A Novel Homozygous Missense Mutation in the Apo A-I Gene With Apo A-I Deficiency

Wei Huang, Jun Sasaki, Akira Matsunaga, Hiroshi Nanimatsu, Kengo Moriyama, Hua Han, Mari Kugi, Takafumi Koga, Kohei Yamaguchi, Kikuo Arakawa

Abstract—We analyzed the genetic defect in a 67-year-old Japanese male patient with apolipoprotein (apo) A-I and high density lipoprotein (HDL) deficiencies, corneal opacities, and coronary artery disease. The plasma concentrations of apoA-I and HDL cholesterol were 2.9 to 7.3 mg/dL and 0.08 to 0.19 mmol/L, respectively. The lecithin:cholesterol acyltransferase (LCAT) activity and cholesterol esterification rate were <40% of normal control values. LCAT mass was ≈50% of normal control. Sequence analysis of polymerase chain reaction–amplified DNA of the proband’s apoA-I gene showed a homozygous T-to-A transition resulting in the substitution of Val 156 with Glu (apoA-I Oita). Direct sequencing of samples obtained from other family members showed that the brother was homozygous, whereas the son was a heterozygous carrier of apoA-I Oita. The heterozygote for apo A-I Oita showed nearly 60% of normal apoA-I and normal HDL cholesterol levels. In vivo turnover studies in rabbits demonstrated that the variant apoA-I was rapidly cleared from plasma compared with normal human apoA-I. Our data suggest that the Val156Glu substitution is associated with apoA-I and HDL deficiency, partial LCAT deficiency, and corneal opacities and that Val156 of apoA-I may play an important role in apoA-I function. (Arterioscler Thromb Vasc Biol. 1998;18:389–396.)

Key Words: HDL deficiency ■ apolipoprotein variant ■ apoA-I Japanese ■ corneal opacities

Subjects

A 67-year-old Japanese man was admitted to Oita Prefectural Hospital because of worsening effort-dependent chest oppression, which was first experienced 3 months before admission. His body mass index was 27.5 kg/m². He had smoked 50 cigarettes per day for 50 years. Physical examination showed bilateral corneal opacities, and the blood pressure was 130/70 mm Hg. There was no tonsillar hypertrophy, tendinous xanthomata, hepatosplenomegaly, or peripheral neuropathy. Serum concentrations of apoA-I and HDL cholesterol were markedly low (2.9 to 7.3 mg/dL and 0.08 to 0.19 mmol/L, respectively). Other laboratory findings were within the normal range. A coronary arteriogram showed 90% stenosis of the left main trunk and 75% stenosis of the proximal portion of the left anterior descending artery and circumflex artery. Aortocoronary bypass surgery was performed. Complete occlusion of the left internal carotid artery, stenosis of the right proximal internal carotid artery, complete occlusion of the left vertebral artery, and stenosis of the right vertebral artery were also detected on angiography. The pedigree for the proband is shown in Fig 1. Examination of other family members showed that the brother (II 1) also had bilateral corneal opacities, but neither the proband’s son (III 1) nor his grandchildren (IV 1, 2) had corneal opacities. The proband’s elder brother (II 1) had no history of diabetes mellitus or hypertension or any symptom suggestive of CAD, and he also had a normal resting electrocardiogram. The proband’s son (III 1) did not suffer from clinical symptoms or signs of CAD, and he had a normal resting and stress electrocardiogram. The proband’s parents had died at 83 and 82 years of age. This study was approved by the Ethics Review Committee of the School of Medicine, Fukuoka University, Fukuoka, Japan.

