Human Apolipoprotein A-I Polymorphism

IDENTIFICATION OF AMINO ACID SUBSTITUTIONS IN THREE ELECTROPHORETIC VARIANTS OF THE MÜNSTER-3 TYPE

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Variant forms of apolipoprotein A-I (apo-A-I) have been shown to exist in the human population. One mutant form, referred to as apo-A-I-Münster-3, is one charge unit more basic than normal apo-A-I on isoelectric focusing gels. This variant has the same immunologic characteristics and molecular weight as normal apo-A-I. The apo-A-I-Münster-3 from subjects in three unrelated families (in two of which the trait has been shown to be transmitted as an autosomal co-dominant) has been analyzed by partial amino acid sequencing to define the cause of the electrophoretic abnormality. In the apo-A-I of family A, the abnormality was shown to occur in the smallest cyanogen bromide fragment, CB-2 (residues 87–112), and amino acid sequencing revealed asparagine instead of the usual aspartic acid at residue 103. Subjects with this mutant form have shown no signs of dyslipoproteinemia. The N\(\text{H}_2\)-terminal cyanogen bromide fragment (CB-1, residues 1–86) from the apo-A-I of family B was shown to differ electrophoretically from normal CB-1, and amino acid sequencing revealed that a substitution of arginine for proline at residue 4 was responsible for this variant form. Analysis of the plasma lipids of one affected family B member demonstrated that the percentage of the total cholesterol that was esterified was somewhat lower than that normally observed. In a third family, family C, a variant having the same electrophoretic abnormality as the other two was determined to have an amino acid substitution at yet a different position. In this variant, histidine was found at residue 3 in the apo-A-I sequence, rather than the usual proline. In all three cases, the substitution could account for the electrophoretic abnormality. It is proposed that these three apo-A-I-Münster-3 variants be designated apo-A-I(Asp\(_{103}\) → Asn), apo-A-I(Pro\(_{4}\) → Arg), and apo-A-I(Pro\(_{4}\) → His), respectively, to indicate the substitution that accounts for the abnormality in isoelectric focusing gels.

Polymorphic forms of human apolipoproteins have been described for apo-E (1), apo-C-III (2), apo-C-II (3), apo-A-I (4–7), and apo-A-IV (4, 7). Some of these polymorphic forms exist due to differences in sialic acid content, as in the case of apo-C-III (2) and apo-E (8, 9), while apo-E also demonstrates genetic polymorphism due to variations in primary structure (10–12).

Human apo-A-I exhibits a few rare mutant forms: A-I Milano (5, 6, 13), A-I-Marburg and A-I-Giessen (7), and A-I-Münster-1–3 (4). The amino acid substitution that is responsible for the occurrence of the A-I Milano variant has been identified (13). Patients with either A-I Milano or A-I-Marburg often have mild forms of hypertriglyceridemia (5, 7).

Because of the possibility of metabolic defects caused by various apolipoprotein mutations, as has been demonstrated for apo-E (11, 12), it is of particular interest to establish which specific regions of the apolipoproteins are affected by mutations and to study their possible impact on lipoprotein metabolism. In this paper, three different mutations that are responsible for the occurrence of three distinct apo-A-I-Münster-3 variants are reported.

EXPERIMENTAL PROCEDURES

Subjects—Subjects with apo-A-I electrophoretic variants were detected by screening large populations (4).

Materials—Plasma for preparative isolation of lipoprotein fractions was obtained by plasmapheresis. Acrylamide, \(N\,N\,N',N'\)-tetramethylenediamine, and \(N\,N'\)-methylenebisacrylamide were purchased from Bio-Rad. Sodium decyl sulfate was obtained from Eastman Kodak, and ampholytes were acquired from Serva (Heidelberg, W. Germany) and LKB (Bromma, Sweden).

