Case Report

Potential Effects of \textit{NPC1L1} Polymorphisms in Protecting against Clinical Disease in a Chinese Family with Sitosterolaemia

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Sitosterolaemia is caused by mutations in either \textit{ABCG5} or \textit{ABCG8}. Chinese and Japanese individuals usually have mutations in \textit{ABCG5}. We herein report a known and a novel mutation in \textit{ABCG8} and their potential interaction with \textit{NPC1L1} polymorphisms in a Chinese family with sitosterolaemia. We sequenced \textit{ABCG5} and \textit{ABCG8} and measured the levels of plasma plant sterols in a 15-year-old Chinese girl with clinical sitosterolaemia (xanthomas with elevated low-density lipoprotein cholesterol (LDL-C) and plant sterols) and her apparently healthy family members. \textit{NPC1L1} was sequenced in the genetically affected sibling and other family members. A known mutation, c.490C>T (p. Arg164\*) in exon 4 and a novel mutation, c.1949T>G (p.Leu650Arg) in exon 13 of \textit{ABCG8} were detected in the proband and her sister, who had elevated sterols but low LDL-C levels and no xanthomas. The genetically affected sister, but not the proband, carried two additional heterozygous changes in \textit{NPC1L1} (rs2072183 C>G, rs2301935 A>C), which were inherited from the mother, who also had a low LDL-C level. In this study, we detected a known and a novel mutation in \textit{ABCG8} in a Chinese patient with sitosterolaemia. The same mutations were found in her clinically normal sister, suggesting that the contrasting features with the proband may be related to different variants in \textit{NPC1L1} and/or some other undetermined lipid-related genetic factors.


Key words: Sitosterolaemia, Plant sterols, LDL-C, ABCG8, NPC1L1

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Received: March 6, 2014
Accepted for publication: June 5, 2014

Introduction

Sitosterolaemia (MIM #210250) is a very rare autosomal recessive disease characterized by excessive absorption and high plasma levels (>30-fold) of plant sterols, particularly sitosterol and campesterol, the two major plant-derived sterols\textsuperscript{1,2}. The clinical manifestations include tendon and tuberous xanthomas and premature atherosclerotic disease as a result of sterol deposition in the skin, tendons and coronary arteries\textsuperscript{1,3}. Patients with sitosterolaemia, in whom there is increased absorption and decreased biliary excretion of cholesterol (similar to plant sterols), usually have normal to moderately elevated plasma cholesterol levels, despite the downregulation of endogenous cholesterol synthesis in hepatocytes\textsuperscript{4}. Intestinal sterol/cholesterol absorption is governed by three key transporter molecules, including the two ATP-binding cassette (ABC) half transporters, ABCG5 and ABCG8 (previously known as sterolin-1 and -2, respectively), which heterodimerise to form a sterol efflux transporter in the liver and intestine to transport sterols (cholesterol and plant sterols) and the Niemann-Pick C1-like 1 (NPC1L1) transporter, a polytopic transmembrane protein that mediates intestinal absorption of cholesterol and sterols\textsuperscript{5}. Sitosterolaemia is caused by mutations in either \textit{ABCG5} or \textit{ABCG8}\textsuperscript{6}. It has been estimated that sitosterolaemia occurs in 1 in 5 million people, although the true
prevalence of the disease is unknown because a significant number of cases remain undiagnosed. Caucasians usually carry mutations in ABCG8, whereas Chinese and Japanese patients are more likely to have mutations in ABCG5.

Several mutations in ABCG5 responsible for sitosterolaemia in Chinese individuals have been described. We herein report a novel mutation in ABCG8 in a 15-year-old Chinese girl with clinical sitosterolaemia and her apparently normal sibling and discuss how polymorphisms in NPC1L1 may modify the manifestations of the disease.

