A Mutation in the Human Apolipoprotein A-I Gene

DOMINANT EFFECT ON THE LEVEL AND CHARACTERISTICS OF PLASMA HIGH DENSITY LIPOPROTEINS*

Samir S. Deeb‡‡‡, Marian C. Cheung‡‡, Reiling Peng‡, Anitra C. Wolf‡‡, Ralph Stertn, John J. Albers‡‡, and Robert H. Knoppt

From the ‡Department of Medicine, University of Washington School of Medicine, the ‡‡Northwest Lipid Research Laboratories and the ‡‡‡Northwest Lipid Research Clinic, Seattle, Washington 98195

Epidemiologic and genetic data suggest an inverse relationship between plasma high density lipoprotein (HDL) cholesterol and the incidence of premature coronary artery disease. Some of the defects leading to low levels of HDL may be a consequence of mutations in the genes coding for HDL apolipoproteins A-I and A-II or for enzymes that modify these particles.

A proband with plasma apoA-I and HDL cholesterol that are below 15% of normal levels and with marked bilateral arcus senilis was shown to be heterozygous for a 45-base pair deletion in exon four of the apoA-I gene. This most likely represents a de novo mutation since neither parent carries the mutant allele. The protein product of this allele is predicted to be missing the third amphipathic helical domain. The HDL of the proband and his family were studied. Using anti-A-I and anti-A-II immunosorbents we found three populations of HDL particles in the proband. One contained both apoA-I and A-II, Lp(A-I w A-II); one contained apoA-I but no A-II, Lp(A-I w/o A-II); and the third (an unusual one) contained apoA-II but no A-I. Only Lp(A-I w A-II) and (A-I w/o A-II) were present in the plasma of the proband’s parents and brother. Analysis of the HDL particles of the proband by sodium dodecyl sulfate-polyacrylamide gel electrophoresis revealed two protein bands with a molecular mass differing by 6% in the vicinity of 28 kDa whereas the HDL particles of the family members exhibited only a single apoA-I band. The largely dominant effect of this mutant allele (designated apoA-ISeattle) on HDL levels suggests that HDL particles containing any number of mutant apoA-I polypeptides are catabolized rapidly.

Apolipoprotein (apo) A-I, a polypeptide of 243 amino acids, is synthesized in the liver and small intestine and secreted into plasma in association with lipoprotein particles. apoA-I is the principal protein of high density lipoprotein (HDL), and its biological functions include activation of lecithin-cholesterol acyltransferase, the enzyme responsible for cholesterol esterification in plasma, and solubilization of lipids in an aqueous environment (for reviews see Refs. 1 and 2).

Considerable interest has been generated in the metabolism of apoA-I and HDL mainly because of results of both epidemiologic and genetic studies which revealed an inverse relationship between low plasma HDL and apoA-I levels and the incidence of premature coronary heart disease (8–12). These results support the hypothesis that HDL particles promote the transport of cholesterol from peripheral cells to the liver for eventual removal into the biliary tract (13). HDL particles are heterogeneous with respect to size, density, lipid, and lipoprotein content. Immunoaffinity chromatography identifies two major classes of HDL particles based on apolipoprotein content: particles containing apoA-I without apoA-II and particles containing apoA-I and apoA-II (14).

The complete absence of HDL particles has been reported in three kindreds. One of these was shown to be caused by a deletion of the entire apoA-I/C-III/A-IV gene complex (15); another was a result of a rearrangement (inversion) involving the apoA-I and C-III genes (16); and the third was caused by the synthesis of a truncated protein, presumably due to premature termination of translation (18).

