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Apolipoprotein A-I (Glu 198→Lys): A Mutant of the Major Apolipoprotein of High-Density Lipoproteins Occurring in a Family with Dyslipoproteinemia

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ABSTRACT. To detect genetic mutants of apo A-I, the major structural protein of human HDL, we screened 530 unrelated Austrian probands (168 children, 362 adults). An apo A-I mutant characterized by an exchange of the acidic amino acid Glu in position 198 with the basic amino acid Lys was identified in the serum of the mother of a hyperlipoproteinemic girl. So far only two patients with this mutant, referred to as apo A-I (Glu 198→Lys) have been described. We detected six new patients (two children and four adults) with apo A-I (Glu 198→Lys) among 20 members in three generations of the affected family. An autosomal codominant inheritance of the apolipoprotein variant could be established. All affected individuals were heterozygous for the mutant. Among the six new subjects with apo A-I (Glu 198→Lys) two children and one adult presented with high-density lipoprotein (HDL) cholesterol concentrations below the fifth percentile for age and sex and with low serum apo A-I and A-II. Although there was no consistent relationship of the mutant with low serum HDL in this family, a moderate effect of apo A-I (Glu 198→Lys) on HDL levels cannot be ruled out. Hyperlipoproteinemia of types IIa, IIb, and IV was observed in eight of the 20 family members studied, but did not cosegregate with the mutant apo A-I. There was no association of apo A-I (Glu 198→Lys) with premature clinical manifestations of atherosclerosis. The mutation occurred in a part of the apo A-I molecule, which is thought to be involved in lipid binding. The mutant apo A-I, however, was almost exclusively bound to HDL, similar to normal apo A-I. Although apo A-I (Glu 198→Lys) does not appear to be related to dyslipoproteinemia or premature atherosclerosis, this apolipoprotein variant may be of interest in studying the interaction of HDL with cell surface receptors and for genetic mapping studies. (*Pediatr Res* 24: 222–228, 1988)

Abbreviations

VLDL, very low-density lipoproteins (d > 1.006)
LDL, low-density lipoproteins (d 1.006–1.063)
HDL, high-density lipoproteins (d 1.063–1.21)
C, cholesterol
TG, triglycerides
VLDL-C, LDL-C, HDL-C, VLDL-, LDL-, HDL-cholesterol
apo A-I, A-II, B, C-III, E: apolipoproteins A-I, A-II, B, C-III, E
LCAT, lecithin:cholesterol acyltransferase
SDS, sodium dodecylsulfate
HPLC, high-performance liquid chromatography

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It is well established that disorders of lipoprotein metabolism play a central role among the risk factors for premature coronary heart disease (1). In adult populations elevated serum levels of total cholesterol and LDL-C and increased levels of HDL-C are associated with an increased risk for coronary heart disease (1). Recently, it has been proposed that the apolipoproteins, the protein moieties of the lipoprotein particles, may be even better predictors of atherosclerosis than HDL- or LDL-C (2). In adult patients, coronary heart disease is positively correlated to the serum levels of apo B, the protein constituent of LDL and inversely related to apo A-I, the predominant protein of HDL (3, 4).

Serum lipoprotein levels in children are closely related to the extent of early atherosclerotic lesions (5) and to the prevalence of premature coronary artery disease in their parents (6). The levels of apo A-I and B in children appear to be even more closely associated with the prevalence of myocardial infarction in the parents than LDL-C or HDL-C (7).

Apo A-I is a polypeptide of 243 amino acids of known sequence (8). Apo A-I is secreted into the bloodstream by the liver and intestine as a proprotein, which is then rapidly converted to mature apo A-I (9). Two major isoforms of mature normal apo A-I, which arise by deamidation, can be separated in human serum (10). The protective role of apo A-I in the pathogenesis of atherosclerosis is not completely understood. It is assumed that apo A-I participates in the transport of cholesterol from peripheral tissues to the liver (11), the only organ capable of C disposal. In this transport process, apo A-I acts as cofactor of the enzyme LCAT, which catalyzes the formation of C esters in serum (12) and may also be involved in the binding and uptake of HDL by specific cell surface receptors (13).

Structural mutants of apolipoproteins can be used to study the role of the apolipoproteins in lipoprotein metabolism and atherosclerosis. Recently, several rare variants of human apo A-I have been discovered and some of them have been characterized concerning their difference in primary structure as compared to normal apo A-I (14–23). Here, we report on an apo A-I variant

that we discovered in the family of a hyperlipoproteinemic girl during a screening program.

