We report about a brother and sister having clinical symptoms similar to those of homozygous familial hypercholesterolemia (FH) but surprisingly who have normal low density lipoprotein (LDL) receptor activities (M. Harada-Shiba et al, J Jpn Atheroscler Soc 1991;19:227–242). The LDL receptor activities in the cultured fibroblasts of the patients were compared with those of FH heterozygotes and homozygotes for the LDL receptor mutation. The LDL receptor activities in the cultured fibroblasts of the patients were in the normal range, but their plasma cholesterol concentrations were similar to patients with homozygous FH. After the plasma LDL was removed by plasmapheresis in both patients, plasma cholesterol levels started to increase. The “rebound” of plasma cholesterol was compared with those for heterozygous and homozygous FH. The plasma cholesterol levels of the patients, which were >410 mg/dl 2 weeks after plasmapheresis, were much higher than those of FH heterozygotes (232–311 mg/dl) but similar to those of FH homozygotes (345–464 mg/dl). The urinary mevalonate excretion rate, which reflects the rate of whole-body cholesterol synthesis, was higher for the brother (patient 1, 32.6 nmol/kg · day$^{-1}$) than for the normal subjects (17.7±4.1 nmol/kg · day$^{-1}$) but was similar to those of FH homozygotes (31.2±4.3 nmol/kg · day$^{-1}$) and heterozygotes (29.8±10.9 nmol/kg · day$^{-1}$). To estimate the catabolic and production rates of cholesterol in the brother, the time course for the increment in the total cholesterol level after plasmapheresis was analyzed by the two-compartment model. The fractional catabolic rates and the synthesis rates of cholesterol were estimated to be 0.102 pool/day and 19.4 mg/kg · day$^{-1}$ for the brother, 0.101 pool/day and 29.2 mg/kg · day$^{-1}$ for homozygotes, and 0.280 pool/day and 21.2 mg/kg · day$^{-1}$ for heterozygotes. The results suggest that the patient has a disorder not in the regulation of the production of cholesterol but in the catabolism of cholesterol, seemingly other than that caused by the LDL receptor mutation. (Arteriosclerosis and Thrombosis 1992;12:1071–1078)

**Key Words** • low density lipoprotein receptor • cholesterol synthesis • familial hypercholesterolemia • plasmapheresis

The elevation of plasma low density lipoprotein (LDL) is caused by a delay in the removal of LDL from the plasma because of the impaired function of LDL receptors (familial hypercholesterolemia [FH] in a narrow sense),$^1,2$ mutation of apolipoprotein (apo) B-100 (a ligand for the LDL receptor),$^3,4$ endocrinologic disorders,$^5,6$ or autoimmune diseases.$^7,8$ Among them, an abnormality in the LDL receptor gene in the homozygous form demonstrates the most severe symptoms of hypercholesterolemia. FH homozygotes show plasma cholesterol levels five times higher than normal and suffer from prominent cutaneous and tendon xanthomas and fatal coronary artery disease in the first to third decades of life. Their plasma cholesterol levels respond poorly to dietary and drug therapies. The final diagnosis of homozygous FH can be made by analysis of the LDL receptor activity in cells obtained from the patients and subsequently cultured.

Recently, we have reported about a brother and sister displaying clinical symptoms similar to those of a homozygote with an LDL receptor disorder.$^9$ However, the cultured fibroblasts from both patients showed normal LDL receptor activity, synthesized the normal-size LDL receptor protein, and demonstrated normal downregulation of LDL receptor activity when loaded with either LDL or free cholesterol.$^1$ The LDL receptor genes of the brother and sister showed different haplotypes,$^8$ which indicates that they do not have the homozygous form of the LDL receptor gene mutation. There were no signs of abnormality in LDL binding to the LDL receptor, the presence of autoantibodies against LDL or the LDL receptor, or disorders in their endocrine systems.$^8$ Because the brother and sister manifested almost the same symptoms, it is highly possible that the disease is “familial” hypercholesterolemia.

