Japanese Sisters Associated with Pseudohomozygous Familial Hypercholesterolemia and Sitosterolemia

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Pseudohomozygous familial hypercholesterolemia is a rare condition of unknown etiology. Sitosterolemia is a rare autosomal recessively inherited disorder that is characterized by premature coronary artery disease, cutaneous xanthomas, and increased plasma plant sterols and 5α-stanols. Only a few cases of both sitosterolemia and pseudohomozygous familial hypercholesterolemia have been reported. In this study, we report two sisters with both conditions. With a low-cholesterol diet (<250 mg/day), serum cholesterol concentration decreased rapidly to an almost normal level and cutaneous xanthomas gradually regressed and finally disappeared; however, plant sterol levels did not change during the period. Plant sterols should be measured in patients considered to have pseudohomozygous familial hypercholesterolemia. The two conditions in this family may have been the results of a single gene mutation. The findings also indicate that low cholesterol diet therapy is effective for the treatment of hypercholesterolemia but not of sitosterolemia in this family. J Atheroscler Thromb, 2000; 7: 33-38.

Key words: Cutaneous xanthoma, Plant sterol, Diet therapy, Inheritance

Introduction

Pseudohomozygous familial hypercholesterolemia (phFH) or pseudohomozygous type II hypercholesterolemia is characterized by the following abnormalities: [1] severe hypercholesterolemia (total plasma cholesterol level of 350 to 700 mg/dl) due to a selective elevation in low density lipoprotein (LDL); [2] a normal triglyceride level; [3] cutaneous planar xanthomas of the type seen in homozygous familial hypercholesterolemia (FH); [4] normal or slightly elevated plasma cholesterol levels in both parents; and [5] a striking response to treatment with dietary restriction of cholesterol and the use of cholestyramine, resulting in complete regression of xanthomas (1). In this rare condition, the high plasma cholesterol values and xanthomas are readily treated by dietary cholesterol restriction and the use of a binding resin, cholestyramine. phFH is clinically similar to LDL receptor-deficient homozygous familial hypercholesterolemia although LDL receptor in phFH is normal (2) and the condition is sporadic and does not involve a family history of dyslipidemia. However, it is important to point out that the clinical presentation of phFH is also quite similar to that of sitosterolemia.

Sitosterolemia, also known as phytosterolemia, is a rare autosomal recessive genetic disorder of sterol metabolism, which was first described in 1974 by Bhattacharyya and Connor (3, 4). Extensive tendon and cutaneous xanthomas usually develop during childhood despite normal levels of plasma cholesterol. The condition is characterized by high levels of plant sterols in the plasma and tissues. Increased absorption of plant sterols, possibly combined with impaired excretion, has been suggested as the cause of the disease (5, 6).

Almost half the patients of sitosterolemia have plasma cholesterol concentrations within the normal range. In most of the remaining cases, moderate hypercholesterolemia has been reported (7). Only in one patient,
plasma cholesterol has been reported to be as high as 750 mg/dl (8). Hyperapobetalipoproteinemia combined with normal or mildly increased plasma total LDL-cholesterol levels was described in several cases. However, two Chinese patients have been reported to have a combination of elevated plant sterol levels and phFH (9). In the present study, we report two Japanese sisters with phFH who also have elevated serum sitosterol levels.

**Materials and Methods**

Blood was drawn from each subject after an overnight fast. The lipoprotein fractions were isolated serially by ultracentrifugation, according to the procedure described by Hatch and Lees (10).

Serum total cholesterol (TC), triglycerides (TG), and high density lipoprotein-cholesterol (HDL-C) were determined using enzymatic assay kits (Kyowa Medex, Japan). Apolipoprotein (Apo) A1, AII, B, CII, CIII and E were measured by turbidimetric immunocassay kits (Daichi Pure Chemicals, Japan). Lipoprotein (a) (Lp(a)) was determined by a latex immunoassay method (Daichi Pure Chemicals, Japan). Remnant-like particles cholesterol (RLP-C) was determined by an immune adherence method (Japan Immunoresearch Laboratories, Japan). Agarose gel immunoelectrophoresis was performed with a commercial kit (Helena Laboratories, Japan). Polyacrylamide gel lipoprotein disc electrophoresis was performed using a commercial kit (Quantimetrix, USA). Plant sterols and cholestanol were analyzed by gas chromatography as described by Kuksis et al. (11). Lymphocyte low density lipoprotein (LDL) receptor activity was measured by the method described by Ranganathan et al. (12). All studies were approved by the institutional review board in Nakatsugawa Municipal Hospital, and written consent was obtained from the participants or, in the case of minors, from their parents.

