

Clinical features and genetic analysis of childhood sitosterolemia

Two case reports and literature review

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Abstract

Rationale: Sitosterolemia is a rare autosomal recessive disorder of dyslipidemia due to mutations of genes *ABCG5* and *ABCG8*, leading to highly elevated plasma levels of plant sterols and expanded body pools of cholesterol.

Patient concerns: We present a 9-year-old and a 7-year-old Chinese boy with hypercholesterolemia and xanthomas of sitosterolemia due to *ABCG5* gene mutations. We also make a literature review of another 30 sitosterolemic children cases that have been reported with virulence *ABCG5* gene mutations.

Diagnosis: We took peripheral blood samples from 2 patients and their parents to conduct genetic analysis by next-generation sequencing (NGS) technologies.

Interventions: The 2 patients received dietary modifications without pharmaceuticals treatment.

Outcomes: A c.1166G>A (Arg389His) homozygosis mutation in exon 9 was observed in case 1, whereas a c.751C>T (Gln251*) homozygosis mutation in exon 6 was found in case 2. Literature review found another 30 pediatric cases with sitosterolemia due to *ABCG5* gene mutation. The lipid profile was normalized and xanthomas got smaller with combined therapy of a combined low-cholesterol and low-phytosterols diet.

Lessons: These suggested that in patients (especially Asian patients) with multiple xanthomas, severe hypercholesterolemia, or elevated low-density lipoprotein-cholesterol, sitosterolemia should be considered in the differential diagnosis. Early diagnosis is important, and restriction of both cholesterol and phytosterols diet should suggested for these patients.

Abbreviations: ABC = adenosine triphosphate-binding cassette, ABCA1 = ATP-binding cassette subfamily A member 1, *ABCG5* = ATP-binding cassette subfamily G member 5, *ABCG8* = ATP-binding cassette subfamily G member 8, APOA1 = apolipoprotein A1, APOA5 = apolipoprotein A5, ApoB = apolipoprotein B, APOE = apolipoprotein E, FH = familial hypercholesterolemia, GC-MS = gas chromatography-mass spectrometry, HAMP = hepcidin antimicrobial peptide, HDL-C = high-density lipoprotein-cholesterol, HFE = homeostatic iron regulator, LCAT = lecithin-cholesterol acyltransferase, LDL-C = low-density lipoprotein-cholesterol, LDLR = low-density lipoprotein receptor, LDLRAP1 = low-density lipoprotein receptor adaptor protein 1, LIPA = lipase A, LIPC = lipase C, hepatic type, LIPI = lipase I, LPL = lipoprotein lipase, NGS = next-generation sequencing, PCSK9 = proprotein-convertase subtilisin/kexin type 9, SLC40A1 = solute carrier family 40 member 1, TC = total cholesterol, TFR2 = transferrin receptor 2, USF1 = upstream transcription factor 1.

Keywords: *ABCG5* gene, hypercholesterolemia, low-density lipoprotein-cholesterol, phytosterol, sitosterolemia, xanthomas

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Ethical approval: Informed written consent was obtained from the parent for publication of this case report and accompanying images. The study was proved by the Ethical Committee of the Children Hospital of Zhejiang University School of Medicine. The ethical approval number: 2015-HP-025.

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1. Introduction

Sitosterolemia is a rare autosomal recessive disease, manifested by elevated plant sterols levels.^[1,2] The clinical features are various such as xanthoma, arthritis, thyroid dysfunction, premature atherosclerotic disease, hematologic manifestations including unexplained hemolytic anemia and macrothrombocytopenia, splenomegaly, and abnormal bleeding.^[3,4] The mechanisms are considered as intestinal hyperabsorption of all sterols and impaired ability to excrete sterols into bile,^[5] which caused by mutations in 2 half-size ATP-binding cassette (ABC) transporters on chromosome 2p21, sterolin-1 and sterolin-2 encoded by *ABCG5* and *ABCG8*, respectively.^[6,7] As a result of elevated phytosterol, sitosterolemia patients are at an increased risk of cardiovascular disease at early ages.^[8] Contrasted with familial hypercholesterolemia (FH), sitosterolemia has a good response to a low-cholesterol diet intervention, but not statins.^[9] Early molecular diagnosis is important and prompt treatment benefit clinical care and minimize the progression of premature coronary heart diseases.^[1,10,11] Although about 100 cases with sitosterolemia have been reported since first reported in 1974 by Bhattacharyya,^[12] sitosterolemia is easier to be misdiagnosed as FH without accurate plasma sterols analysis,^[13,14] as most of

them presented as severe hypercholesterolemia and xanthomas.^[15] It remains challenging to diagnose sitosterolemia in childhood. Herein, we report 2 children with sitosterolemia due to *ABCG5* mutations and reviewed the literatures on pediatric patients with sitosterolemia due to *ABCG5* mutations to highlight this rare condition.

