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Sitosterolaemia in Switzerland: molecular genetics links the US Amish-Mennonites to their European roots

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Abstract

Sitosterolaemia is a rare autosomal recessive disease characterized by increased intestinal absorption of plant sterols, decreased hepatic excretion into bile and elevated concentrations in plasma phytosterols. Homozygous or compound heterozygous loss of function mutations in either of the ATP-binding cassette (ABC) proteins ABCG5 and ABCG8 explain the increased absorption of plant sterols. Here we report a Swiss index patient with sitosterolaemia, who presented with the classical symptoms of xanthomas, but also had mitral and aortic valvular heart disease. Her management over the last 20 years included a novel therapeutic approach of high-dose cholesterol feeding that was semi-effective. Mutational and extended haplotype analyses showed that our patient shared this haplotype with that of the Amish-Mennonite sitosterolaemia patients, indicating they are related ancestrally.

Keywords

Sitosterolaemia; plant sterols; Amish-Mennonites; ABC95; ABC98; valvular xanthomas

Sitosterolaemia is a rare autosomal recessive disease, first described in 1974 (1). The characteristic feature of this disease is the presence of large quantities of plant sterols in the plasma and tissues measured by using either capillary gas liquid or high-performance liquid chromatography, because routine enzymatic cholesterol determination does not distinguish between phytosterols or cholesterol (2–4). The normal western diet contains daily similar amounts of cholesterol and plant sterols. Normal individuals absorb approximately 40–50% of intestinal (from diet and bile) cholesterol, but less than 5% of plant sterols are absorbed and principally excreted by the liver into bile (5–8). Patients with sitosterolaemia absorb more than 50% of plant sterols and show an inability to excrete these sterols in their bile (5,9–12).

Clinical features of sitosterolaemia include the presence of tendon and tuberous xanthomas, especially on Achilles tendon and on extensor tendons of the hands, premature atherosclerotic disease, arthritis and arthralgia and haemolytic anaemia (1,2,9,13,14).

Pedigree assembly and genetic analyses facilitated the localization of the disease locus (*STSL*) to chromosome 2p21 (15,16). Not one but two genes comprise the *STSL* locus, *ABCG5* and *ABCG8*, and homozygous or compound heterozygous mutations in either gene cause the disease (15,17,18). The proposed function of these proteins is to pump plant sterols

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and cholesterol back out into the biliary system or the intestinal lumen (18,19). Clinical management of sitosterolaemia has included a low-phytosterol and low-cholesterol diet, resin therapy and ileal bypass (2,5,9,20–27).

Ezetimibe is the most promising new agent for management in sitosterolaemia, as it specifically inhibits all dietary sterol absorption from the intestinal lumen (28–30).

Here we report the management of a young sitosterolaemic patient over a period of 20 years. Because the absorption of both plant sterols and cholesterol is dependent upon solubilization into micelles and that these sterols compete with each other for solubilization (31,32), we hypothesized that high-dose cholesterol should be able to prevent plant sterols from absorption. This therapy was semi-effective. Mutational analysis showed that our patient was homozygous for a mutation that has been described in the Amish-Mennonite community in the USA (16, 33). Extended haplotype analyses showed a remarkable degree of conservation, suggesting that our patient's ancestors and the Amish-Mennonite ancestors are connected. Lineage tracing of our proband's family suggests that there may be more sitosterolaemia cases in a limited geographic area around the Swiss border.

Materials and methods

All clinical analyses other than plant sterols, cholesterol and genetic analysis were performed using standard methods at the Laboratory of the University Hospital of Bern, Switzerland. Cholesterol and plant sterols analyses were performed by gas chromatography – mass spectroscopy as previously described (30). For genotyping, genomic DNA was isolated from whole blood. Genomic DNA was amplified with microsatellite marker-specific radiolabelled primers as previously described (15,17). Single nucleotide polymorphisms (SNPs) were determined by restriction enzyme digestion pattern or direct sequencing as previously described (16,17).

Case report

Our patient was born in 1976. The diagnosis of sitosterolaemia was recorded at the age of 10 years because of increasing tendon xanthomas by effective low-cholesterol therapy. Unfortunately, no data are available from the time of this diagnosis from her medical records. Consequently, therapy with cholestyramine (4 g daily) was initiated, and the patient was advised to adhere to low-phytosterol and low-cholesterol diets.

