Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.0050-22
Intern Med 62: 251-259, 2023
http://internmed.jp

【 CASE REPORT 】

Children with Severe Hypercholesterolemia Caused by a Pathogenic Mutation in *ABCG5*

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Abstract:
We herein present a case series of hypercholesterolemia caused by a pathogenic mutation in the ATP-binding cassette sub-family G member 5 (*ABCG5*). Three unrelated infantile patients who were breastfed and had extremely elevated low-density lipoprotein (LDL) cholesterol levels were referred to our hospital. Their LDL cholesterol levels decreased significantly after weaning. Panel sequencing revealed a pathogenic mutation in *ABCG5* in each patient. An 8-year-old girl was also referred due to suspected familial hypercholesterolemia. Panel sequencing revealed a pathogenic mutation in *ABCG5*. A cholesterol-reduced diet alone significantly reduced the LDL cholesterol levels. Moreover, the administration of ezetimibe was found to be beneficial.

Key words: familial hypercholesterolemia, ABCG5, LDLR, LDL cholesterol

(Intern Med 62: 251-259, 2023)
(DOI: 10.2169/internalmedicine.0050-22)

Introduction

Familial hypercholesterolemia (FH) is a codominant disorder caused by an LDL receptor (*LDLR*) and its associated genes (1). Commonly, the LDL cholesterol level of patients with FH is extremely high, and it is challenging to reduce via a cholesterol-restriction diet alone (2). In contrast, sitosterolemia is a very rare recessive disorder caused by the ATP-binding cassette sub-family G member 5 (*ABCG5*) or ATP-binding cassette sub-family G member 8 (*ABCG8*) mutations. Moreover, this condition is characterized by extremely elevated LDL cholesterol levels that can be reduced via a cholesterol-restriction diet (3, 4). Although sitosterolemia with biallelic pathogenic mutations is rare, carriers with one pathogenic mutation have elevated LDL cholesterol levels by 26 mg/dL per one allele, and are at elevated risk for coronary artery disease by 2-fold per one allele (5). Moreover, we have previously shown that patients with sitosterolemia and carriers with one pathogenic mutation exhibited disturbed post-prandial lipoprotein metabolism (6), thus suggesting that phenotypes of sitosterolemia and carriers with one pathogenic mutation are greatly affected by their dietary habits. We herein present a case series of elevated LDL cholesterol levels, which were as high as those in FH, in three infants and an adolescent. However, their cholesterol levels significantly decreased with weaning and a cholesterol-restriction diet alone. Panel sequencing, which covers the genes associated with monogenic lipid disorders, including FH and sitosterolemia, was performed. The results revealed that the patients demonstrated carriers of sitosterolemia with one pathogenic mutation in *ABCG5*.

Methods

Study Participants

This study included three infants (8-month-old girl and 9- and 12-month-old boys) and an 8-year-old girl with extremely high LDL cholesterol levels who were referred to our hospital as well as their family members, including parents and grandparents. None of the parents showed any evidence of a consanguineous marriage. Table 1 shows the characteristics of the participants.

Biochemical Analysis

Fasting blood samples were collected for assay without any lipid-modifying treatments, except in a grandmother who had been receiving statin therapy. The serum total cholesterol, triglyceride, and HDL and LDL cholesterol concentrations were assessed enzymatically. Further, the serum si-

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Received: March 30, 2022; Accepted: May 2, 2022; Advance Publication by J-STAGE: June 14, 2022
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Table 1. Characteristics of Family 1.

Subject (sex)	I.1 (male)	II.1 (female)	II.2 (female)
Genotype*	W/M	W/W	W/M
Age (years)	32	30	0 (8 months)
Total cholesterol level (mg/dL)	227	198	430
Triglyceride level (mg/dL)	140	112	130
HDL cholesterol level (mg/dL)	54	60	45
LDL cholesterol level (mg/dL)	146	116	359
Sitosterol level (μg/mL)	7.2	3.0	8.4

*W: wild type, M: c.1166G>A (p.Arg389His) (*ABCG5*)

I.1, II.1, and II.2 are individual IDs, that correspond to those illustrated in Fig. 1.

tosterol levels were determined using a highly sensitive gas chromatography method (7).

