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Human Genetics

Apolipoprotein $AI_{Marburg}$: Studies on Two Kindreds with a Mutant of Human Apolipoprotein AI

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Summary. Three probands heterozygous for a mutant of apolipoprotein AI (apo $AI_{Marburg}$, Utermann et al. 1982a) were detected by screening of 2282 unrelated individuals resulting an a frequency estimate of about 1/750 in the German population. All three probands with apo $AI_{Marburg}$ had hypertriglyceridemia (triglyceride above 250 mg/dl) and subnormal HDL-cholesterol (below 30 mg/dl), but no other lipoprotein abnormalities. The kindreds of two probands with $AI_{Marburg}$ were studied. The family data are consistent with an autosomal codominant inheritance of the trait. A total of 16 heterozygous blood relatives with the mutant $AI_{Marburg}$ were detected in these kindreds.

Analysis of the plasma lipid and lipoprotein levels in relation to the apo AI phenotype was complicated by the high prevalence of diabetes mellitus and thyroid disease in one kindred and of hyperlipidemia in both kindreds. No consistent relationship between plasma lipid and lipoprotein levels, and the mutant apo AI could be demonstrated. Instead the mutant apo AI and the dyslipoproteinemia seem to co-exist independently in these kindreds. Three sibs with the homozygous apo E-2/2 phenotype were detected in one kindred, and all three sibs had subnormal LDL-cholesterol and beta-VLDL, e.g., the lipoprotein abnormality characterizing primary dysbetalipoproteinemia. Genetic apo E phenotypes and the apo AI mutant segregated independently, indicating that the structural gene loci for apo E and apo AI are not closely linked.

Introduction

The protein moiety of plasma lipoproteins consists of several distinct polypeptides called apolipoproteins. These proteins serve different specific functions including enzymatic cofactor activity for lipases and LCAT[1] (Schaefer et al. 1978), recognition of cell surface receptors (Brown et al. 1981), and exchange of cholesterylester between lipoproteins (Chajek and Fielding 1978). Mutations affecting the structure and function of apolipoproteins may impair the metabolism of lipoproteins and result in certain forms of genetic dyslipoproteinemia that might be termed apolipoproteinopathies (Utermann et al. 1982). Two forms of apolipoproteinopathies have recently been described. One of these is familial hyperlipoproteinemia type III, where patients are homozygous for a mutant of apolipoprotein E (phenotype apo E-2/2; Utermann et al. 1977, 1979b, 1982b; Zannis et al. 1981). The mutant apo E from some patients with hyperlipoproteinemia type III is functionally abnormal and does not bind to specific cell surface receptors (Schneider et al. 1981; Weisgraber et al. 1982) resulting in an impaired catabolism of apo E and of cholesterylester-rich remnant lipoproteins (Havel et al. 1980; Gregg et al. 1981). A second form of apolipoproteinopathia was recently described in an Italian family (apo AI_{Milano}, Franceschini et al. 1980). Patients with apo AI_{Milano} are heterozygous for a mutant of apo AI that contains cysteine, an amino acid not present in the normal AI sequence (Weisgraber et al. 1980; Utermann et al. 1982a). The mutant is associated with hypertriglyceridemia and low HDL-cholesterol (Franceschini et al. 1980).

In analogy to human hemoglobin, mutants of apolipoproteins will be powerful tools to study structure-function relationships of these proteins and, in addition, may elucidate the function of apolipoproteins such as apo AII or apo AIV, that at present are not known. However until recently no method existed that permitted screening for variants of apolipoproteins. We have recently developed simple and rapid procedures to identify and characterize genetic variants of apolipoproteins AI, AII, and AIV without ultracentrifugation, and have identified mutants of apolipoproteins AI and AIV which we have designated apo $AI_{Marburg}$, apo $AI_{Giessen}$, and apo$AIV_{Marburg}$ (Utermann et al. 1982). Here we report on studies of two kindreds with apo $AI_{Marburg}$, that were undertaken to establish the genetic nature of the variant and to investigate its possible association with dyslipoproteinemia.

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1 *Abbreviations and nomenclature: VLDL:* very low density lipoproteins; *LDL:* low density lipoproteins; *HDL:* high density lipoproteins; *apo:* apolipoprotein; *LCAT:* lecithin-cholesterol-acyltransferase; *SDS-PAGE:* polyacrylamide-gel electrophoresis in sodium dodecylsulfate; *Apo E* isoforms, phenotypes and genes are designated according to a new comprehensive nomenclature (Zannis et al., to be published)

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Materials and Methods

Study Population and Probands

Blood samples were collected without additives from 542 healthy blood donors from the Blood Transfusion Service at the University of Giessen, from 520 hyperlipidemic patients (cholesterol above 300 mg/dl and/or triglyceride above 250 mg/dl) and from 62 hypocholesterolemic patients (cholesterol below 130 mg/dl) of the Policlinic for Internal Medicine of the University of Marburg, from 461 patients with various neurologic disorders from the Neurological Clinic at the University of Marburg, from 358 coronary angiographed patients from the University of Göttingen, from 239 patients with myocardial infarction from the Rehabilitation Center at Bad Krotzingen, and from 100 diabetic patients from the Diabetes Institute at the University of Düsseldorf. Three unrelated probands were detected in these groups that had a phenotypically identical apo AI variant designated apo $AI_{Marburg}$ (Utermann et al. 1982a).