Isoelectric focusing and Two-dimensional Gel Electrophoresis Techniques—Isoelectric focusing of serum was performed as previously described (1). Isoelectric focusing of apo-HDL or apo-A-I was performed as described (13), except that 0.75-mm-thick vertical slab gels were used rather than rod gels. For two-dimensional electrophoresis, the discontinuous system of Neville was used (14). Immunological detection of apo-A-I after isoelectric focusing was conducted by immunoelectrophoresis in the second dimension (4). Lipoproteins were isolated by ultracentrifugation by the method of Havel et al. (15). After dialysis against 0.9% NaCl, the HDL were delipidated with ethanol ether (21) and pure ether (16). The apo-HDL were solubilized in 1% decyl sulfate in 0.01 M Tris-HCl, pH 8.2, and subjected to flatbed preparative isoelectric focusing in a pH gradient of 5 to 7 in 6 M urea (17). Protein bands were eluted from the gel with 1% decyl sulfate in 0.01 M Tris-HCl pH 8.2, and checked for purity by isoelectric focusing.

The abbreviations used are: apo, apolipoprotein; HDL, high density lipoproteins; LDL, low density lipoproteins; VLDL, very low density lipoproteins; PTH, phenylthiohydantoin.

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A polypeptide variant was identified in two of the three families (A and C) demonstrated the transmission of apo-A-I-Münster-3 (Fig. 4). Both males and females expressed the abnormal A-I. Every person with apo-A-I-Münster-3 also had normal apo-A-I, and the variant A-I was present in two generations of each family. The variant form is therefore transmitted by an autosomal co-dominant trait in these two families. Transmission has not yet been documented in family C.

Representative lipid values for affected members of the families are shown in Table I. Values for affected members of family A and C appeared to be within normal limits. However, the analysis of the plasma lipids in family B suggested that the percentage of the total cholesterol that was esterified was lower than that which is normally observed (54% versus a normal value of 65 to 80%).

To investigate the cause of the electrophoretic abnormality, the variant apo-A-I from the delipidated HDL of one individual (I-1, family A) was isolated by preparative isoelectric focusing procedure. An alternative preparative isoelectric focusing procedure was performed using the LKB Immobilie system, pH 4.9 to 5.9 (LKB Application Note 321). Modifications included the substitution of an isokinetic gradient for a linear gradient, the inclusion of 6 M urea in the gel solutions, and the use of vertical slab gels. Purified apo-A-I or delipidated HDL was solubilized in 0.1 M Tris-HCl, pH 8.0, containing 1% deoxycholate, 20% sucrose, 6 M urea, and loaded onto the top of the gel, which was previously rinsed with the same buffer. The samples were then overlaid with 10% sucrose followed by a layer of 0.01 M NaOH (upper electrolyte buffer). The lower electrolyte buffer consisted of 0.01 M glutamic acid (Sigma). The gels were electrophoresed overnight at 4°C at a constant power of 5 watts. The focused apolipoproteins were visualized by soaking the gels in water (18). Individual visualized bands were then excised and eluted with 0.1 M Tris-HCl, pH 7.4, containing 4 M guanidine and 1 mM EDTA. Eluted proteins were exhaustively dialyzed against 5 mM NH₄HCO₃, lyophilized, and resolubilized in 0.1 M NH₄HCO₃.

Cyanogen Bromide Hydrolysis—For analytical purposes, the pure apo-A-I isoforms were precipitated with 10% trichloroacetic acid to remove the ampholytes and washed 3 times with 10% trichloroacetic acid at 0°C. The trichloroacetic acid was removed from the precipitate by ice-cold acetone. The protein was solubilized in 1% formic acid and 1% decyl sulfate, and CNBr was added in a 500-fold molar excess to the methionine content (1,500 molar ratio to apo-A-I) and the mixture kept for 24 h at 25°C. The CNBr peptides were lyophilized and subjected to isoelectric focusing in a pH gradient of 3.5 to 10. After separation by isoelectric focusing, the peptides were analyzed in the second dimension by sodium dodecyl sulfate gel electrophoresis (14) to determine their molecular weight.