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Lipid, Apolipoprotein, and Lipoprotein Analyses

Blood samples were collected in tubes containing disodium EDTA after a 12-hour fast. Plasma concentrations of cholesterol and TG were determined by an enzymatic method. Apolipoprotein concentrations were measured by single radial immunodiffusion, and plasma lipoproteins were separated by sequential ultracentrifugation. The following density fractions were obtained: VLDL (d = 1.006 to 1.030 g/mL), LDL (d = 1.031 to 1.063 g/mL), and HDL (d = 1.063 to 1.21 g/mL). Fractionation of plasma lipoproteins by Superox 6HR 10/30 gel chromatography was performed with a fast protein liquid chromatography system (Pharmacia Fine Chemicals). The column matrix was equilibrated with PBS containing 1 mmol/L EDTA and 0.02% NaN3 (pH 7.4). Fifty 0.5-mL fractions were collected at a flow rate of 0.5 mL/min. Electrophoresis of lipoproteins from the different plasma density fractions was performed by 12.5% SDS-PAGE, and the distribution of apoA-I was visualized by Coomassie brilliant blue G-250 staining. Protein transfer to nitrocellulose was performed using a semidry blotting system (Amersham Life Science). The resulting bands were identified by immunoblotting with a polyclonal rabbit antiserum (Extracyte, Seattle, Wash.35) raised against recombinant human apoA-I. The apoA-I bands were identified by immunoblots with a polyclonal rabbit antiserum to human apoA-I antiserum.

Isolation of ApoA-I

HDLs (d = 1.063 to 1.21 g/mL) were isolated from a 50 mL plasma sample from the proband and his family and was performed as described previously. In brief, 1 mL plasma was incubated at room temperature for 1 hour with 30 mL of 10 mmol/L Tris HCl (pH 8.2) containing 1% sodium deoxycholate, 2% ampholytes (pH 4 to 6), and 10% (w/v) bovine serum albumin. The nondenaturing gel system (Bio-Rad Laboratories) was used for IEF. The pH gradient ranging from 4 to 6 used for IEF. A two-dimensional gel electrophoresis of plasma, consisting of IEF followed by SDS-PAGE, was performed as described previously. The apoA-I bands were identified by immunobots with a polyclonal rabbit anti-human apoA-I antiserum. ApoE genotypes were determined by using HhaI digestion of the apoE gene after amplification by PCR as reported in our laboratory. The apoA-I gene was amplified using a high molecular weight calibration kit, Pharmacia LKB Biotechnology. HDL particle sizes were determined by nondenaturing gradient PAGE on 4% to 30% gels (Pharmacia LKB Biotechnology). Plasma with a d < 1.21 g/mL was obtained by ultracentrifugation at 100 000 rpm for 2 hours (Beckman TL-100 ultracentrifuge). This was followed by the addition of 20 mg/mL bovine albumin to the gel. Protein was visualized with 0.05% Coomassie brilliant blue G-250, and then the gel was scanned with a personal densitometer SI (Molecular Dynamics Inc). The gel was calibrated with a standard protein mixture (high-molecular-weight calibration kit, Pharmacia LKB Biotechnology). HDL particle sizes were calculated from the calibration curve of the peak migration distance versus that of the standard.

CER, LCAT Activity, and LCAT Mass Measurements

CER indicates the esterification of endogenous cholesterol, which was determined by the method of Stokke and Norum. LCAT activity was measured by using a proteoliposome as a substrate. LCAT mass was kindly assayed by Prof John J. Albers, University of Washington, Department of Medicine, Northwest Lipid Research Laboratories, Seattle, Wash.36