Despite the continued therapy and a normal TS[1] level (166 mg/dl), at the age of 19 (1995), xanthomas reached a maximal thickness of 5 cm on both elbows and of 3 cm on both Achilles tendons. The patient admitted a poor compliance for the proposed low-phytosterol diet. Furthermore, she discontinued cholestyramine treatment in 1998 because of increased bloating and constipation. The cholesterol-poor diet alone maintained TS levels at levels lesser than 200 mg/dl. Because both cholesterol and phytosterols compete with each other for entry into micelles, it was reasoned that a high-cholesterol intake may reduce phytosterol absorption, and thus xanthomas progression. At the age of 23 years, empiric therapy with cholesterol tablets, 500 mg/day for 6 weeks, increasing to 1000 mg/day was initiated. Initially TS levels peaked to 276 mg/dl, but after 2 months, they returned to pre-treatment levels (<200 mg/dl). Unfortunately, we do not have cholesterol and plant sterol values before initiation of therapy, but blood sample analysed by GC 3 weeks after initiation showed a cholesterol value of 239 mg/dl and a sitosterol value of 26.4 mg/dl, confirming sitosterolaemia. Interestingly, with

[1]We use the term TS to indicate all sterols measured enzymatically (the standard 'cholesterol' test) to distinguish between gas chromatography (GC) determined cholesterol or plant sterols.

continued therapy, there was progressive fall in cholesterol (25%) and sitosterol (33%) (Fig. 1). High-density lipoprotein sterol (HDL-S) followed a similar pattern as TS (Fig. 1). The TS/HDL-S ratio gradually increased from 3.7 to 5.2. During this therapy, her xanthomas remained unchanged and large.

From October 2000 onwards, cholesterol therapy was discontinued; despite biochemical improvement, there was no clinical improvement. Until April 2001, she received no medication, and her xanthomas remained stable, although TS (245 mg/dl) and HDL-S rose by 25%.

In May 2001, she began therapy with ezetimibe 10 mg/day as part of a clinical trial (30). As a result of this therapy, her TS (176 mg/dl), cholesterol, sitosterol and TS/HDL-S decreased by 28%, 13%, 50% and 32%, respectively (Fig. 1). During ezetimibe therapy, her xanthomas were overall reduced to less than 1 cm.

At the age of 20 (1997), a new 2/6 grade diastolic murmur was noticed. Colour-flow Doppler echocardiography showed a thickening and sclerosis of the mitral valve ring and of the aortic valve leaflets associated with grade I valvular regurgitation across both valves (Fig. 2). There was no history of rheumatic fever. From 1997 until 2001, yearly echocardiograms showed a slight increase in the aortic regurgitation to grade II. Since the beginning of ezetimibe in 2001, her cardiac findings showed neither progression nor regression.

Mutational analysis showed that our patient was homozygous for the Gly574Arg mutation in *ABCG8*. This mutation has been found previously in the Amish-Mennonite patients where a founder effect has been reported (34). Haplotype analyses using microsatellite markers spanning 4.9 cM on chromosome 2 showed that our patient and the two Amish-Mennonite probands were identical for markers *D2S2174, D2S1761, D2S4009, D2S4014, D2S4015* and *D2S4016* encompassing the *STSL* locus (33) (data reviewed but not shown). We have previously reported a number of SNPs of the *STSL* locus (16,17). We compared poly SNPs at the *STSL* locus of our patient to that of two Amish-Mennonite probands (two families). The Swiss proband and one Amish proband shared identical SNPs, with a minor difference between the two Amish-Mennonite probands (Table 1).

A family history from the patient indicated that most of her relatives lived during the last 300 years in a small region between the northeast of German-speaking Switzerland and southern Germany (between the lake of Constance and the Obersaxen in the Canton of Grison). These data support the contention that the Gly574Arg mutation in the Amish-Mennonite originated in Europe and is likely more than 250 years old.

Discussion

Initially, the patient adhered to a cholesterol-poor diet alone or in combination with cholestyramine, but this was insufficient to relieve her symptoms and led to increased side-effects. Every attempt to introduce a phytosterol-poor diet was unsuccessful. We reasoned if plants sterols compete with cholesterol for micellar solubilization when given in high concentrations (and this reduce absorption), the reverse would also be true (31,32). This is the first report of using high doses of cholesterol to reduce plant sterol levels in sitosterolaemia. Despite the decrease of phytosterols in the blood, some caution about this therapy should be expressed. In contrast to the following treatment with ezetimibe, our patient did not report any significant improvement in her xanthomas (despite the relatively short term), and the TS/HDL-S ratio worsened.

In our patient also, we report valvular heart disease limited to the aortic and mitral valves. After exclusion for other causes, we presume that this is a clinical manifestation of her

sitosterolaemia. Deposition of phytosterols-enriched macrophages into the valvular tissue might have caused the valvular heart disease in this patient. Xanthomatosis involving the mitral valve and the entire aorta with occlusion of coronary ostia has been reported at autopsy in a sitosterolaemic girl (35). Interestingly, the valvular regurgitations have remained stable over the 3 years since therapy with ezetimibe was initiated.

Finally, our proband and two probands from the Amish-Mennonite community in the US share the same homozygous mutation of *ABCG8*, and haplotype analysis links these patients (34). We have reported previously that there may be a founder effect for the mutation in the US Amish-Mennonite community (34). Interestingly, while similar founder effects have been proposed for other diseases (36,37), this is the first direct evidence linking two existing communities. If this is true, there may be more undiagnosed individuals in this region, and attempt to identify at-risk individuals should be made.