SUBJECTS AND METHODS

A total of 530 unrelated Austrian probands (168 children, 362 adults) was screened for apo A-I variants. The probands had been referred to the lipid outpatient clinic for suspected hyperlipidemia, for follow-up of known hyperlipoproteinemia or study during cardiovascular rehabilitation. A total of 210 of these probands was hyperlipidemic with serum C <250 mg/dl and/or triglycerides >300 mg/dl in adults or with serum C >220 mg/dl and/or TG >250 mg in children, 320 were normolipidemic. A total of 159 of the subjects was survivors of myocardial infarction or suffered from coronary artery disease.

Among this study population one family with an apo A-I mutant was detected. A medical history was obtained and a short physical examination was performed on all members of this family. A routine clinical chemistry program including tests for hepatic and renal function, serum electrolytes, blood sugar, and blood count was carried out. The family members affected with the apo A-I variant were examined for signs of atherosclerosis by ECG and Doppler sonography of the great arteries of the lower limbs and of the carotid arteries.

Blood samples were collected after an overnight fast. Blood was allowed to clot at room temperature and serum was separated by low speed centrifugation. Sera were stored at 4°C for up to 1 wk before lipid and lipoprotein determination or at −20° C until analyzed for apo A-I mutants.

Serum C and TG were determined by enzymatic methods (24, 25). In accordance with Lipid Research Clinic methods (26) serum lipoproteins were separated by a combination of ultracentrifugation and polyanionprecipitation for the determination of lipoprotein C. Serum apo A-I, apo A-II, and apo B were determined by electroimmunoassay using commercially available monospecific antibodies (Immuno Diagnostika, Vienna, Austria) as described previously (27).

Screening for variants of apo A-I was performed by isolectric focusing as described by Menzel *et al.* (14). Native serum, after incubation with decylsulfate and mercaptoethanol, was focused on vertical 7.5% polyacrylamide slab gels containing 6 M urea in a pH gradient from 4 to 6. The immunoreactivity of the protein bands separated by isoelectric focusing was established by immunoelectrophoresis against monospecific anti apo A-I and anti apo A-II in the second dimension and by two-dimensional gel electrophoresis (14) of immunoprecipitates prepared from whole serum with monospecific anti apo A-I (28). Mol. wt. was determined by second dimension SDS gel electrophoresis using the discontinuous gel system of Neville (29).

VLDL (d < 1.006), LDL (d 1.006–1.063), and HDL (d 1.063–1.21) were isolated by sequential preparative ultracentrifugation (30). The fractions were dialyzed against 0.15 M NaCl, pH 7.4 and delipidated twice with ethanol/diethylether (3 + 1) and once with diethylether alone. Apolipoproteins were dissolved in 0.02 M Tris/HCl, pH 8.2, 1.5% decylsulfate, and 8 M urea in the presence of mercaptoethanol. The occurrence of the mutant apo A-I in the isolated lipoprotein fractions was studied by isoelectric focusing in a gradient from pH 4–6 as described above. Apo E phenotypes were determined by isoelectric focusing of delipidated VLDL in 7.5% polyacrylamide slab gels containing 6 M urea in a pH gradient from 4–7.5 (31).

For the determination of the amino acid substitution in the mutant apo A-I normal and mutant apo A-I was isolated from HDL by preparative isoelectric focusing on Immobiline gel (32). The fractions were eluted electrophoretically and dialyzed extensively against 0.05 M sodium bicarbonate, pH 7.4 and then subjected to digestion with trypsin (Sigma, Munich, West Germany) in a ratio of 1:40 at 37° C overnight. The tryptic peptides were separated by reverse phase HPLC using a C18 column with a gradient (1–40% in 60 min) of 0.1% trifluoroacetic acid and acetonitrile and analyzed by time-of-flight secondary ion mass spectrometry (32). Microsequence analysis of HPLC-separated peptides was performed by the 4-N,N-dimethylaminoazobenzene-4′isothiocyanate/phenylisothiocyanate double coupling method as described earlier (32).

The Mann Whitney U test was used for statistical comparison of the lipoprotein and apolipoprotein values in the probands with the apo A-I mutant with those in their unaffected family members because the data did not follow a normal distribution.