More than a dozen structural variants of apoA-I have been detected by virtue of altered electrophoretic mobility and later shown to be the result of single amino acid substitutions (1). Only two of these variants, however, were associated with diminished HDL levels. The first, referred to as apoA-IMilan, resulted from the substitution of cysteine for arginine at position 173 (17, 18). Individuals heterozygous for this variant had plasma HDL and apoA-I levels approximately 6% and 60% of normal, respectively, but no evidence of atherosclerosis. Low HDL levels were shown to be most likely caused by a more rapid catabolism of the abnormal apoA-I allele. Heterozygotes for the second variant, Pro→Arg, were observed in six unrelated families to have significantly lower HDL and apoA-I levels (58 and 62%, respectively) relative to noncarriers, with no associated hypertriacylglycerolemia (19). In this report we describe a deletion mutation in the apoA-I gene which has a dominant effect on plasma apoA-I and HDL.

* This study was supported by National Institutes of Health Grant HL 30886 and by Clinical Nutrition Research Unit Grant 35816. The costs of publication of this article were defrayed in part by the family members of the proband.

‡ To whom correspondence and reprint requests should be sent: Division of Medical Genetics, RG-25, University of Washington, Seattle, WA 98195. Tel.: 206-543-4001 or 1706; Fax: 206-543-0754.

§ The abbreviations used are: apo, apolipoprotein; bp, base pair(s); HDL, high density lipoprotein; LDL, low density lipoprotein; VLDL, very low density lipoprotein; SDS, sodium dodecyl sulfate; PAGE, polyacrylamide gel electrophoresis; PCR, polymerase chain reaction; Lp, lipoprotein.

Vol. 260, No. 21, Issue of July 25, pp. 13654-13660, 1991
Printed in U.S.A.
levels and the characteristics of the HDL particles in the proband and his family.

MATERIALS AND METHODS

Subjects—Proband A. S. is a Caucasian male of northern European origin. He first came to medical attention at age 34 when a university health service physician in Montana noted arcus senilis. His plasma cholesterol, triglyceride, apoA-I, and A-I1 at that time were 154, 231, 35, and 13 mg/dl, respectively. Low density lipoprotein (LDL) cholesterol was 99 mg/dl, and HDL cholesterol was 9 mg/dl. On physical examination at the Northwest Lipid Research Clinic 3 years later, arcus senilis was marked bilaterally, but there were no other physical abnormalities. Thyroid gland and function were normal; there was no cardiac murmur; no bruits over carotid, abdominal, or femoral arteries; no tendinous xanthomas or evidence of peripheral neuropathy. At an 18-min exercise treadmill test showed a normal electrocardiogram pattern. Plasma, LDL, and HDL cholesterol were 163, 113, and 7 mg/dl, respectively. Plasma triglyceride was 216 mg/dl, and apoA-I was 14 mg/dl. A. S. was 6 feet (183 cm) tall and weighed 160 pounds (73 kg). His medical history included tonsillectomy twice in childhood (no abnormal coloration reported), and hay fever-type allergies to a variety of allergens determined by skin testing. He reported smoking four cigarettes and drinking a glass of wine or beer daily. Lipid analyses of blood samples obtained at this and three subsequent visits in 3.5 years showed a gradual increase in plasma cholesterol (163, 181, 216, and 209 mg/dl) and triglyceride (216, 286, 345, and 411 mg/dl) and a slight decrease in HDL cholesterol (7.7, 6.7, and 4 mg/dl). ApoA-I and A-I1 repeatedly measured within the 10-18 mg/dl range.

The family history disclosed no evidence of premature cardiovascular disease in either parent. The father (H. S.), age 67, was treated with diiltiazem HCl for hypertension and allopurinol for hyperuricemia. A radionuclide exercise test had raised the possibility of a prior undetected myocardial infarction but was not definite. The mother (J. S.), age 62, had mild diabetes treated with insulin and hypothyroidism treated with thyroid hormone replacement. She also took lovastatin (Mevacor) and postmenopausal estrogen, which may have increased her HDL. Other medications included the antidepressants nortriptyline (Pamelor) and amitriptyline (Elavil), and Bactrim antibiotic therapy. The brother (B. S.), age 36, had no known clinical disease. Paternal and maternal grandmothers reportedly died of heart failure at ages 72 and 64, and the grandfathers on the paternal and maternal sides died of trauma and tuberculosis, respectively. There was no known consanguinity.