Genetic Analyses

We isolated the genomic DNA of each participant from peripheral white blood cells using a standard DNA extraction protocol. DNA was pooled, selected according to size, ligated to sequencing adapters, and amplified to enrich the targets sequenced using the Kapa DNA Library Preparation. A customized NimbleGen in-solution DNA capture library (Roche NimbleGen, Madison, USA) was designed to capture all coding exons in 21 dyslipidemia-related Mendelian genes, including those associated with primary hypercholesterolemia [*LDLR*, apolipoprotein B (*APOB*), LDL receptor adaptor protein 1 (*LDLRAP1*), and proprotein convertase subtilisin/kexin type 9 (*PCSK9*)] and sitosterolemia (*ABCG5* and *ABCG8*). The details are described in a previous study (8).

Ethical Considerations

A genetic analysis was approved by the Ethics Committee of Kanazawa University. All procedures were performed in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the 1975 Declaration of Helsinki, as revised in 2008. Informed consent for genetic analyses was obtained from all participants.

Determination of Causative Mutations

A pathogenic mutation was defined as a mutation that met any of the following criteria: a) rare (minor allele frequency of <1% among the East Asian population) protein truncating variants (premature stop, insertions or deletions that shift frames, or canonical splice-sites); b) rare damaging missense variants, defined as those predicted as damaged by all five according to the silico software program (SIFT, Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster-2, and LRT); c) Chinar-registered pathogenic or likely pathogenic mutations causing sitosterolemia.

Case Reports

Case 1

Table 1 shows the baseline characteristics of the patient. Fig. 1 depicts the family tree. Case 1 involved an 8-month-old girl who was referred to our hospital due to an extremely elevated LDL cholesterol level. Her initial LDL cholesterol level was 359 mg/dL. She had been completely breastfed at that time, and her father also exhibited hyper LDL cholesterolemia (LDL cholesterol level=146 mg/dL). At the initial evaluation, FH was suspected. However, she and her father did not exhibit cutaneous xanthomas. Thus, a genetic analysis was performed using panel sequencing that covers the FH and other genes associated with other Mendelian lipid disorders, including sitosterolemia. The results showed a pathogenic mutation in the *ABCG5* [NM_022436.3:c.1166G>A (p.Arg389His)] in the proband and her father. This mutation has been shown to be the most common pathogenic mutation as sitosterolemia in Japan, and it is considered to be a loss-of function mutation (9). We did not identify any other mutations in these genes, including the FH genes. The serum sitosterol levels of the patient (8.4 μg/mL) and her father (7.2 μg/mL) were significantly high. Interestingly, weaning alone significantly reduced the LDL cholesterol level from 359 to 169 mg/dL. However, the serum sitosterol level increased from 8.4 to 11.3 μg/mL (Fig. 2). No abnormal findings were observed in her blood count.

Case 2

Table 2 shows the baseline characteristics of the patient. Fig. 3 depicts the family tree. Case 2 involved a 9-month-old boy who was referred to our hospital due to an extremely elevated LDL cholesterol level. His initial LDL cholesterol level was 602 mg/dL. He had been completely breastfed at that time. Both of his parents did not exhibit hyper LDL cholesterolemia. At the initial evaluation, he did not present with cutaneous xanthomas. Thus, sitosterolemia was suspected because of hyper LDL cholesterolemia and the lack of apparent family history of hyper LDL cholesterolemia. A genetic analysis was performed using panel sequencing, and the results identified a pathogenic mutation in the *ABCG5* [NM_022436.3: c.1336C>T (p.Arg446Ter)] in the proband and his mother. This mutation has been shown to be the pathogenic mutation closely associated with sitosterolemia in Japan, and it is considered to be a loss-of function mutation (9). We did not identify any other mutations in these genes, including the FH genes. The patient's serum

