



CLINICAL REPORT

Family sitosterolemia: Report of two cases in Colombia

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Abstract Sitosterolemia is an autosomal recessive and very rare disease. Its main characteristic is that there is a greater absorption and a decrease in the excretion of sterols, which leads to them being deposited in tissues. It is given by mutations in the ABCG5 or ABCG8 genes found on chromosome 2p21. In this clinical note, we describe the first two patients with familial sitosterolemia described in Colombia, brothers, one of them with xanthomas in extremities as the only symptom, and the other, completely asymptomatic. Genetic studies were performed as a diagnostic test in both patients, where a pathogenic homozygous variant could be identified in the ABCG8 gene in the first case (symptomatic), and a heterozygous variant in the ABCG8 gene in the second case (asymptomatic), the first patient has responded to treatment with ezetimibe. In conclusion, xanthomas should be studied in depth in pediatric age as they may be the only visible sign of such complex and hereditary diseases as familial sitosterolemia, which can be controlled and prevent cardiovascular complications of the disease.

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PALABRAS CLAVE

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Sitosterolemia familiar: reporte de dos casos en Colombia

Resumen La sitosterolemia es una enfermedad autosómica recesiva y muy infrecuente. Su principal característica es que se presenta una mayor absorción y una disminución en la excreción de esteroides, lo que conlleva a que se depositen en los tejidos. Está dada por mutaciones en los genes ABCG5 ó en el ABCG8 que se encuentran en el cromosoma 2p21. En esta nota clínica, se describen los dos primeros pacientes con sitosterolemia familiar descritos en Colombia, hermanos, uno de ellos con nódulos xantomatosos en extremidades como único síntoma, y

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el otro, completamente asintomático. Se realizaron estudios genéticos como prueba diagnóstica en ambos pacientes, donde se pudo identificar una variante homocigota patogénica en el gen ABCG8 en el primer caso (sintomático), y una variante heterocigótica en el gen ABCG8 en el segundo caso (asintomático), el primer paciente ha respondido al tratamiento con ezetimibe. En conclusión, los xantomas deben ser estudiados a profundidad en la edad pediátrica ya que pueden ser el único signo ostensible de enfermedades tan complejas y hereditarias como la sitosterolemia familiar, la cual puede ser controlada y evitar las complicaciones cardiovasculares propias de la enfermedad.

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Introduction

Sitosterolemia is considered a rare autosomal hereditary recessive disorder of lipid storage, and its origin is due to homo or heterozygous mutations in the ABC genes that encode the efflux transporters of the hepatic and intestinal heterodimer G5 and G8 located on chromosome 2p21.¹ The ABCG5 and ABCG8 genes encode transporters expressed in the biliary tract and the enterocyte, whose function is to transport sterols out of the cells to the intestinal lumen or into the bile. It is characterised by greater absorption and a decrease in the biliary excretion of plant sterols and cholesterol, resulting in high concentrations of sterols of plant origin, such as sitosterol, campesterol and stigmasterol, resulting in their deposition in tissues.² Healthy people absorb only about 5% of the average 200 to 300 mg of plant sterols consumed each day. Almost all of the absorbed sitosterol is rapidly excreted into the bile, so that only traces of sitosterol and other plant sterols remain in the blood. In contrast, people with sitosterolemia absorb between 15% and 60% of ingested sitosterol and excrete only a fraction in the bile.¹

The clinical manifestations of sitosterolemia include: the presence of cutaneous xanthomas, atherosclerosis, arthritis, hepatomegaly and splenomegaly, lipid-type red blood cells, macroplatelets and thrombocytopenia.³ In addition to dietary measures, where it is essential to avoid foods containing phytosterols, the use of the sterol absorption inhibitor, ezetimibe, possibly in combination with cholestyramine, a bile acid-binding resin, is the most effective option for its treatment.³ The diagnosis of sitosterolemia in childhood is a major challenge, since, without an accurate analysis of plasma sterols, it is easier to misdiagnose it as familial hypercholesterolemia.⁴