Case Presentation

Patients

The studied family comprised five family members, including three siblings and their parents (Fig. 1). The proband was a 15-year-old Han Chinese girl, the third child of healthy unrelated parents (subject II-3, Fig. 1A), who was referred for an evaluation of hypercholesterolaemia due to the presence of xanthomas on the hands and Achilles tendons that caused some discomfort. She had noticed xanthomas on the extensor tendons of the fingers since 11 years of age, while a new xanthoma had developed more recently on the extensor tendon to the left thumb. An ultrasound examination of the xanthomas on the hands (Fig. 2A) revealed focal thickening to over twice the normal size of the extensor tendon expansions overlying the metacarpophalangeal joints of the middle and ring fingers bilaterally (Fig. 2B). Ultrasound of both Achilles tendons also demonstrated diffuse tendon thickening bilaterally, particularly of the proximal to mid-portion of the tendon, with more discrete areas of tendon hypoechochogenicity (Fig. 2C). The maximum Achilles tendon cross-sectional area was 1.9 cm² (normal 0.54 ±0.11 cm²). The patellar tendons were normal. Magnetic resonance imaging of the Achilles tendons showed similar findings, with tendon thickening and heterogeneity primarily involving the proximal to mid-third of the Achilles tendons (Fig. 2D, 2E).

The proband was previously well, with the exception of one episode of convulsions, with no specific cause, at 2 years of age. Her body weight and height were in the lower part of the normal range (5th and 25th percentiles, respectively) of the growth curves for Hong Kong children. She had reached menarche at 14 years of age and experienced regular menses thereafter. The patient had elevated plasma total cholesterol (7.5 mmol/L) and LDL-C (4.6 mmol/L) levels (Table 1), whereas both of her parents and two siblings had normal or low low-density lipoprotein cholesterol (LDL-C) levels, and there was no family history of premature coronary artery disease or xanthomas. All family members were clinically euthyroid, and thyroid function tests in the proband and her sister with a low LDL-C level showed the plasma sensitive thyroid-
Sitosterolaemia in a Chinese Family

stimulating hormone (sTSH) levels to be within the normal range (1.16 and 1.11 mIU/L, respectively; local age-related reference range: 0.27-4.20 mIU/L). The proband as well as her mother and sister were thin and underweight (body mass index <18.5 kg/m²; Table 1). Since the inheritance pattern of the hypercholesterolaemia in this family was most consistent with a recessive disorder, and, based on our experience, Chinese patients with heterozygous dominant familial hypercholesterolaemia do not have xanthomas with such a level of LDL-C at this age, sitosterolaemia rather than autosomal recessive hypercholesterolaemia appeared to be the most likely diagnosis.

The Joint Clinical Research Ethics Committee of
Table 1. Characteristics of each study subject

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>I-1</th>
<th>I-2</th>
<th>II-1</th>
<th>II-2</th>
<th>II-3 (Proband)</th>
<th>II-3 on ezetimibe for 6 weeks</th>
<th>II-3 on ezetimibe for 12 weeks</th>
<th>Ref Range</th>
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<tr>
<td>Age (years)</td>
<td>49</td>
<td>47</td>
<td>24</td>
<td>22</td>
<td>15</td>
<td></td>
<td></td>
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<tr>
<td>Body mass index (kg/m²)</td>
<td>22.6</td>
<td>17.6</td>
<td>23.0</td>
<td>17.8</td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total cholesterol (mmol/L)</td>
<td>6.1</td>
<td>5.0</td>
<td>5.8</td>
<td>4.3</td>
<td>7.5</td>
<td>5.4</td>
<td>4.9</td>
<td>&lt;5.2</td>
</tr>
<tr>
<td>HDL-C (mmol/L)</td>
<td>2.0</td>
<td>3.0</td>
<td>1.8</td>
<td>2.1</td>
<td>2.3</td>
<td>2.3</td>
<td>1.4</td>
<td></td>
</tr>
<tr>
<td>Triglycerides (mmol/L)</td>
<td>2.1</td>
<td>0.7</td>
<td>1.1</td>
<td>0.5</td>
<td>1.4</td>
<td>1.4</td>
<td>0.8</td>
<td></td>
</tr>
<tr>
<td>LDL-C (mmol/L)</td>
<td>3.2</td>
<td>1.7</td>
<td>3.5</td>
<td>1.9</td>
<td>4.6</td>
<td>2.5</td>
<td>3.1</td>
<td>&lt;4.1</td>
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<tr>
<td>Beta-sitosterol (μmol/L)</td>
<td>10.0</td>
<td>15.0</td>
<td>9.0</td>
<td>497</td>
<td>582</td>
<td>420</td>
<td>341</td>
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<tr>
<td>Campesterol (μmol/L)</td>
<td>21.1</td>
<td>23.4</td>
<td>13.0</td>
<td>246</td>
<td>354</td>
<td>249</td>
<td>202</td>
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<td>Stigmasterol (μmol/L)</td>
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<td>&lt;0.1</td>
<td>&lt;0.1</td>
<td>17.2</td>
<td>24.4</td>
<td>22.5</td>
<td>25.4</td>
<td>≤3.5</td>
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<td>Cholesterol (μmol/L)</td>
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<td>11.7</td>
<td>7.7</td>
<td>23.0</td>
<td>66.1</td>
<td>51.4</td>
<td>46.0</td>
<td>≤13</td>
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<tr>
<td>NPC1L1 rs2072183 C&gt;G</td>
<td>CC</td>
<td>CG</td>
<td>CC</td>
<td>CC</td>
<td>CC</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NPC1L1 rs2301935 A&gt;C</td>
<td>AA</td>
<td>AC</td>
<td>AA</td>
<td>AC</td>
<td>AA</td>
<td></td>
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<tr>
<td>NPC1L1 rs4720470 G&gt;A</td>
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<td>AA</td>
<td>GA</td>
<td>GA</td>
<td>GA</td>
<td></td>
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</tbody>
</table>

