Internal Medicine
The Japanese Society of Internal Medicine
doi: 10.2169/internalmedicine.3811-19
Intern Med 59: 3033-3037, 2020
http://internmed.jp

【 CASE REPORT 】

# Sitosterolemia Exhibiting Severe Hypercholesterolemia with Tendon Xanthomas Due to Compound Heterozygous *ABCG5* Gene Mutations Treated with Ezetimibe and Alirocumab

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**Abstract:**

We herein report a rare case presenting with severe hypercholesterolemia, massive Achilles tendon xanthomas, and multi-vessel coronary artery disease. Initially, the patient was misdiagnosed with familial hypercholesterolemia. However, a genetic analysis using our custom sequencing panel covering genes associated with Mendelian lipid disorders revealed him to have a genetic basis of sitosterolemia with compound heterozygous mutations in the *adenosine triphosphate binding cassette subfamily G5* (*ABCG5*) gene. A comprehensive genetic analysis can be particularly useful for diagnosing cases with severe phenotypes, leading to appropriate and medical therapies. Our patient was refractory to statins, whereas ezetimibe and PCSK9 inhibitor with a low-plant-sterol diet successfully reduced his serum levels of low-density lipoprotein cholesterol.

**Key words:** sitosterolemia, *ABCG5*, PCSK9 inhibitor

**(Intern Med 59: 3033-3037, 2020)**
**(DOI: 10.2169/internalmedicine.3811-19)**

## Introduction

Sitosterolemia is a rare autosomal recessive disorder caused by loss of function mutations in *adenosine triphosphate binding cassette subfamily G5 or G8* (*ABCG5 or ABCG8*). *ABCG5* and *ABCG8* are the causative genes for sitosterolemia but have also been recognized as minor causative genes for familial hypercholesterolemia (FH) by next-generation DNA sequencing (1).

ABCG5 and ABCG8 form heterodimers, which act as efflux pumps to preferentially export free sterols from both hepatocytes and enterocytes, respectively, into the bile and intestinal lumen (2, 3).

Sitosterolemic patients have markedly increased concentrations of plasma and tissue plant sterols, which result in tendon xanthomas, premature atherosclerosis, hemolytic anemia, and macrothrombocytopenia (4). To date, approximately 100 cases of sitosterolemia have been reported worldwide (5).

We herein report a patient with sitosterolemia exhibiting severe hypercholesterolemia, massive Achilles tendon xanthomas, and multi-vessel coronary artery disease who was initially misdiagnosed with FH.

## Case Report

A 60-year-old man was referred to our hospital with a complete right bundle branch block by electrocardiography and a short run of ventricular premature contractions on 24-h Holter monitoring. He had experienced left-sided anterior chest and shoulder numbness and taken pregabalin at 58 years old, and at the same time, he had been diagnosed with hypercholesterolemia [serum levels of total cholesterol (TC) 354 mg/dL, low-density lipoprotein cholesterol (LDL-C) 270 mg/dL, high-density lipoprotein cholesterol (HDL-C) 46 mg/dL, and triglyceride (TG) 188 mg/dL] and hypothyroidism at an annual health checkup, for which he'd been prescribed

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Received: August 21, 2019; Accepted: May 14, 2020; Advance Publication by J-STAGE: July 21, 2020
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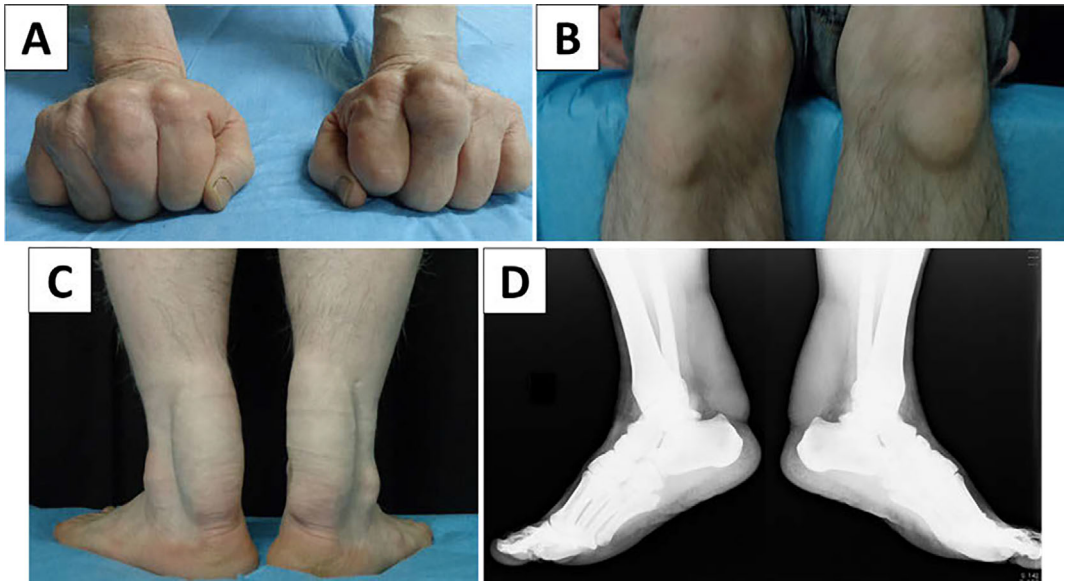