Clinical case 1

A 7-year-old patient presented with grouped xanthomatous nodules, located on the knees, of three years' duration, which were not painful, and with no other initially associated symptoms (Fig. 1). Laboratory tests had been performed that revealed the following lipid values: total cholesterol (TC): 495 mg/dL, and low-density lipoprotein cholesterol (LDLc): 427 mg/dL. It was initially interpreted as a case of familial hypercholesterolemia (according to the

Dutch Lipid Clinic Network [DLCN] criteria for the diagnosis of familial hypercholesterolemia. The score given was 14, (with the knowledge that a result >8 would be considered as a certainty in the diagnosis), and for which he received treatment with atorvastatin for 6 months, with a decrease in serum lipid values. However, new xanthomatous nodules appeared located on the elbows and hindfoot at the level of the Achilles tendons (Fig. 1). There was no family history of hypercholesterolemia and/or cardio or cerebrovascular events. After an exhaustive search, again investigating the family history some time after the initial suspicion of familial hypercholesterolemia, it was discovered that the paternal grandfather had died at the age of 42 due to an acute coronary event.

Physical examination revealed a weight of 24.5 kg, height of 124 cm, Z score (P/E: .28, T/E: .34, BMI: .08 kg/m²), anthropometric parameters appropriate for age. Blood pressure was normal in both extremities. Multiple xanthomatous nodules were identified in both knees, in the elbows, in the second metacarpal of both hands and in the hindfoot, which involved both Achilles tendons (Fig. 1). No lymph nodes were identified.

Initial laboratory results revealed TC: 495 mg/dL, triglycerides: 87 mg/dL, high-density lipoprotein cholesterol (HDL-C): 51 mg/dL, and LDL-C: 427 mg/dL (Table 1). No abnormalities were found in liver function, kidney function, or blood glucose.

A colour Doppler echocardiogram was performed, in which no cardiac pathologies were identified. Abdominal ultrasound was normal. The peripheral blood smear reported: red series with hypochromia, light anisocytosis with microcytes and light poikilocytosis with dianocytes, white and red series normal in number and morphology. A biopsy was performed on one of the lesions located in the knee, reporting a population of histiocytes with CD68, concluding non-Langerhans cell histiocytosis.

Genetic testing was performed by sequencing and NGS copy number variant (CNV) analysis on genes related to primary dyslipidemias at the Gencell Advanced Genetics Center. 26 genes were evaluated: ABCG5, ABCG8, APOA1, APOA2, APOA5, APOB, APOC2, APOC3, APOE, APTX, CREB3L2, CYP27A1, EPHX2, GHR, GPD1, GPIHBP1, ITIH4, LDLR, LDLRAP1, LIPA, LIPI, LMF1, LPL, LRP6, PCSK9 and PPP1R17. A pathogenic homozygous variant was identified in the ABCG8 gene that consists of the duplication of 11



Figure 1 Skin lesions. A) Xanthomas on elbows. B) Xanthomas on knuckles. C) Xanthomas in the rearfoot, at the level of the bilateral Achilles tendon. D) Xanthomas on both knees.

Table 1 Evolution of plasma lipids after the definitive diagnosis of patient 1.

	Initial	3 months	8 months	13 months	15 months
CT (mg/dl)	495	155	154	261	165
LDL-C (mg/dl)	427	101	94	216	112
HD-CL (mg/dl)	52	47	51	57	46
Triglycerides (mg/dl)	87	36	47	54	76

nucleotides (GGGTGAGCGCA) between positions 647 and 657 of exon 5/13 of the cDNA of the gene (c.647_657dup), which at the protein level produces the frameshift change, leading to a premature stop codon in amino acid 256 (p.Arg220ValfsTer37) in a 673 amino acid protein.

After the definitive diagnosis, patient 1 was administered: ezetimibe 10 mg/day and rosuvastatin 10 mg/day, accompanied by a low-fat diet low in plant sterols and regular physical activity. Regular follow-up was carried out in paediatric clinics, every 6–12 weeks. After 15 months of this treatment, taking into account the reduction in lesions and normal serum lipid values, it was decided to discontinue rosuvastatin, and to date the patient continues to receive ezetimibe.