For preparative purposes, the isoforms were dissolved in 70% formic acid and reacted 24 h at 25°C with a 30-fold weight excess (7,900 molar ratio to apo-A-I) of CNBr (Pierce). The peptides were chromatographed on a Sephadex G-50 column and analyzed in the Beckman 121 MB analyzer equipped with a model 126 data system. No corrections were made for hydrolytic destruction or incomplete release.

Sequence Analysis—Sequence analysis was performed on a Beckman 8900C Sequencer equipped with a cold trap accessory. The lyophilized samples were dissolved in 0.5 ml of 30% acetic acid and applied along with 2 mg of Polybrene (Sigma). A standard 0.1 M Quadrol program (No. 122974) was used. After conversion in 1 N HCl at 80°C for 10 min and extraction with ethyl acetate, PTH amino acids were identified and quantified on a Beckman model 332 liquid chromatograph system that was equipped with a CRlA integrator recorder. The mobile phase, chromatography parameters, and criteria for identification have been described (10).

Lipid Analysis—Levels of cholesterol, triglycerides, and phospholipids were determined with a commercially available test kit (Boehringer Mannheim). Cholesterol ester levels were determined by thin layer chromatography.

RESULTS

Isoelectric focusing of serum or apo-HDL in a pH gradient of 4 to 6 revealed in some rare cases (~0.1% of the population studied) the existence of an additional band with one more basic charge than normal apo-A-I (Fig. 1). As determined by two-dimensional electrophoresis, this band had a molecular weight identical with that of normal apo-A-I, Mw = 28,000 (Fig. 2). After establishing that the abnormal protein was an apo-A-I isoform, it was designated apo-A-I-Münster-3 [4]. After isoelectric focusing, this abnormal band was shown to react with a monospecific antibody to apo-A-I by immunoelectrophoresis (Fig. 3). By visual inspection of the peak (Fig. 3A), a ratio of nearly 1:1 for normal and variant apo-A-I was estimated. This electrophoretic variant has been identified in three families. Pedigree analysis in two of the three families (A and B) demonstrated the transmission of apo-A-I-Münster-3 (Fig. 4). Both males and females expressed the abnormal A-I. Every person with apo-A-I-Münster-3 also had normal apo-A-I, and the variant A-I was present in two generations of each family. The variant form is therefore transmitted by an autosomal co-dominant trait in these two families. Transmission has not yet been documented in family C.

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FIG. 3. Two-dimensional immunoelectrophoresis against specific anti-apo-A-I. A, with serum from subject 1-1 of family A; B, with normal serum.

FIG. 4. Pedigrees of two families with apo-A-I-Münster-3. Individuals with normal apo-A-I (○, □); individuals with both normal and Münster-3 apo-A-I (△, ◄); n.d., not determined. In each family, the proband is individual 1-1.

FIG. 5. Preparative Immobiline gel of apo-HDL from subject 1-1 of family A. As described under ‘Experimental Procedures,’ ~25 mg of apo-HDL were applied to a preparative gel. The focused isoform bands appeared as opaque bands in the gel after it was immersed in deionized water. The eluted isoforms were used for amino acid analysis, CNBr cleavage, and sequencing.

FIG. 6. Two-dimensional electrophoresis of CNBr peptides of normal apo-A-I (upper) and the apo-A-I-Münster-3 from individual 1-1 of family A (lower). Included is a molecular weight standard with eight different marker proteins whose molecular weights are indicated. The nomenclature of CNBr peptides is according to Brewer et al. (19), and identification was based on molecular weight determination and isoelectric focusing position. Asterisks mark the abnormal CNBr peptides.