the Chinese University of Hong Kong and New Territories East Cluster approved the study. Written informed consent was obtained from the parents of the proband and all the other adult participants enrolled in the study for the genetic and other analyses to determine the cause for hypercholesterolaemia in the proband and identify the pattern of inheritance.

**Determination of the Plasma Sterol Levels**

The plasma concentrations of plant sterols, including cholestanol, campesterol, stigmasterol and β-sitosterol, were measured in all participants using gas chromatography-mass spectrometry at the Clinical Biochemistry Unit, Queen Mary Hospital, Hong Kong. The plasma sterol concentrations in the proband were markedly elevated, with β-sitosterol and campesterol levels of approximately 50-fold and 20-fold higher, respectively, than the upper limits of normal (Table 1). Meanwhile, the proband’s parents exhibited slight elevation of the β-sitosterol and campesterol levels, while her brother had normal values of these parameters. Interestingly, her elder sister, who had a low LDL-C level and no evidence of xanthomas clinically or on ultrasound examinations, also had high levels of plasma sterols (Table 1). As a surrogate marker for atherosclerosis, the carotid intima media thickness was assessed using ultrasound and found to be normal in all family members.

**Molecular Genetics**

Genomic DNA was extracted from peripheral white blood cells in all enrolled subjects. All coding exons and flanking introns of the ABCG5 and ABCG8 genes were amplified via standard polymerase chain reaction (PCR) (the primer sequences and PCR protocol are available on request) and then directly sequenced in both directions according to the dideoxy-terminator method on an automated capillary sequencer. The GenBank reference sequences NM_022436.2 and NM_022437.2 were used for ABCG5 and AGCB8, respectively.

The mutation analysis of the proband showed no pathogenic mutations in the ABCG5 gene, but two heterozygous changes c.490C>T and c.1949T>G were identified in the ABCG8 gene. The predicted change for c.490C>T is the creation of a premature stop codon at amino acid position 164 (p.Arg164*). The c.1949T>G is a novel missense change that is predicted to cause a substitution of leucine at position 650 by arginine (p.Leu650Arg). This mutation was not detected in 180 Hong Kong Chinese subjects. Compound heterozygosity of ABCG8 c.490C>T and c.1949T>G in the proband was confirmed on an analysis of the parents. The c.490C>T mutation was inherited from the mother and the c.1949T>G mutation from the father. The asymptomatic elder sister with elevated plant sterols had the same ABCG8 genotype as the proband (Table 1). The elder brother with normal plant sterols was a carrier of the maternal ABCG8 c.490C>T mutation.