RESULTS

Characterization of mutant apo A-I. During the screening project, a 15-yr-old girl with type IIa hyperlipoproteinemia (III-9 in Fig. 1) was referred to the lipid outpatient clinic. When her first degree relatives were studied, a variant of apo A-I was identified in the serum of the girl's mother, a 32-yr-old woman with type IIa hyperlipoproteinemia (II 7 in Fig. 1). All of the other 529 patients screened for apo A-I mutants had a normal apo A-I isoprotein pattern. All available relatives of the proband with the mutant were investigated. Twenty family members from three generations of the kindred with the apo A-I mutant participated in this family study (Fig. 1). Five blood relatives of the proband (II-7) were found to be affected with the mutant apo A-I. Among them there was an identical twin (II-8) of the proband (II-7).

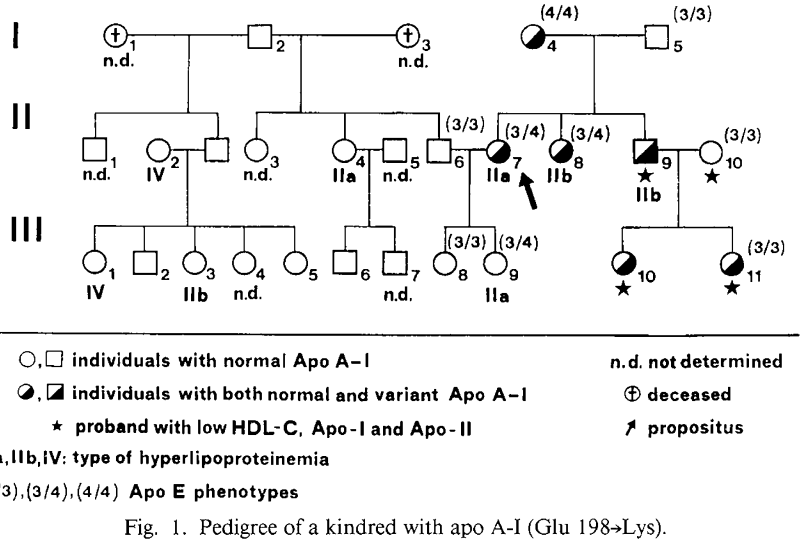

Fig. 1. Pedigree of a kindred with apo A-I (Glu 198→Lys).

On isoelectric focusing gels of native serum from the individuals with the apo A-I mutant, two additional protein bands appeared that differed from the two major normal apo A-I isoproteins (apo A-I-1 and apo A-I-2) (14) by a charge difference of +2 units (Fig. 2). The immunoreactivity of the abnormal bands with monospecific apo A-I antiserum could be established (Fig. 2). Immunoelectrophoresis of the serum of the proband (II-7) showed that the normal and the variant apo A-I were present in approximately equal amounts. The mol. wt. of the mutant protein was identical to that of normal apo A-I (approximately 28.6 kDa). This was determined by two dimensional SDS gel electrophoresis of native serum (not shown) or immunoprecipitates prepared from whole serum using monospecific apo A-I antibody (Fig. 3). The chromatograms obtained by reverse phase HPLC of the tryptic peptides of the mutant apo A-I were characterized by the disappearance of peak T32 (mol. wt. 1215Da) containing amino acids 196–206 of normal apo A-I (Table 1) and the presence of an abnormal peak designated as T32x with a molecular mass of 913 Da, as determined by time-of-flight secondary ion emission mass spectrometry. These findings suggested the introduction of a new trypsin cleavage site at position 198 by an amino acid exchange of Glu by Lys. Manual microsequence analysis of the abnormal peptide confirmed this amino acid exchange (Table 1). The mutant is therefore referred to as apo A-I (Glu 198→Lys).

Figure 4 shows a helical wheel presentation (33) of the segment of normal and mutant apo A-I containing amino acids 190–207. In this part of the sequence the amino acids are arranged in an amphipatic helix, *i.e.* a helical structure with one side that is apolar and carries hydrophobic amino acids, whereas the other face is polar (34). In the model of the sequence neighboring the mutation the amphipathic structure appears intact. In normal apo A-I, three Glu residues are located on the hydrophilic face of this helix. This acidic region of the molecule is interrupted by the basic Lys at position 198 in the mutant.

Lipoprotein distribution of apo A-I (Glu 198→Lys). VLDL, LDL, HDL, and the d > 1.21 fraction of serum from three probands with the mutant and from an unaffected relative were isolated by preparative ultracentrifugation and analyzed by isoelectric focusing in a pH gradient from 4–6 as described above. The mutant apo A-I was almost exclusively bound to HDL (Fig. 5). The isoprotein pattern of apo C-III, which is closely linked to apo A-I in the genome (35), was normal in the patients with apo A-I (Glu 198→Lys).