DNA Amplification by PCR

Genomic DNA was isolated from 120 mL peripheral blood as described in the Genomix kit manual (Talent srL). Ten microliters of this extract was used as a template for PCR. Oligonucleotide primers...
for the apoA-I gene were synthesized on the basis of published data (model 380B DNA synthesizer, Applied Biosystems). The primers for the apoA-I gene are listed in Table 1. PCR was performed according to the protocol published by the manufacturer with the use of a Perkin Elmer–Cetus thermal cycler. Amplification was performed by initial denaturation at 96°C for 10 minutes, followed by 30 cycles at 96°C for 30 seconds, 60°C for 30 seconds, and 72°C for 1 minute, and a final extension for 7 minutes at 72°C. A reaction volume of 100 mL contained 2.5 U Taq polymerase (Perkin Elmer–Cetus), 150 ng genomic DNA, 20 pmol of each primer, and 200 mol/L KCl, 15 mmol/L MgCl$_2$, and 0.1% (wt/vol) gelatin, as reaction buffer containing 0.1 mol/L Tris HCl, pH 8.3, 0.5 Taq contained 2.5 U Taq polymerase (Perkin Elmer–Cetus), 150 ng genomic DNA, 20 pmol of each primer, and 200 pmol of each dNTP in 10× reaction buffer containing 0.1 mol/L Tris HCl, pH 8.3, 0.5 mol/L KCl, 15 mmol/L MgCl$_2$, and 0.1% (wt/vol) gelatin, as described previously.

DNA Sequence Analysis
The PCR-amplified DNA products were purified with a GeneClean kit (Bio101), and purified DNA fragments were ligated to the pT7Blue-T vector (Novagen). Twelve clones of both strands in opposite directions were sequenced by the dye deoxy chain-termination method using Sequenase (US Biochemical). The DNA fragment around the mutation in exon 4 of apoA-I was amplified and subjected to direct sequencing by using a DNA sequencing kit (dye terminator cycle sequencing ready reaction, Perkin Elmer) with an ABI 373 DNA sequencer.

Statistical Analysis
Data are expressed as mean±SD. Differences in PCR in the turnover study were evaluated for statistical significance by Student’s t test. A value of P < .05 denoted statistical significance.

Results

DNA Sequence Analysis
Three pairs of oligonucleotide primers were used to amplify exons and exon-intron boundaries of the apoA-I gene by PCR. Nucleotide numbers were based on the published sequence for apoA-I DNA. Fig 2A shows an autoradiogram of the sequencing results around region 1762 in all 12 clones of the subcloned DNA fragment from the proband, resulting in a single amino acid change (Glu for Val) at residue 156. Since this substitution has not been previously reported, we termed this apoA-I variant (Val156Glu) “apoA-I Oita” owing to the residence of the proband. Other parts of the apoA-I gene were identical to the wild type, except for a −75 G- to- A variation in the promoter region. Further direct sequence analysis showed that the brother of the proband was also homozygous for apoA-I Oita, whereas the proband’s son was heterozygous (Fig 2B); however, the two grandchildren did not carry this mutant allele.

Lipid, Apolipoprotein, and Lipoprotein Profiles
The concentrations of lipids, lipoproteins, and apolipoproteins in members of the family with apoA-I Oita are shown in Tables 2 and 3. The concentrations of total cholesterol, TG, and LDL cholesterol in the proband were within normal levels. The free cholesterol–total cholesterol ratio in the proband was slightly higher in the total, VLDL, and LDL fractions but threefold higher in the HDL fraction than those of nonaffected family members, indicating that esterification of cholesterol was reduced mainly in the HDL fraction (Table 4). The proband and his brother had <10% and <15%, respectively, of normal apoA-I and HDL cholesterol levels. The proband’s son had nearly 60% of normal apoA-I and HDL cholesterol levels, whereas these two parameters were normal in other family members. Although cholesterol concentrations in both HDL$_2$ and HDL$_3$ subfractions were markedly reduced, the degree of reduction in the HDL$_2$ subfraction was higher than that in HDL$_3$ in either homozygotes or heterozygotes. Plasma lipoproteins separated by gel chromatography from the proband showed a rearrangement of TGs among plasma lipoproteins (Fig 3). The TG of the proband was distributed mainly in the LDL fraction instead of in VLDL, a characteristic similar to that of normal control subjects. The cholesterol level in the HDL fraction was markedly reduced, whereas TG was only slightly decreased. The distribution of apoA-I was shifted to a higher density fraction of HDL (Fig 3). ApoC-II and apoC-III were at moderately low levels in the homozygote, whereas apoB and apoE concentrations were within the normal range. apoE genotype was E3/E3 in all family members tested in the present study.