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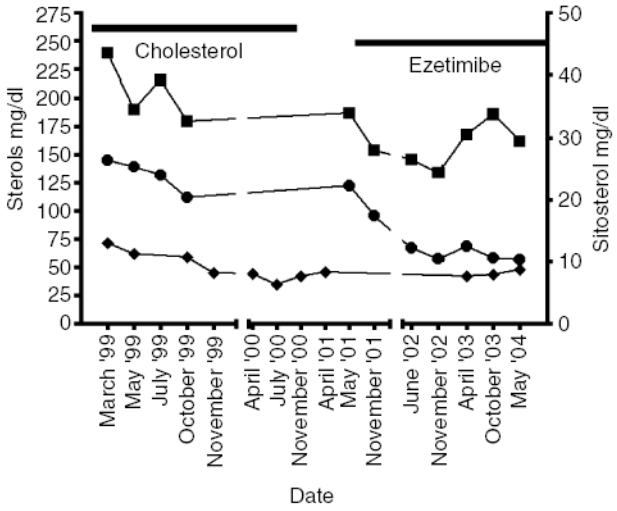

Fig. 1.

Cholesterol, plant sterol and high-density lipoprotein sterol (HDL-S) profiles on oral cholesterol and ezetimibe therapies. The cholesterol (mg/dl) (▪) and sitosterol (mg/dl) (◦) profiles, as determined by gas chromatography (GC), on samples during oral cholesterol therapy (indicated by the horizontal line) and on ezetimibe monotherapy. Because the enzymatic test was used to determine the HDL-cholesterol values (mg/dl) (♦), the term HDL-S is used. After an initial decrease, the plasma cholesterol values remained around 190 mg/dl on cholesterol tablets and 170 mg/dl on ezetimibe. In contrast, note that the plant sterol values fell on oral cholesterol therapy. The greatest and most persistent falls (more than 50%) were during therapy with ezetimibe, with lowering of sitosterol to the lowest values our patient has ever achieved (10 mg/dl).

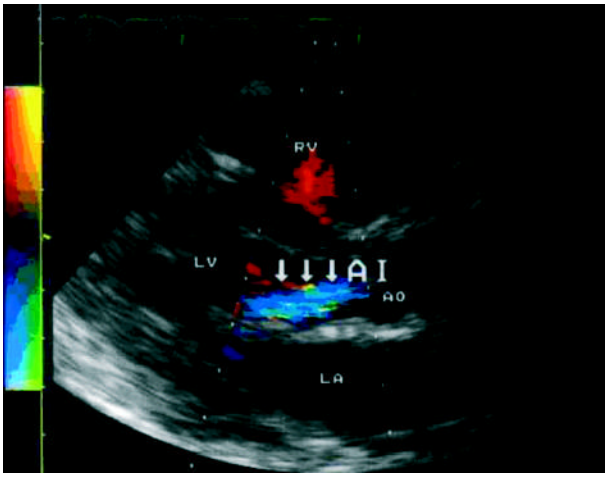

Fig. 2.
Doppler colour echocardiogram demonstrating the valvular heart disease in our patient. The left ventricle (LV), left atrium (LA), right ventricle (RV) and the aorta (Ao) are as indicated. The colour flow patterns show the aortic valve regurgitation (blue and indicated by the arrows).

Table 1

Single nucleotide polymorphisms (SNP) of *ABCG5* and *ABCG8* of the Swiss/German proband and the two Amish-Mennonite probands

Gene	SNP	Position	refSNP	Swiss/German	Amish 1	Amish 2
ABCG5	EXI	P9P		CC	CC	CC
	EX2	R50C	rs6756629	CC	CC	CC
	EX11	V523I		AA	AA	AA
	EX13	C600Y		GG	GG	GG
	EX13	Q604E	rs6720173	GG	GG	GG
	EX13	V622M		AA	AA	AA
ABCG8	5′UTR	41		CC	CC	CC
	5′UTR	19	rs11887534	GG	GG	TT[a]
	EX1	P17P		CC	CC	CC
	EX1	D19H		GG	GG	GG
	INT1	21		CA	CA	CA
	INT1	7		CT	CT	TT[a]
	EX2	C54Y	rs4148211	GG	GG	GG
	EX6	E238L		GG	GG	GG
	EX6	A259V		CC	CC	CC
	EX7	Q340E		GG	GG	GG
	EX8	T400K	rs9282575	CC	CC	CC
	INT9	19		CC	CC	CC
	INT10	50		CC	CC	CC
	EX11	A565A	rs4148217	CC	CC	CT[a]
	EX11	G575R		GG	GG	GG
	EX13	A632V	rs6544718	CC	CC	CC

[a]The minor differences in one of the Amish-Mennonite proband.

Note the high degree of SNP conservation as well as homozygosity of the SNPs at the *STSL* locus.