Genetics of apo A-I (Glu 198→Lys). Vertical transmission of the trait over three generations of the affected kindred could be demonstrated (Fig. 1). Inspection of the pedigree suggests that apo A-I (Glu 198→Lys) is inherited as autosomal codominant trait. All affected individuals were heterozygotes having normal apo A-I in addition to the mutant.

Apo A-I (Glu 198→Lys) and dyslipoproteinemia. In the kindred with apo A-I (Glu 198→Lys) described here, three of the six family members with the mutant (two children and one adult) presented with serum HDL-C levels below the fifth percentile for age and sex (36, 37) (II-9, III-10, III-11 in Fig. 1 and Table 2). In contrast, only one of the 15 unaffected siblings had low HDL-C (II-10 in Fig. 1 and Table 2). The apo A-I and apo A-II concentrations in the three patients with apo A-I (Glu 198→Lys) and decreased HDL-C appear to be low (Table 2) when compared to those of the unaffected family members. The apo A-I levels of both

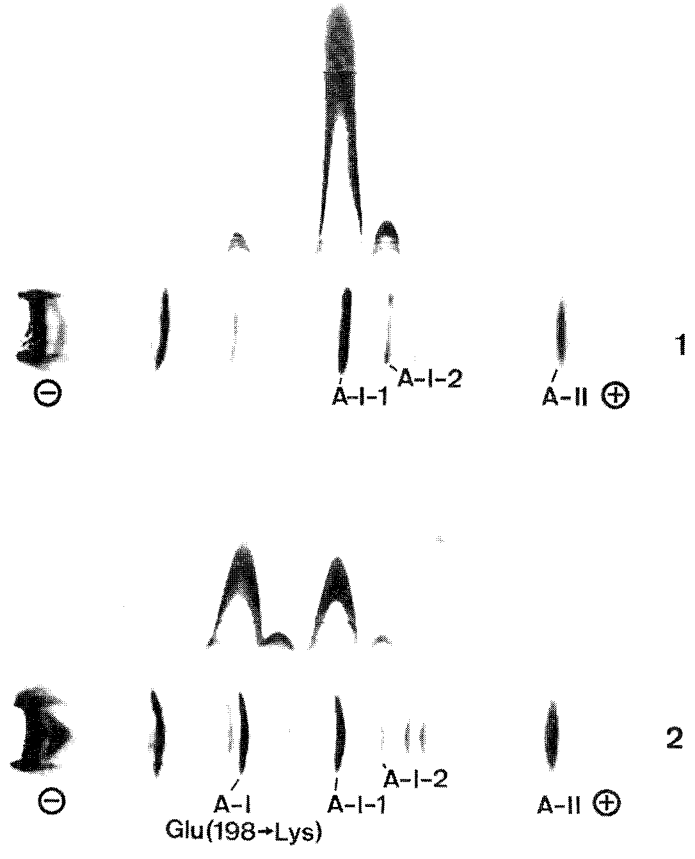

Fig. 2. Isoelectric focusing (pH 4–6) (horizontal) of native serum followed by immunoelectrophoresis (vertical) using monospecific anti apo A-I. *Upper panel*, control; *lower panel*, patient with apo A-I (Glu 198→Lys).

children affected with the mutant (III-10, III-11 in Fig. 1 and Table 2) were below the fifth percentile of reference values in a large pediatric population determined by a method comparable to the one used here (38). The mean values for serum HDL-C apo A-I and apo A-II for all six probands with the mutant, however, did not differ significantly from those for their relatives without it (Table 2).

No consistent association of apo A-I (Glu 198→Lys) with hyperlipoproteinemia could be established (Fig. 1). The proband identified during the screening (II-7) had type IIa hyperlipoproteinemia. Her twin sister (II-8) and her brother (II-9), both also heterozygous for the mutant, presented with type IIb hyperlipoproteinemia. In contrast, the three other blood relatives with apo A-I (Glu 198→Lys), one adult (I-4) and two children (III-10 and III-11), were not hyperlipoproteinemic. Moreover, hyperlipoproteinemia was diagnosed in five of the 14 members of the kindred, who were unaffected by the mutant apo A-I (II-2, II-4, III-1, III-3, III-9). The mean serum levels of total C, TG, LDL-C, VLDL-C, and apo B did not differ significantly between family members with apo A-I (Glu 198→Lys) and their relatives without the mutant (Table 1).

