modulating the function of a protein called Smoothened (SMO). Several mechanisms by which SHH signaling might be impaired in SLOS have been proposed, including interaction of the amino terminus of *DHCR7* with SMO to regulate SHH signaling, and modulation of SMO function via PTCH-mediated transport of vitamin D3. Additional work is necessary to reconcile various experimental observations.

Because SLOS is autosomally recessive in nature, a rapid and correct diagnosis is important to protect the health of the affected newborn as well as the mother. The first prenatal diagnosis of SLOS was made by Johnson et al.[19], who detected multiple anomalies in a fetus that was diagnosed with SLOS after birth. However, many fetal anomalies are associated with, but not specific to, SLOS. Moreover, ultrasound abnormalities may not be sufficient to indicate SLO in pregnancies that have no family history of the disorder. McGaughran et al.[20] used prenatal biochemical testing to successfully diagnose SLO syndrome with intrauterine growth retardation. Today, SLOS can be diagnosed before birth by measuring 7DHC levels in amniotic fluid or CVS, or via direct mutational analysis of DNA isolated from aminocytes or CVS. Furthermore, measurement of uE3 levels in maternal serum or urine samples is a promising non-invasive new method for detecting SLOS in the fetus during pregnancy. Synthesis of uE3 depends on cholesterol produced by fetal tissues. Low levels of uE3 have been observed in urine, amniotic fluid and serum samples from pregnant women carrying fetuses with SLOS. In the case described here, a maternal serum triple marker was screen-positive for trisomy 18, and revealed low maternal levels of uE3 (i.e., 0.17 MoM).

We have provided the first report of a Korean patient with SLOS who was diagnosed using *DHCR7* mutation analysis. The patient's low plasma cholesterol levels were accompanied by a severe SLOS phenotype that included ambiguous genitalia and cardiac malformations. In pregnancies where ultrasound imaging reveals intrauterine growth retardation with physical anomalies, low maternal uE3 levels, and normal karyotypes, clinicians need a higher index of suspicion for SLOS. Early recognition, appropriate diagnosis and therapeutic intervention may improve the quality of life in patients with SLOS.

한 글 요 약

7-dehydrocholesterol reductase (*DHCR7*) 변이로 진단된 Smith-Lemli-Opitz 증후군 1예

*울산의대 서울아산병원 소아청소년과, 의학유전학클리닉**

박미림 · 고정민 · 전종근 · 김구환* · 유한욱

Smith-Lemli-Opitz 증후군은 콜레스테롤 합성 과정의 장애로 발생하는 상염색체 열성으로 유전되는 드문 질환으로 다양한 기형을 동반한다. 이는 *DHCR7* 유전자 변이로 인한 활성도 저하로 발생하는 질환으로 7DHC, 8DHC의 증가 및 체내 콜레스테롤의 감소에 따른 임상상을 특징으로 한다. 저자들은 국내에서 최초로 SLO 증후군을 유전자 분석을 통하여 진단하였기에 이를 문헌 고찰과 함께 보고하는 바이다.

