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## Mutations in the *ABCG8* gene are associated with sitosterolaemia in the homozygous form and xanthelasmas in the heterozygous form

**Background:** Sitosterol is the most abundant plant sterol found in our diet. Sitosterolemia (OMIM 210250), also known as phytosterolaemia, is a rare autosomal recessive disease caused by the inability to efficiently excrete plant sterol, and is characterized by cutaneous xanthomas and accelerated atherosclerosis. Sitosterolaemia is caused by homozygous or compound heterozygous mutations in either *ABCG5* or *ABCG8* (both on chromosome 2p21), which encode the sterol efflux transporter ABCG5 (sterolin-1) and ABCG8 (sterolin-2), respectively. **Objectives:** To investigate a Tunisian family with several members who manifested with generalized cutaneous xanthomas, whereas others had only isolated xanthelasmas. **Materials & methods:** Genetic analysis was performed based on exome sequencing of DNA obtained from five affected individuals and one unaffected individual from a Tunisian family. **Results:** A novel mutation in the *ABCG8* gene, designated c.965-1G>C, was identified by exome sequencing in the members of this family. The homozygous form was associated with generalized cutaneous xanthomatosis while the heterozygous form was linked to isolated xanthelasmas. **Conclusion:** Our results indicate a gene dosage effect of *ABCG8* and suggest that individuals at risk should be followed closely.

**Key words:** *ABCG8*, phytosterolaemia, sitosterolaemia, xanthelasma, xanthoma

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**S**itosterolaemia (OMIM 210250), also known as phytosterolaemia, is a rare autosomal recessive disease caused by the inability to efficiently excrete plant sterol and is characterized by cutaneous xanthomas and accelerated atherosclerosis [1]. Sitosterolaemia is caused by homozygous or compound heterozygous mutations in either *ABCG5* or *ABCG8* (both on chromosome 2p21), which encode the sterol efflux transporter ABCG5 (sterolin-1) and ABCG8 (sterolin-2), respectively. ABCG5 and ABCG8 transporters pump sterols into the intestinal lumen or into bile which are subsequently excreted by the body. These transporters are expressed in enterocytes, in the proximal small intestine and hepatocytes. Sitosterolaemia is considered a disorder of increased intestinal absorption and decreased biliary excretion of plant sterols [2-4]. It is still not clear whether the liver or the intestine has a greater role in maintaining the balance of non-cholesterol sterols, yet two anecdotal reports suggest a predominant role for the liver in the regulation of non-cholesterol sterols [5, 6]. The prevalence of sitosterolaemia is unknown. To date, 40 cases have been reported [7]. However, sitosterolaemia may be more frequent than previously thought, as its true prevalence is unknown because most clinicians are unaware of it [8].

The clinical manifestations of sitosterolaemia vary widely and encompass a wide spectrum of phenotypic hetero-

geneity. Some individuals with homozygous mutations may appear almost totally asymptomatic. On the other hand, other patients may manifest variable symptoms as severe as sudden and premature death due to cardiovascular complications [9, 10]. The major clinical manifestations of sitosterolaemia are tendinous or tuberous xanthomas on extensor areas (such as the Achilles tendon), extensor tendons of the hand, elbows, and knees [11, 12]. These xanthomas can occur in childhood or manifest in adults and can present in various and sometimes unusual locations such as the heels, knees, buttocks, and elbows [1].

Minor trauma plays an important role in the development of xanthomas, explaining their predominance on the extensor surfaces [13]. Xanthomas may begin to appear at an early age in patients with sitosterolaemia, even during the first year of life [14, 15]. Intertriginous xanthomas are a very rare type of planar xanthomas that have been reported to be pathognomonic for homozygous familial hypercholesterolaemia [16]. However, they have also been reported in patients with sitosterolaemia [15].

The pathophysiology of development of xanthomas seems to resemble that of early stages of atherosclerotic plaques, *i.e.* as clusters of foam cells; this sheds light on the inherently increased cardiovascular risk in patients with xanthomas [17]. Even xanthelasmas (which were previously

considered to be only a cosmetic lesion) have been found to be associated with an increased cardiovascular risk and reduced average lifespan [18]. In contrast to normolipemic xanthomas, those in patients with sitosterolaemia regress and may completely disappear as plasma cholesterol levels decrease (although plant sterol levels may still be relatively high) [15, 19-23]. However, some patients may be completely asymptomatic and the only manifestation in some cases is haematological changes which include stomatocytic haemolysis, large platelets, splenomegaly, and abnormal bleeding [24-28].