Apo E phenotypes and dyslipoproteinemia. Apo E phenotypes could be determined in nine individuals of the kindred with apo A-I (Glu 198→Lys) (Fig. 1). Hyperlipoproteinemia occurred in

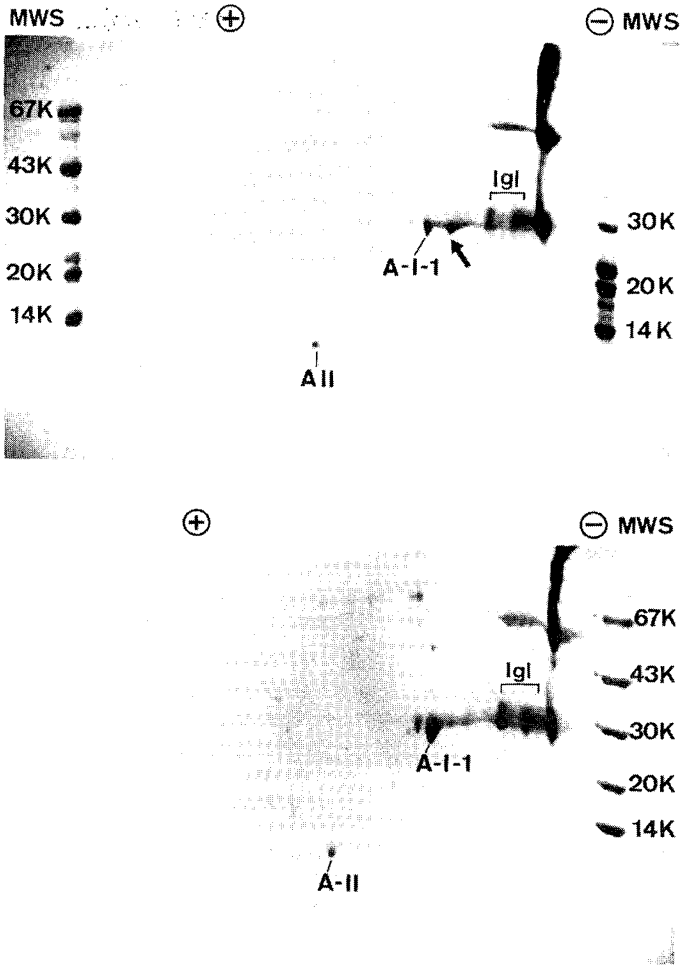

Fig. 3. Two-dimensional SDS gel electrophoresis of immunoprecipitates prepared from normal serum (*lower panel*) and from serum of a patient heterozygous for apo A-I (Glu 198→Lys) (*upper panel*) using monospecific anti apo A-I. *MWS*, molecular weight standard; *K*, kilodalton; *Igl*, immunoglobulin light chains. *Arrow* denotes apo A-I (Glu 198→Lys).