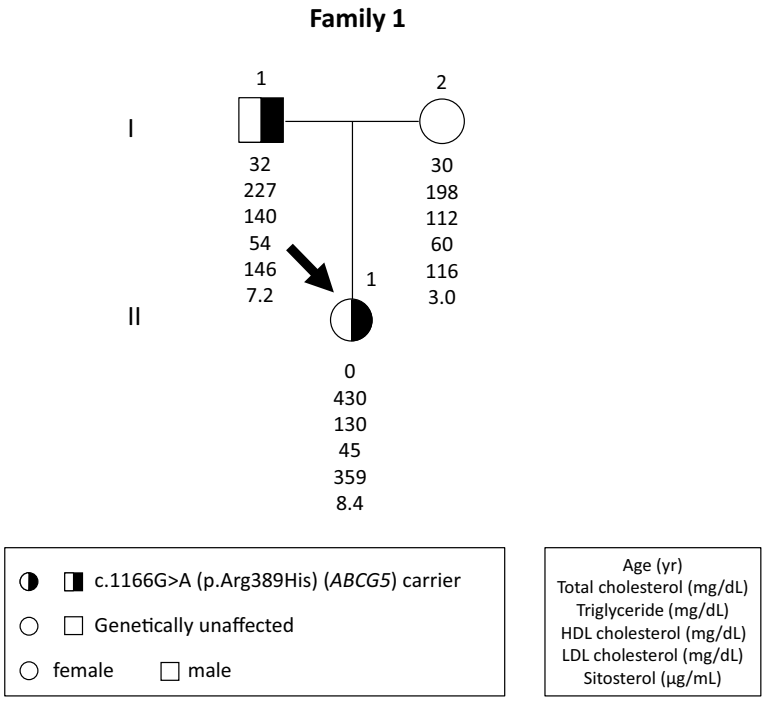

Figure 1. Family tree (family 1). The arrow indicates the proband. The black color indicates the mutation carrier of c.1166G>A (p.Arg389His) (*ABCG5*).

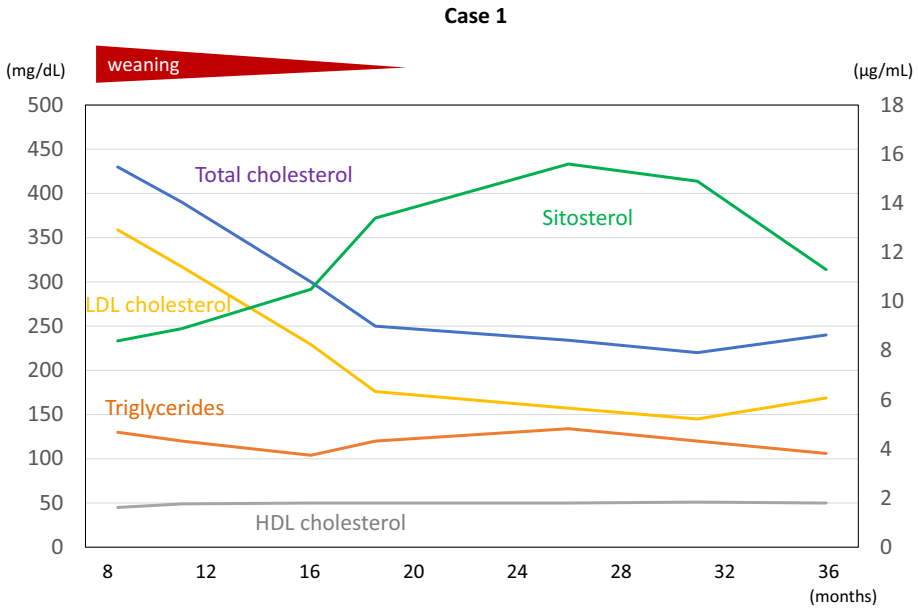

Figure 2. Clinical course of Case 1. The purple color indicates the total cholesterol level. The yellow color indicates the low-density lipoprotein cholesterol level. The orange color indicates the triglyceride level. The gray color indicates the high-density lipoprotein cholesterol level. The green color indicates the sitosterol level.

sitosterol level (2.2 μg/mL) was within the normal range (0.99-3.88 μg/mL). However, his mother's sitosterol level (9.7 μg/mL) was significantly elevated. Interestingly, weaning alone substantially reduced the LDL cholesterol level from 602 to 89 mg/dL. Nevertheless, the serum sitosterol level increased from 2.2 to 9.6 μg/mL (Fig. 4). No abnormal findings were identified in his blood count.

Case 3

Table 3 shows the baseline characteristics of the patient. Fig. 5 depicts the family tree. Case 3 involved a 12-month-old boy who was referred to our hospital due to an extremely elevated LDL cholesterol level. His initial LDL cholesterol level was 365 mg/dL. He had been completely

Table 2. Characteristics of Family 2.