Proband K.M. is a 45-year-old male detected in the hyperlipidemic group. He was admitted to the Policlinic for Internal Medicine because of hyperacidity of the stomach and duodenitis. The obese patient had hepatomegaly probably due to alcoholic fatty liver. He had glaucoma of both eyes detected in 1973 and the right eye had to be enucleated. In 1979 he had had severe stenocardic complaints but there was no evidence for myocardial infarction. Laboratory examination showed an elevation of triglycerides (352 mg/dl), low HDL-cholesterol (21 mg/dl), and a moderate elevation of γGT (35 units/ml).

Proband K.So. is a 38-year-old healthy male blood donor. Laboratory examination revealed hypertriglyceridemia (267 mg/dl) and low HDL-cholesterol (28 mg/dl).

Proband B.B. is a 70-year-old female with a known history of hypertension since 1960. In 1960 she had an apoplectic insult with aphasia. In 1979 she had a myocardial infarction and she has suffered from resting angina pectoris since 1980. Coronary angiography was performed in 1980 and showed a 70% stenosis of the R. circumflexus and a total stenosis of the R. interventricularis anterior of the A. coronaria sinistra.

All available blood relatives of probands K.M. and K.So. and their spouses were studied. From all family members, a medical history was obtained with the main emphasis on alcohol consumption, smoking habits, medications, changes in body weight, and ischemic vascular disease. A brief physical examination was done and clinical-chemical tests measuring hepatic, renal and thyroid functions, and fasting blood-glucose levels were performed.

Methods

Blood was allowed to clot at room temperature (1–2 h) and the blood clot was removed by low speed centrifugation. Sera were used either directly for lipid, lipoprotein, and apolipoprotein analysis (including all sera from the family studies), or were frozen immediately and stored at −20°C until analyzed for apolipoprotein mutants.

Screening for variants of apolipoproteins AI, AII, and AIV was performed by a variant of the "double-one-dimensional electrophoresis" (Altland and Hackler 1980) involving agarose gel electrophoresis in detergent followed by isoelectric focusing of separated apolipoproteins in a pH gradient from 4–6.5 as outlined in detail elsewhere (Utermann et al. 1982a). Apolipoprotein E phenotypes were determined by isoelectric focusing of heparin/Mg^{++} precipitated lipoproteins after delipidation as described (Utermann et al. 1977).

Density gradient ultracentrifugation of sera was performed by the method of Redgrave et al. (1975) with minor modifications (Utermann et al. 1980) using the SW42 rotor (Beckman Instruments). After centrifugation 0.5 ml fractions were collected from the bottom of the tube using the Beckman equipment for fractionation. Total cholesterol was determined directly in density fractions and in total serum by an enzymatic method using a commercial test kit (Boehringer Mannheim, Federal Republic of Germany). Cholesterol concentrations in lipoprotein fractions were calculated as percent of total cholesterol determined in the gradient and converted to mg/dl serum by taking the cholesterol in serum as 100%, thus correcting for losses during manipulation of the samples.

HDL-cholesterol was determined alternatively after precipitation of apo B containing lipoproteins from serum by phosphotungstate using a commercial test kit (Boehringer, Mannheim, Federal Republic of Germany)[2]. The distribution of apolipoprotein AI in serum was determined by two-dimensional immunoelectrophoresis using monospecific anti-apolipoprotein AI following the procedure outlined by Beisiegel and Utermann (1979) or by immunochemical analysis of apo AI in fractions from density gradient ultracentrifugation. Density fractions were dialysed against 0.15 *M* NaCl-0.01 *M* EDTA, pH 7.4, and apo AI concentrations were determined by electroimmunodiffusion.

The apoprotein composition of lipoprotein fractions was analyzed by electrofocusing and SDS-PAGE. Fractions from the density gradient ultracentrifugation were dialysed against 0.15 *M* NaCl, 0.01 *M* EDTA, pH 7.4 and extracted with acetone-ethanol 1:1 (v/v) at −20°C. For electrofocusing apolipoproteins were solubilized in 0.02 *M* ethylmorpholine-HCl buffer pH 8.6, 8 *M* urea. Electrofocusing was performed in 7.5% polyacrylamide slab gels containing ampholytes (Pharmacia, pH 4–6.5) and 6 *M* urea. Prior to SDS-PAGE apolipoproteins were solubilized in 0.02 *M* ethylmorpholine-HCl, pH 8.6 buffer, 2% SDS and heated in boiling water for 2 min. SDS-PAGE was performed in 11% slab gels following the procedure of Neville (1971). Two-dimensional gel electrophoresis of apolipoproteins was performed by a modification (Utermann et al. 1982b) of the O'Farrell (1975) procedure. Agarose gel electrophoresis of serum and VLDL was done according to the method of van Melsen et al. (1975). Triglycerides were determined enzymatically with a commercial test kit (Boehringer, Mannheim, Federal Republic of Germany).