In this study, we evaluated a Tunisian family with several members who had clinical manifestations of generalized cutaneous xanthomas while others had only isolated xanthelasma. We found a novel mutation in the *ABCG8* gene that accounts for the observed phenotypes with a gene dosage effect.

## Subjects and methods

### Patients

A Tunisian family presenting with generalized xanthomatosis in two of its members and isolated xanthelasma in three of its members was recruited to this study as part of the Genodermatology-approved Institution Review Board (IRB) protocol der.mk.01. All individuals signed a consent form for blood sample collection. Skin biopsy was taken from the elbow of one of the affected members with generalized xanthomatosis.

### Exome sequencing

Genomic DNA (gDNA) was isolated from peripheral blood lymphocytes collected from the family members and unrelated healthy control individuals. We performed exome sequencing on DNA obtained from five affected individuals and one unaffected individual from a Tunisian family. The exome capture method used was the V6 Sure Select Kit from Agilent, and the libraries were run on a HiSeq4000 platform from Illumina at Macrogen-South Korea. The generated Fastq files were mapped to reference genome using the Burrows-Wheeler Alignment Tool (BWA), and subsequently insertion/deletion realignment was carried out using the Genome Analysis Tool Kit (GATK) software which was also used for variant calling and filtering. Variant annotation was carried out using SnpEff and results were recorded in Excel alongside the BAM and VCF files. For all five samples, the total number of analysed bases was within the  $7-7.8 \times 10^6$  range. The average throughput depth of target regions was 128.5 with more than 70% coverage of  $>50\times$ . We first filtered the  $\sim 100,000$  single nucleotide polymorphisms (SNPs) and indels by eliminating the synonymous variants and those variants in the non-coding regions of the genes to reach around 12,000 SNPs and indels. The latter were then filtered out again to keep only the variants with less than a minor allele frequency of 10% (MAF < 10%). Cross comparative analysis between members of the same family was performed using the variant call software from Illumina, taking into consideration an autosomal recessive pattern of inheritance, focusing thus on only variants shared between the two affected siblings

and inherited on both alleles using an ABI3500 platform at the Molecular Core Facility of the American University of Beirut, as previously described [29].

## Results

### Clinical phenotypes

We examined six patients from a Tunisian family presenting with cutaneous xanthomatosis of different severities. Two of the patients showed generalized cutaneous xanthomatosis affecting elbows, knees, intertriginous areas, the eyelids, as well as the scapula. Three other individuals had isolated xanthelasma (eyelid xanthomatosis) and another individual was unaffected (*figure 1*). None of the family members had any systemic involvement or history of early-onset coronary artery disease.

A skin biopsy obtained from the elbow of the 25-year-old woman with disseminated xanthomatosis showed foamy histiocytes in the dermis confirming the clinical diagnosis of xanthomatosis (*figure 2*).

We subsequently performed blood laboratory tests, including lipid profiling, which were all within the normal range (the lipid profile did not include plant sterols). Serum and urine protein electrophoresis were performed to rule out plasma cell dyscrasias and were also normal. In the setting of the clinical, histological, and laboratory findings, a diagnosis of either familial idiopathic xanthomatosis or sitosterolaemia was considered.

### Exome sequencing results

After filtration and analysis of the exome sequencing data, we identified a homozygous mutation in the *ABCG8* gene, designated c.965-1G>C (splice acceptor) in the two individuals with generalized cutaneous xanthomatosis (NM\_0022437.2). The other three individuals with xanthelasma were heterozygous for the mutation, and the unaffected member was wild-type for both alleles (*figure 3*). The mutation was predicted to be damaging based on both Polyphen-2 and SIFT in-silico analysis. The allele frequency for the mutation was close to zero in the international database, as well as in 200 control Lebanese and Arab individuals. Sanger sequencing of DNA from the six family members confirmed the exome sequencing results.

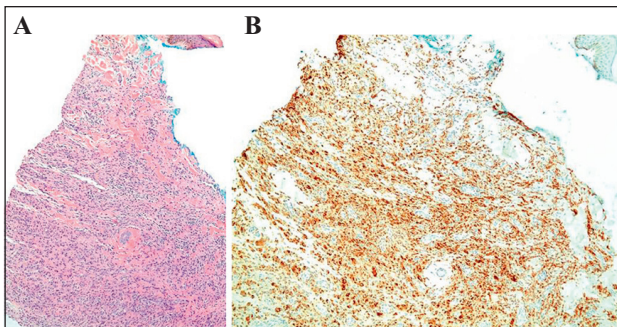
## Discussion

Sitosterol is the most abundant plant sterol found in our diet, and is the predominant form in patients with sitosterolaemia [24, 30]. The most common sources of plant sterols include nuts, seeds, chocolate, avocados, vegetable oils, wheat germs, and margarine [1, 26]. Plant sterols are structurally similar to cholesterol but differ due to the presence of an ethyl group (sitosterol), methyl group (campesterol), or a double bond (stigmasterol) [31].