Subject (sex)	I.1 (male)	II.1 (female)	II.2 (male)
Genotype*	W/W	W/M	W/M
Age (years)	34	34	0 (9 months)
Total cholesterol level (mg/dL)	170	229	742
Triglyceride level (mg/dL)	44	41	216
HDL cholesterol level (mg/dL)	63	100	39
LDL cholesterol level (mg/dL)	106	120	602
Sitosterol level (μg/mL)	1.9	9.7	2.2

*W: wild type, M: c.1336C>T (p.Arg446Ter) (ABCG5)

I.1, II.1, and II.2 are individual IDs, that correspond to those illustrated in Fig. 3.

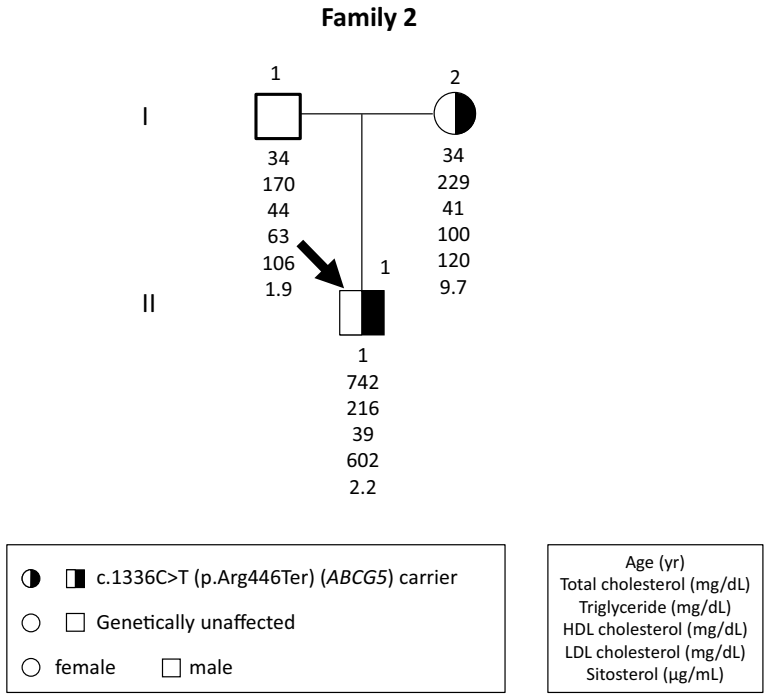

Figure 3. Family tree (family 2). The arrow indicates the proband. The black color indicates the mutation carrier of c.1336C>T (p.Arg446Ter) (*ABCG5*).

breastfed at that time, and his father also exhibited hyper LDL cholesterolemia (LDL cholesterol level=144 mg/dL). His grandparents, except for his paternal grandfather who died of myocardial infarction at 60 years of age, were evaluated. The results showed that his paternal grandmother and maternal grandfather exhibited hyper LDL cholesterolemia (LDL cholesterol level=140 and 153 mg/dL, respectively). At the initial evaluation, FH was suspected. However, the patient and his father did not exhibit any cutaneous xanthomas. A genetic analysis was performed using panel sequencing, and the results identified a pathogenic mutation in the *ABCG5* [NM_022436.3: c.751C>T (p.Gln251Ter)] in the proband and his father. This mutation has not been previously identified as a pathogenic mutation associated with sitosterolemia; however, this is considered to be a nonsense mutation, and thus it is expected to be a loss-of function mutation. We did not identify any other mutations in these genes, including the FH genes. The patient's serum sitosterol level (1.9 μg/mL) was within the normal range (0.99-3.88 μg/mL). Nevertheless, the sitosterol level (4.7 μg/mL) of the patient's father was slightly elevated. Interestingly, weaning alone substantially reduced the LDL cholesterol level from 365 to 98 mg/dL. Nonetheless, the serum sitosterol level increased from 1.9 to 7.8 μg/mL (Fig. 6). No abnormal findings were observed in his blood count.