**Figure 1. Multiple tendon xanthomas on the hands (A) and the knee (B). Extremely enlarged 'Club-shaped' Achilles' tendons (C). Maximum thickness of the Achilles' tendon by radiography was 36 mm on the right and 37 mm on the left (D).**

rosuvastatin (5 mg daily) and levothyroxine (87.5 μg daily). He had never smoked and had no history of hypertension or diabetes mellitus. He was born from a second-cousin marriage, and his parents and younger brother had also exhibited hypercholesterolemia.

A physical examination revealed xanthomas on his hands, knees, and Achilles tendon (right side: 36 mm, left side: 37 mm), as confirmed by radiography (Fig. 1). Thus, he was initially diagnosed with FH according to the diagnostic criteria established by the Japan Atherosclerosis Society (6).

Coronary computed tomography and coronary angiography revealed total occlusive lesions in the proximal left anterior descending and high lateral arteries, and a 50% stenotic lesion was noted in the proximal right coronary artery (Fig. 2). Percutaneous coronary intervention using three drug-eluting stents was performed in the proximal left anterior descending artery with favorable angiographic results.

His peripheral platelet count was decreased to 97,000/μL, and his mean platelet volume was increased to 12.4 fL. In addition, his serum LDL-C levels exhibited resistance to statin therapy (rosuvastatin 5 mg daily) at 196 mg/dL. His thyroid function normalized following supplementation of levothyroxine (87.5 μg/day). Titration of rosuvastatin 10 mg daily as monotherapy and ezetimibe 5 mg daily were discontinued due to drug-induced liver toxicity and statin-induced myopathy, respectively. Ezetimibe was discontinued, and other statins and colestimide (3.62 g daily) were prescribed to reduce lipids in our patient. However, the LDL-C target was not achieved. Furthermore, the serum creatinine kinase and transaminase levels were chronically elevated. He received combination therapy using a statin and proprotein covertase subtilisin/kexin 9 (PCSK9) inhibitor (alirocumab 75 mg Q2W), which inadequately reduced his LDL-C level to 130 mg/dL (Fig. 3).

As our patient was highly resistant to available lipid-lowering therapies, we performed a genetic analysis using a custom panel, including genes associated with Mendelian dyslipidemias (7). We sequenced the exome region of 21 dyslipidemia-related Mendelian genes, including 3 FH genes (*LDLR, PCSK9,* and *APOB*) and other LDL-altering genes (*ABCG5, ABCG8, APOE,* and *LDLRAP1*). The details were described previously (8). Target-enriched products were sequenced using the MiSeq, Illumina's integrated next generation sequencing instrument. The target coverage for each subject was ≥20-fold in ≥98% of all targeted exons. We defined a pathogenic variant if it met any of the following criteria: a) rare (minor allele frequency <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 damaging by all five *in silico* software programs (SIFT, Polyphen2-HDIV, Polyphen2-HVAR, MutationTaster-2, and LRT); and c) ClinVar-registered pathogenic or likely pathogenic variants. In addition, we evaluated whether or not those variants were classified as pathogenic, at least according to supporting evidence based on the standard ACMG criteria (9).