Dietary cholesterol and non-cholesterol sterols are absorbed from the intestinal lumen via the sterol influx transporter, Nieman Pick C1 Like 1 (NPC1L1), which has higher affinity to cholesterol than to plant sterols



**Figure 1.** Individuals from the Tunisian family with homozygous mutation in the *ABCG8* gene showing disseminated xanthomatosis involving the scapula, knuckles, elbows, and knees. Patients with heterozygous mutation in the *ABCG8* had isolated xanthelasmas.

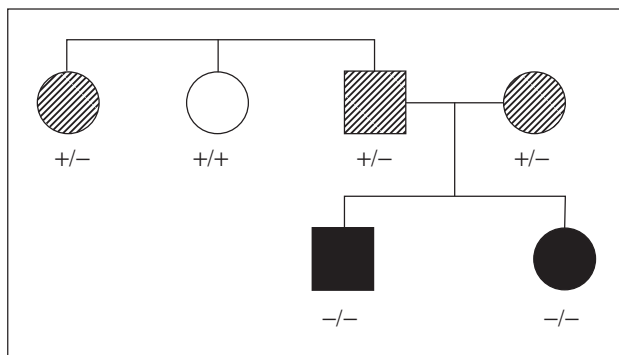


**Figure 2.** Skin biopsy taken from the elbow of a 25-year-old woman with disseminated xanthomatosis shows foamy histiocytes in the dermis (A). Immunoperoxidase stain for CD68 is positive in histiocytes (B).

[26, 32, 33]. In enterocytes, about 50-60% of absorbed cholesterol is esterified by acetyl-sterol O-acyltransferase 2 (SOAT2), which is transported to the liver and packed in chylomicrons [34]. Un-esterified cholesterol or plant sterols are pumped back into the intestinal lumen by the sterol efflux transporters ABCG5 and ABCG8 [26]. Plant

sterols not pumped back to the intestinal lumen then become part of the chylomicrons, are transported to the liver, and are eventually pumped out into the bile by the hepatic ABCG5/ABCG8 transporters [26, 30].

In our study, we identified a novel mutation in the *ABCG8* gene, designated c.965-1G>C, in a Tunisian family. The mutation most likely results in the abolition of the splice acceptor site, resulting in subsequent exon skipping, premature termination, and mRNA decay. Interestingly, in the homozygous form, the mutation was associated with generalized tuberous, tendinous and intertriginous xanthomatosis while for the heterozygous pattern, the patients showed isolated xanthelasmas. Xanthomas most commonly occur in the setting of hyperlipidaemia, less commonly in association with monoclonal gammopathy, and the remaining cases are considered idiopathic. On the other hand, isolated xanthelasmas are rarely associated with hyperlipidaemia, and are considered idiopathic. Here, we showed that at least a subset of idiopathic xanthomatosis and xanthelasmas are linked to mutations or altered function of the ABCG8 pump protein. Little is known about the prevalence of mutations in *ABCG5/8* and their associated phenotypes worldwide, and whether or not such mutations could be linked to defects in LDLR and hypercholesterolaemia. A better



**Figure 3.** Pedigree of the family with sitosterolaemia with corresponding genotype/phenotype. The core family was composed of a boy and a girl in their 20s, both diagnosed with severe xanthomatosis (black circle and square), who were found to harbour the c.965-1G>C variant in the *ABCG8* gene on both alleles (-/-). Their parents, as well as a parental aunt, were heterozygous for the mutation (-/+) and had only isolated xanthelasma (dashed circles and square). The second paternal aunt was healthy (white circle) with no mutation in *ABCG8* (+/+).

understanding of the function of *ABCG8* and the possible crosstalk with other lipid-related molecules, such as LDL, will contribute to uncovering the pathophysiology of cutaneous xanthomatosis, as well as simple xanthelasma. Defects in either *ABCG5* or *ABCG8* result in increased intestinal absorption and decreased biliary excretion of plant sterols, leading to extremely high plasma levels of plant sterols (50 to 200-fold) [2, 3]. These are carried in low-density lipoprotein (LDL) and very-LDL particles [2, 11]. The levels of plant sterols cannot be measured in the blood using regular blood tests used to measure total cholesterol, LDL, and HDL. Gas chromatography-mass spectrometry (GC-MS) or liquid chromatography-mass spectrometry are reliable methods to measure plant serum sterol levels and screen sitosterolaemia, in which increased plant sterol levels and sitosterol:cholesterol levels are almost invariably observed [31].