The coding exons and flanking introns of the NPC1L1 gene were subsequently sequenced to examine the possibility that mutations in NPC1L1 may have contributed to the contrasting features of the proband and her genetically affected sibling. Sequencing of NPC1L1 in the genetically affected sister identified three heterozygous changes in NPC1L1 (rs2072183 C>G, rs2301935 A>C, rs4720470 G>A), whereas...
the proband only carried one of these heterozygous changes (rs4720470 G>A) (Table 1). The heterozygosity for the NPC1L1 rs2072183 C>G, rs2301935 A>C mutations in the asymptomatic genetically affected sister was inherited from the mother.

**Management**

Based on the DNA test results and plasma sterol levels, a diagnosis of sitosterolaemia was made for the proband and her sister. Both patients were advised to reduce their intake of plant sterols; however, the consumption of a sterol-restricted diet for four weeks did not improve the plasma cholesterol or sterol levels in the proband. The proband was subsequently treated with ezetimibe at a dose of 10 mg daily, and her plasma LDL-C and sterol levels gradually improved thereafter, although the sterol concentrations remained elevated (Table 1). Her affected sibling has not been treated with any medical interventions for the time being. Both patients receive regular follow-up at the lipid research clinic.

**Discussion**

Previous studies have demonstrated that most Japanese and Chinese probands with sitosterolaemia have defects in ABCG5, ABCG8, although mutations of Arg263Gln/Pro231Thr in ABCG8 have been reported in one Chinese patient. Similarly, only mutations in ABCG5 but not ABCG8, were found to be responsible for sitosterolaemia in two previously reported local cases and in five cases reported from Taiwan. However, among the present cases of sitosterolaemia, we found one known mutation (p.Arg164*) in exon 4, and a novel mutation, p.Leu650Arg, in exon 13 of ABCG8. The Arg164* mutation in ABCG8 has previously been reported in a South African family of Indian Asian origin, whereas this is the first report of the p.Leu650Arg mutation in patients with sitosterolaemia. The presence of this mutation in our patient and her sibling with high plasma sterol levels confirmed that the mutation is causative.

Clinically, there are no apparent differences between patients with mutations in ABCG5 or ABCG8. It has been shown that there is considerable heterogeneity in the clinical features, particularly atheromatous and skin manifestations, and biochemical parameters of patients with sitosterolaemia caused by the same mutations. In the current cases, the proband and her affected sibling also had different phenotypes. The proband exhibited elevated plasma levels of sterols and LDL-C and tendon xanthomas, whereas her genetically affected sister displayed low LDL-C with 15% and 25% lower levels of plasma sitosterol and campesterol, respectively. The level of LDL-C observed in the proband does not usually cause xanthomas in young heterozygous familial hypercholesterolaemia patients, and the sibling of the proband was free of xanthomas, suggesting that the deposition of both sterols and cholesterol contributes to the formation of xanthomas in the setting of sitosterolaemia, as previously reported.

The disparate phenotypes of the two sisters may also be explained by other genetic and/or environmental factors that modulate the clinical manifestations of the disease. For example, the dietary sterol intake may influence the plasma sterol levels, although the two patients lived in the same household and reported consuming a similar diet. Animal studies shown that the genetic inactivation of NPC1L1 protects against sitosterolaemia in mice lacking Abcg5/Abcg8. In humans, rare variants in NPC1L1 have been reported to be associated with reduced sterol absorption and plasma LDL-C levels. It is unclear whether the common synonymous mutation rs2072183 1735C>G and the intronic SNP rs2301935 in NPC1L1 are functional and contributed to the differences in plasma sterol levels between the two sisters. The rs2072183 polymorphism is the top SNP within the NPC1L1 gene most significantly associated with an increased plasma LDL-C level according to a genome-wide analysis of Caucasians. However, this polymorphism appears to be associated with reduced LDL-C levels in East Asians, and other recent studies have demonstrated that the rs2072183 polymorphism is associated with increased cholesterol levels in men only and may interact with dietary cholesterol to determine the cholesterol levels. It is also possible that mutations in NPC1L1 or polymorphisms in other genes influencing cholesterol absorption or other pathways inherited from the mother, who also had a low LDL-C level (1.7 mmol/L), may have contributed to the reduced plasma sitosterol, campesterol and LDL-C levels in the affected sibling relative to the proband. Potential genetic factors responsible for the low LDL-C level in the sister of the proband include mutations in the LDL receptor (LDLR), proprotein convertase subtilisin/kexin type 9 (PCSK9) or apolipoprotein B (APOB) genes, three well-known lipid-related genes, and/or other candidate genes, such as apolipoprotein E (APOE), microsomal triglyceride transfer protein (MTP), myosin regulatory light chain interacting protein (MYLIP/IDOL), sterol response element-binding protein (SREBP), LDLR adaptor protein 1 (LDLRAP1/ARH), angiopoietin-like 3 (ANGPTL3), 3-hydroxy-3-methylglutaryl-CoA reducta-
tase (HMGCR) and other lipid-related genes identified in genome-wide association studies\(^7\). Furthermore, it is possible that the differences in the LDL-C levels observed between the two sisters were due to some other novel lipid-related genes that have not yet been identified. However, we did not perform investigations of other possible genetic factors accounting for the differences in the LDL-C level noted in the two sisters; this is one of the limitations of this study.