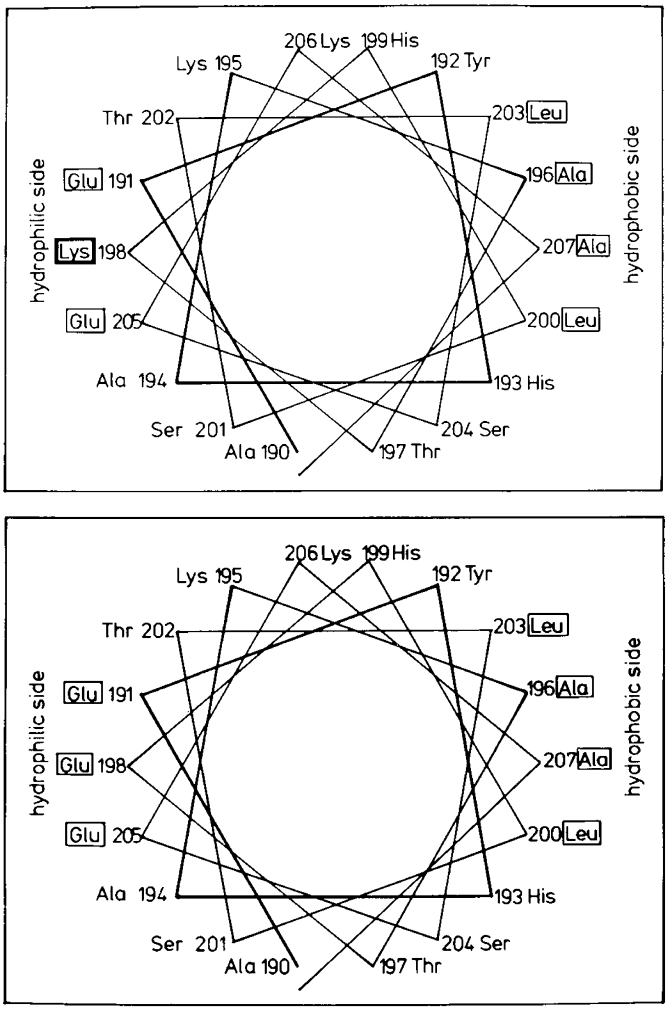

Fig. 4. Helical wheel representation of a segment of normal apo A-I (*lower panel*) and apo A-I (Glu 198→Lys) (*upper panel*) containing amino acids 190–207. The helix extends clockwise through the plane of the page (from Ala 190 to Ala 207).

Table 1. *Amino acid substitution in variant Apo A-I**

(A) Tryptic peptide T32 of normal apo A-I:

Ala – Thr – *Glu*[198] – His – Leu – Ser – Thr – Leu – Ser – Glu – Lys

mol. wt. = 1215 Da

(B) Tryptic peptide T32x of variant apo A-I:

Ala – Thr – *Lys*[198] | † His – Leu – Ser – Thr – Leu – Ser – Glu – Lys

mol. wt. = 913 Da

* The mol. wt. (as determined by time-of-flight secondary ion mass spectroscopy) and amino acid sequences (determined by manual microsequencing) of tryptic peptide T32 of normal apo A-I (A) and of the abnormal tryptic peptide T32x of apo A-I (Glu 198→Lys) (B) are given.

† Denotes new tryptic cleavage site introduced by the exchange of Glu 198 with Lys in the mutant.

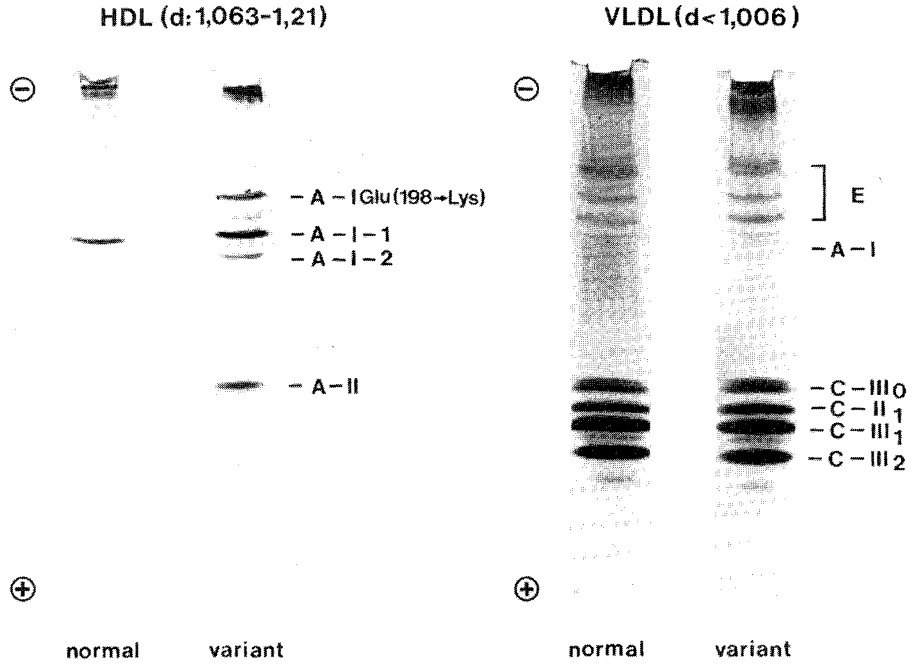

Fig. 5. Isoelectric focusing (pH 4–6) of delipidated HDL and VLDL from a proband with normal apo A-I (*left*) and from a patient heterozygous for apo A-I (Glu 198→Lys) (*right*).