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Position*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (20-mer)</td>
<td>5'-GCTTGGTTGTCCACCTGTC-3'</td>
<td>−177 to −158</td>
</tr>
<tr>
<td>2 (19-mer)</td>
<td>5'-CGACCTCTCTCTCGAGCTC-3'</td>
<td>22 to 3</td>
</tr>
<tr>
<td>3 (23-mer)</td>
<td>5'-TCACCTGGCGAAATGAGTGGG-3'</td>
<td>344 to 366</td>
</tr>
<tr>
<td>4 (23-mer)</td>
<td>5'-GATATTAGGTGAGGACTCGCC-3'</td>
<td>908 to 905</td>
</tr>
<tr>
<td>5 (23-mer)</td>
<td>5'-GTGACTGGAATGCTAGGACG-3'</td>
<td>1308 to 1330</td>
</tr>
<tr>
<td>6 (23-mer)</td>
<td>5'-GCTTTATCTGACCCGAAA-3'</td>
<td>2069 to 2047</td>
</tr>
</tbody>
</table>

*Nucleotide numbering is based on published data by Shoulders et al.
Plasma CER, LCAT Activity, and LCAT Mass
Analysis of CER, LCAT activity, and LCAT mass showed that these parameters were \( \approx 50\% \) lower in homozygous apoA-I Oita carriers than in normal control subjects but were not different from those in heterozygous carriers (Table 5). These data indicate that the partial reductions in LCAT activity and mass in this case may be secondary to a Val156Glu substitution in apoA-I rather than a defect in the LCAT gene itself.

Electrophoresis and Immunoblots
IEF of plasma at a pH range of 4 to 6 followed by immunoblotting showed no apoA-I-4 band in the proband (II 2) or his brother (II 1) but rather showed apoA-I-3 and apoA-I-5 bands. In contrast, a plasma sample from the proband’s son (III 1) showed apoA-I-2, apoA-I-4, and faint apoA-I-3 and apoA-I-5 bands (Fig 4). Two-dimensional electrophoresis (IEF and SDS) followed by immunoblotting showed that the molecular weights of apoA-I-3 and apoA-I-5 bands of the proband were similar to that of normal apoA-I (data not shown). The nondenaturing gradient gel electrophoresis of HDL particles followed by scanning with a personal densitometer SI is shown in Fig 5. In the homozygous proband (II 2), the HDL fraction was hardly observed. In the heterozygote (III 1), HDL particle size was smaller than that in normal control subjects.

Haplotype Analysis
The results of apoC-III SstI, apoA-I MspI, and apoA-I promoter (−75G/A) polymorphisms are shown in Table 3. Haplotypes S1S1, M1M1, and AA were observed in both the proband (II 2) and his brother (II 1). The distribution pattern of these three markers in the apoA-I Oita family was compatible with that of an apoA-I Oita mutant allele in the pedigree.

In Vivo ApoA-I Kinetic Study
Plasma decay curves of \(^{125}\text{I}-\)labeled normal and variant apoA-I in rabbits are shown in Fig 6. The turnover of \(^{125}\text{I}-\)labeled apoA-I Oita was markedly faster than that of \(^{125}\text{I}-\)labeled normal human apoA-I. The mean FCR of \(^{125}\text{I}-\)labeled apoA-I Oita was more than double that of \(^{125}\text{I}-\)labeled normal human apoA-I (Table 6).

