Case Report

Effect of Statin Therapy in 4-Year-Old Dichorionic Diamniotic Twins with Familial Hypercholesterolemia Showing Multiple Xanthomas

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Familial hypercholesterolemia (FH) is characterized by a high level of low-density lipoprotein cholesterol (LDL-C) and is inherited as an autosomal dominant trait. We report 4-year-old dichorionic diamniotic twins (boy and girl) with FH who presented with multiple xanthomas on the face, both knees, both feet, and buttocks. Family history revealed vertical transmission of hypercholesterolemia from father to patients, thereby suggesting dominant inheritance. Lipid data of their mother did not match the criteria of FH. However, lipid data of maternal grandmother and maternal sister matched the criteria of FH. LDL receptor gene analysis of the family revealed that their father was heterozygous for a missense mutation, L547V, their mother was heterozygous for a nonsense mutation, C675X, and patients were compound heterozygous for L547V and C675X. After 10 months of treatment with pitavastatin (2 mg/day) and ezetimibe (10 mg/day), LDL-C decreased from 595 mg/dL to 267 mg/dL in the boy and from 530 mg/dL to 182 mg/dL in the girl. These findings suggest that lipid-lowering therapy with statin may be considered in pediatric patients with compound heterozygous FH (hetero FH) before inducing LDL apheresis, and gene analysis for true diagnosis in pediatric patients with multiple xanthomas should be considered, though they appear to be hetero FH from the family history and lipid data of parents.

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Introduction

Familial hypercholesterolemia (FH) is one of the most common inborn errors of metabolism, and it is inherited as an autosomal dominant trait¹. The homozygote and heterozygote frequencies are estimated to be one in 1 million and one in 500 in the general population, respectively². Based on the recent annual birth rate in Japan, it is estimated that approximately 2,000 children with heterozygous FH (hetero FH) and one child with homozygous FH (Homo FH) are born every year. Homo FH is characterized by the presence of a total cholesterol (TC) level of >600 mg/

dL and xanthomas and coronary vascular disease from childhood, with both parents being hetero FH³. Therefore, the clinical diagnosis of homo FH is usually not difficult for a pediatrician. In contrast, hypercholesterolemia is the only clinical finding in children with hetero FH in the first decade of their life⁴. Diagnostic criteria recently published for hetero FH in Japanese children comprises the following two items: 1) hypercholesterolemia: untreated low-density lipoprotein cholesterol (LDL-C) level of >140 mg/dL and 2) an family history of FH or premature coronary artery diseases in the patient's second-degree relatives^{3, 5}. In the previous study, we reported an 11-year-old boy with compound hetero FH^{6, 7}. His LDL-C level decreased from 440 mg/dL to 220 mg/dL after 12 months statin therapy⁶. LDL-C level of his mother was within the normal range, but LDL receptor gene analysis showed a heterozygous missense mutation (L547V). This mutation may show milder phenotype than other mutations^{8, 9}. Recently, we examined

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Table 1. Lipid profiles of the patient's family

	Age (ys)	Total Cholesterol	Triglyceride	HDL-C	LDL-C
Patient (boy)	4	671	105	55	595
Patient (girl)	4	597	98	47	530
Father	32	283	139	58	197
Mother	32	248	297	55	140
Sister	13	139	61	50	77
Brother	1	243	94	34	190

Values are expressed as mg/dL

4-year-old dichorionic diamniotic (DD) twins (boy and girl) with FH showing multiple xanthomas. Similar to the previous report, LDL-C level of their mother was within the normal range. Here we report these cases.

Case Presentation

Four-year-old DD twins (boy and girl) were referred to our hospital from a regional hospital; they presented with multiple xanthomas on the face, both knees, both feet, and buttocks and high levels of total cholesterol (boy: 671 mg/dL; girl: 597 mg/dL). They did not test their serum lipids before visiting regional hospital. Their father had been diagnosed simply as hypercholesterolemia, although his grandfather died because of a coronary heart disease at 60 years of age. Furthermore, two of his paternal uncles had acute myocardial infarction at 35 and 44 years of age. He was initially treated with statin, but neglected to continue treatment with statin because serum concentrations of his cholesterol reduced to normal levels. Maternal grandmother and aunt of the twin patients had hypercholesterolemia, which matched the criteria of FH in Japan^{3,5}. However, lipid data of the twin's mother did not match the criteria of FH. **Table 1** shows the familial lipid profiles of the twin patients. Younger brother also shows hypercholesterolemia. The family tree of patients is shown in **Fig. 1**.