TABLE I
Lipid values for affected family members

| Age/sex | TC* | CE (%) | PL (%) | TG (%) | VLDL# | LDL | HDL |
|---------|-----|--------|--------|--------|-------|-----|-----|
| Family A |     |        |        |        |       |     |     |
| 1-1     | 39/F | 199    | 65     | 234    | 198   | 25  | 160 |
| 1-2     | 39/F | 199    | 65     | 234    | 198   | 25  | 160 |
| Family B |     |        |        |        |       |     |     |
| I-1     | 56/M | 231    | 54     | 265    | 255   | 51  | 221 |
| 1-1     | 41/M | 152    | 76     | ND     | ND    | ND  | ND  |

* Total cholesterol (TC) in milligrams/dl; cholesterol ester (CE) in percentage of total cholesterol; phospholipids (PL) in milligrams/dl; triglycerides (TG) in milligrams/dl. The values obtained were within normal limits for age- and sex-matched controls (24), except for the cholesterol ester value for individual 1-1 of family B.

# The VLDL, LDL, and HDL were isolated at d < 1.006, d = 1.006 to 1.063, and d = 1.063 to 1.21, respectively. All determinations were made on fasting samples; ND = not determined.

The purified variant isoform was fragmented with the “analytical” CNBr method described under “Experimental Procedures.” This method gave relatively high yields of partial digestion products, which were desirable in this case because the small CNBr peptides of apo-A-I (CB-2 and CB-3) did not fix or stain well using these procedures. Although not conclusive, comparison of two-dimensional gels of normal and variant apo-A-I CNBr fragments suggested that some bands from apo-A-I-Münster-3 apparently focused more cathodically than those from normal A-I (Fig. 6). The tentative peptide designations were made on the basis of both pl (CB-4 is much more basic than CB-1 (19)) and approximate molecular weight. The difference in the CNBr peptides of focusing (Fig. 5).
normal and variant apo-A-I (family A) apparently occurred not in the large peptides (CB-4 and CB-1) but in the partial digestion fragments that contained CB-2 (CB-1-2* and CB-2*-3-4).

The CNBr peptides from the variant isoform of family A were prepared and isolated by Sephadex G-50 chromatography (Fig. 7). The amino acid analysis of neither the whole variant apo-A-I-Munster-3,A nor its individual CNBr peptides gave a strong indication of an amino acid substitution (Tables II and III). Although amino acid analysis of CB-2* did not reveal any dissimilarity to normal CB-2, the sequence analysis of CB-2* (Table IV) demonstrated a single substitution at cycle 17 (asparagine in apo-A-I-Munster-3,A as opposed to aspartic acid in normal apo-A-I). This corresponds to residue number 103 in apo-A-I (19).

When the methods described above were applied to the isolated apo-A-I-Munster-3 of individual I-1 of family B, it was observed that the CB-1 fragment of this variant form had a more basic pI value than that of normal CB-1 (Fig. 8). Furthermore, amino acid analysis of the variant apo-A-I-Munster-3,B (Table II) and its CNBr peptides (Table V) suggested that there was probably one less proline but one more arginine residue compared to either normal apo-A-I or the normal apo-A-I isomorph from this subject. Therefore, sequence analysis of peptide CB-1* was undertaken. This analysis revealed that the occurrence of arginine instead of proline at residue 4 was the probable cause of the different pI value of this mutant (Table VI).

Sequence analysis of the first six residues of the intact apo-A-I-Munster-3,B variant isoform (2000 pmol) from the same subject confirmed the substitution at residue 4: step 1, Asp (850 pmol); step 2, Glu (1200 pmol); step 3, Pro (910 pmol); step 4, Arg (<200 pmol; Pro carryover to cycle 4 was 11%); step 5, Glu (550 pmol); step 6, Ser (trace). The amino acid sequence of the isolated peptide CB-2 (family B) was identical with that of normal apo-A-I, i.e., aspartic acid at residue 103 (Table VII), while the sequence of the first five residues of peptide CB-1 (family A) revealed proline at residue 4, the same as in normal A-I (data not shown). Therefore, it is definite that these two families do not share the same mutation.