2. Methods

As both of our cases had xanthomas, elevated total cholesterol (TC), and low-density lipoprotein-cholesterol (LDL-C) levels without family history, sitosterolemia or other autosomal recessive hypercholesterolemia was suspected. To aid early diagnosis, we took peripheral blood samples from patients and their parents after informed consent obtained. DNA was extracted from peripheral blood and powerful next-generation sequencing (NGS) technologies^[16] be used to decode completely the genome of patients. The flow of NGS work grounded on a panel was designed for sequencing the most prevalent dyslipidemia causing genes (*LDLR*, *APOB*, *LDLRAP1*, *PCSK9*, *ABCG5*, *ABCG8*, *LIPA*, *LPL*, *APOA5*, *LIPI*, *USF1*, *APOE*, *LIPC*, *ABCA1*, *LCAT*, *APOA1*, *HAMP*, *HFE*, *HFE2*, *SLC40A1*, *TFR2*). All mutations found by NGS were confirmed by direct sequencing.

3. Case reports

3.1. Case 1

Case 1 was a 9-year-old Chinese boy who presented as cutaneous xanthomas for 6 years. His development had been within the normal range, except for yellow streaks were noted on fingers when he was about 3 years old. Elevated TC and LDL-C were found and was misdiagnosed with FH in local hospital. No history of anemia, bleeding, arthralgia, and arthritis was provided. He was G₂P₂ of healthy parents with a healthy older

sister. No family history of hypercholesterolemia or premature coronary artery disease was reported.

Physical examination showed that the height and weight were both around 50th percentiles, according to Chinese references.^[17] Dermal plaques with a diameter of about 0.3 to 0.5 cm were noted on the fingers, elbows, and gluteal folds (Fig. 1). There were no signs of premature cardiovascular disease with normal heart examination. Lung, liver, spleen, and lymph nodes were unremarkable.

Serum lipid profiles showed an elevated TC of 557 mg/dL (normal values <170 mg/dL), LDL-C of 430 mg/dL (normal values <110 mg/dL), and apolipoprotein B of 229 g/dL (normal values <90 mg/dL) with normal high-density lipoprotein-cholesterol (HDL-C, 62 mg/dL; normal values >45 mg/dL), total triglycerides (24 mg/dL; normal values <75 mg/dL, age ≤ 9; normal values <90 mg/dL, age ≥ 10), apolipoprotein A1 (126 mg/dL, normal values >120 mg/dL). There was a normal range both in liver enzymes, creatinine, and blood and urine analysis in routine tests. No hepatosplenomegaly and liver steatosis was found in abdominal ultrasound examination.

Molecular genetic analysis revealed a c.1166G>A homozygous mutation in the exon 9 of *ABCG5* gene in case 1. Both his parents were heterozygous carriers for this mutation (Fig. 3). Bioinformatics analysis showed that it was a missense variant led to arginine substitute to histidine (Arg389His) at 389th amino acid locus. Moreover, a compound heterozygous mutation, c.3121C>G (Leu1041Val) in the exon 22 and c.5002G>A (Val1668Ile) in the exon 37 of *ABCA1* gene, was reported, which was found in his father. Unfortunately, his sister did not take part in molecular test.