Preparation and Southern Blot Analysis of DNA—DNA was extracted from peripheral blood leukocytes by the proteinase K phenol method on an Applied Biosystems (Foster City, CA) model 340A nucleic acid extractor according to the protocol provided by the manufacturer. Conditions for Southern blot analysis were as described (24, 25, Table I). Primer 1 DNA (DNA and RNA) (pAI-1153) (3) was a kind gift of J. Breslow (Rockefeller University, New York).

PCR Amplification—Oligodeoxynucleotide primers used in amplification and sequencing were synthesized on an Applied Biosystems model 380B DNA synthesizer. The sequence and position of these primers are shown in Table I. Three fragments of the apoA-I gene of a normal control and of the proband were PCR amplified (22) using a Perkin-Elmer Cetus Instruments thermal cycler and amplification kit. Fragment 1 included 199 bp of the 5' upstream regulatory region, exon 1, and the 5' 17 bp of intron 1 (using primers 1 and 2). Fragment 2 encompassed exons 2 and 3 and the intron exon junctions (using primers 3 and 4). Fragment 3 contained exon 4 and the intron 3-exon 4 junction (using primers 5 and 6). Thirty cycles of amplification were employed with 1 min of each of denaturation at 94°C, annealing at 58°C, and extension at 72°C.

Direct Sequencing of PCR-amplified DNA—PCR products were purified from the unincorporated dNTPs and nonspecific DNA products by electrophoresis in a low melting point agarose gel (SeaPlaque agarose, FMC BioProducts). The isolated gel slice containing the PCR product was then extracted twice with phenol to remove the agarose and the DNA was then precipitated with ethanol and 0.3 M NaOAc.

Reagents of the Sequenase kit (U. S. Biochemical Corp.) were used for sequencing reactions according to the following procedure. About 1 pmol of the double-stranded PCR product was mixed with 5 pmol of sequencing primer (either one of the PCR primers) in 10 µl of sequencing buffer (40 mm Tris-HCl, pH 7.5, 20 mm MgCl2, and 50 mm NaCl). The template-primer mix was heated in a boiling water bath for 5 min followed by quick chilling on ice. After a quick spin to bring down the condensation, the template-primer was mixed with 5 µl of 10X PCR mix (20 µm each of dATP, dGTP, dCTP, dTTP, dGTP, dATP, dTTP, dCTP, plus 8 µM of one of the four deoxyribonucleotides), and incubated at 37°C for 5 min. Three µl of stop solution (95% formamide, 20 µM EDTA, 0.05% bromphenol blue, 0.05% xylene cyanol) was then added to the mixture. The samples were heated to 75°C for 2 min immediately before loading on an 8% polyacrylamide, 7 M urea gel for sequence analysis. At least 200 bases could be read after an overnight exposure.

Lipoprotein Fractionation—Fractionation of very low density lipoproteins (VLDL), LDL, and HDL was performed as described (23). Briefly, plasmas were subjected to ultracentrifugation at 105,000 x g for 18 h without prior density adjustment. LDL was recovered in the top fraction by tube slicing technique. LDL and HDL were recovered in the bottom fraction. LDL was separated either from plasma or the d > 1.006 plasma fraction by precipitation with dextran sulfate and magnesium. Lipid in each fraction was quantitated as described below. LDL lipid was calculated as the difference between the lipid in the d > 1.006 plasma fraction and the dextran sulfate-magnesium supernatant fraction. VLDL lipid was calculated as the difference between lipid in plasma and the d < 1.006 plasma fraction.
resis (PAGE) according to the method of Laemmli (32). HDL particle sizes were determined by nondenaturing gradient PAGE using precast 4–30% gels (Pharmacia) as described (33). Proteins in SDS and nondenaturing gels were visualized with 0.1% Coomassie Blue R-250 and G-250, respectively. Gels were scanned with a laser densitometer and integrated with the LKB 2400 Gelscan software. Chemical cross-linking studies were performed with dimethylsuberimidate according to the method of Swaney and O’Brien (34). The cross-linked products were delipidated and analyzed with SDS-PAGE as described (35).