**Case Reports**

Patient 1, a girl, was born in 1993 as the first child of nonconsanguineous parents. She was first noted to have cutaneous xanthomas on both knees at 10 months of age. Her parents consulted a dermatologist. In the clinic, hypercholesterolemia was detected, showing serum TC of 803 mg/dl, HDL-C of 39 mg/dl, and TG of 91 mg/dl, and she was referred to Nakatsugawa Municipal Hospital. Streak-like cutaneous xanthomas were noted in the creases of her feet, hands, and buttocks (Figs 1A, 1B, and 1C). Nodular xanthomas were found on the knees (Fig. 1D). Biopsy of a xanthoma showed the accumulation of foam cells in the dermis (Fig. 2). The tendon xanthomas and palpebral xanthelasmas were not found. The results of the remainder of the physical examination were unremarkable. Results of the following investigations were normal: complete blood count, serum chemistry including liver, renal, and thyroid function tests, urinalysis, chest radiography, electrocardiography, and echocardiography. Serum TC was 704 mg/dl and apolipoprotein B was 356 mg/dl (Tables 1, 2). However, homozygous FH was excluded because her parents showed normal LDL-cholesterol levels and did not have Achilles tendon thickening. LDL receptor activity of lymphocytes was 84%, suggesting that it was normal. Gas chromatographic analysis of serum sterols showed that sitosterol was 155.8 μg/ml, indicating the presence of sitosterolemia (Table 1, Fig. 3). The patient was put on a low-cholesterol diet (<250 mg/day); the serum cholesterol concentration decreased rapidly to an almost normal level (Fig. 4). Xanthomas progressed during the 7 months after diet therapy was started, then regressed gradually, and almost disappeared after 2 years. However, the serum sitosterol level remained high during the diet therapy (172.7 μg/ml after 3 years).

Patient 2, a girl, was born in 1997 as the second child of the same parents. At the age of 6 months, when she was admitted to the hospital because of bronchopneumonia, she was first noted to have hypercholesterolemia. Serum TC was 527 mg/dl, and sitosterol was 46.8 μg/ml (Table 1). During the feeding of maternal milk, serum TC was in the range between 700 and 900 mg/dl. At 1 year of age, cutaneous xanthomas appeared. After stopping intake of maternal milk, low-cholesterol diet therapy was started.

No hemolytic period was observed in these girls.

Family background. Serum lipids of their parents, who are obligate heterozygotes of sitosterolemia, were also analyzed (Tables 1, 2). Gas chromatographic analysis of their father’s sterols showed that sitosterol was 3.7 μg/ml, indicating that the value was slightly higher than that of control volunteers (2.4±0.7 μg/ml). Gas chromatographic analysis of their mother’s sterols showed that sitosterol was 9.0 μg/ml, campesterol was 10.3 μg/ml, and cholestanol was 5.8 μg/ml, indicating that these values were higher than those of control volunteers (2.4±0.7 μg/ml, 4.9±1.4 μg/ml and 2.4±0.7 μg/ml, respectively).

Serum lipid profiles of the sisters and their parents are shown in Tables 1, 2. The relatives of the sisters were also investigated. There was no record of consanguinity for three generations. The serum lipid profile is summarized in Table 1. Three additional members (cases 6, 7 and 9) were presumed to be heterozygous for sitosterolemia because of their abnormally high serum sitosterol levels. Proposed inheritance of the disease is shown in Fig. 5. Patient 7 had angina pectoris. Others examined had no evidence of coronary heart disease.

**Discussion**

Sitosterolemia, characterized by an increase of plasma
A

B

C

D

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phytosterol levels, is not necessarily accompanied by hypercholesterolemia. Hidaka et al. (13) reported that in homozygotes of sitosterolemia, plasma TC was 195 ± 18.5 mg/dl, and in heterozygotes, 195 ± 41.7 mg/dl, and about half of the homozygous patients were normolipidemic.

Beaty et al. (14) reported that in heterozygotes, plasma sitosterol levels were normal; however, Hidaka et al. (13) reported that in Japanese heterozygotes, slight increases of sitosterol were found. In our study, their mother showed slight increases of serum sitosterol, campesterol, stigmasterol and cholestanol. However, their father showed only a marginal increase of serum sitosterol. These results are consistent with the cases reported by Hidaka et al. (13).

In phFH, high plasma cholesterol values and xanthomas are readily treated by dietary cholesterol restriction and the use of cholestyramine (15, 16). Because our patients were children, we did not use cholestyramine and chose diet therapy, and started to restrict cholesterol intake because it was impossible to restrict both cholesterol and plant sterols. Hypercholesterolemia of Patient 1 markedly responded to diet therapy. However, sitosterolemia did not respond to the therapy, consistent with the results reported by Hidaka et al. (13). Although serum sitosterol levels did not decrease by the therapy, cutaneous xanthomas decreased gradually, suggesting that hypercholesterolemia is mainly involved in the formation of cutaneous

Fig. 1. Cutaneous xanthomas in areas of the ankle (A), wrist (B), buttocks (C) and knee (D). Arrows in A, B, and C show streak-like cutaneous xanthomas. Arrow in D shows the region of the nodular xanthoma biopsied (Fig. 2).