In this report we suggest that the patients may have a disorder not in the production of cholesterol but in the catabolism of cholesterol inside the cells, other than that mediated by the LDL receptor.
Case Summaries

Patient 1 (T.H.), a 38-year-old man, noticed xanthomas in his elbows and knees at the age of 9–10 years. The total plasma cholesterol level was 600 mg/dl at that time. Clofibrate was administered but was withdrawn several years later because of an allergic reaction. At the age of 30, coronary angiography revealed 50% stenosis in both the proximal portion of the left anterior descending coronary artery and the distal portion of the right coronary artery. He has been treated with plasmapheresis therapy once every 2 weeks since then. At the age of 35, he was reexamined by coronary angiography and progression of the coronary artery lesions was found (No. 2, 25%; No. 3, 50%; No. 6, 89%; No. 7, 75%; and No. 12–1, 99%, according to the classification by the American Heart Association). Percutaneous transluminal coronary angioplasty was performed five times for the lesion in No. 6.

Patient 2 (K.A.), the 43-year-old sister of patient 1, was diagnosed as deaf when she was 1 year old. She also had severe hypercholesterolemia (total cholesterol, 533 mg/dl) and multiple xanthomas since the age of 14 years. She was diagnosed as having polymyositis and was found to have a fatty liver by muscle and liver biopsy when she was 32 years old. She was treated with corticosteroids, which caused an elevation of her plasma cholesterol level in a dose-dependent manner.

Both patients were born from a consanguineous marriage between an uncle and a niece (see Figure 1). Their father died at the age of 61. There was no evidence that he had hypercholesterolemia or xanthomas. Their mother, 64 years old, was healthy with a normal plasma cholesterol level (200 mg/dl) and no xanthomas. The patients had no relatives with coronary heart disease. The functions of the thyroid and adrenal glands of the patients were normal. Isoforms of apo E were 3/3, and the levels of lipoprotein(a) were 8.5 and 8.0 mg/dl for patients 1 and 2, respectively (normal value is <10 mg/dl). The thickness of their Achilles tendons was 23 mm in the right and 28 mm in the left in patient 1 and 18.5 mm in the right and 20 mm in the left in patient 2.

Methods

Cell Culture and 125I-LDL Binding Study

Na225I was purchased from New England Nuclear. LDL (1.019<d<1.063 g/ml) and lipoprotein-deficient serum (LPDS, d>1.215 g/ml) were prepared from serum by sequential ultracentrifugation as described. LDL was iodinated with 125I by the iodine monochloride method. The specific activity of 125I-LDL was >200 cpm/ng protein. The fibroblasts used in this study were obtained from skin biopsy specimens from patients 1 (T.H.) and 2 (K.A.), from 16 homozygotes and 40 heterozygotes with clinically diagnosed FH, and from several normal subjects (volunteers). The FH heterozygotes and homozygotes were diagnosed by their plasma cholesterol levels, family history, and the thickness of their Achilles tendons. All the fibroblast cell lines were established in our laboratory from the biopsy specimens as described previously. Stock cultures were maintained as monolayers in a humidified incubator (5% CO2) at 37°C in a culture medium containing Eagle’s minimal essential medium (MEM) supplemented with penicillin (100 units/ml), streptomycin (100 μg/ml), and 10% (vol/vol) fetal calf serum. Cells were used for the experiments before the 20th passage. For the experiments, on day 0 7.5×105 cells were seeded into 60-mm-diameter plastic dishes containing 3 ml culture medium. On day 3 the medium was replaced with 3 ml fresh culture medium. On day 5 the medium was changed to MEM containing 10% (vol/vol) LPDS. After incubation for 48 hours the medium was removed, and the cells received 2 ml fresh MEM containing 10% (vol/vol) LPDS, 10 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid (HEPES, pH 7.4), and 10 μg/ml 125I-LDL and were incubated at 4°C for 2 hours on a rotatory shaker. After incubation the cells were washed, the bound 125I-LDL was released with dextran sulfate (4 mg/ml), and the radioactivity was measured in a gamma well-type scintillation counter as described. The cell protein was determined by the method of Lowry et al.