RESULTS

DNA Analysis—Genomic DNA from the proband and a normolipidemic control were first examined for major rearrangements by Southern blot analysis. DNA fragments (approximately 2.5–14 kilobases in length) generated by digestion with the restriction enzymes BglII, PstI, HindIII, SstI, XmnI, and XbaI did not appear to be different from normal. MspI digests, however, revealed that the proband had both the fragment of expected length (1.08 kilobases) and another that was shorter by approximately 50 bp (Fig. 1). This shorter fragment was not present in either the parents or the brother of the proband.

PCR-amplified DNA fragments encompassing the apoA-I gene of the proband were next subjected to gel electrophoretic analysis. Fragment 1 included 199 bp of 5' upstream sequences, exon 1, and the 5'-17 bp of intron 1. Fragment 2 contained exons 2 and 3 and the intron-exon junctions. Fragment 3 contained exon 4 and the intron 3-exon 4 junction. Two distinct fragments, differing in size by 40–50 bp, were observed when the proband's DNA was used as a template for the oligodeoxynucleotide primers flanking exon 4 (Fig. 1). Only one fragment, corresponding in length to the wild-type allele, was obtained when DNA samples from other members of the nuclear family and several other normolipidemic individuals were used as templates in the amplification reaction. These results are consistent with those of Southern blot analysis after digestion with MspI and furthermore show that the proband is heterozygous for a deletion in exon 4.

All amplified DNA fragments were then purified by electrophoresis on agarose gels and sequenced directly by the dideoxy chain termination method as described in detail under “Materials and Methods.” Sequencing of both strands of the shortened exon 4 DNA fragment of the proband revealed the existence of a 45-bp deletion in this exon extending (5' → 3') from either nucleotide 1572 or 1574 (Fig. 2). The discrepancy is caused by the presence of the dinucleotide GC at both ends of the deletion. In either case this deletion would be expected to result in the synthesis of a protein missing 15 amino acid residues (Glu146 to Arg160) (Fig. 3). Exon 4 of the apoA-I gene contains six homologous tandem repeats (66 bp in length) that code for six 22-amino acid segments (3). The 45-bp deletion described above occurred within repeat number three.

![Fig. 2. Sequence of the normal and mutant apoA-I alleles. Autoradiographs of sequencing gels of the normal and deleted apoA-I alleles. Deleted bases are indicated with a bracket on the autoradiograph and a box on the sequence. Sequencing both strands was primed with primers 7 or 8 (Table I). The sequence shown is that of the sense strand.](image)

![Fig. 3. Location of the deletion on the apoA-I gene. The structure of the apoA-I gene is shown on top. Exons are indicated with thick hatched boxes. Positions of primers used in PCR amplification are indicated by arrows. Amino acid and nucleotide sequences spanning the deletion are shown below. The dotted line indicates alternative deletion points. Amino acid residue numbers 146 and 160 are indicated above the sequence.](image)
The sequence of the normal length exon 4 PCR fragment as well as of the other two fragments of the proband's ApoA-I gene was identical to that of a normal control and to the sequence published previously (3, 5).

Since neither parent is a carrier of the mutant allele, the apoA-I deletion represents a de novo mutation. The inheritance of alleles of several polymorphic markers in the family was investigated to test this hypothesis. These included hypervariable regions 3' at the apoB (21, 36) and the α-globin gene (37) loci and YNHH24 (38) in addition to six dimorphic restriction fragment length polymorphisms at the apoA-I/C-III/A-IV locus (XmnI, MspI (promoter), MspI (intron 3), XbaI).

The paternity index (λ) and inclusion probability (W) for the proband's father were determined (39) from genotypes at three hypervariable regions (Table II). The calculated cumulative inclusion probability of 0.999 strongly favors the hypothesis of a de novo mutation.