Fig. 2. Histologic appearance of xanthoma from the knee of Patient 1. ×500.
The cases reported by Low et al. (9) and by us suggest that sitosterolemia and phFH can be associated. Goldstein et al. (1) suggested that since the clinical features of the two conditions were virtually identical, many, if not all, of the children diagnosed as having phFH may actually have sitosterolemia. Molecular bases of these disorders remain to be elucidated. Segregation analyses for the genes for sterol regulatory binding proteins, acyl coenzyme A : cholesterol acyltransferase, microsomal triglyceride transfer protein, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, HMG-CoA synthase and LDL-receptor excluded these as sites of mutation (17). Very recently, Patel et al. (18) localized the genetic defect to chromosome 2p21 by studying families with sitosterolemia. The abnormality in the absorption of sterols may be a plausible mechanism underlying these diseases. In usual phytosterolemic patients, the abnormality is mainly the absorption of plant sterols, although the absorption of cholesterol has also been reported to be in the high normal range (19). However, in sitosterolemic patients associated with phFH, the increase of cholesterol absorption may be more pronounced than that of plant sterol absorption. As proposed by Goldstein et al. (1), plant sterols should be measured in all patients considered to have phFH.

Table 1. Serum lipid profile of the family members investigated. Normal ranges of serum phytosterols and cholestanol were determined from healthy volunteers (mean ± SD, n=17). ND: not determined.

<table>
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<tr>
<th>Case number</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
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<tr>
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<td>63</td>
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<td>63</td>
<td>61</td>
<td>34</td>
<td>11</td>
<td>9</td>
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<tr>
<td>TC (mg/dl)</td>
<td>704</td>
<td>527</td>
<td>165</td>
<td>253</td>
<td>212</td>
<td>215</td>
<td>210</td>
<td>225</td>
<td>305</td>
<td>209</td>
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<tr>
<td>TG (mg/dl)</td>
<td>108</td>
<td>61</td>
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<td>36</td>
<td>145</td>
<td>132</td>
<td>67</td>
<td>119</td>
<td>54</td>
<td>255</td>
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<td>HDL-C (mg/dl)</td>
<td>36</td>
<td>50</td>
<td>51</td>
<td>102</td>
<td>50</td>
<td>77</td>
<td>78</td>
<td>69</td>
<td>80</td>
<td>60</td>
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<tr>
<td>Sitosterol (µg/ml)</td>
<td>155.8</td>
<td>46.8</td>
<td>3.7</td>
<td>9.0</td>
<td>1.9</td>
<td>7.0</td>
<td>6.6</td>
<td>3.1</td>
<td>13.8</td>
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<td>2.6</td>
<td>2.4±0.73</td>
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<td>Campesterol (µg/ml)</td>
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<td>19.4</td>
<td>5.5</td>
<td>10.3</td>
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<td>10.5</td>
<td>8.8</td>
<td>ND</td>
<td>11.4</td>
<td>ND</td>
<td>ND</td>
<td>4.9±1.4</td>
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<td>Sitostanol (µg/ml)</td>
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<td>3.2</td>
<td>&lt;1.0</td>
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<td>ND</td>
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<td>Cholestanol (µg/ml)</td>
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<td>35.6</td>
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<td>5.8</td>
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<td>1.7</td>
<td>ND</td>
<td>4.8</td>
<td>ND</td>
<td>ND</td>
<td>2.4±0.7</td>
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</table>

Table 2. Apolipoproteins, RLP-C, and Lp(a) of Cases 1, 2, their father (Case 3) and mother (Case 4).

<table>
<thead>
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<td>112</td>
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<td>194</td>
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<tr>
<td>Apo A-II (mg/dl)</td>
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<td>23.8</td>
<td>36.1</td>
<td>33.1</td>
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<td>Apo B (mg/dl)</td>
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<td>264</td>
<td>77</td>
<td>98</td>
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<tr>
<td>Apo C-II (mg/dl)</td>
<td>4.2</td>
<td>3.0</td>
<td>4.6</td>
<td>2.4</td>
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<td>Apo C-III (mg/dl)</td>
<td>12.3</td>
<td>6.8</td>
<td>11.3</td>
<td>9.1</td>
</tr>
<tr>
<td>Apo E (mg/dl)</td>
<td>16.9</td>
<td>8.3</td>
<td>3.5</td>
<td>6.4</td>
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<tr>
<td>RLP-C (mg/dl)</td>
<td>34.9</td>
<td>11.9</td>
<td>4.6</td>
<td>3.5</td>
</tr>
<tr>
<td>Lp(a) (mg/dl)</td>
<td>6</td>
<td>4</td>
<td>6</td>
<td>20</td>
</tr>
</tbody>
</table>

Fig. 3. Gas chromatographic analysis of serum sterols in Patient 1. Cholestanol is contained in the cholesterol peak in this graph.
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References