Case 1 was kept a low cholesterol diet in the early stages with poor response. Although the TC and LDL-C decreased to 262 and 166 mg/dL, his xanthomas enlarged tuberous xanthomas with a diameter of about 5 cm on left elbow after 18 months of low-cholesterol diet (Fig. 1A, D). B-ultrasound showed about

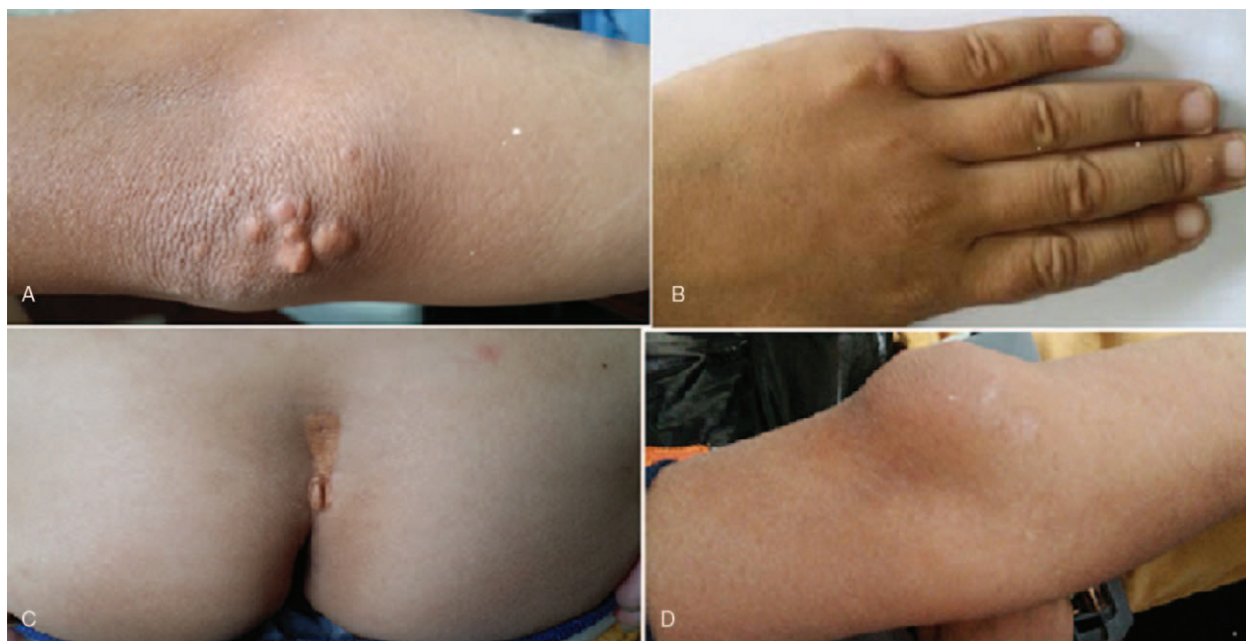


Figure 1. Cutaneous features of case 1. (A) Several small xanthomas on elbow at age 9 years old; (B) xanthomas on fingers at age 9 years old; (C) xanthomas on gluteal folds at age 9 years old; (D) The xanthomas enlarged on one elbow at age 10.5 years old.