Plasmapheresis

Patients. The characteristics of the patients who underwent plasmapheresis are shown in Table 1. All of the FH homozygotes in Table 1 belonged to the LDL receptor-negative type; they were diagnosed by their LDL receptor activities in cultured fibroblasts. The homozygous patients, 4 and 6, were medicated with probucol, but patients 3 and 5 were not receiving medication. The heterozygotes were treated with cholestyramine and a 3-hydroxy-3-methylglutaryl coen-
TABLE 1. Profiles of Familial Hypercholesterolemia Patients Who Underwent Plasmapheresis

<table>
<thead>
<tr>
<th>No.</th>
<th>Initials</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Body weight (kg)</th>
<th>Initial TC* (mg/dl)</th>
<th>Drugs†</th>
<th>Xanthomas (cutaneous/tendon)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T.H.</td>
<td>31</td>
<td>M</td>
<td>69.5</td>
<td>600</td>
<td>Probucol, RI</td>
<td>+/+</td>
</tr>
<tr>
<td>2</td>
<td>K.A.</td>
<td>36</td>
<td>F</td>
<td>45.0</td>
<td>533</td>
<td>None</td>
<td>+/+</td>
</tr>
<tr>
<td>3</td>
<td>K.K.</td>
<td>11</td>
<td>F</td>
<td>30.0</td>
<td>694</td>
<td>None</td>
<td>+/-</td>
</tr>
<tr>
<td>4</td>
<td>Y.S.</td>
<td>15</td>
<td>F</td>
<td>50.0</td>
<td>903</td>
<td>Probucol</td>
<td>+/-</td>
</tr>
<tr>
<td>5</td>
<td>K.M.</td>
<td>19</td>
<td>M</td>
<td>58.5</td>
<td>680</td>
<td>None</td>
<td>+/-</td>
</tr>
<tr>
<td>6</td>
<td>S.H.</td>
<td>23</td>
<td>M</td>
<td>60.0</td>
<td>472</td>
<td>Probucol</td>
<td>+/-</td>
</tr>
<tr>
<td>7</td>
<td>H.T.</td>
<td>55</td>
<td>F</td>
<td>47.7</td>
<td>354</td>
<td>CT, RI</td>
<td>-/+</td>
</tr>
<tr>
<td>8</td>
<td>T.F.</td>
<td>57</td>
<td>F</td>
<td>49.0</td>
<td>366</td>
<td>CT, RI</td>
<td>-/+</td>
</tr>
<tr>
<td>9</td>
<td>J.K.</td>
<td>35</td>
<td>M</td>
<td>59.0</td>
<td>335</td>
<td>CT, RI</td>
<td>+/+</td>
</tr>
<tr>
<td>10</td>
<td>M.T.</td>
<td>51</td>
<td>F</td>
<td>49.0</td>
<td>452</td>
<td>CT, RI, probucol</td>
<td>-/+</td>
</tr>
</tbody>
</table>

*Initial total plasma cholesterol (TC) levels were measured when the patients first visited the hospital and before any medication was prescribed.
†Drugs are the kinds of medication used during plasmapheresis: CT, cholestyramine; RI, 3-hydroxy-3-methylglutaryl-coenzyme A reductase inhibitor. "+,” "−" in the last column indicates the presence or absence of types of xanthomas.

zyme A (HMG CoA) reductase inhibitor. Patient 10 was also treated with probucol. Patient 1 was treated with probucol and an HMG CoA reductase inhibitor. No antilipidemic drug was administered to patient 2 because of her allergic reaction.