Case 4

Table 4 shows the baseline characteristics of the patient. Fig. 7 depicts the family tree. Case 4 involved an 8-year-old girl who was referred to our hospital due to an extremely elevated LDL cholesterol level. Her initial LDL cholesterol level was 277 mg/dL. Her father also exhibited hyper LDL cholesterolemia (LDL cholesterol level=204 mg/dL). At the initial evaluation, FH was suspected. However, the patient and her father did not exhibit any cutaneous xanthomas. Thus, a genetic analysis was performed using panel sequencing, and the results showed a pathogenic mutation in the *ABCG5* [NM_022436.3:c.1166G>A (p.Arg389His)] in

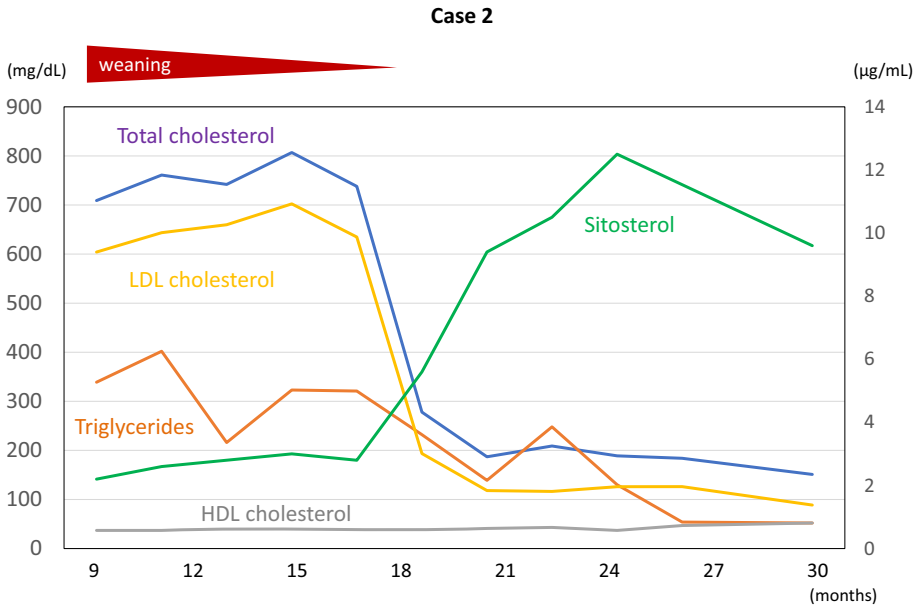

Figure 4. **Clinical course of Case 2. The purple color indicates the total cholesterol level. The yellow color indicates the low-density lipoprotein cholesterol level. The orange color indicates the triglyceride level. The gray color indicates the high-density lipoprotein cholesterol level. The green color indicates the sitosterol level.**

Table 3. **Characteristics of Family 3.**

Subject (sex)	I.2 (female)	I.3 (male)	I.4 (female)	II.1 (male)	II.2 (female)	III.1 (male)
Genotype*	W/W	W/W	W/W	W/M	W/W	W/M
Age (years)	68	66	64	30	30	0 (12 months)
Total cholesterol level (mg/dL)	224	226	182	204	164	467
Triglyceride level (mg/dL)	116	151	83	75	93	193
HDL cholesterol level (mg/dL)	66	43	80	45	63	63
LDL cholesterol level (mg/dL)	140	153	86	144	82	365
Sitosterol level (μg/mL)	1.9	1.8	2.6	4.7	1.7	1.9

*W: wild type, M: c.751C>T (p.Gln251Ter) (*ABCG5*)

I.2, I.3, I4, II.1, II2, and III.1 are individual IDs, that correspond to those illustrated in Fig. 5.

the proband and her father. This mutation has been shown to be the most common pathogenic mutation associated with sitosterolemia in Japan, and it is considered to be a loss-of function mutation (9). We did not identify any other mutations in these genes, including the FH genes. The serum sitosterol levels of the patient (5.4 μg/mL) and her father (6.6 μg/mL) were significantly elevated. Interestingly, a cholesterol-restriction diet alone significantly reduced the LDL cholesterol level from 277 to 181 mg/dL. Moreover, the use of ezetimibe reduced the LDL cholesterol from 181 to 106 mg/dL and the serum sitosterol level from 6.7 to 2.9 μg/mL with a plant sterol-restriction diet (avoiding nuts, chocolate, and avocado) (Fig. 8). No abnormal findings were identified in her blood count.

Discussion

We herein describe four children with a single pathogenic mutation in the *ABCG5* who exhibited severe hyper LDL cholesterolemia, which was significantly reduced by weaning from breastfeeding or a cholesterol-restriction diet alone. Based on the results, the following conclusions were obtained: 1) some patients present with hyper LDL cholesterolemia caused by a pathogenic mutation in the *ABCG5*, 2) hyper LDL cholesterolemia attributed to a pathogenic mutation in *ABCG5* can be remarkably attenuated by the cholesterol intake, and 3) the serum sitosterol level is a good biomarker for identifying a single pathogenic mutation in *ABCG5*.