Table 2. *Serum lipids, lipoprotein-C, apo A-I, A-II, and B in a kindred with apo A-I (Glu 198→Lys) (mg/dl, means ± SD)*

1) Siblings with apo A-I(Glu 198→Lys), n = 6										
No.	Age	Sex	C	TG	LDL-C	HDL-C	VLDL-C	A-I	A-II	B
I-4	67	F	136	112	78	44	14	129	28	76
II-7	33	F	288	114	226	37	25	107	37	188
II-8	33	F	373	131	255	74	44	168	49	307
II-9	35	M	271	225	212	20	48	85	28	173
III-10	11	F	119	59	75	31	12	74	24	80
III-11	14	F	173	59	137	32	04	94	28	98
$\bar{x}$ ± SD	33 ± 20 yr		227 ± 100	117 ± 61	164 ± 78	40 ± 19	25 ± 18	110 ± 34	32 ± 9	154 ± 89

2) Siblings without apo A-I(Glu 198→Lys), *n* = 14 (6M, 8F)									
	Age	C	TG	LDL-C	HDL-C	VLDL-C	A-I	A-II	B
$\bar{x}$ ± SD	30 ± 21	240 ± 60	186 ± 165	163 ± 52	46 ± 13	31 ± 29	116 ± 49	38 ± 15	127 ± 54
p	NS	NS	NS	NS	NS	NS	NS	NS	NS

NS, $p > 0.05$, as compared to probands with apo A-I (Glu 198→Lys), Mann-Whitney U test.

three of four probands carrying the E4 allele (II-7, II-8, III-9), but in none of five patients without an apo E4 allele.

Clinical features of the patients with apo A-I (Glu 198→Lys). Clinical examination and routine laboratory tests revealed liver cirrhosis, obesity, and lichen urticatus in patient I-4. Cholelithiasis was diagnosed in proband II-7. Patient II-9 was obese and reported to smoke 30 cigarettes daily. Both children affected with the mutant were apparently healthy.

Neither medical history, clinical examination, ECG, or Doppler sonography of the carotid arteries and the arteries of the lower extremities revealed any symptoms or signs of atherosclerosis in the family members heterozygous for apo A-I (Glu 198→Lys).

DISCUSSION

The results of our study indicate that the apo A-I variant detected in the course of our screening program differs from normal apo A-I by a substitution of the basic amino acid Lys for the acidic amino acid Glu at position 198. This amino acid exchange accounts for the charge difference of +2 units as compared to normal apo A-I on isoelectric focusing gels. An apo A-I mutant with a similar electrophoretic mobility has recently been reported (16). This mutant is characterized by a substitution of Lys for Glu at position 136, and is thereby clearly distinct from the one described here (23).

So far only two patients with apo A-I (Glu 198→Lys) have been described (17). Apo A-I (Glu 198→Lys) has been identified for the first time in a hyperlipoproteinemic man during a population study carried out in Münster (GFR) and was referred to as apo A-I Münster 4 (17). One additional subject with this apo A-I variant was detected in Marburg (GFR). No family study of apo A-I (Glu 198→Lys) has been reported.

In our study we describe a kindred with six new individuals affected with apo A-I (Glu 198→Lys). We could establish the genetic transmission of this mutant, which appears to be inherited in an autosomal codominant way. A familial nature of the mutation has also been reported for several of the other apo A-I mutants and an autosomal codominant inheritance has been proposed for some of them (14, 16, 20, 21).

The kindred with apo A-I (Glu 198→Lys) described here also provided the first opportunity to investigate if there exists a relationship of this apo A-I mutant with dyslipoproteinemia or atherosclerosis. This question appeared to be of particular interest, as both the patient with apo A-I (Glu 198→Lys) identified in our screening study and the proband first described in Münster (17) were hyperlipoproteinemic. Although a high percentage of both the adults (five of 10) and the children (three of nine) in the affected kindred had hyperlipoproteinemia of types IIa, IIb, or IV, no consistent relationship between apo A-I (Glu 198→Lys) and hyperlipoproteinemia could be demonstrated (Fig. 1). The occurrence of hyperlipoproteinemia of types IIa, IIb, and IV in the same family suggests the diagnosis of familial combined hyperlipoproteinemia (39). Although the apo E phenotype could be determined only in part of the family, hyperlipoproteinemia appeared to be more common in patients with the E4 allele than in others. This finding agrees with reports on an increased prevalence of the apo E4 allele in patients with hypercholesterolemia (40).