Procedures. To remove LDL from the patients’ plasma by plasmapheresis, two techniques were used, the double-membrane filtration method10 and the LDL-adsorption method.11 In the double-membrane filtration method, a polyvinyl alcohol (Plasmacure, Kuraray, Osaka, Japan) or cellulose acetate hollow-fiber membrane filter (Plasmaflo, Asahi Medical, Tokyo, Japan) was used for the separation of plasma from blood cells, and an ethylene-vinyl alcohol copolymer filter (Evaflux 4A, Kuraray) or cellulose diacetate filter (Cascadeflo, Asahi Medical) was used to trap the LDL in plasma as a second membrane. In the LDL-adsorption method, a polysulfone hollow fiber (Sulflox, Kanegafuchi, Osaka, Japan) was used as a plasma separator and dextran sulfate–cellulose (Liposorbal or Liposorber, Kanegafuchi) as an adsorbent for LDL. Plasma cholesterol was determined just before and after plasmapheresis by enzymatic methods.19-20 The patients were treated by plasmapheresis once every 2 weeks.

Measurement of Urinary Mevalonate Excretion Rate

Urine samples were taken every 24 hours from patient 1, FH homozygotes, FH heterozygotes, and normal subjects; collected under acidic conditions; and stored at −20°C. Mevalonate was isolated and measured as reported previously by Takizawa et al.21 The urine samples were neutralized with 6N NaOH and adsorbed by an ion-exchange resin (Bio-Rad AG1). The mevalonate was eluted with 0.1N formic acid. The eluted mevalonate was separated and quantified by gas chromatography–mass spectrometry. DL[4,5-14C]mevalonate was added to the samples as an internal standard.

Mathematical Model

To estimate the kinetics for the change of the cholesterol concentration in plasma in patient 1, the time course of the plasma cholesterol increment was analyzed by the two-compartment model22 (Figure 2). The model demonstrates that cholesterol is cleared from the plasma at a rate k (fractional catabolic rate, FCR), flows into the plasma at a rate R (production rate, PR; milligrams per day), and exchanges between the plasma pool and the tissue pool at rates k12 and k21. The quantitative relations are expressed as follows:

\[
dC_1/dt = R/V_1 - (k_{12} + k_{21})C_1 + k_{21}C_2
\]
\[
dC_2/dt = k_{12}C_1 - k_{21}C_2
\]
is the plasma volume (in milliliters), which is calculated from the following formula:

$$V_p = \left(1 - \frac{\text{Hct}}{100}\right) \times \left[16.52 \times \text{height} + 38.46 \times \text{weight} - 1369\right]$$  \hspace{1cm} (3)

where $V_p$ is in milliliters, hematocrit (Hct) is in percent, height is in centimeters, and weight is in kilograms.

## Results

### $^{125}$I-LDL Binding in the Patients' Fibroblasts

The patients' total plasma cholesterol levels were measured when they first visited our hospital before starting any treatments. The patients' fibroblast cell lines were established after they were diagnosed as FH heterozygotes or homozygotes from their clinical features, and the $^{125}$I-LDL binding activities were measured. The relation between the patients' total plasma cholesterol levels and the $^{125}$I-LDL binding activities of their cultured fibroblasts are shown in Figure 3. The $^{125}$I-LDL binding activities in the heterozygotes tended to decrease as the total cholesterol levels increased, although the values for the FH heterozygotes were distributed over a wide range. The plasma cholesterol levels of patients 1 and 2 before the commencement of treatment by plasmapheresis were much higher than those of most of the heterozygotes but were in the range of those of the homozygotes. In contrast, the $^{125}$I-LDL binding activities of patients 1 and 2 at 4°C were 86% and 128% of the normal level, respectively (Figure 3). In addition, the activities of binding, incorporation, and degradation of $^{125}$I-LDL of the cultured fibroblasts at 37°C in patients 1 and 2 showed normal values (104%, 86%, and 87%, respectively, in patient 1; 140%, 94%, and 112%, respectively, in patient 2).9