Previous case reports have suggested that sitosterolaemia is associated with rapidly progressive atherosclerosis and premature coronary heart disease, and the administration of effective long-term therapy is advised in such cases in order to halt the progression of atherosclerotic disease\(^20, 21\). In the general population, there are conflicting data as to whether mild to moderately elevated plasma plant sterol levels are a risk factor for cardiovascular disease\(^22\). However, the full clinical spectrum of sitosterolaemia is likely not known due to under-reporting and/or underdiagnosis of the condition in patients with mild or no clinical features, such as the affected sibling of the proband\(^9\). Whether these patients, particularly those with normal plasma LDL-C levels, should receive long-term treatment to reduce the plasma sterol levels at an early age remains uncertain, although it has been reported that patients with sitosterolaemia have developed coronary heart disease after the plasma LDL-C, but not sterol, level was treated and controlled\(^21\). It is worth noting that the mother of the proband and the two affected sisters each exhibited elevated LDL-C levels, particularly the mother, who had a markedly elevated HDL-C level of 3.0 mmol/L, a value compatible with the complete loss of the (cholesteryl ester transfer protein) CETP activity\(^22\). Large-scale prospective studies have demonstrated a strong inverse relationship between the plasma HDL-C level and risk of coronary artery disease\(^23\). Therefore, whether the plasma HDL-C level modulates the risk of coronary artery disease in patients with sitosterolaemia warrants further investigation.

Current therapies for sitosterolaemia, including bile acid sequestrants and ezetimibe, an NPC1L1 inhibitor, have moderate effects (20-50%) in reducing the levels of plasma sterols, and these parameters remain substantially elevated in most treated patients\(^9, 21\). However, the plasma cholesterol levels are usually well controlled with ezetimibe. In the current study, the plasma LDL-C level in the proband was reduced by nearly 50%, which is much greater than that seen in patients with primary hypercholesterolaemia (<20%). This finding may be related to the complex role of NPC1L1 in governing cellular cholesterol homeostasis (e.g., cholesterol absorption and endogenous cholesterol synthesis) and its interaction with the disease process\(^4\). Therefore, a diagnosis of sitosterolaemia may be considered in patients with an elevated LDL-C level who exhibit an unusually large LDL-C response to ezetimibe.

In summary, we detected a known mutation and novel mutation in ABCG8 in a Chinese patient with clinical sitosterolaemia. The same mutations were found in her clinically normal sister with elevated plasma sterol levels. The contrasting features of the proband and her genetically affected sibling may be related to mutations in NPC1L1 and/or some other as yet undetermined lipid-related genetic factors. The two cases described in this report highlight the diverse clinical spectrum of this disease.

**Acknowledgements**

We thank the family for participating in this study and Dr. SC Tam, Clinical Biochemistry Unit, Queen Mary Hospital, Hong Kong for carrying out the measurements of the plasma sterol levels.

**Conflicts of Interest**

The authors declare that they have no known conflicts of interest.

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