Clinical case 2

Patient 1's sister was 5 years old when her brother's definitive diagnosis was made. She had lipid profile controls taken annually for three years, where no alterations in the results were evident. However, due to consanguinity, genetic analysis by sequencing and CNV analysis by NGS was performed on genes related to primary dyslipidemias

at the Gencell advanced genetics centre. 26 genes were evaluated: ABCG5, ABCG8, APOA1, APOA2, APOA5, APOB, APOC2, APOC3, APOE, APTX, CREB3L2, CYP27A1, EPHX2, GHR, GPD1, GPIHBP1, ITIH4, LDLR, LDLRAP1, LIPA, LIPI, LMF1, LPL, LRP6, PCSK9 and PPP1R17. The heterozygous variant c.647.657dup was identified in the ABCG8 gene. The lipid parameters of the first-degree relatives are represented in Fig. 2 and in Table 2. The anthropometric measurements correspond to a weight of 16.6 kg, height of 110 cm, score Z (P/E: −.89, T/E: −.26, BMI: −1.13 kg/m²), with risk of thinness, but the rest of the anthropometric parameters were age-appropriate. Her physical examination showed no pathological findings, nor was the presence of xanthomas identified. To date, she has not developed associated clinical manifestations. Taking into account the absence of symptoms and the recent diagnosis, additional studies have not yet been performed on the lipid profile nor has treatment been administered.

Discussion

Sitosterolemia is a rare Mendelian (autosomal recessive) disorder of lipid metabolism, characterised by increased

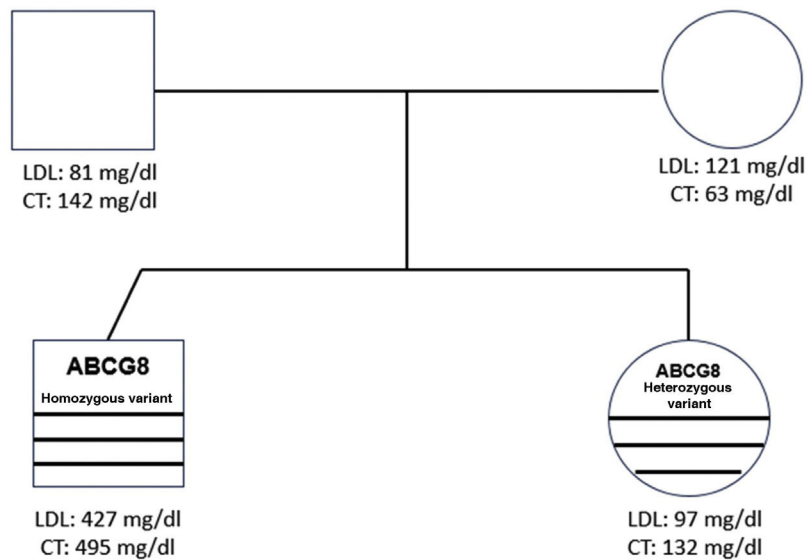


Figure 2 Family tree of the index patient.

Table 2 Evolution of plasma lipids after the definitive diagnosis of patient 2.

	Initial	9 months	20 months
CT (mg/dl)	132	136	130
LDL-C (mg/dl)	97	90	82
HDL-C (mg/dl)	34	41	47
Triglycerides (mg/dl)	58	49	36

absorption and decreased biliary excretion, primarily of plant sterols (sitosterol, campesterol, and stigmasterol) and cholesterol, resulting in an increase of their serum concentrations.⁴ Under normal conditions, only 5% of the phytosterols that are absorbed remain in the body. It was first described in 1974 by Bhattacharyya and Connor.⁵ This disorder is related to the appearance of xanthomatous nodules, which are usually found on the heels, knees, elbows, and buttocks. It is associated with complications such as premature atherosclerosis, cardiovascular diseases, and haematological disorders such as haemolytic anaemia, stomatocytosis, giant platelets, splenomegaly, arthralgia and arthritis.⁶ All of these features resemble familial hypercholesterolemia (FH).