### Plasmapheresis

The average total cholesterol levels just after plasmapheresis were 99–148 mg/dl and 99–105 mg/dl in the FH homozygotes and heterozygotes, respectively (Table 2). The total cholesterol level 2 weeks after treatment was greater in the FH homozygotes (345–464 mg/dl) than in the heterozygotes (232–311 mg/dl), and the differences became more significant 3 weeks after treatment (Table 2). In the FH heterozygotes the total cholesterol levels increased to almost the original levels 2 weeks after plasmapheresis. In contrast, the total cholesterol levels in the FH homozygotes did not reach original levels even 3 weeks after plasmapheresis, although their plasma cholesterol values reached greater levels than those for the heterozygotes. It seems possible to distinguish FH heterozygotes from homozygotes by their cholesterol “rebound” patterns. Patient 1 demonstrated a greater rebound level compared with the heterozygotes, but his rebound profile resembled those of the homozygotes (Table 2). From the rebound patterns of their plasma cholesterol, patients 1 and 2 can be classified as being similar to homozygous FH patients.

### Urinary Mevalonate Excretion Rate

The high rebound levels of the plasma cholesterol in patients 1 and 2 must be due to either a low catabolic process or a high PR of plasma cholesterol. Because the LDL receptor activities were normal in patients 1 and 2, we suspected that the cholesterol PRs in the patients were increasing. The amount of mevalonate excretion into urine during 24 hours has been reported to be a good marker for estimating the rate of whole-body cholesterol synthesis.24–26 The amount of urinary mevalonate excretion was measured in normal subjects, FH homozygotes, FH heterozygotes, and patient 1. Table 3 shows the parameters and the data for mevalonate excretion in these subjects. All four homozygotes were under plasmapheresis treatment, and one of them was medicated with probucol while the others received no antilipidemic drugs. All values for the 10 heterozygotes were obtained when they were treated with neither plasmapheresis nor antilipidemic drugs. The data for patient 1 were obtained while he was being treated with plasmapheresis and probucol. Both FH homozygotes (31.2±4.3 nmol/kg·day$^{-1}$) and heterozygotes (29.8±10.9 nmol/kg·day$^{-1}$) showed greater values of mevalonate urinary excretion than did the control subjects (17.7±4.1 nmol/kg·day$^{-1}$). Patient 1 produced
TABLE 2. Rebound Profiles of Plasma Cholesterol Levels After Plasmapheresis

<table>
<thead>
<tr>
<th>No.</th>
<th>Initials</th>
<th>Procedure*</th>
<th>0 Week</th>
<th>1 Week</th>
<th>2 Weeks</th>
<th>3 Weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>T.H.</td>
<td>DF</td>
<td>192±35 (36)</td>
<td>ND</td>
<td>470±38 (36)</td>
<td>ND</td>
</tr>
<tr>
<td>2</td>
<td>K.A.</td>
<td>DF</td>
<td>156±12 (22)</td>
<td>ND</td>
<td>433±28 (22)</td>
<td>ND</td>
</tr>
<tr>
<td>FH homozygotes</td>
<td>3</td>
<td>K.K.</td>
<td>DS</td>
<td>116±14 (21)</td>
<td>ND</td>
<td>464±30 (17)</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>Y.S.</td>
<td>DF</td>
<td>112±33 (85)</td>
<td>273±32 (39)</td>
<td>428±51 (46)</td>
</tr>
<tr>
<td></td>
<td>5</td>
<td>K.M.</td>
<td>DF</td>
<td>137±26 (109)</td>
<td>ND</td>
<td>386±31 (97)</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>S.H.</td>
<td>DF</td>
<td>148±32 (27)</td>
<td>ND</td>
<td>364±31 (27)</td>
</tr>
<tr>
<td>FH heterozygotes</td>
<td>7</td>
<td>H.T.</td>
<td>DS</td>
<td>102±12 (35)</td>
<td>ND</td>
<td>249±21 (30)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>T.F.</td>
<td>DS</td>
<td>99±6 (29)</td>
<td>ND</td>
<td>232±9 (18)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>J.K.</td>
<td>DS</td>
<td>103±10 (20)</td>
<td>ND</td>
<td>274±27 (13)</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>M.T.</td>
<td>DF</td>
<td>105±15 (33)</td>
<td>ND</td>
<td>311±21 (29)</td>
</tr>
</tbody>
</table>

ND, not determined. Values are mean±SD. Numbers in parentheses show the number of plasmapheresis treatments.