This analysis revealed novel compound heterozygous mutations in exons 8 (NM_022436.3:c.1108_1118+2del) as well as a mutation in exon 12 (NM_022436.3:c.1673_1677 delCTTTT) of the *ABCG5* gene. At the same time, measurements of plasma plant sterol levels revealed high concentrations of sitosterol (132 μg/mL; reference range 1.67-3.13 μg/mL) and campesterol (82 μg/mL; reference range 2.65-4.45 μg/mL). Genetic testing of the patient's parents and brother confirmed the segregation pattern of the pathogenic mutations of the *ABCG5* gene (Fig. 4). Based on these findings, we prescribed low-cholesterol and low-plant-sterol dietary

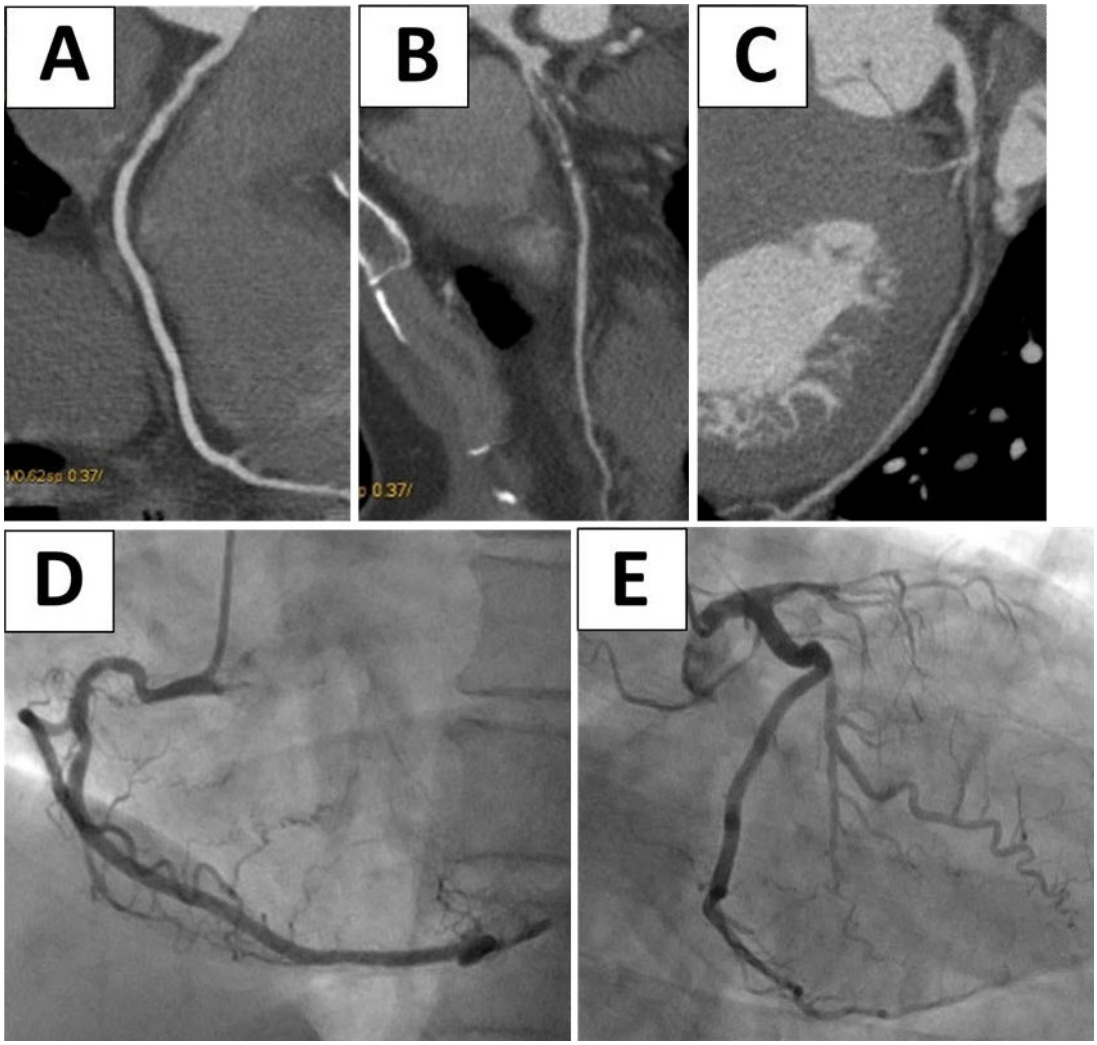


**Figure 2.** **Coronary CT angiography (A, B, C) and coronary angiography (D, E) reveals multivessel coronary atherosclerosis with total occlusion of the proximal left anterior descending artery (B, E) and a high lateral branch (C, E). The right coronary artery has diffuse plaques (A, D).**

therapy along with medical therapy using ezetimibe (10 mg daily), rosuvastatin (5 mg daily), and alirocumab (75 mg injection every 2 weeks). As a result, his LDL-C level was effectively reduced to less than 50 mg/dL (Fig. 3).