A third subject (family C) was identified who also had the apo-A-I-Munster-3 variant (as well as normal apo-A-I). Amino acid analysis of the variant apo-A-I isomorph isolated from this individual suggested that there was probably one less proline but one more histidine residue compared to nor-

### Table II

**Amino acid compositions of apo-A-I variants**

|               | Normal A-I, from sequence | Normal A-I (n = 11) | Munster-3,A | Munster-3,B | Munster-3,C |
|---------------|---------------------------|--------------------|-------------|-------------|-------------|
|               | Variant isoform (n = 2)    | Normal isoform (n = 2) | Variant isoform (n = 2) | Normal isoform (n = 2) | Variant isoform (n = 5) | Pro-apo-A-I (variant) |
| Asp           | 21                        | 20.8               | 21.0        | 21.1        | 21.0        | 20.6        | 21.0              |
| Thr           | 10                        | 9.6                | 9.6         | 9.6         | 9.6         | 9.4         | 9.8               |
| Ser           | 15                        | 13.5               | 13.7        | 13.6        | 13.5        | 13.3        | 13.7              |
| Glu           | 46                        | 46.2               | 46.4        | 46.2        | 46.3        | 46.6        | 47.9              |
| Pro           | 10                        | 10.6               | 10.7        | 9.7         | 10.6        | 9.7         | 9.8               |
| Gly           | 10                        | 10.6               | 10.7        | 10.5        | 10.4        | 10.4        | 10.8              |
| Ala           | 19                        | 19.1               | 19.2        | 19.3        | 19.1        | 19.2        | 19.4              |
| Cys           | 0                         | ND                 | ND          | ND          | ND          | ND          | ND                |
| Val           | 13                        | 13.7               | 13.5        | 13.6        | 13.5        | 13.8        | 13.4              |
| Met           | 3                         | 2.8                | 2.7         | 2.6         | 2.6         | 2.6         | 2.8               |
| Ile           | 0                         | 0                  | 0           | 0           | 0           | 0           | 0                 |
| Leu           | 37                        | 37.3               | 37.4        | 37.0        | 37.3        | 37.4        | 37.2              |
| Tyr           | 7                         | 7.0                | 7.1         | 6.9         | 6.8         | 6.9         | 6.8               |
| Phe           | 6                         | 6.2                | 6.1         | 6.3         | 6.3         | 6.1         | 7.1               |
| Lys           | 21                        | 20.7               | 20.8        | 21.0        | 20.9        | 20.7        | 20.7              |
| His           | 5                         | 4.9                | 5.0         | 5.0         | 4.8         | 5.9         | 6.7               |
| Trp           | 4                         | ND                 | ND          | ND          | ND          | ND          | ND                |
| Arg           | 16                        | 15.8               | 15.9        | 17.0        | 16.1        | 16.2        | 16.8              |

*Ref. 19.

*Compositions are given in residues/mol. Calculations are based on 239 residues/mol (Trp excluded), except for Munster-3,C pro-apo-A-I variant, for which 244 residues/mol was used (Trp excluded). Boxed residues highlight differences in composition that were confirmed by sequence analysis; ND = not determined.
The amino acid at residue 103 in normal apo-A-I is aspartic acid, as determined from both protein sequence (19) and apo-A-I cDNA sequence (25, 26).

Because there are three proline residues in the first seven amino acid residues of the normal apo-A-I polypeptide (19), the NH2-terminal sequence of the intact apo-A-I-Munster-3, C variant isoform was determined first. At residue 3, where there is normally proline in apo-A-I, histidine was found (Table VIII). This histidine substitution was the probable cause of the electrophoretic abnormality in this isoform.