*Procedures were those employed for plasmapheresis: DS, LDL-adsorption method; DF, double-membrane filtration method.
†Durations after plasmapheresis treatments.

32.6 nmol/kg·day⁻¹, which was almost the same level of mevalonate excretion as in FH homozygotes and heterozygotes. The result suggests that cholesterol synthesis in patient 1 just as in the FH heterozygotes or homozygotes was high compared with the normal subjects.

Kinetics of Net Cholesterol Rebound After Plasmapheresis

To evaluate the catabolic rate of plasma cholesterol, the plasma cholesterol levels were monitored after plasmapheresis in patient 1, an FH homozygote (LDL receptor-negative type treated by plasmapheresis and probucol), and an FH heterozygote (patient 10 in Tables 1 and 2). The values were plotted in Figure 4. The time courses for the rebound plasma cholesterol levels were analyzed, and k and R (cholesterol PR) were calculated by the nonlinear regression program of the Multi Runge-Kutta-Gill as described elsewhere. The FCRs and PRs of cholesterol were calculated to be 0.101 pool/day and 29.2 mg/kg·day⁻¹ in the FH homozygote and 0.280 pool/day and 21.2 mg/kg·day⁻¹ in the FH heterozygote. As shown in Table 3, the FCR of cholesterol in patient 1 (0.102 pool/day) was similar to that of the homozygote (0.101 pool/day), and the PR (19.4 mg/kg·day⁻¹) was similar to that of the heterozygote (21.2 mg/kg·day⁻¹). The calculated FCR of patient 1 suggests that he has a disorder in the catabolism of plasma cholesterol, i.e., plasma LDL. Again, the PR of cholesterol in patient 1 was calculated to be similar to either the FH heterozygote or homozygote, agreeing with the results from the measurement of the mevalonate excretion rate (Table 4).

Discussion

In the present study, we report about a brother and sister who have had severe hypercholesterolemia, multiple xanthomas, and premature atherosclerosis since childhood. Because they were born from a consanguineous marriage, they were clinically diagnosed initially as FH homozygotes. We previously reported that the LDL receptor activities, the molecular size of the synthesized LDL receptor proteins, and the suppression of the LDL receptor syntheses by cholesterol or LDL were normal in their cultured fibroblasts. The LDL receptor activities of the patients were even higher than those of FH heterozygotes (Figure 2). We previously reported that their haplotypes of the LDL receptor gene were different from each other, indicating that they were not homozygous for the LDL receptor mutation. Further-

TABLE 3. Characteristics of Patients and Measurement of Their Urinary Excretion of Mevalonate

<table>
<thead>
<tr>
<th>n</th>
<th>Age (years)</th>
<th>Body weight (kg)</th>
<th>Body mass index*</th>
<th>Total cholesterol (mg/dl)</th>
<th>Mevalonate excretion rate (μmol/day)</th>
<th>Mevalonate excretion rate (nmol/kg·day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>4</td>
<td>37±14</td>
<td>56.3±10.5</td>
<td>19.9±1.2</td>
<td>192±39</td>
<td>1.00±0.25</td>
</tr>
<tr>
<td>FH homozygotes</td>
<td>4</td>
<td>12±6</td>
<td>38.9±18.9</td>
<td>15.9±3.8</td>
<td>430±143</td>
<td>0.91±0.42</td>
</tr>
<tr>
<td>FH heterozygotes</td>
<td>11</td>
<td>55±11</td>
<td>54.3±6.8</td>
<td>21.2±2.7</td>
<td>340±81</td>
<td>1.62±0.61</td>
</tr>
<tr>
<td>Patient 1</td>
<td>1</td>
<td>38</td>
<td>70.0</td>
<td>24.8</td>
<td>390</td>
<td>2.28</td>
</tr>
</tbody>
</table>

FH, familial hypercholesterolemia. Values except those for patient 1 are mean±SD. n, Number of individuals for whom mevalonate values were available.