**Lipid and Apolipoprotein Profiles**—The plasma cholesterol and triglyceride levels of proband A. S. were 209 and 411 mg/dl, respectively, at the time this HDL characterization study was performed. His mother (J. S.), who is mildly diabetic and is on insulin treatment, had elevated plasma cholesterol and triglyceride, but the lipid profiles of his father (H. S.) and brother (B. S.) were normal (Table III). In A. S. and J. S., most of the triglyceride was associated with VLDL although their LDL lipid composition also revealed 7–10% more triglyceride than that of H. S. and B. S. The HDL cholesterol of A. S. was about 10% and below the fifth percentile of that reported for the Lipid Research Clinic Prevalence Study norms (40). His HDL triglyceride and phospholipid, although also low, were disproportionately high for the amount of cholesterol in that fraction. The HDL lipid levels of his parents and brother were normal.

The plasma apoA-I and A-II concentrations of A. S. were about 12 and 36% of controls, respectively (Table IV). Thus his plasma A-II/A-I ratio was considerably higher than that in population controls. His apoD and lecithin-cholesterol acyltransferase, two other proteins normally found in HDL, were also reduced. In contrast, the levels of lecithin-cholesterol acyltransferase, apoA-I, A-II, and D were between normal and high normal in the parents and brother. The plasma apoB values of A. S. and his parents were high, above the 95th percentile of control values.

**Characterization of HDL Particles**—Three populations of HDL particles were found in the plasma of the proband: one contained both apoA-I and A-II, Lp(A-I w A-II); one contained apoA-I but no A-II, Lp(A-I w/o A-II); and the third contained apoA-II but no A-I, Lp(A-II). In the relatives, Lp(A-I w A-II) and Lp(A-I w/o A-II) were also present, but there was no evidence for the presence of any significant amounts of Lp(A-II). When the HDL particles were examined by SDS-PAGE, one protein band corresponding to the position of purified apoA-I was observed in the HDL particles of the relatives. However, two protein bands at positions around purified apoA-I were observed in the proband's Lp(A-I w A-II) and Lp(A-I w/o A-II) (Fig. 4). The calculated molecular weights of these two protein bands differed by about 6%. Consistent with this observation, the molecular weight of mutant A-I would be predicted to be about 6% lower than normal A-I, assuming deletion of 15 amino acids in the mutant A-I. No protein band was detected in the vicinity of 28 kDa in the proband's Lp(A-II), and a 17-kDa band corresponding to apoA-II was absent in all Lp(A-I w/o A-II). Thus, SDS-PAGE confirmed the absence of apoA-II in all Lp(A-I w/o A-II) and the absence of apoA-I in A. S.'s Lp(A-II). Varying amounts of several proteins larger than apoA-I were consistently found in all HDL particles. Such proteins have also been found in the Lp(A-I w A-II) and Lp(A-I w/o A-II) of normal individuals (41, 42). Consistent with our earlier observations, protein bands with molecular weights similar to the apocls were seen in the HDL particles of the relatives and were more abundant in Lp(A-I w A-II). However, these protein bands were not detected in the HDL particles of the proband under similar experimental conditions. The lipid and protein compositions of the HDL particles are shown in Table V. Compared with the HDL of his relatives, the HDL particles of the proband were enriched in triglyceride and depleted of cholesterol ester. Furthermore, the Lp(A-I w A-II) and Lp(A-I w/o A-II) of the proband had relatively lower lipid contents (37 and 34%) than those of his parents and brother (40–52%), suggesting that they were denser HDL particles. His Lp(A-II), however, contained about equal amounts of lipid and protein. The amount of lipid in Lp(A-II) represented nearly half of the total HDL-associated lipids.