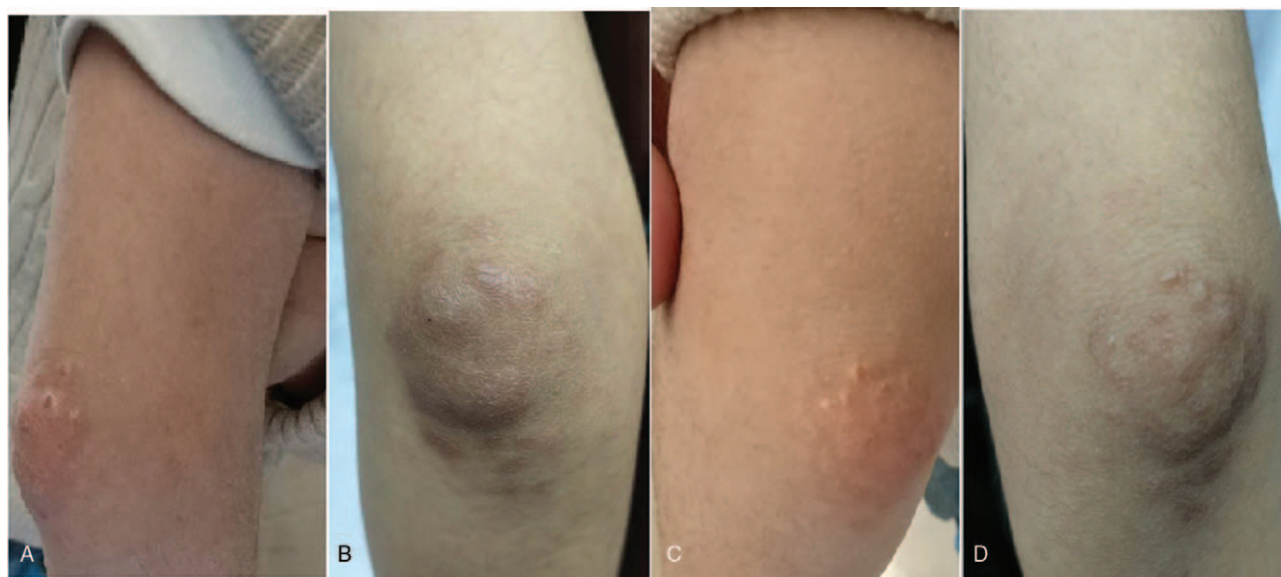


Figure 2. Cutaneous features of case 2. (A) Several small xanthomas on the right elbow at age 7 years old; (B) the xanthomas enlarged into 3 bulks on the right elbow at age 8 years old; (C) xanthomas on the left elbow at age 7 years old; (D) the xanthomas on the left elbow have few changes at age 8 years old.

53 × 36 × 12 mm hypoechoic mass with small amount of blood flow signal on his left elbow. The lipid profile was normalized and xanthomas got smaller with combined therapy of a combined low-cholesterol and low-phytosterols diet until 30 months later.

3.2. Case 2

Case 2 was a 7-year-old boy who presented to our department as multiple xanthomas. He was G₁P₁ of unremarkable history parents. His parents first noted the presence of xanthomas on his elbows about 6.5 years old. No history of anemia, bleeding, arthralgia, and arthritis was provided. His parents were not related to dyslipidemia or premature atherosclerosis, as well.

Physical examination showed a height of 120.7 cm and a weight of 20.8 kg that was between -1SD to median for Chinese boys in the same age.^[17] Dermal xanthomas with a diameter of about 0.5 cm were noted on his buttocks and elbows (Fig. 2). There were no signs of premature cardiovascular disease with normal heart examination. Lung, liver, spleen, and lymph nodes were unremarkable as well.

Serum lipid profiles showed an elevated TC of 478 mg/dL, LDL-C of 359 mg/dL, apolipoprotein B of 184 mg/dL with a critical level of HDL-C (41 mg/dL), normal total triglycerides (29 mg/dL), and a low level of apolipoprotein A1 (103 mg/dL). Liver and kidney function, creatinine and routine blood and urine analysis were all in normal range.

Molecular genetic analysis found a c.751C>T homozygous mutation in the exon 6 of *ABCG5* gene in case 2. His father and mother were heterozygous carriers for this mutation (Fig. 4). Bioinformatics analysis showed an amino acid codon (glutamic acid) to a stop codon at codon 251 (Gln251*), thus leading to early termination of protein translation. No related mutations were found in his younger sister.

Case 2 did not give any pharmacologic therapy as well. His TC (258 mg/dL) and LDL-C (159 mg/dL) were significantly decreased after 12 months of low-cholesterol diet. The xanthomas enlarged into 3 bulks on the right elbow at the age of 8 years

(Fig. 2A–B), whereas the size of xanthomas on the left elbow had no significant change (Fig. 2C–D).

4. Discussion

A literature search was performed using the electronic English database “PubMed” and Chinese database “CNKI and Wan-Fang” for all patients’ reports in the literature. Besides our 2 cases, there were 30 pediatric cases with *ABCG5* mutations sitosterolemia. Excluding the gender of 1 case unknown, they were 23 girls and 8 boys aged from 3 months to 18 years with a median age of 7 years at the time of diagnosis. It was notable that only 3 cases were non-Asian, Chinese and Japanese were predominant, which accounted for 37.50% and 34.38%, respectively. Among these 32 cases, 26 (78.80%) had xanthomas. Interestingly, xanthomas were noted in 2 breast-fed infants, although several cases >10 years did not report this feature. Moreover, 27 (81.81%) presented marked hypercholesterolemia, only 4 (12.50%) had hematologic manifestations, and 3 (93.75%) had atherosclerotic complication. Among these patients, a total of 22 mutations were reported and most of these are located in exons 9. Among these mutations, the most common was c.1166G>A (R389H) mutation which noted in 13 cases (40.62%, including our case one), followed by c.1336C>T (R446*) in 9 cases (28.12%), as shown in Table 1.