References

1) Smith DW, Lemli L, Opitz JM. A Newly recognized syndrome of multiple congenital anomalies. J Pediatr 1964;64:210-7.
2) Ryan AK, Bartlett K, Clayton P, Eaton S, Mills L, Donnai D, et al. Smith-Lemli-Opitz syndrome: a variable clinical and biochemical phenotype. J Med Genet 1998;35:558-65.
3) Jezela-Stanek A, Ciara E, Malunowicz EM, Korniszewski L, Piekutowska-Abramczuk D, Popowska E, et al. Mild Smith-Lemli-Opitz syndrome: Further delineation of 5 Polish cases and review of the literature. Eur J Med Genet 2008;51:124-40.
4) Curry CJ, Carey JC, Holland JS, Chopra D, Fineman R, Golabi M, et al. Smith-Lemli-Opitz syndrome-type II: multiple congenital anomalies with male pseudohermaphroditism and frequent early lethality. Am J Med Genet 1987;26:45-57.
5) Yu H, Lee MH, Starck L, Elias ER, Irons M, Salen G, et al. Spectrum of Delta(7)-dehydrocholesterol reductase mutations in patients with the Smith-Lemli-Opitz (RSH) syndrome. Hum Mol Genet 2000;9:1385-91.
6) Loeffler J, Utermann G, Witsch-Baumgartner M. Molecular prenatal diagnosis of Smith-Lemli-Opitz syndrome is reliable and efficient. Prenat Diagn 2002;22:827-30.
7) Bialer MG, Penchaszadeh VB, Kahn E, Libes R, Krigsman G, Lesser ML. Female external genitalia and Mullerian duct derivatives in a 46,XY infant with the SmithLemli-Opitz syndrome. Am J Med Genet 1987;28:723-31.
8) Kelley RI, Hennekam RC. The Smith-Lemli-Opitz syndrome. J Med Genet 2000;37:321-35.
9) Irons M, Elias ER, Salen G, Tint GS, Batta AK. Defective cholesterol biosynthesis in Smith-Lemli-Opitz syndrome. Lancet 1993;341:1414.
10) Fitzky BU, Witsch-Baumgartner M, Erdel M, Lee JN, Paik YK, Glossmann H, et al. Mutations in the Delta7-sterol reductase gene in patients with the Smith-Lemli-Opitz syndrome. Proc Natl Acad Sci U S A 1998;95:8181-6.
11) Tint GS, Salen G, Batta AK, Shefer S, Irons M, Elias ER, et al. Correlation of severity and outcome with plasma sterol levels in variants of the Smith-Lemli-Opitz syndrome. J Pediatr 1995;127:82-7.
12) Cunniff C, Kratz LE, Moser A, Natowicz MR, Kelley RI. Clinical and biochemical spectrum of patients with RSH/Smith-Lemli-Opitz syndrome and abnormal cholesterol metabolism. Am J Med Genet 1997;68:263-9.
13) Witsch-Baumgartner M, Clayton P, Clusellas N, Haas D, Kelley RI, Krajewska-Walasek M, et al. Identification of 14 novel mutations in DHCR7 causing the Smith-Lemli-Opitz syndrome and delineation of the DHCR7 mutational spectra in Spain and Italy. Hum Mutat 2005;25:412.
14) Waye JS, Eng B, Nowaczyk MJ. Prenatal diagnosis of Smith-Lemli-Opitz syndrome (SLOS) by DHCR7 mutation analysis. Prenat Diagn 2007;27:638-40.
15) Correa-Cerro LS, Porter FD. 3beta-hydroxysterol Delta7-reductase and the Smith-Lemli-Opitz syndrome. Mol Genet Metab 2005;84:112-26.
16) Witsch-Baumgartner M, Gruber M, Kraft HG, Rossi M, Clayton P, Giros M, et al. Maternal apo E genotype is a modifier of the Smith-Lemli-Opitz syndrome. J Med Genet 2004;41:577-84.
17) Kelley RL, Roessler E, Hennekam RC, Feldman GL, Kosaki K, Jones MC, et al. Holoprosencephaly in RSH/Smith-Lemli-Opitz syndrome: does abnormal cholesterol metabolism affect the function of Sonic Hedgehog? Am J Med Genet 1996;66:478-84.
18) Porter JA, Young KE, Beachy PA. Cholesterol modification of hedgehog signaling proteins in animal development. Science 1996;274:255-9.
19) Johnson JA, Aughton DJ, Comstock CH, von Oeyen PT, Higgins JV, Schulz R. Prenatal diagnosis of Smith-Lemli-Opitz syndrome, type II. Am J Med Genet 1994;49:240-3.
20) McGaughran J, Donnai D, Clayton P, Mills K. Diagnosis of Smith-Lemli-Opitz syndrome. N Engl J Med 1994;330:1685-6; author reply 7.