From Ref. 19. The amino acid at residue 103 in normal apo-A-I is aspartic acid, as determined from both protein sequence (19) and apo-A-I cDNA sequence (25, 26).
Asparagine at position 103 occurs in a region with a predicted α-helix, which may be important in apo-A-I interaction with lipids (21).

In the apo-A-I variant of family B, the amino acid arginine occurs instead of proline at residue 4, while in the apo-A-I variant of family C, histidine occurs instead of proline at residue 3. These variants can also be explained by a point mutation in the apo-A-I gene, and each substitution is sufficient to explain the electrophoretic abnormality.

No known function has been ascribed to the NH2-terminal segment of apo-A-I. In normal human A-I and in primate A-I, consecutive proline residues are present at positions 3 and 4. In all other species, one of these prolines is absent (reviewed in Ref. 22), but this apparently does not affect the function of the protein. At this time, it is unclear what effect, if any, the substitution at residue 3 or 4 in the apo-A-I might have on lipoprotein metabolism. The family C individual apparently has normal lipid values. However, there is a possibility that the observed lower level of esterified cholesterol in one affected family B member might be related to the presence of the apo-A-I variant. Because all individuals so far identified as having one of these three variants are heterozygous for a variant apo-A-I and normal apo-A-I, it is possible that the presence of normal apo-A-I in these subjects may mask or compensate for any lipid abnormality caused by the variant apo-A-I. Therefore, studies of the possible effects of these apo-A-I variants on protein-lipid and protein-protein interaction, and on activation of lecithin:cholesterol acyltransferase, must be carried out on the isolated variant isoform in vitro. Such studies are currently in progress.

The data presented here show that the identical electrophoretic abnormalities of apo-A-I variants are not necessarily caused by the same mutation. The designation of these var-

### Table VI

| Residue No.* | Cycle No. | PTH amino acid identified | Yield \((n + 1)/n\) | nmol |
|-------------|-----------|----------------------------|----------------|------|
| 1           | 1         | Asp                        | 7.1            | 0.18 |
| 2           | 2         | Glu                        | 13.4           | 0.06 |
| 3           | 3         | Pro                        | 9.2            | 0.04 |
| 4           | 4         | Arg                        | 3.7            | ND   |
| 5           | 5         | Gln                        | 4.3            | 0.16 |
| 6           | 6         | Ser                        | 2.3            | 0.30 |
| 7           | 7         | Pro                        | 4.9            | 0.08 |
| 8           | 8         | Trp                        | 5.5            | 0.11 |
| 9           | 9         | Asp                        | 3.7            | 0.19 |
| 10          | 10        | Arg                        | 1.5            | ND   |
| 11          | 11        | Val                        | 10.5           | 0.18 |
| 12          | 12        | Lys                        | 5.4            | 0.16 |
| 13          | 13        | Asp                        | 3.3            | 0.21 |
| 14          | 14        | Leu                        | 9.2            | ND   |
| 15          | 15        | Ala                        | 7.2            | 0.19 |
| 16          | 16        | Thr                        | 2.7            | 0.11 |
| 17          | 17        | Val                        | 7.4            | 0.30 |
| 18          | 18        | Tyr                        | 5.3            | 0.19 |
| 19          | 19        | Val                        | 7.5            | 0.29 |
| 20          | 20        | Asp                        | 4.5            | 0.38 |
| 21          | 21        | Val                        | 6.9            | 0.27 |
| 22          | 22        | Leu                        | 7.3            | 0.38 |
| 23          | 23        | Lys                        | 4.0            | 0.25 |
| 24          | 24        | Asp                        | 2.5            | 0.36 |
| 25          | 25        | Ser                        | 2.2            | 0.09 |
| 26          | 26        | Gly                        | 4.8            | 0.33 |
| 27          | 27*       | Arg                        | 1.6            | ND   |
| 28          | 28        | Asp                        | 1.8            | 0.35 |
| 29          | 29        | Tyr                        | 3.4            | 0.26 |
| 30          | 30        | Val                        | 4.2            | 0.53 |

*From aqueous phase analysis; ND = not determined.