The results of physical examination were as follows: boy; height, 99.6 cm (height SD score: -0.17 SD); weight, 14.0 kg (the percentage of standard weight: -8.8%); body mass index (BMI), 14.1 kg/m²; and blood pressure, 99/57 mmHg. Xanthomas were present on the face, both knees, both feet, and buttocks (**Fig. 2**). Girl: height, 100.5 cm (height SD score: 0.28 SD); weight, 13.3 kg (the percentage of standard weight: -14.3%); body mass index (BMI), 13.2 kg/m²; and blood pressure, 97/53 mmHg. Xanthomas were present on the face, both knees, both feet, and buttocks (**Fig. 2**). Radiographs of the Achilles

tendons of both the patients revealed no thickening (right: 6 mm and left: 6 mm). As shown in **Table 2**, serum concentrations of TC, triglyceride (TG), LDL-C, and apolipoprotein B were very high in the patients and that of HDL-C were within the normal range. Serum concentration of lipoprotein (a) [Lp(a)] in the boy was very high, but that in girl was within the normal range. Serum concentrations of plant-derived sterols such as sitosterol and cholestanol in the patients were within the normal range. Arterial intima-media thickness (IMT) was assessed by carotid echogram, which revealed no plaque formation and no hyperplasia of the intima-media complex (maximum thickness <0.5 mm).

Gene Analysis of LDL-Receptor

Genomic DNA was extracted from whole white blood of the patients using automated DNA extract machine (NA-3000; KURABO, Osaka, Japan). Primers covering all the exons and exon-intron boundary sequence of LDLR (18 exons) were designed¹⁰. They were also designed including 100–150 bases upstream or downstream of each exon sequence. The polymerase chain reaction (PCR) was conducted in a reaction mixture containing 20 ng genomic DNA, forward and reverse primers (5 pmol), and 0.5 U Hot-StarTaq® Master Mix Kit (Qiagen) with the corresponding buffer. After PCR, the amplification products were purified using ExoSAP-IT® (GE healthcare, Waukesha, WI). The designed primers were added in the M-13 sequence (5'-AAAACGACGGCCAGT-3') for the convenience of sequencing analysis. Direct sequencing was performed using BigDye Terminator v1.1 Cycle Sequencing kit (Life technologies, Foster, CA) by ABI Prism 3130 DNA Analyzer (Life Technologies). We conducted the LDL receptor gene analysis of our patients and both their parents. Informed consent was obtained from both the parents. It revealed that their father was heterozygous for a missense mutation, L547V, their mother was heterozygous for a nonsense mutation, C675X, and patients

Fig. 1. Family tree of the patients. Lipid data of the patients' family are shown in Table 1 (patients, older sister, younger brother, and parents). Lipid data of people who agreed with lipid analysis are provided.

were compound heterozygous for L547V and C675X in the LDL receptor gene.

Effect of Lipid Lowering Therapy

Initially, in addition to FH, we considered the possibility of sitosterolemia. Patients were administered ezetimibe (5 mg/day), but there was no remarkable reduction in LDL-C. After the serum concentrations of plant-derived sterols were determined, the patients were tentatively diagnosed as severe hetero FH based on the lipid data of both the parents and began treatment with HMG-CoA reductase inhibitor (pitavastatin 1 mg/day) and ezetimibe (5 mg/day). After 2 weeks of therapy, the pitavastatin dose increased on the basis of the LDL-C levels to 2 mg/day in both the patients. After 2 months of therapy, the LDL-C level decreased by 50% in the boy and by 56% in the girl. After 6 months of therapy, the ezeti-

mibe dose increased to 10 mg/dL because the LDL-C level increased to 346 mg/dL in the boy and 308 mg/dL in the girl. After 10 months of therapy, LDL-C level decreased from 595 mg/dL to 267 mg/dL in the boy and from 530 mg/dL to 182 mg/dL in the girl. Thus far, we have not observed any adverse effect of statin therapy. Sizes of all xanthomas decreased after 10 months of therapy. However, their LDL-C levels did not fall below 180 mg/dL. We are going to adjust the pitavastatin dosage by carefully checking the side effects.

Discussion

Xanthomas with hypercholesterolemia are usually found in pediatric patients with homo FH, sitosterolemia, or cerebrotendinous xanthomatosis (CTX)^{11, 12)}. Similar to sitosterolemia, serum concentrations of

Fig. 2. Xanthomas on both the feet and buttocks in patients.

cholestanol increase in patients with CTX¹²). Therefore, after determining the serum concentrations of cholestanol and sitosterol, sitosterolemia and CTX were excluded from differential diagnosis in our patients (**Table 2**).