ABCG5 plays a vital role in the excretion of sterols, including cholesterol and sitosterol in the human intestine (10). Therefore, a dysfunction caused by genetic mutations will lead to elevated serum sterol levels. Sitosterolemia caused by critical biallelic genetic mutations is a recessive disorder characterized by extremely high LDL cholesterol and sitosterol levels (11-18). However, there are emerging data showing that a single mutation in *ABCG5* can exceed the clinical threshold, and this is referred to as hyper LDL

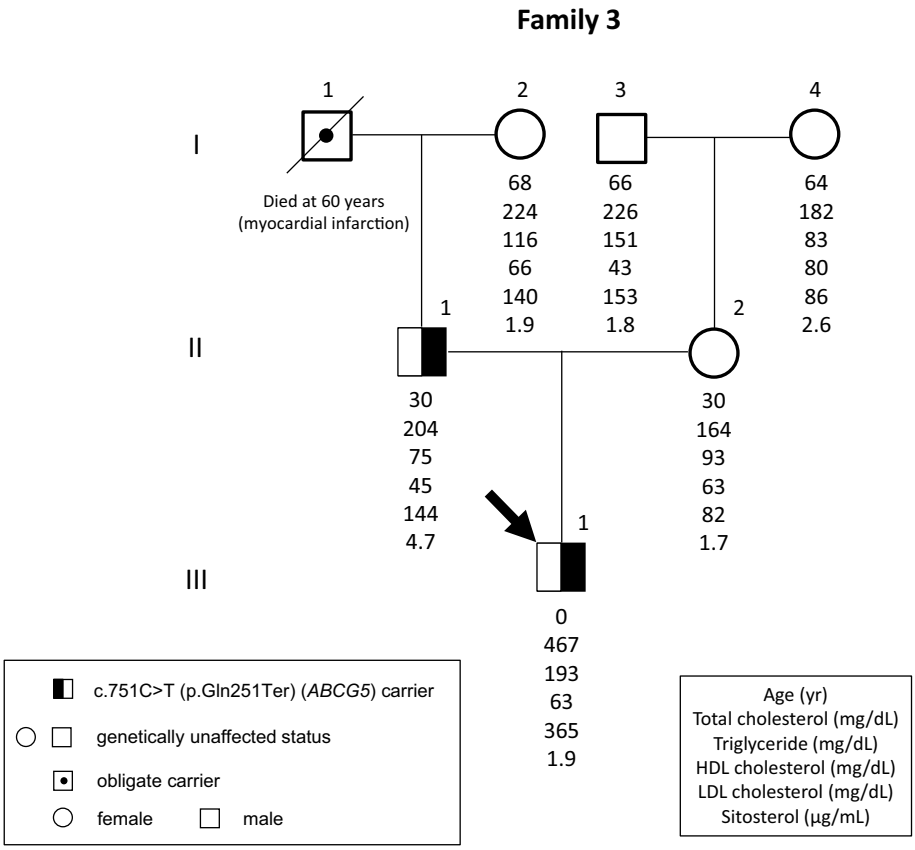

Figure 5. **Family tree (family 3). The arrow indicates the proband. The black color indicates the mutation carrier of c.751C>T (p.Gln251Ter) (*ABCG5*).**