Among these 32 cases, we noted most were female with a female-to-male ratio of 2:1. Whether this difference of sex is associated with higher absorption of cholesterol in female is required further study. The clinical features of sitosterolemia are heterogeneous. Similar to current cases, dermatologic manifestation, xanthomas may be the only feature of sitosterolemia, which is unfamiliar to many pediatricians. It was reported that higher TC and phytosterols levels were noted in pediatric cases as their immature intestine might absorb higher amounts of cholesterol phytosterols than that of adults.^[18] Without the analysis of plasma cholesterol levels, this rare condition may misdiagnose as FH, even elevated TC and LDL-C were found. Moreover, few sitosterolemia reported previously did not present xanthomas or

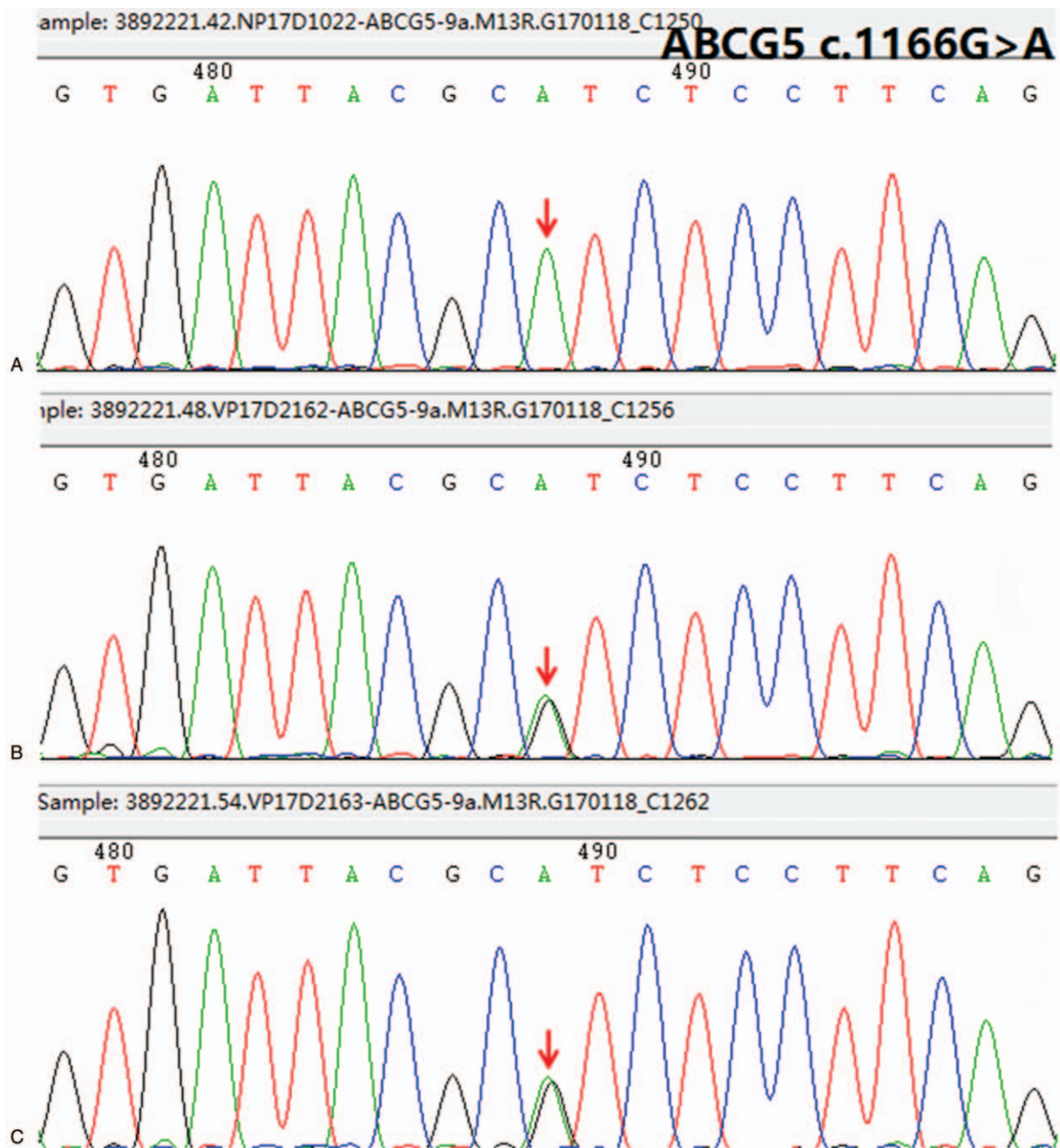


Figure 3. DNA mutations in the family of case 1. (A) Homozygous c.1166G>A mutation in *ABCG5* gene in the proband; (B) heterozygous c.1166G>A mutation in *ABCG5* gene in his father; (C) heterozygous c.1166G>A mutation in *ABCG5* in his mother.