Heterozygous patients are clinically normal and may present slightly elevated plasma phytosterol levels. Cholesterol levels may be normal. However, they are usually significantly elevated, even in breast-feeding patients, apparently before phytosterols rise in the blood.⁷

The accumulation of plant sterols in these particular patients is the main cause of atherogenesis. Cases of acute myocardial infarction and sudden death have been described at very early ages due to secondary atherosclerosis, as well as paraplegia due to compression of the spinal canal due to xanthomas and adrenal insufficiency.⁸

There is a mutation of the ABCG5 or ABCG8 genes on chromosome 2p21 that code for two intestinal transporters, called ABCG5 and ABCG8. These genes encode two proteins (Sterolin-1 and Sterolin-2), which form a heterodimer trans-

porter located in the endoplasmic reticulum of the brush border membrane of enterocytes and the canalicular membrane of hepatocytes, and this is where they act, intervening in the transport of absorbed sterol from the intracellular to the extracellular space.⁹

The cutaneous manifestations of FH are the clinical key to reaching an early diagnosis. These include cutaneous xanthomas, tuberous xanthomas, and tendon xanthomas, with the latter two having a strong association with HHH when they begin in childhood. These skin manifestations can be slowly reversed by correcting blood cholesterol levels, but they often require surgical removal. The diagnosis is confirmed with genetic study. If this is not available or if the case raises doubts, the diagnosis can be supported by the clinical picture, family history and laboratory testing. Currently, the diagnosis of sitosterolemia is mainly based on the level of serum plant sterols and gene sequencing. It is true that these plant sterols cannot be distinguished from cholesterol by the methods normally used, and consequently it is necessary to perform gas chromatography and mass spectrometry (GC/MS) to identify them. In institutions providing health services, it is unusual to have this type of technology, which is why sitosterolemia is often misdiagnosed as hypercholesterolemia. The main diagnostic technique, and more accessible than GC/MS, is gene sequencing. It has been found that mutations in ABCG5 are twice as frequent as those in ABCG8 in cases of sitosterolemia. The main sites where the ABCG5 gene mutation is observed are exon mutations. However, there are a small number of patients who have intron mutations.¹⁰

Based on the latest published guidelines, HHH treatment should be instituted early and its objective is to reduce LDL-C levels, ideally to values <70 mg/dl, to avoid atheromatosis and irreversible cardiac damage. All patients should be prescribed lifestyle modifications, with a diet rich in fruits, vegetables and whole grains, low in saturated fat and cholesterol, combined with physical activity. Mention is also made of a diet low in sterols and low fat. Dietary restriction of plant sterols should be the first line strategy, but a Mediter-

anean diet is appropriate and is not low in fat (exceeds 35% of total caloric value (TCV) at the expense of monounsaturated fat (MUF), whose source is olive oil).

Standard pharmacological management is based on the use of ezetimibe and cholestyramine. The combination of statin and ezetimibe is usually insufficient, and is ineffective if there is no residual LDL receptor activity. Patients with sitosterolemia generally do not respond to statins because HMG-CoA reductase activity is already maximally inhibited in sitosterolemia. However, statins are effective in reducing LDL-C, at least in some sitosterolemic patients.² However, it would not reduce serum plant sterol levels and therefore statin therapy alone is not an appropriate treatment for sitosterolemia. In short, the introduction of a statin associated with ezetimibe is possible, but its withdrawal due to an improvement in the lipid profile does not seem appropriate, in the absence of side effects. Finally, genetic counselling is important to diagnose the heterozygous variant in other family members and consider the risk in offspring. The cases presented are the first report of sitosterolemia in paediatric patients in Colombia, and although it was not possible to determine serum sterols, the clinical picture and the genetic test led to the diagnosis and treatment began with the standard recommended regimen, combined with lifestyle modifications, and good clinical and laboratory control.

These cases highlight the importance of knowledge and timely screening for tuberous xanthomas as the first manifestation of HHF. The first to detect its clinical manifestations will be primary care physicians, paediatricians and dermatologists, so recognition, timely referral and multidisciplinary management are essential to prevent accelerated atherosclerosis and early death in the patient and their relatives.

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Conflict of interests

The authors have no conflict on interests applicable to this publication.

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