In clinical cardiology practice, we and others have identified a number of young patients (of less than 40 years) developing coronary artery disease with supposedly unidentified risk factors, yet upon coronary artery catheterization, they showed cholesterol plaques. We are currently in the process of studying these patients to see whether a subset of them may have elevated plant sterols. This may alter the diet generally prescribed for patients with metabolic syndrome which generally relies on an increased intake of plant derivatives, including plant sterols, as some may carry aberrations in *ABCG5* and *ABCG8*, increasing the risk of worsening conditions.

In conclusion, we identified a novel mutation in the *ABCG8* gene, which in the homozygous form was associated with generalized xanthomatosis, and in the heterozygous form was associated with isolated xanthelasma. Future work will identify whether plant sterols constitute a risk factor for coronary artery disease in a subset of patients with malfunctioning *ABCG5/ABCG8* proteins. ■

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## References

1. Merkens LS, Myrie SB, Steiner RD, Mymin D. Sitosterolemia. In: Pagon RA, Adam MP, Ardinger HH, et al. *GeneReviews*(R). Seattle (WA), 1993.
2. Berge KE, Tian H, Graf GA, et al. Accumulation of dietary cholesterol in sitosterolemia caused by mutations in adjacent ABC transporters. *Science* 2000; 290: 1771-5.
3. Lu K, Lee MH, Hazard S, et al. Two genes that map to the STSL locus cause sitosterolemia: genomic structure and spectrum of mutations involving sterolin-1 and sterolin-2, encoded by *ABCG5* and *ABCG8*, respectively. *Am J Hum Genet* 2001; 69: 278-90.
4. Wang J, Mitsche MA, Lutjohann D, Cohen JC, Xie XS, Hobbs HH. Relative roles of *ABCG5/ABCG8* in liver and intestine. *J Lipid Res* 2015; 56: 319-30.
5. Baila-Rueda L, Mateo-Gallego R, Lamiquiz-Moneo I, Cenarro A, Civeira F. Severe hypercholesterolemia and phytosterolemia with extensive xanthomas in primary biliary cirrhosis: role of biliary excretion on sterol homeostasis. *J Clin Lipidol* 2014; 8: 520-4.
6. Miettinen TA, Klett EL, Gylling H, Isoniemi H, Patel SB. Liver transplantation in a patient with sitosterolemia and cirrhosis. *Gastroenterology* 2006; 130: 542-7.
7. Kidambi S, Patel SB. Sitosterolaemia: pathophysiology, clinical presentation and laboratory diagnosis. *J Clin Pathol* 2008; 61: 588-94.
8. Wilund KR, Yu L, Xu F, et al. No association between plasma levels of plant sterols and atherosclerosis in mice and men. *Arterioscler Thromb Vasc Biol* 2004; 24: 2326-32.
9. Mannucci L, Guardamagna O, Bertucci P, et al. Beta-sitosterolaemia: a new nonsense mutation in the *ABCG5* gene. *Eur J Clin Invest* 2007; 37: 997-1000.
10. Yoo EG. Sitosterolemia: a review and update of pathophysiology, clinical spectrum, diagnosis, and management. *Ann Pediatr Endocrinol Metab* 2016; 21: 7-14.
11. Salen G, Horak I, Rothkopf M, et al. Lethal atherosclerosis associated with abnormal plasma and tissue sterol composition in sitosterolemia with xanthomatosis. *J Lipid Res* 1985; 26: 1126-33.
12. Bhattacharyya AK, Connor WE. Beta-sitosterolemia and xanthomatosis. A newly described lipid storage disease in two sisters. *J Clin Invest* 1974; 53: 1033-43.
13. Sethuraman G, Sugandhan S, Sharma G, et al. Familial homozygous hypercholesterolemia: report of two patients and review of the literature. *Pediatr Dermatol* 2007; 24: 230-4.
14. Rios J, Stein E, Shendure J, Hobbs HH, Cohen JC. Identification by whole-genome resequencing of gene defect responsible for severe hypercholesterolemia. *Hum Mol Genet* 2010; 19: 4313-8.
15. Park JH, Chung IH, Kim DH, Choi MH, Garg A, Yoo EG. Sitosterolemia presenting with severe hypercholesterolemia and intertriginous xanthomas in a breastfed infant: case report and brief review. *J Clin Endocrinol Metab* 2014; 99: 1512-8.
16. Manchanda Y, Sharma VK. Intertriginous xanthomas: a marker of homozygous type IIa hyperlipoproteinemia. *Int J Dermatol* 2004; 43: 676-7.
17. Zak A, Zeman M, Slaby A, Vecka M. Xanthomas: clinical and pathophysiological relations. *Biomed Pap Med Fac Univ Palacky Olomouc Czech Repub* 2014; 158: 181-8.