## Discussion

The current case was initially misdiagnosed as FH due to severe hyper-LDL-cholesterolemia, massive systemic xanthoma, and severe coronary artery disease. His LDL-C level was inadequately controlled with statins and a PCSK9 inhibitor. Comprehensive genetic testing resulted in an accurate diagnosis, and additional ezetimibe with a low-plant-sterol diet resulted in adequate lipid control.

Sitosterolemia is caused by homozygous or compound heterozygous mutations in one or both of the *ABCG5* and *ABCG8* genes, located on human chromosome 2p 21 (10, 11). In the current case, we found compound heterozygous mutations in the *ABCG5* gene and carefully checked them with gnomAD (http://gnomad.broadinstitute.org/) and ClinVar (http://www.ncbi.nlm.nih.gov/).One was a novel mutation (NM_022436.3:c.1108_1118+2del), and the other was a known mutation (NM_022436.3:c.1673_1677 delCTTTT) (12). The patient was born from a second-cousin marriage, but his genetic abnormality was not a homozygous mutation but rather compound heterozygous ones that were unrelated to consanguinity. Because sitosterolemia is considered a recessive disorder, the reason why his families, heterozygous carriers of *ABCG5* gene, showed hypercholesterolemia is unclear. However, some studies have shown that heterozygous mutations in the *ABCG5/8* genes can be a genetic cause of hyper-LDL cholesterolemia (12).

Sitosterolemic patients have markedly increased concentrations of plasma and tissue plant sterols owing to intestinal hyperabsorption and low bile excretion (5, 13). The pathogenicity of plant sterols in the development of atherosclerosis remains unclear. One proposed mechanism is that plant sterols directly contribute to the atherogenic process (14), while another suggests that there is no direct atherogenic effect of the plant sterols but that the associated level of cholesterols in atherogenic lipoproteins, such as LDL-C, is the main driver of atherosclerosis (15).