*Body mass index was calculated by dividing the body weight in kilograms by the square of height in meters.

Total plasma cholesterol (TC) levels were measured when the measurements of urinary mevalonate were performed.
more, because they have no relatives with hypercholesterolemia or xanthomas, it is not possible that they are typical heterozygotes for the LDL receptor mutation, the genetic transmission of which is known to be dominant. Nevertheless, patients 1 and 2 had severe hypercholesterolemia.

Patients 1 and 2 have been treated with plasmapheresis for 7 years to reduce their plasma cholesterol levels. As shown in Table 2, the rebound levels of total cholesterol 2 weeks after plasmapheresis were much higher in the FH homozygotes than the heterozygotes. The cholesterol levels returned to the original level in the heterozygotes 2 weeks after plasmapheresis, whereas the levels had not returned to their original levels in FH homozygotes after 2 weeks (Table 2). This is compatible with the fact that homozygotes have no or a very limited number of LDL receptors. The rebound profile of the plasma cholesterol in patient 1 was similar to that of the homozygotes (Table 2). Their abnormal cholesterol metabolism does not appear to be due to a defect in the LDL receptor gene itself, as the patients' haplotypes of the LDL receptor genes were different from each other. Abnormality in apo B-100 is also known to cause mild hypercholesterolemia, the genetic transmission of which is dominant. However, the LDL from patient 1 was able to compete with normal LDL for binding to the LDL receptor, and the LDL from patient 2 was internalized and degraded normally by the fibroblasts. Autoantibodies against LDL or the LDL receptor are also known to cause hypercholesterolemia. We could not detect any inhibitor activities in the patients' LDL fraction, in which the specific antibodies that inhibit LDL binding to the LDL receptor might be expected to occur. Patient 1 was initially treated by plasmapheresis by the double-membrane method, and later the treatment was switched to the LDL-adsorption method. The second membrane in the double-membrane filtration method removes 33% of immunoglobulin G and 42% of immunoglobulin A from the plasma. However, the double-membrane filtration method had no effect on the rebound level of cholesterol in patient 1 (Table 2). The fact that removal of the immunoglobulin fraction did not affect the plasma cholesterol levels in patient 1 gives further evidence that the high plasma cholesterol level in patient 1 was not caused by autoantibodies. Patient 2 has had polymyositis since the age of 32, and she has been intermittently medicated with corticosteroids. This drug increased her cholesterol level in a dose-dependent manner. When her polymyositis worsened, Evaflux 2A, which has a smaller pore size than that of Evaflux 4A and removes 50% of immunoglobulin G from the plasma, was used as the second membrane for plasmapheresis. The rebound level of the plasma cholesterol was not affected by this type of membrane. Therefore, it is unlikely that an autoimmune mechanism is involved in the pathogenesis of their hypercholesterolemia.

Pseudohomozygous type II hyperlipoproteinemia has been reported to show severe hypercholesterolemia in childhood without a family history. Recently, it has been reported that in this disorder LDL catabolism is normal and the LDL PR is accelerated to four times the normal level. The patients with this disease can decrease their plasma cholesterol levels easily by diet and medication. However, patients 1 and 2 did not respond to either of these treatments, indicating that their disorder was different from this type of disease.

The factors that determine total plasma cholesterol level are the balance of the degradation and the PRs of cholesterol within the body. For estimating the amount of whole-body cholesterol synthesis, the amount of mevalonate excreted into urine has been reported to reflect de novo cholesterol production. The amount of mevalonate excreted in urine during 24 hours was measured, and the amounts in the FH heterozygotes and homozygotes were found to be higher than those in the normal subjects (Table 3). There was no difference between FH homozygotes and heterozygotes in the present study (details are in preparation and will be submitted elsewhere). The urinary mevalonate excretion in patient 1 (32.6 nmol/kg·day⁻¹) was as high as that in the FH homozygotes (31.2±4.3 nmol/kg·day⁻¹) and heterozygotes (29.8±10.9 nmol/kg·day⁻¹) (Table 3). The rate of whole-body cholesterol synthesis in patient 1 was estimated to be less than those of the homozygotes. This was surprising to us because the LDL receptor activities were normal in patients 1 and 2 in their cultured fibroblasts. We expected that choles-