Discussion
The major finding of the present study was the identification of a novel, homozygous, missense point mutation, apoA-I

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**TABLE 2. Lipid and Lipoprotein Profiles of the Apo A-I Oita Family**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex/Age, y</th>
<th>Cholesterol, mmol/L</th>
<th>VLDL</th>
<th>LDL</th>
<th>HDL</th>
<th>HDL2</th>
<th>HDL3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Homozygote</td>
<td></td>
<td>Total</td>
<td>3.98</td>
<td>0.54</td>
<td>3.23</td>
<td>0.19</td>
<td>0.05</td>
</tr>
<tr>
<td>II 2*</td>
<td>M/67</td>
<td></td>
<td>3.67</td>
<td>0.28</td>
<td>3.13</td>
<td>0.24</td>
<td>0.06</td>
</tr>
<tr>
<td>II 1</td>
<td>M/71</td>
<td></td>
<td>5.43</td>
<td>0.96</td>
<td>3.93</td>
<td>0.78</td>
<td>0.45</td>
</tr>
<tr>
<td>Heterozygote</td>
<td></td>
<td></td>
<td>5.66</td>
<td>0.59</td>
<td>3.31</td>
<td>1.74</td>
<td>1.25</td>
</tr>
<tr>
<td>III 1</td>
<td>M/47</td>
<td></td>
<td>5.66</td>
<td>1.01</td>
<td>3.05</td>
<td>1.58</td>
<td>1.05</td>
</tr>
<tr>
<td>Noncarrier</td>
<td></td>
<td></td>
<td>4.68</td>
<td>0.49</td>
<td>2.48</td>
<td>0.41</td>
<td>1.34</td>
</tr>
<tr>
<td>IV 1</td>
<td>M/12</td>
<td></td>
<td>4.68</td>
<td>0.18</td>
<td>2.48</td>
<td>0.36</td>
<td>1.04</td>
</tr>
<tr>
<td>IV 2</td>
<td>M/8</td>
<td></td>
<td>4.68</td>
<td>0.18</td>
<td>2.48</td>
<td>0.36</td>
<td>1.04</td>
</tr>
<tr>
<td>Normal control</td>
<td>(n=15)</td>
<td></td>
<td>4.68</td>
<td>0.49</td>
<td>2.48</td>
<td>0.41</td>
<td>1.34</td>
</tr>
</tbody>
</table>

*Indicates the proband.
†Values are mean ± SD.

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**TABLE 3. Apolipoprotein Profiles and Haplotype Markers of the ApoA-I Oita Family**

<table>
<thead>
<tr>
<th>Subjects</th>
<th>Sex/Age, y</th>
<th>Apolipoprotein, mg/dL</th>
<th>Apo E Genotype</th>
<th>Haplotype Markers</th>
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</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>A-I</td>
<td>A-II</td>
<td>B</td>
</tr>
<tr>
<td>Homozygote</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II 2*</td>
<td>M/67</td>
<td>11</td>
<td>3.6</td>
<td>116</td>
</tr>
<tr>
<td>II 1</td>
<td>M/71</td>
<td>11</td>
<td>8.5</td>
<td>98</td>
</tr>
<tr>
<td>Heterozygote</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>III 1</td>
<td>M/47</td>
<td>85</td>
<td>28.9</td>
<td>135</td>
</tr>
<tr>
<td>Noncarrier</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IV 1</td>
<td>M/12</td>
<td>116</td>
<td>28.9</td>
<td>98</td>
</tr>
<tr>
<td>IV 2</td>
<td>M/8</td>
<td>127</td>
<td>31.4</td>
<td>90</td>
</tr>
<tr>
<td>Normal control</td>
<td>(n=15)</td>
<td>138</td>
<td>32.7</td>
<td>84</td>
</tr>
</tbody>
</table>

*Indicates the proband.
†Values are mean ± SD.
(Val156Glu) Oita, in exon 4 of the apoA-I gene that was found to be associated with markedly low levels of apoA-I and HDL cholesterol, partial LCAT deficiency, corneal opacities, and CAD. The plasma concentrations of apoA-I and HDL cholesterol in the homozygous apoA-I Oita subjects were <15% of those in normal control subjects but were ≈60% of the control values in the heterozygote. We also observed a gene-dosage effect of the apoA-I Oita mutant allele on apoA-I and HDL cholesterol levels in this family.