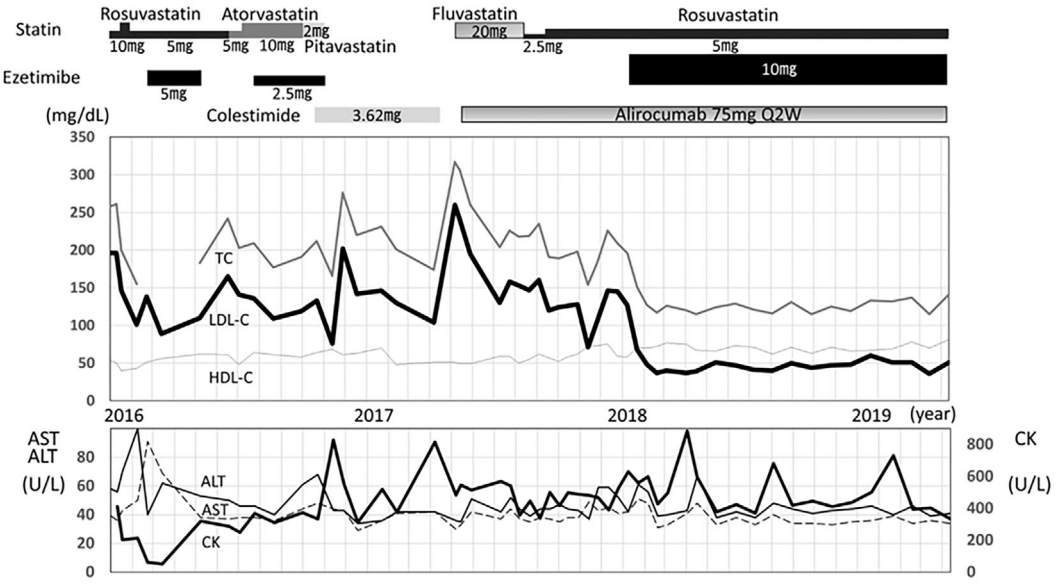


**Figure 3.** **Clinical course of lipid lowering therapy. Titration of rosuvastatin monotherapy and combination therapy with ezetimibe were discontinued due to drug-induced liver toxicity and statin-induced myopathy. Additionally, using other statins and colestimide, the LDL-C target goal could not be achieved and serum creatinine kinase and transaminase were chronically elevated. Administration of ezetimibe (10 mg daily), rosuvastatin (5 mg daily), and alirocumab (75 mg every 2 weeks) successfully reduced LDL-C. TC: total cholesterol, LDL-C: low-density lipoprotein cholesterol, HDL-C: high-density lipoprotein cholesterol, CK: creatinine kinase, AST: aspartate aminotransferase, ALT: alanine aminotransferase**

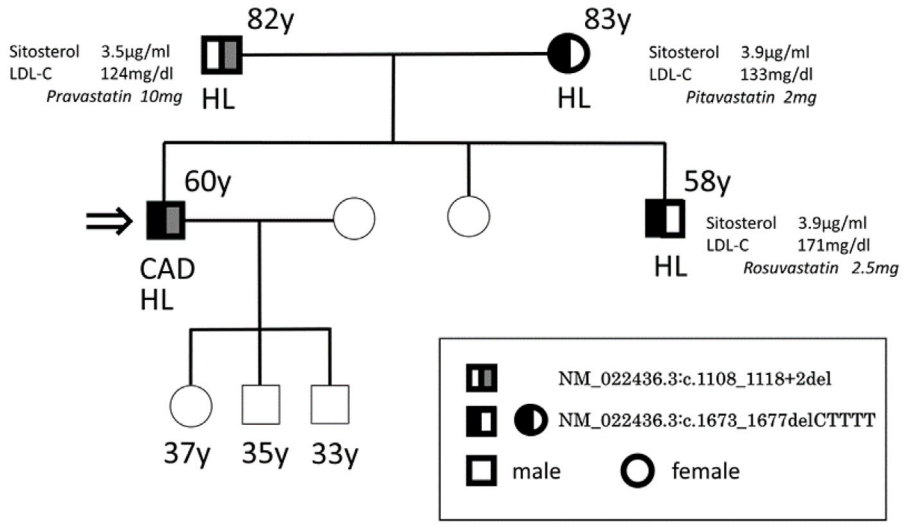


**Figure 4.** **Family history pedigree. The proband is indicated by an arrow and affected subjects are indicated by solid symbols. Subjects indicated by non-bold frames did not undergo genetic testing. HL: hyperlipidemia, CAD: coronary artery disease, LDL-C: low-density lipoprotein cholesterol, y: years old**

A mainstay of therapy is dietary restriction of both cholesterol and plant sterols. Foods rich in plant sterols, including vegetable oils, wheat germs, nuts, seeds, avocado, margarine, shortening, chocolate, shellfish, and seaweed, should be avoided (5, 16). Bile acid sequestrants were reported to reduce plasma plant sterol levels (17, 18). At present, ezetimibe is used as a first-line medical therapy for sitosterolemic patients (19, 20). It acts by inhibiting the absorption of dietary and biliary cholesterol and plants sterol by blocking Niemann-Pick-C1-like 1 protein transporter, subsequently reducing the delivery of intestinal sterols to the liver and resulting in reduced plasma sterol concentrations in sitosterolemic patients (21). Statins have not been shown to be effective for reducing plasma plant sterol concentrations in

sitosterolemia, as the synthesis of liver cholesterol is very low, and the further inhibition of HMG-CoA reductase does not upregulate the LDL receptor expression (21). In sitosterolemic patients, there is currently no evidence supporting the effectiveness of PCSK9 inhibitor for reducing plant sterol levels, and the effectiveness of reducing sitosterol levels to prevent atherosclerosis remains controversial. However, we believe that beneficial evidence concerning the efficacy of statins as well as PCSK9 inhibitors along with a low-plant-sterol diet can be applied even in patients with sitosterolemia.

In summary, the clinical manifestations in sitosterolemic patients are similar to those in patients with FH. As shown in the present patient, sitosterolemia can be misdiagnosed as FH when accompanied by hyper-LDL-cholesterolemia. Sitosterolemia must be considered in the differential diagnosis of FH, particularly in cases with a severe phenotype and/or among those with a poor response to statins.

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

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