### Table VII

| Residue No.* | Cycle No. | PTH amino acid identified | Yield \((n + 1)/n\) | nmol |
|-------------|-----------|----------------------------|----------------|------|
| 87          | 1         | Ser                        | 17.6           | 0.07 |
| 88          | 2         | Lys                        | 20.3           | 0.07 |
| 89          | 3         | Asp                        | 15.4           | 0.05 |
| 90          | 4         | Leu                        | 22.4           | 0.06 |
| 91          | 5         | Glu                        | 22.7           | 0.14 |
| 92          | 6         | Glu                        | 23.7           | 0.12 |
| 93          | 7         | Val                        | 25.9           | 0.10 |
| 94          | 8         | Lys                        | 17.7           | 0.16 |
| 95          | 9         | Ala                        | 22.7           | 0.15 |
| 96          | 10        | Lys                        | 15.2           | 0.14 |
| 97          | 11        | Val                        | 23.5           | 0.13 |
| 98          | 12        | Glu                        | 8.3            | 0.18 |
| 99          | 13        | Pro                        | 12.6           | 0.17 |
| 100         | 14        | Tyr                        | 14.0           | 0.21 |
| 101         | 15        | Leu                        | 18.8           | 0.23 |
| 102         | 16        | Asp                        | 8.5            | 1.12 |
| 103         | 17        | Asp                        | 9.5            | 0.21 |
| 104         | 18        | Phe                        | 14.0           | 0.24 |
| 105         | 19        | Glu                        | 6.1            | 0.32 |
| 106         | 20        | Lys                        | 11.3           | 1.09 |
| 107         | 21        | Lys                        | 12.3           | 0.23 |
| 108         | 22        | Trp                        | 6.2            | 0.32 |
| 109         | 23        | Glu                        | 3.4            | 0.44 |
| 110         | 24        | Glu                        | 8.4            | 0.65 |
| 111         | 25        | Glu                        | 5.5            |      |

*From Ref. 19. The amino acid at residue 103 in normal apo-A-I is aspartic acid, as determined from both protein sequence (19) and apo-A-I cDNA sequence (25, 26). In addition, our analyses of the comparable peptide from several normal individuals gave the same sequence as above (not shown).

**DISCUSSION**

In this study, the nature of the difference between normal apo-A-I and apo-A-I-Münster-3 has been examined. It has been shown that at least three variant forms of the apo-A-I-Münster-3 type exist, which differ from one another in both the nature and sites of amino acid substitutions. For the variant form of family A, a substitution occurs at position 103, where asparagine replaces aspartic acid. This single substitution is sufficient to explain the more basic PI value of this apo-A-I-Münster-3. A single base change (point mutation) in the normal apo-A-I gene corresponding to this position could account for the occurrence of apo-A-I-Münster-3.A. The substitution apparently has no effect on lipid metabolism in affected individuals, since all lipid values were in the normal range and the variant was present in the same lipoprotein fractions as normal apo-A-I. The asparagine at position 103 occurs in a region with a predicted α-helical conformation (21). Since both asparagine and aspartic acid have a polar side chain, the substitution probably does not alter the nature of the hydrophobic or hydrophilic face of this presumed α-helix, which may be important in apo-A-I interaction with lipids (21).
The amino acid at residue 3 in normal apo-A-I is proline, as determined from both protein sequence (19) and apo-A-I cDNA analysis; ND = not determined.

The following nomenclature is proposed for the apo-A-I-Münster-3 of family A, apo-A-I(Pro3 → Arg); and for the apo-A-I-Münster-3 of family B, apo-A-I(Pro4 → Arg); and for the apo-A-I-Münster-3 of family C, apo-A-I(Pro → His). In like fashion, the previously documented apo-A-I_Milano variant (13) can be described as apo-A-I(Arg171 → Cys).

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