More than 10 cases of homozygous apoA-I defects caused by a nonsense mutation, a deletion, and an insertion have been reported to be associated with apoA-I and HDL deficiency. The clinical manifestation in these cases was variable and included premature atherosclerosis, xanthomatosis, and corneal opacities. Premature CAD was clearly reported in only four cases.6–8,13 In contrast, ≈40 structural apoA-I variants have been isolated from plasma, and LCAT cofactor activity of the variant has been demonstrated to be ≈60% of that in control apoA-I.22 In another study, isolated apoA-I (Pro165Arg) from the proband’s plasma was reconstituted into artificial discoidal HDL. The apoA-I (Pro165Arg) variant exhibited ≈30% to 70% of LCAT cofactor activity compared with that of normal apoA-I.40 Low LCAT activity was also reported in apoA-I (Arg151Cys) Paris and apoA-I (Arg160Leu) Oslo.20,21 These data indicate the important role of residues 143 to 164 in apoA-I function, particularly the LCAT activation process.1,39 A naturally occurring mutation in apoA-I suggest that residues 143 to 164 of apoA-I are involved in the LCAT activation process.1,39 A naturally occurring mutation in apoA-I (Val156Glu) substitution in homozygosity influences HDL metabolism and results in clinical complications are probably complex. In vitro experiments with monoclonal antibodies and mutagenized apoA-I suggest that residues 143 to 164 of apoA-I are involved in the LCAT activation process.1,39 A naturally occurring mutation, apoA-I Seattle, with a deletion from Gln146 to Arg160, in homozygosity was reported to be associated with a reduction in apoA-I and HDL cholesterol levels to below 15% of control values.11 Four of six reported heterozygoses for apoA-I variants in this domain with a dominant effect on HDL metabolism are associated with reduced LCAT cofactor activity or LCAT activity per se. ApoA-I (Pro143Arg) Giesen has been isolated from plasma, and LCAT cofactor activity of the variant has been demonstrated to be ≈60% to 70% of that in control apoA-I.22 In another study, isolated apoA-I (Pro165Arg) from the proband’s plasma was reconstituted into artificial discoidal HDL. The apoA-I (Pro165Arg) variant exhibited ≈20% to 60% of LCAT cofactor activity compared with that of normal apoA-I.40 Low LCAT activity was also reported in apoA-I (Arg151Cys) Paris and apoA-I (Arg160Leu) Oslo.20,21 These data indicate the important role of residues 143 to 164 in apoA-I function, particularly the LCAT activation process. The reduced CER, LCAT activity, and LCAT mass but normal LCAT specific activity observed in the apoA-I Oita homozygote indicates a partial LCAT deficiency. The reduced LCAT activity in the apoA-I Oita homozygote indicates a partial LCAT deficiency.