The particle size profile of HDL was studied by nondenaturing PAGE. As shown in Fig. 5, the two populations of apoA-I-containing particles in the proband and his family were heterogeneous in size. However, the proband's Lp(A-I w A-II) and Lp(A-I w/o A-II) were significantly enriched with small (7–8-nm hydrated Stokes diameter) and very small (<7-nm) particles. In contrast, the proband's Lp(A-II) contained a major size species with Stokes diameter of about 8.0 nm

### Table II

**Genotype Frequency Inclusion probability (W)**

| VNTR DNA marker | Genotype | Frequency of shared allele* | Paternity index (λ) | Inclusion probability (W) |
|-----------------|----------|----------------------------|---------------------|--------------------------|
| 1. 3′-ApoB      | 36/50    | 0.017 (974)                | 29                  | 0.967                    |
| 2. D2S44        | 2.2/3.2  | 0.068 (952)                | 7.3                 | 0.880                    |
| 3. 3′-α-globin  | 2.4/3.4  | 0.057 (471)                | 8.8                 | 0.898                    |

* Values in parentheses denote the number of alleles used to calculate frequency.

### Table III

**Lipid profile of proband and family**

| Subject | Age/sex | Plasma | VLDL | LDL | HDL |
|---------|---------|--------|------|-----|-----|
|         |         | TC     | FC   | TG  | PL  | TC | FC | TG  | PL  | TC | FC | TG  | PL  |
| A. S.   | 39/M    | 209    | 66   | 411 | 240 | 51 | 28 | 336 | 84  | 154 | 37 | 68  | 120 | 4  | 1  | 6  | 36  |
| H. S.   | 67/M    | 237    | 57   | 132 | 231 | 32 | 14 | 91  | 45  | 163 | 37 | 31  | 94  | 42 | 7  | 11 | 92  |
| J. S.   | 62/F    | 284    | 79   | 490 | 376 | 71 | 41 | 414 | 146 | 149 | 31 | 53  | 105 | 64 | 9  | 22 | 146 |
| B. S.   | 36/M    | 215    | 50   | 159 | 220 | 33 | 17 | 127 | 59  | 133 | 26 | 22  | 68  | 49 | 7  | 10 | 94  |
Distribution of ApoA-I, A-II, D, and Lecithin-cholesterol Acyltransferase in Plasma—The distribution of these proteins weight equivalent to four molecules of apoA-I (Fig. 6). Cross-linking of Lp(A-II) with dimethylsuberimidate followed by delipidation and SDS-PAGE showed that this major size species contained proteins with a total molecular weight equivalent to four molecules of apoA-II (Fig. 6). (One molecule of apoA-II is defined as two identical peptides of 77 amino acids.)

Distribution of ApoA-I, A-II, D, and Lecithin-cholesterol Acyltransferase in Plasma—The distribution of these proteins in the various HDL particles, Lp(B) (materials bound to dextran sulfate cellulose), and lipoprotein-deficient plasma immunosorbents) is shown in Table VI. In the proband, and A-I by dextran sulfate-cellulose, anti-A-11, and anti-A-I antibodies, only 60% of plasma apoA-I and A-II existed in separate HDL particles, and A-I 11 by dextran sulfate-cellulose, anti-A-11, and anti-A-I antibodies, only 60% of plasma apoA-I and A-II existed in separate HDL particles, and A-I 11 by dextran sulfate-cellulose, antibodies, only 60% of plasma apoA-I and A-II existed in separate HDL particles.

Table IV

| Subject | A-I | A-II | B | D | Lechithin-cholesterol acyltransferase |
|---------|-----|------|---|---|-----------------------------------|
| A. S.   | 15  | 12   | 177| 5.2| 1.9                              |
| H. S.   | 124 | 32   | 156| 5.5| 5.4                              |
| J. S.   | 194 | 53   | 168| 5.8| 7.4                              |
| B. S.   | 138 | 36   | 120| 5.6| 6.8                              |
| Control |     |      |   |   |                                   |
| Male    | 120 ± 20 | 33 ± 5 | 102 ± 24 | 6.2 ± 1.1 | 5.6 ± 0.9 |
| Female  | 135 ± 26 | 36 ± 6 | 98 ± 23 | 5.9 ± 1.3 | 5.9 ± 1.1 |