high cholesterol, but hematologic or other manifestations. Hence, in patients with xanthomas, higher TC, and LDL-C, sitosterolemia should be considered in the differential diagnosis. Sitosterolemia is a risk of atherosclerotic cardiovascular disease. There are 3 pediatric cases presented atherosclerosis at an early age and myocardial infarction has been reported in children as young as 5 years old.^[19] Hence, it is very important to early diagnose and monitor for cardiovascular occurrences, even in pediatric patients. Phytosterols measurement and genetic analysis should be performed early,^[20] and genetic counseling is necessary for the family members of proband.

Most Chinese daily diets contain lot of sterols. Normal individuals absorb <5% of phytosterols and principally excreted

into bile. In sitosterolemic patients, approximately 50% phytosterols was absorbed and the ability to excrete into their biliary system was impaired. In human, ABC transporters function well in maintaining sterol balance.^[6] *ABCG5* and *ABCG8* are subgroups of ABC transporters. Defects of these 2 genes may cause intestinal hyperabsorption of all sterols (about 50%) and impaired ability to pump sterols out into the bile system and intestinal lumen.^[21,22] There are hot mutation in *ABCG5* gene mutations, including c.1166G>A (R389H) and c.1336C>T (R446*). Moreover, 4 cases presented hematological abnormalities, even when the cholesterol is generally normal. The mechanisms remain unclear. It may be implied that blood cells could be a target for the toxic effect of plasma phytosterols.^[23]

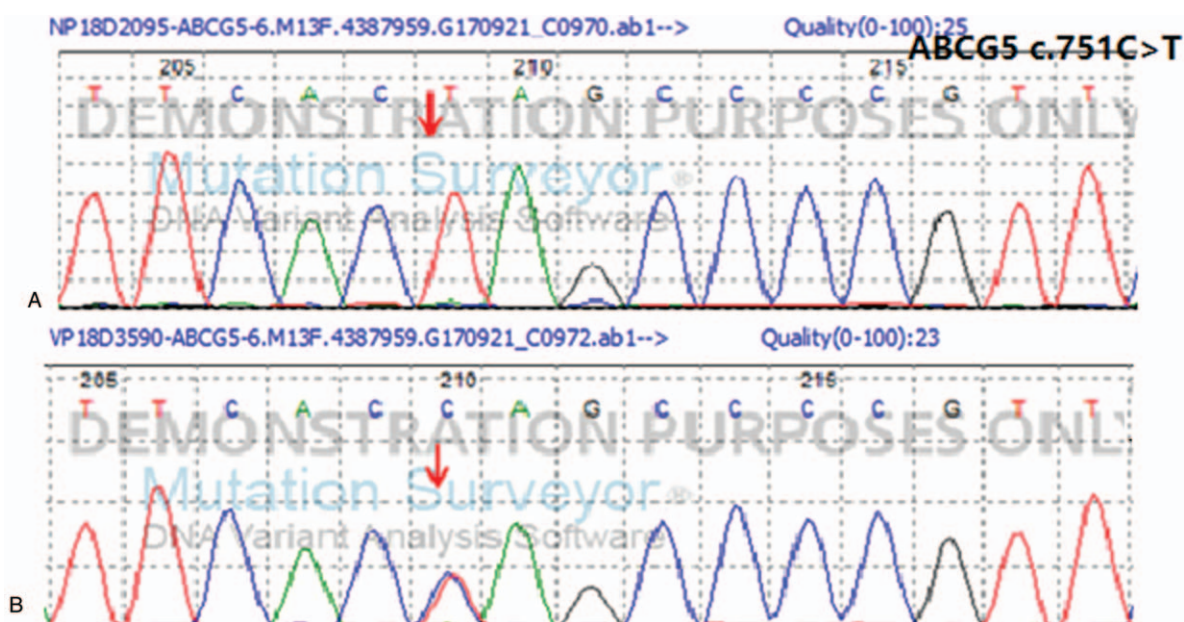


Figure 4. DNA mutations in the family of case 2. (A) Homozygous c.751C>T mutation in *ABCG5* gene in the proband; (B) heterozygous c.751C>T mutation in *ABCG5* gene in his father.