The mechanisms by which the apoA-I (Val156Glu) substitution in homozygosity influences HDL metabolism and results in clinical complications are probably complex. In vitro experiments with monoclonal antibodies and mutagenized apoA-I suggest that residues 143 to 164 of apoA-I are involved in the LCAT activation process.1,39 A naturally occurring mutation, apoA-I Seattle, with a deletion from Gln146 to Arg160, in homozygosity was reported to be associated with a reduction in apoA-I and HDL cholesterol levels to below 15% of control values.11 Four of six reported heterozygoses for apoA-I variants in this domain with a dominant effect on HDL metabolism are associated with reduced LCAT cofactor activity or LCAT activity per se. ApoA-I (Pro143Arg) Giesen has been isolated from plasma, and LCAT cofactor activity of the variant has been demonstrated to be ≈60% to 70% of that in control apoA-I.22 In another study, isolated apoA-I (Pro165Arg) from the proband’s plasma was reconstituted into artificial discoidal HDL. The apoA-I (Pro165Arg) variant exhibited ≈30% to 70% of LCAT cofactor activity compared with that of normal apoA-I.40 Low LCAT activity was also reported in apoA-I (Arg151Cys) Paris and apoA-I (Arg160Leu) Oslo.20,21 These data indicate the important role of residues 143 to 164 in apoA-I function, particularly the LCAT activation process. The reduced CER, LCAT activity, and LCAT mass but normal LCAT specific activity observed in the apoA-I Oita homozygote indicates a partial LCAT deficiency. The reduced LCAT activity in the apoA-I Oita homozygote is consistent with the elevated free cholesterol-to-total cholesterol ratio observed in the HDL fraction. Because the substitution of Val for Glu at residue 156 is positioned within residues 143 to 164 of apoA-I, partial LCAT deficiency in the present case is secondary to this substitution and in part is the cause of low plasma HDL levels observed in patients with apoA-I Oita.

Recently, the central amphipathic α-helices have been identified as the functional domains for cholesterol efflux. Monoclonal antibodies with epitopes recognizing amino acid residues 149 to 186 of apoA-I reduce the binding of reconstituted HDL to Hela cells and of cholesterol efflux from adipocytes.31 On the other hand, the naturally occurring heterozygous apoA-I (Pro165Arg) variant failed to promote cellular cholesterol efflux in murine adipocytes and peritoneal macrophages.12 These in vitro studies suggest that central
amphipathic α-helices of apoA-I contain several discontinuous, specific regions responsible for the efflux of cellular cholesterol, probably by selectively interacting with the cell surface binding sites. Because apoA-I Oita is located within epitopes of monoclonal antibodies that inhibit cellular cholesterol efflux by apoA-I, apoA-I Oita may be associated with impaired efflux of cellular cholesterol. Hence, low levels of HDL cholesterol in the apoA-I Oita proband may be associated with impaired cholesterol efflux from cells.

Brinton et al. have recently demonstrated that the level of plasma apoA-I in humans is mainly determined by the FCR of apoA-I. More recently, Miettinen and coworkers showed that the presence of apoA-I (Leu159Arg) Fin was associated with markedly reduced concentrations of plasma apoA-I and HDL cholesterol and increased catabolism of apoA-I in affected heterozygous subjects. They speculated that the moderate hypertriglyceridemia in affected subjects with apoA-I (Leu159Arg) Fin may have contributed to the increased apoA-I FCR and markedly reduced concentration of serum HDL cholesterol. Increased catabolism of apoA-I was also observed in apoA-I (Arg173Cys) Milano, apoA-I (Arg26Gly) Iowa, apoA-I (Lys107→0), and in subjects with low HDL cholesterol concentrations. Turnover studies of isolated apoA-I Milano showed an increased catabolism of the variant apoA-I due to heterodimerization or homodimerization of mutant apoA-I. On the other hand, apoA-I Iowa was shifted to a higher density HDL fraction (HDLd, d>1.21 g/mL), whereas the turnover of apoA-I Iowa was faster than that of normal apoA-I in the high-density HDL fraction. In the present case, the distribution of the proband’s apoA-I was shifted to a higher density lipoprotein fraction, and gradient gel electrophoresis of HDL particles indicated that HDL particle size was smaller in the apoA-I Oita heterozygote than in normal control subjects. We isolated variant apoA-I Oita from the plasma of the proband and studied its turnover rate in rabbits. The turnover study demonstrated that apoA-I Oita has an accelerated catabolic rate in rabbits compared with normal human apoA-I. The shift of apoA-I to a higher density HDL fraction in the proband with apoA-I Oita may be related to faster catabolism of apoA-I, which may contribute to apoA-I deficiency and low HDL cholesterol levels in our patient.