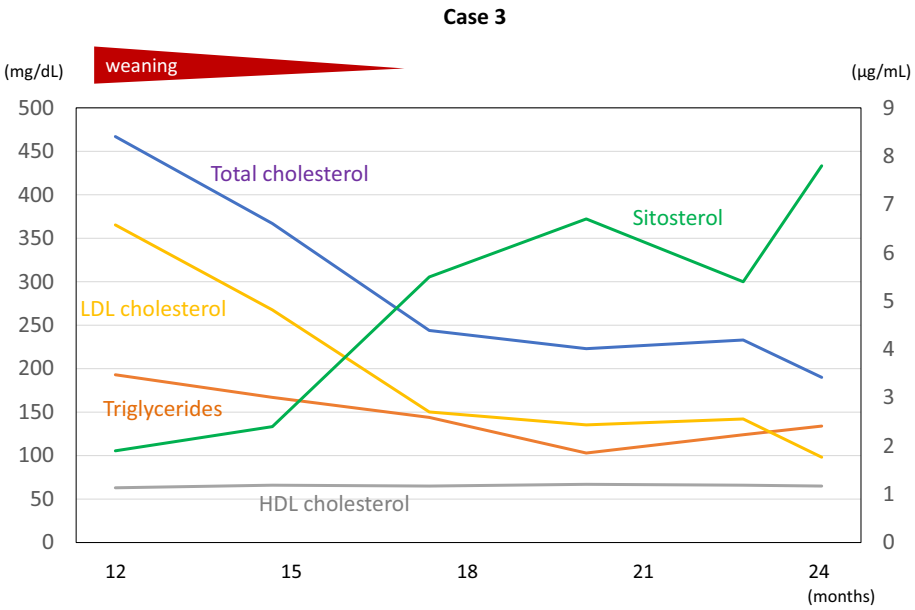

Figure 6. **Clinical course of Case 3. The purple color indicates the total cholesterol level. The yellow color indicates the low-density lipoprotein cholesterol level. The orange color indicates the triglyceride level. The gray color indicates the high-density lipoprotein cholesterol level. The green color indicates the sitosterol level.**

cholesterolemia. Individuals with a deleterious genetic mutation in *ABCG5* have elevated LDL cholesterol levels and are at risk for coronary artery disease. However, the extent of these effects was significantly lower than that of the effects of FH caused by a *LDLR* or *PCSK9* mutation (5). However, individuals with such variations exhibit vulnerability to die-

Table 4. Characteristics of Family 4.

Subject (sex)	I.1 (male)	II.1 (female)	II.2 (female)
Genotype*	W/M	W/W	W/M
Age (years)	41	39	8
Total cholesterol level (mg/dL)	277	178	347
Triglyceride level (mg/dL)	156	102	70
HDL cholesterol level (mg/dL)	48	68	56
LDL cholesterol level (mg/dL)	204	90	277
Sitosterol level (μg/mL)	6.6	2.5	5.4

*W: wild type, M: c.1166G>A (p.Arg389His) (*ABCG5*)

I.1, II.1, and II.2 are individual IDs, that correspond to those illustrated in Fig. 7.

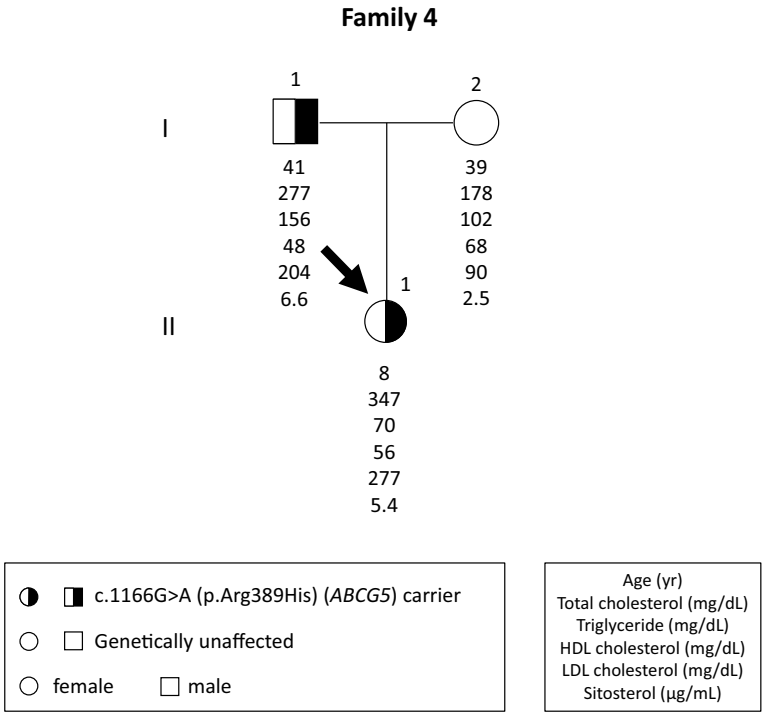

Figure 7. Family tree (family 4). The arrow indicates the proband. The black color indicates the mutation carrier of c.1166G>A (p.Arg389His) (*ABCG5*).

tary habits (6). It is interesting to note that the serum sitosterol levels of infantile cases were elevated after their weaning, probably due to an increased intake of sitosterol in their diet.