Table 1

A summary 32 cases of clinical findings, laboratory serum lipid profiles, and *ABCG5* mutations in each analyzed sitosterolemia patients.

No.	Age/Sex/Ethnicity	Location	Mutation	Xantho- mas	Hemato- logic feature	TC	LDL-C	HDL-C	Phytosterl	Others	Ref.
1	9 y/F/Chinese	Exon 9	1166G>A (R389H)	+	-	↑	-	-	ND		Current
2	7 y/F/Chinese	Exon 6	751C>T (Q251*)	+	-	↑	-	-	ND		Current
3	8 y/F/Chinese	Exon 8	987C>A (Y329X)	+	-	↑	↑	-	ND		[28]
4	18 mo/F/Chinese	Exon 9 Exon 10	1166G>A (R389H) 1336C>T (R446*)	+	-	↑	↑	-	↑		[28]
5	3 mo/F/Chinese	Exon 9 Exon 10	1166G>A (R389H) 1336C>T (R446*)	-	-	↑	↑	-	↑		[28]
6	23 mo/F/Chinese	Exon 9	1166G>A (R389H)	+	-	↑	↑	-	↑		[28]
7	12 y/F/Chinese	Exon 9	1166G>A (R389H)	+	-	↑	↑	-	↑		[28]
8	10 y-/Chinese	Exon 7	850G>A (G269R)	ND	-	↑	↑	ND	ND		[22]
9	7 y/M/Chinese	Exon 9	1256G>A (R419H)	+	-	↑	↑	-	↑		[29]
10	4 y/M/Chinese	Intron 12 Exon 9	IVS12+1G>A 1256G>A (R419H)	+	-	↑	↑	-	↑		[30]
11	10 y/F/Iranian	Exon10	1336C>T (R446*)	+	-	↑	↑	-	↑	Mitral and tricuspidal leaflets thickening; family history	[31]
12	16 y/M/Japanese	Exon 9 Exon 13	1166G>A (R389H) 1949 C>G (Q604E)	+	-	↑	↑	-	↑	Subclinical progression of atherosclerosis	[32]
13	13 y/F/Japanese	Exon 9	1756 A>C (R550S)	+	-	↑	↑	-	↑	Coronary atherosclerosis with percutaneous coronary intervention	[32]
14	16 mo/F/Japanese	Exon 9	1166G>A (R389H)	+	-	↑	↑	ND	↑		[33]
15	14 y/M/Turkish	Exon10	1336C>T (R446*)	ND	-	↑	↑	-	↑	Thyroid dysmorphogeneses; family history	[34]
16	13 y/M/Turkish	Intron 10	IVS10-1G>T	-	+	-	-	-	↑	Growth retardation	[35]
17	15 mo/F/Korean	Exon7 Exon10	904+1G>A (M302Asnfs*82) 1336C>T (R446*)	+	-	↑	↑	ND	↑	Family history	[11]
18	11 mo/F/Romanian	Exon1 Exon10	47C>T (Q16X) 1336C>T (R446*)	+	-	↑	↑	-	↑		[36]

(continued)

Table 1
(continued).