Table V

| Subject | Lp(A-I w/o A-II) | Lp(A-I w/o A-II) | Lp(A-II) |
|---------|------------------|------------------|----------|
| FC      | CE               | TG               | PL       | Protein | FC | CE   | TG | PL | Protein |
| A. S.   | 1.8              | 5.1              | 7.3      | 22.5    | 63.3| 2.7 | 3.7 | 7.4 | 19.9 | 66.3| 2.0 | 6.2 | 11.3 | 33.8 | 46.7 |
| H. S.   | 2.5              | 16.3             | 2.3      | 26.2    | 52.7| 2.3 | 16.2| 4.9  | 28.1 | 48.5| 11.3 | 33.8 | 46.7 |
| J. S.   | 1.8              | 17.4             | 3.9      | 27.7    | 49.2| 1.2 | 14.5| 4.4  | 23.6 | 56.3| 11.3 | 33.8 | 46.7 |
| B. S.   | 1.7              | 20.8             | 2.5      | 24.3    | 50.7| 2.0 | 13.7| 3.2  | 20.4 | 60.7| 11.3 | 33.8 | 46.7 |

DISCUSSION

An individual with highly diminished plasma apoA-I and HDL cholesterol levels (hypoalphalipoproteinemia) was shown by both Southern blot analysis and sequencing of PCR-amplified DNA fragments to be heterozygous for a 45-bp deletion in exon 4 of the apoA-I gene. The parents and brother of the proband, none of whom had reduced apoA-I and HDL levels, were shown not to carry the mutant allele. This, together with results of analysis of the inheritance of several polymorphic markers in this family, strongly suggest that this represents a de novo mutation. It is predicted that the protein product of this mutant allele would be missing 15 amino acids (7 of which are charged) from the third 22-residue repeat. Consistent with this, two protein bands in the vicinity of purified apoA-I with molecular weight differing by 6% were observed in an SDS gel. Six homologous 22-residue tandem repeats comprise most of the carboxyl-terminal half of the mature apoA-I polypeptide. These repeats, which also exist in other apolipoproteins (apoA-II, A-IV, C-11, and C-111), represent segments of amphipathic α-helices that are important for lipid binding and maintenance of the structural integrity of HDL particles (45).

The presence of this mutant apoA-I missing 15 amino acids had a profound effect on the quantity and characteristics of plasma apoA-I and A-II in the proband were HDL-associating acyltransferase, respectively, in the relatives, only 48% of the plasma apoD and 45% of lecithin-cholesterol acyltransferase in the proband were HDL-associated. Non-HDL apoD was found predominantly in the Lp(B) fraction whereas 84–97% of non-HDL lecithin-cholesterol acyltransferase was located in the lipoprotein-deficient plasma fraction.
plasma HDL in the proband. In normal individuals, approximately two-thirds of plasma apoA-I and nearly all apoA-II are found in some HDL particles. However, in the proband, 60% of plasma apoA-I and A-II were found in separate HDL particles. The occurrence of substantial amounts of plasma HDL particles containing apoA-II as the major protein component is very unusual. Such quantities of Lp(A-II) have only been reported in Tangier plasma (46, 47) and in another HDL-deficient subject we have studied (48). Chemical cross-linking studies showed that most of the Lp(A-II) particles contained proteins with total molecular weight equivalent to four molecules of apoA-II. Interestingly, in vitro complexing of apoA-II with HDL lipids also resulted in HDL with four molecules of apoA-II/particle (49).

All of the HDL particles in the proband were rich in triglyceride and poor in cholesterol ester with the proportion of core to surface lipid being normal. This suggests that most of the HDL particles were probably spherical. Despite the relatively low content of cholesterol ester in HDL, the free cholesterol/cholesterol ester ratio in the proband’s plasma was normal, suggesting that cholesterol esterification by lecithin-cholesterol acyltransferase was not affected significantly by the low level of apoA-I or by the presence of mutant apoA-I.

We have studied the electrophoretic mobility of the proband’s HDL particles on an agarose gel. Because of the small amounts of materials available and the low lipid/protein ratio of some of the particles, we were only able to see, with certainty, α-migrating Lp(A-I w A-II) and Lp(A-II) when the gel was stained for lipid with Sudan black. Staining the gel for protein with Coomassie G-250 revealed both α- and pre-β-materials in Lp(A-I w/o A-II).