- 18.** Menotti A, Puddu PE, Lanti M, Maiani G, Fidanza F. Cardiovascular risk factors predict survival in middle-aged men during 50 years. *Eur J Intern Med* 2013; 24: 67-74.
- 19.** Niu DM, Chong KW, Hsu JH, *et al.* Clinical observations, molecular genetic analysis, and treatment of sitosterolemia in infants and children. *J Inherit Metab Dis* 2010; 33: 437-43.
- 20.** Belamarich PF, Deckelbaum RJ, Starc TJ, Dobrin BE, Tint GS, Salen G. Response to diet and cholestyramine in a patient with sitosterolemia. *Pediatrics* 1990; 86: 977-81.
- 21.** Parsons HG, Jamal R, Baylis B, Dias VC, Roncari D. A marked and sustained reduction in LDL sterols by diet and cholestyramine in beta-sitosterolemia. *Clin Invest Med* 1995; 18: 389-400.
- 22.** Salen G, Starc T, Sisk CM, Patel SB. Intestinal cholesterol absorption inhibitor ezetimibe added to cholestyramine for sitosterolemia and xanthomatosis. *Gastroenterology* 2006; 130: 1853-7.
- 23.** Cheng WF, Yuen YP, Chow CB, Au KM, Chan YW, Tam SC. Sitosterolaemia and xanthomatosis in a child. *Hong Kong Med J* 2003; 9: 206-9.
- 24.** Escola-Gil JC, Quesada H, Julve J, Martin-Campos JM, Cedo L, Blanco-Vaca F. Sitosterolemia: diagnosis, investigation, and management. *Curr Atheroscler Rep* 2014; 16: 424.
- 25.** Rees DC, Iolascon A, Carella M, *et al.* Stomatocytic haemolysis and macrothrombocytopenia (Mediterranean stomatocytosis/macrothrombocytopenia) is the haematological presentation of phytosterolaemia. *Br J Haematol* 2005; 130: 297-309.
- 26.** Othman RA, Myrie SB, Jones PJ. Non-cholesterol sterols and cholesterol metabolism in sitosterolemia. *Atherosclerosis* 2013; 231: 291-9.
- 27.** Neff AT. Sitosterolemia's stomatocytosis and macrothrombocytopenia. *Blood* 2012; 120: 4283.
- 28.** Goodyer M, Lovey J, Menetrey MJ. Peripheral blood features of phytosterolaemia. *Br J Haematol* 2015; 171: 669.
- 29.** El-Rassy I, Bou-Abdallah J, Al-Ghadban S, Bitar F, Nemer G. Absence of NOTCH2 and Hey2 mutations in a familial Alagille syndrome case with a novel frameshift mutation in JAG1. *Am J Med Genet A* 2008; 146: 937-9.
- 30.** Izar MC, Tegani DM, Kasma SH, Fonseca FA. Phytosterols and phytosterolemia: gene-diet interactions. *Genes Nutr* 2011; 6: 17-26.
- 31.** Salen G, Shefer S, Nguyen L, Ness GC, Tint GS, Shore V. Sitosterolemia. *J Lipid Res* 1992; 33: 945-55.
- 32.** Yu L. The structure and function of Niemann-Pick C1-like 1 protein. *Curr Opin Lipidol* 2008; 19: 263-9.
- 33.** Kwon HJ, Palnitkar M, Deisenhofer J. The structure of the NPC1L1 N-terminal domain in a closed conformation. *PLoS One* 2011; 6: e18722.
- 34.** Lee RG, Willingham MC, Davis MA, Skinner KA, Rudel LL. Differential expression of ACAT1 and ACAT2 among cells within liver, intestine, kidney, and adrenal of nonhuman primates. *J Lipid Res* 2000; 41: 1991-2001.