It seems remarkable, however, that both children affected with apo A-I (Glu 198→Lys) (III-10, III-11) and their father (II-9), also heterozygous for the mutant, had decreased serum HDL-C, apo A-I, and apo A-II levels. Moreover, the serum concentrations of these HDL constituents were considered to be at the lower border of the normal range in the proband with the mutant, who was initially identified in our screening program (II-7). However, the HDL-C, apo A-I, and apo A-II concentrations were normal in two of the remaining six individuals with apo A-I (Glu 198→Lys). The interpretation of these results is complicated by the occurrence of several factors in the kindred that are known to affect HDL-C and apo A-I levels, such as cigarette smoking, obesity, and liver disease (41, 21). In addition, all family members with the mutant were heterozygotes, in whom the normal apo A-I allele could compensate for a possible effect of the mutant allele on serum HDL. Based on the family data presented here, it is therefore neither possible to show a consistent relation of apo A-I (Glu 198→Lys) with low HDL-C, apo A-I or apo A-II nor to exclude a moderate effect of the variant on HDL levels. The first patient with apo A-I (Glu 198→Lys) reported from Münster (GFR) (17) presented with a normal HDL concentration.

As with observations in most other apo A-I variants (15–17, 19–21), there was no evidence for accelerated atherosclerosis in our patients with apo A-I (Glu 198→Lys). In contrast, a congenital deficiency of apo A-I and C-III is associated with severe premature arterial disease (43).

The amino acid substitution in apo A-I (Glu 198→Lys) does not appear to disrupt the structure of the apo A-I molecule. This may explain the lack of a major effect of the mutation on serum lipoprotein levels. The amino acid exchange is located in a region of the apo A-I molecule, which is considered to be important for its lipid binding properties (34) due to its amphipathic helical structure. The apolar face of the amphipatic helix is thought to interact with the fatty acyl residues of phospholipids, whereas the polar face may interact with the polar head groups of phospholipid. In apo A-I (Glu 198→Lys) the mutation occurred on the polar side, without interfering with the amphipatic character of the helix (Fig. 4). The lipid binding properties of the entire apo A-I (Glu 198→Lys) molecule seem to be intact, as we observed a normal distribution of the mutant apo A-I among the lipoprotein density classes. The alteration of the charge on the hydrophilic face of the helix, which is predicted to occur in apo A-I (Glu 198→Lys), could affect the interaction of the mutant protein with cellular HDL receptors. Inasmuch as several of the family members with apo A-I (Glu 198→Lys) had low serum HDL-C, apo A-I, and apo A-II, receptor binding studies of the isolated mutant apo A-I should be carried out.

In contrast to apo A-I (Glu 198→Lys), a mutation resulting in an exchange of Cys for Arg at position 173 (apo A-I Milano) (15) has been shown to disrupt one of the amphipatic helices of apo A-I and is associated with severe dyslipoproteinemia. Replacement of Pro by Arg at position 143 (apo A-I Giessen) (21), appears to result in the elimination of a β-turn between two of the amphipathic helices of apo A-I. A deletion of Lys 107 (apo A-I Münster 2 A or Marburg) (19) is thought to change the orientation of one of the amphipathic helices by about 90°. Both of these apo A-I variants are defective in activating the enzyme LCAT. The LCAT activation by apo A-I (Glu 198→Lys) described here was found to be normal in the patient first detected in Münster.

At the level of the apo A-I gene the mutation in apo A-I (Glu 198→Lys) can be explained by an alteration of the triplet GAG coding for Glu 198 (44) to AAG coding for Lys by a single base exchange in the nucleotide sequence. As all three individuals with apo A-I (Glu 198→Lys) were detected in the neighboring countries of Germany and Austria a common origin of the mutant allele appears possible. To test this hypothesis, studies on the restriction fragment length polymorphisms of the apo A-I gene in the affected subjects are currently underway.

In conclusion the data from this first family study of apo A-I (Glu 198→Lys) suggest that this apo A-I mutant is inherited in an autosomal codominant way and does not appear to be related to either hyperlipoproteinemia or premature atherosclerosis. The mutant probably does not significantly influence HDL levels. Nevertheless apo A-I (Glu 198→Lys) may be of interest for studying the interaction of HDL with specific cell surface receptors and for genetic mapping studies. We suggest that children and adults presenting with decreased serum HDL-C, apo A-I, or A-II should be screened for apo A-I mutants. Regardless of whether or not mutant forms of apo A-I bear a causal relationship to dyslipoproteinemia, they may provide new insight into the role of this apolipoprotein in lipoprotein metabolism.

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