In the present study, plasma TG of the apoA-I Oita proband was observed mainly in the LDL fraction. A high TG content in the LDL fraction was also observed in Tangier disease and in subjects homozygous for apoA-I (Glu136Lys). Enrichment of TG in the LDL fraction indicates an imbalance of lipid exchange with HDL.

A common genetic polymorphism (−75 G/A) in the apoA-I promoter region was found in the apoA-I Oita proband (II 2) as well as his brother (II 1) and son (III 1). Although previous studies about the impact of the −75 G- to-A mutation in the apoA-I promoter region on HDL cholesterol or apoA-I level are controversial, more recent investigations in genetically homogeneous populations have indicated that the G- to-A mutation does not have a significant direct effect on plasma HDL cholesterol or apoA-I level. Thus, the presence of the −75 G/A polymorphism in the apoA-I Oita family is probably not related to low plasma HDL cholesterol levels in this case.

### Figure 4
IEF gel electrophoresis of plasma followed by immunoblotting was performed at pH 4 to 6. Lane N, normal control; lane 1, the proband (II 2); lane 2, his brother (II 1); lane 3, the proband’s son (III 1); and lanes 4 and 5, the proband’s two grandsons (IV 1, IV 2).

### Figure 5
HDL particle size profiles. Densitometric scanning was performed after nondenaturing gradient (4% to 30%) PAGE of HDL particles isolated from the proband homozygous for apoA-I Oita (top, II 2), a heterozygous family member (center, III 1), and a normal subject (bottom). The HDL subpopulation intervals are indicated below the scan profiles.

### Table 5. CER, LCAT Activity, and LCAT Mass of Individuals From the Family With ApoA-I Oita

<table>
<thead>
<tr>
<th>Subject</th>
<th>Free Cholesterol/Total Cholesterol</th>
<th>CER, nmol · h⁻¹ · mL⁻¹</th>
<th>LCAT Activity, nmol · h⁻¹ · mL⁻¹</th>
<th>LCAT Mass, μg/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>S.C. (II 2)</td>
<td>0.42</td>
<td>23.0</td>
<td>273</td>
<td>2.98</td>
</tr>
<tr>
<td>J.C. (II 1)</td>
<td>0.32</td>
<td>17.4</td>
<td>218</td>
<td>2.80</td>
</tr>
<tr>
<td>K.C. (III 1)</td>
<td>0.26</td>
<td>38.6</td>
<td>721</td>
<td>5.87</td>
</tr>
<tr>
<td>M.C. (IV 1)</td>
<td>0.27</td>
<td>39.5</td>
<td>590</td>
<td>5.81</td>
</tr>
<tr>
<td>Y.C. (IV 2)</td>
<td>0.28</td>
<td>48.3</td>
<td>513</td>
<td>6.29</td>
</tr>
<tr>
<td>Normal control subjects</td>
<td>0.27±0.01 (14)</td>
<td>51.2±5.3 (13)</td>
<td>723±26 (19)</td>
<td>5.7±1.0 (19)</td>
</tr>
</tbody>
</table>

Control values represent mean±SD and (number of subjects).
In conclusion, we analyzed a novel, homozygous, missense point mutation in the apoA-I gene. The Val156Glu substitution in exon 4 of the apoA-I gene in both alleles has a serious impact on HDL metabolism, leading to apoA-I and HDL deficiencies, partial LCAT deficiency, and corneal opacities. The proband also presented with clinical features of arteriosclerosis affecting the coronary, carotid, and vertebral arteries. However, the proband’s elder brother, who was homozygous for apoA-I Oita, did not have any symptoms suggestive of CAD. Moreover, the proband had other coronary risk factors apart from his apoA-I deficiency, such as age, smoking, and obesity. Because this family study was limited, the impact of this disorder on coronary atherosclerosis remains unknown at present. Further large-scale family studies are necessary to answer this issue. Nevertheless, the unique case presented in this study provides a good opportunity to understand the structure-function relationship between apoA-I and HDL metabolism.

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References


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