Since the proband is heterozygous for the mutant allele it is intriguing that his plasma apoA-I and HDL cholesterol levels were less than 15% of population means. A likely explanation of this observation may lie in the multimeric nature of HDL particles and a possible enhanced catabolic rate of mutant apoA-I. We have shown that in normal subjects most Lp(A-I w A-II) contained two molecules of apoA-I whereas Lp(A-I w/o A-II) contained either two, three, or four molecules of apoA-I/particle (35). If mutant apoA-I is catabolized faster than normal A-I, the large fraction of HDL particles which is expected to contain both mutant and normal apoA-I would be catabolized more rapidly than normal HDL. Hypercatabolism has been observed in Tangier patients (50) and carriers of A-Iseattle (51) and A-Iowa (52). The catabolic rate of apoA-Iseattle remains to be determined.

Two previously described mutations of apoA-I were shown to be associated with decreased plasma apoA-I and HDL cholesterol levels, APOA-ISea (Arg173 → Cys) and Pro156 → Arg. Individuals heterozygous for these alleles had approximately half the normal levels of plasma apoA-I. The trait was inherited in an autosomal co-dominant fashion (17–19). APOA-ISea in the heterozygous state has a much more profound effect on both plasma apoA-I and HDL levels than apoA-I.

![Figure 5. HDL particle size profiles of proband and family.](image)

**Figure 5.** HDL particle size profiles of proband and family. Densitometric scans of Lp(A-I w A-II) (left panel) and Lp(A-I w/o A-II) (right panel) after nondenatured 4–30% PAGE.

![Figure 6. Particle size profile and protein molecular weight of Lp(A-II) of proband.](image)

**Figure 6.** Particle size profile and protein molecular weight of Lp(A-II) of proband. Densitometric scans of Lp(A-II) after nondenatured 4–30% PAGE (A) and chemically cross-linked Lp(A-II) upon SDS-PAGE (4–30%) (B).

| Plasma fraction | A-I | A-II | D | Lecithin-cholesterol acyltransferase |
|-----------------|-----|------|---|-----------------------------------|
|                 | A.S. | H.S. | J.S. | B.S. | A.S. | H.S. | J.S. | B.S. | A.S. | H.S. | J.S. | B.S. | % total |
| Lp(A-I w A-II)  | 37   | 60   | 72  | 77  | 32   | 99   | 99  | 99  | 21   | 50   | 68  | 74  | 2     | 5     | 22    | 12    |
| Lp(A-I w/o A-II)| 60   | 39   | 27  | 22  | 60   | 60   | 60  | 60  | 15   | 15   | 14  | 16  | 35    | 82    | 64    | 55    |
| Lp(A-II)        |      |      |     |     |      |      |     |     |      |      |     |     |      |       |       |       |
| Lp(B)           | 3    | 1    | 1   | 1   | 8    | 1    | 1   | 1   | 42   | 19   | 12  | 10  | 9     | 2     | 2     | 1     |
| ApoA-I, A-II, B-free plasma |      |      |     |     |      |      |     |     |      |      |     |     |      |       |       |       |

**Table VI**

*Distribution of apoA-I, A-II, D, and lecithin-cholesterol acyltransferase in plasma*

Values are percent of total A-I, A-II, D, or lecithin-cholesterol acyltransferase mass in the plasma of the proband A.S. and his relatives H.S., J.S., and B.S.; (−), undetectable levels.
apoA-I*4Bm20, presumably because of the greater alteration in primary structure (loss of 15 amino acids) and the suspected higher rate of catabolism.

During the past 5 years the proband’s plasma triglyceride levels increased to above the 95th percentile of a control population. Whether this is a consequence of the apoA-I deletion mutation remains to be determined. Although an elevated triglyceride level was observed to be more common among individuals heterozygous for the apoA-I*4Bm20, no consistent pattern of association was detected in other families with other variants (19).

Acknowledgments—We greatly appreciate the contributions of the proband (H. A. S.) and his family to this study.

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