Pedigree of our twin patients shows clear vertical transmission of hypercholesterolemia from father to the twin patients, thereby suggesting an autosomal dominant pattern. In addition, good response to lipid-lowering therapy using statin suggested that they were very likely to be hetero FH. Lipid data of their mother did not match the criteria of FH in Japan. However, the lipid data of the maternal grandmother and aunt matched the criteria of FH in Japan. We previously reported a similar case with compound hetero FH^{6,7}). In that case, LDL-C level of the patient's mother was also within the normal range. However, coronary artery disease was common in the maternal family. LDL receptor gene analysis revealed that the normolipidemic mother was heterozygous for L547V. This suggests that there is a mutation that does not show high LDL-C level in the LDL receptor gene muta-

tions. Thus, compound heterozygotes may be misdiagnosed as heterozygous for FH without LDL receptor gene analysis. Therefore, we conducted the LDL receptor gene analysis. As per the results, twin patients were compound heterozygous for L547V and C675X in the LDL receptor gene. Their father was heterozygous for L547V and their mother was heterozygous for C675X. A nonsense mutation, C675X, was reported in the Korean FH patient, but detailed information of this mutation is not available¹³). The maternal grandmother and aunt started lipid-lowering therapy recently. After 3 months of therapy, their LDL-C levels reduced to the normal level (grandmother, pitavastatin 2 mg/day; from 284 mg to 132 mg and aunt, pitavastatin 1 mg; from 214 mg to 131 mg). LDL-C level of their father also reduced to 140 mg/dL (pitavastatin 2 mg/dL). These data suggest that C675X also shows a milder phenotype and is responsive to statin therapy more than other mutations. Because C675X is close to the C-terminal of the LDL receptor, the function of the LDL receptor may be fairly preserved. Good effect of statin therapy in our

Table 2. Results of laboratory tests on admission

	Boy	Girl	
Total Cholesterol	671	597	(<220 mg/dL)
LDL-Cholesterol	595	530	(<140 mg/dL)
Triglyceride	105	98	(<140 mg/dL)
HDL-Cholesterol	55	47	(>40 mg/dL)
Lp(a)	117	14	(<40 mg/dL)
ApoA-I	125	111	(112-162 mg/dL)
ApoB	337	290	(59-99 mg/dL)
ApoE	10.7	10.3	(3.0-5.5 mg/dL)
ApoE phenotype	E3/E3	E3/E3	
AST	20	23	(14-33 IU/L)
ALT	12	9	(4-24 IU/L)
Cholesterol	7.2	7.9	(1-7.9 μ g/mL)
Sitosterol	7.8	11.2	(3-17 μ g/mL)

Rerference intervals are shown in parenthesis.

twin patients with compound heterozygote may be because of the nature of L547V and C675X mutations of the LDL receptor gene.

In Japan, there are currently no guidelines for the pharmacological treatment of children with hetero FH; however, recent studies of children and adolescents with hetero FH have established the effectiveness and safety of statin therapy¹⁴⁻¹⁶. Based on these data, the American Academy of Pediatrics (AAP) released revised recommendations for the management of hypercholesterolemia¹⁷. According to this report, statins are recommended as first-line pharmacologic agents, and drug therapy may be instituted from 8 years of age. Although AAP recommendations for drug therapy had elicited controversy¹⁸, statin should be prescribed for severe hypercholesterolemic FH children like our patients with caution. Thus far, we have not observed any adverse effect of statin therapy.

In our patients, drug therapy enabled a 50%-60% reduction in LDL-C levels, but these values remained >180 mg/dL. Furthermore, Lp(a) level, which is another risk factor for atherosclerosis in the boy patient was >100 mg/dL. We will control the statin dosage by measuring carotid IMT and the thickness of the Achilles tendons in addition to the serum LDL-C levels. In the future, the girl patient would get married and want to have babies. As suggested in the guideline for homo FH^{3, 5}, it is important to permit her to become pregnant as planned. By 3 months before the planned pregnancy, treatment of lipid-lowering drugs other than bile acid-binding resins should be discontinued. LDL apheresis should be induced during pregnancy. In addition, we should not hesitate to induce LDL apheresis when statin therapy

cannot prevent the progression of atherosclerosis.

In conclusion, lipid-lowering therapy with statin may be considered in pediatric patients with compound hetero FH before inducing LDL apheresis, and gene analysis for true diagnosis in pediatric patients with multiple xanthomas should be considered, although they appear to be hetero FH from the family history and lipid data of the parents. Gene analysis could contribute to the selection of the suitable therapy for such patients.

COI

Yoshitsune Miyagi: Nothing to declare

Mariko Harada-Shiba: Kowa Co, Ltd. – Medical Expert, Astellas Pharma Inc., Kaneka Medics

Takao Ohta: Kowa Co, Ltd. – Advisory Board

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