**Table 4. Calculated Values for Cholesterol Synthesis and Fractional Catabolic Rates in Patient 1**

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>BW (kg)</th>
<th>TC (mg/dl)</th>
<th>Plasma volume (ml)</th>
<th>Rate of cholesterol synthesis (mg/kg·day⁻¹)</th>
<th>FCR (day⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient 1 (T.H.)</td>
<td>31</td>
<td>62</td>
<td>600</td>
<td>2297</td>
<td>53.2</td>
</tr>
<tr>
<td>FH heterozygote</td>
<td>51</td>
<td>49</td>
<td>452</td>
<td>1816</td>
<td>57.2</td>
</tr>
<tr>
<td>FH homozygote</td>
<td>23</td>
<td>49</td>
<td>1003</td>
<td>1846</td>
<td>77.4</td>
</tr>
</tbody>
</table>

BW, body weight; TC, total plasma cholesterol; FCR, fractional catabolic rate (degradation rate of cholesterol); FH, familial hypercholesterolemia.
cause the receptor was normally downregulated by cholesterolemia because they have different haplotypes. The catabolic disorder of LDL receptor expression be-
in the LDL receptor gene. 9 The catabolic disorder of LDL in these patients also cannot be explained by the overproduction of cholesterol but to an abnormality in the LDL catabolism inside the cells. Numbers 1–3 indicate possible sites of metabolic disorders in the patients. Arrows with solid lines show the flow of cholesterol and its derivatives. Dotted lines indicate the negative (○) or positive (©) regulation of each pathway. LDL, low density lipoprotein; CE, cholesteryl ester; ACAT, acyl coenzyme A:cholesterol acyltransferase.

The present results clearly show that the elevated cholesterol or LDL in the patients' cultured fibroblasts. 9 If there is a liver-specific regulation of LDL receptor activity and the activity is suppressed by a mutation (see Figure 5, #1), the symptoms could be similar to those of homozygotes for the LDL receptor gene mutation, but the mutation may show a recessive mode of transmission. A similar catabolic disorder could be caused by an overaccumulation of cholesterol in the hepatocytes. Such an accumulation of cholesterol could occur by abnormalities in 1) cholesterol degradation and discharge through bile acids (Figure 5, #2) or 2) the regulation of cholesterol esterification (Figure 5, #3).

The latter possibility seems to be unlikely because acyl coenzyme A:cholesterol acyltransferase, which is the key enzyme for cholesterol reesterification inside the cells, was normal in the patients' cultured fibroblasts. 9 Unlike ordinary FH homozygotes, patient 2 had a fatty liver diagnosed by ultrasonic measurements, computerized tomography, and liver biopsy. Patient 1 also may have a fatty liver as judged from ultrasonic measurements.

In conclusion, we report about a brother and sister who have the same symptoms as FH homozygotes, but who have an abnormality neither in the LDL receptor nor in LDL itself as shown by in vitro study. The pathogenesis could be the result of oversuppression of the LDL receptor activity due to the aberrant cholesterol catabolism inside the cells.

Acknowledgments

The authors thank Ms. Ikuko Okuno of the National Cardiovascular Center Institute for her technical assistance. We also thank Drs. Satoru Takata of Kanegafuchi Kagaku and Kunio Shiba for their helpful discussion.

References


Sibling with normal LDL receptor activity and severe hypercholesterolemia.
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doi: 10.1161/01.ATV.12.9.1071

Arteriosclerosis, Thrombosis, and Vascular Biology is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
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Print ISSN: 1079-5642. Online ISSN: 1524-4636

The online version of this article, along with updated information and services, is located on the World Wide Web at:
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