No.	Age/Sex/Ethnicity	Location	Mutation	Xanthomas	Hematologic feature	TC	LDL-C	HDL-C	Phytosterol	Others	Ref.
19	16 y/M/Japanese	Exon 9	1166G>A (R389H)	+	-	-	ND	-	↑	Arthralgia, carpal tunnel syndrome	[37]
20	2 y/F/Chinese	Exon 6 Exon10	751C>T (Q251*) 1336C>T (R446*)	+	-	↑	↑	-	↑		[10]
21	10 y/F/Mexican	Exon 2 Exon 11	144 -1G>A 1523 delC	+	-	↑	↑	-	↑		[38]
22	15 y/F/Asian	Exon 2	229G>T (E77X)	-	+	ND	ND	ND	↑	Growth retardation; family history	[39]
23	11 y/M/Caucasian	Intron 12 Exon 4	IVS11+3insT 436G>T (E146X)	-	+	ND	ND	ND	↑	Family history	[39]
24	18 y/F/Bangladesh	Exon 2	229G>T (E77X)	-	+	-	ND	ND	↑	Adrenal insufficiency, family history	[40]
25	18 mo/F/Japanese	Exon 9	1166G>A (R389H)	+	-	↑	-	-	↑		[41]
26	18 mo/F/Japanese	Exon 9	1256G>A (R419H)	+	-	↑	-	-	↑		[18]
27	10 mo/F/Japanese	Exon 9 Exon12	1306G>A (R389H) 1813_1817delCTTTT	-	-	↑	ND	-	↑		[18]
28	13 mo/F/Japanese	Exon 9	1306G>A (R389H)	+	-	↑	-	-	↑		[18]
29	2 y/M/Japanese	Exon 1 Exon10	130C>T (S44A) 1336C>T (R446*)	+	-	↑	-	-	↑		[18]
30	5 y/F/ Japanese	Exon12 Exon 9	1813_1817delCTTTT 1306G>A (R389H)	+	-	↑	ND	ND	↑	Carotid intima media thickening	[42]
31	13 y/F/ Japanese	Exon 9	1256G>A (R419H)	+	-	↑	↑	-	↑		[43]
32	3 y/F/Chinese	ND Exon 9	831_849dup 1336C>T (R446*)	+	-	↑	ND	ND	↑	Orthotopic liver transplantation in 2 y old	[44]

ND = not determined, ↑ = the high levels, - = normal levels, ↓ = the low levels, + = abnormal performances, M = Male, F = Female, *ABCG5* = ATP-binding cassette subfamily G member 5, TC = total cholesterol, LDL-C = low-density lipoprotein-cholesterol, HDL-C = high-density lipoprotein-cholesterol.

Therefore, further studies are required to understand the mechanism. We also find a compound heterozygous mutation of *ABCA1* gene in our case one. *ABCA1* gene mutations have an effect on protein transcription and translation, leading to abnormal HDL-C metabolism with autosomal-dominant pattern.^[24,25] However, case 1 and his father had normal HDL-C concentrations, which implied this compound heterozygous state may have no clinical impacts.

Since the first case of sitosterolemia reported in 1974,^[12] patients with initial missed diagnosis or misdiagnosis were commonly reported.^[26] The median age at onset of 32 patients was 7 years, which there are lack of related statistic data of large sample epidemiological survey. Moreover, initially misdiagnosis as FH was noted in our case 1. Elevated TC, phytosterols, cutaneous xanthomas, hematologic abnormalities, or cardiovascular diseases may be a clue of sitosterolemia. Significantly increased phytosterols is unique marker to distinguish sitosterolemia from other lipid disorders (e.g., familial dysbetalipoproteinemia and FH).^[13] Unfortunately, accurate methods such as gas chromatography-mass spectrometry (GC-MS) are not available in most domestic clinical laboratories. It has been reported the diagnosis should be considered in patients with LDL-C levels ≥ 190 mg/dL (without age limit).^[27] Unlike *ABCG8* patients are mostly Caucasians,^[11] we noted that 27 of 30 pediatric patients with *ABCG5* mutations were Asian patients. This suggested that *ABCG5* mutations had race or region difference and should be mostly considered in Asian patients. Compared with FH patients, plasma cholesterol levels in sitosterolemia patients are mostly sensitive to dietary restriction. However, xanthomas of our case 1 enlarged after cholesterol

reduced diet alone. Restriction of both cholesterol and phytosterols has beneficial effects on mitigating the cholesterol reduction and xanthomas regression. Hence, restriction of both cholesterol and phytosterols diet should be suggested for these patients.

In summary, in patients (especially Asian patients) with multiple xanthomas, severe hypercholesterolemia, or elevated LDL-C, sitosterolemia should be considered in the differential diagnosis. Early diagnosis is important, and restriction of both cholesterol and phytosterols diet should be suggested for these patients.

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Author contributions

Zou CC was responsible for this study. Huang D, Zhou Q, and Chao YQ were responsible for manuscript writing and literature review. All authors read and approved the final manuscript.

Writing – original draft: Huang D, Zhou Q, Chao YQ.

Writing – review & editing: Zou CC.

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