Patients with sitosterolemia who have biallelic pathogenic mutations in either *ABCG5* or *ABCG8* tend to exhibit severe hyper LDL cholesterolemia. However, several differences were observed between the patients demonstrating typical sitosterolemia with biallelic pathogenic mutations and our participants. First, none of the patients with a single pathogenic mutation exhibited tendon and/or cutaneous xanthomas, which are important clinical manifestations of sitosterolemia. Second, the serum LDL cholesterol and sitosterol levels of the patients were lower than those demonstrating typical sitosterolemia with biallelic pathogenic mutations. However, it is important to note that hypercholesterolemia can be caused by specific situations, including breast feeding and a large intake of a cholesterol-risk diet. Currently, there is no consensus nor established evidence regarding when it is best to start treatment for this situation. We believe that an LDL cholesterol level of 180 mg/dL or over 10 years of age would be a good time to start such treatment, based on the guidelines of the pediatric FH which is the "phenocopy" of this disease (19).

In conclusion, four children with a single pathogenic mutation in *ABCG5* exhibited severe hyper LDL cholesterolemia, which was significantly reduced by weaning from breastfeeding or a cholesterol-restriction diet alone. There exists a small group of patients who exhibit a great attenuation of their LDL cholesterol by cholesterol-restriction due to a single pathogenic mutation in *ABCG5*.

The authors state that they have no Conflict of Interest (COI).

Financial Support

This work was supported by a scientific research grant from

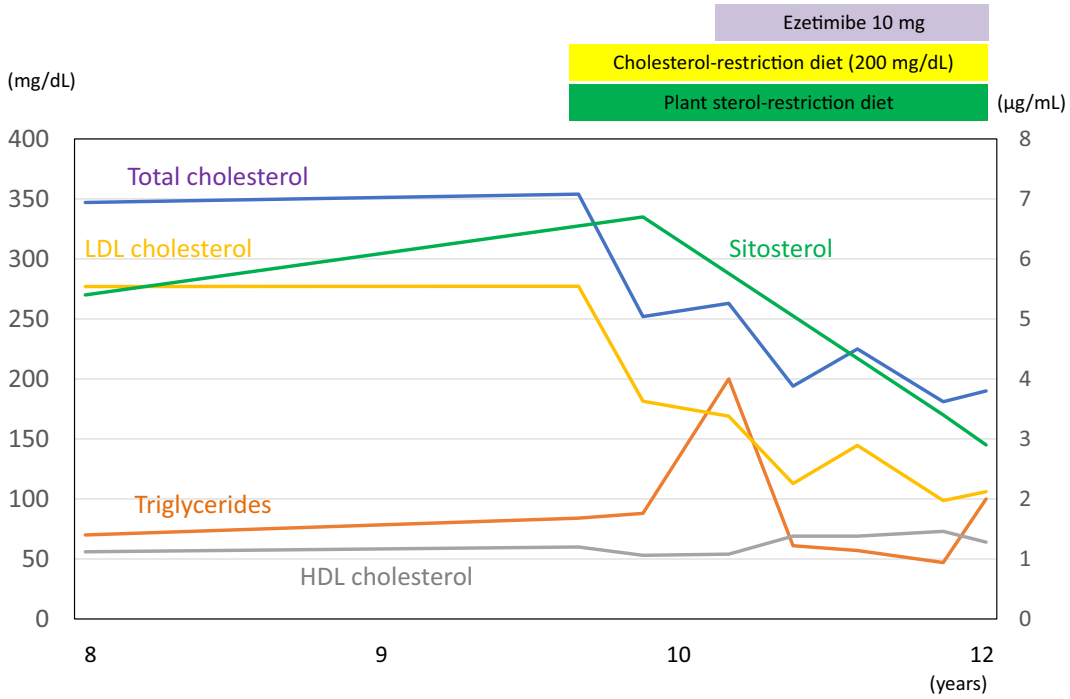

Figure 8. **Clinical course of Case 4. The purple color indicates the total cholesterol level. The yellow color indicates the low-density lipoprotein cholesterol level. The orange color indicates the triglyceride level. The gray color indicates the high-density lipoprotein cholesterol level. The green color indicates the sitosterol level.**

the Ministry of Education, Science, and Culture of Japan (no. 19 K08575).

Acknowledgement

We want to thank Ms. Kazuko Honda and Sachio Yamamoto for providing technical assistance.

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