Results

Frequency of Apo $AI_{Marburg}$

Apolipoprotein AI phenotypes were determined in 2282 unrelated Germans. Four individuals with a mutant of apo AI were detected (frequency about 1:570), three of which exhibited the same focusing phenotype characterized by an additional more acidic set of apo AI isoforms (Fig. 1). Identification of these

2 HDL-cholesterol values in the text will always refer to the value determined by the precipitation method, in order to facilitate comparison with the literature. HDL-cholesterol was considered descreased when it was at, or below, the 10th percentile of the LRC-study values for the same age and sex group (Heis et al. 1980)

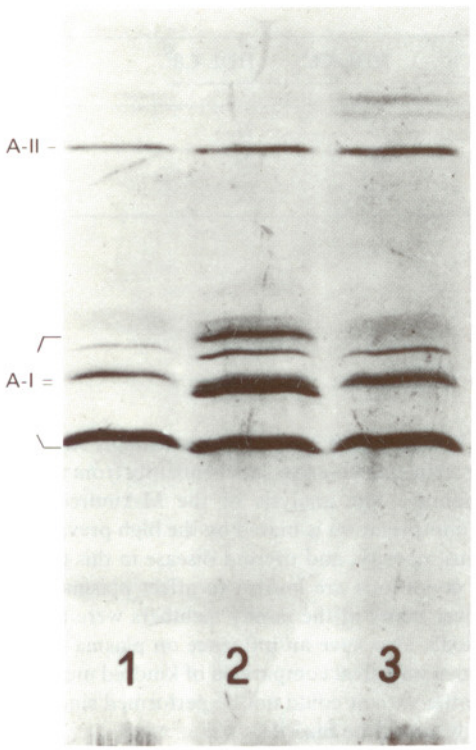

Fig. 1. Isoelectric focusing of individual apolipoproteins prepurified by agarose gel electrophoresis. 1, 3: controls; 2: apo $AI_{Marburg}$; Cathode is at the bottom

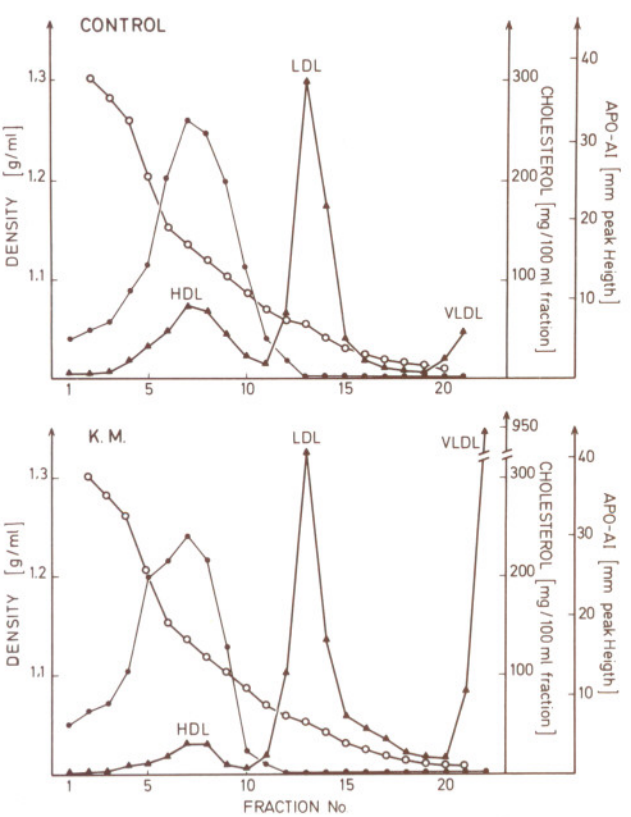

Fig. 2. Density gradient ultracentrifugation of sera from a control individual *(upper part)* and from proband K.M. with apo $AI_{Marburg}$ *(lower part)*. ○——○ density; ▲——▲ cholesterol; ●——● apo AI. For apo AI determination fractions were diluted 1:40 (control) or 1:20 (proband K.M.)

proteins as apo AI was done by immunofixation and crossed immunofocusing using anti apo AI and by two-dimensional electrophoresis (Utermann et al. 1982a). The population studied is not a representative sample of the German population but includes a high proportion of individuals with various disorders. Assuming that there is no association of the mutant with any of these disorders the frequency of apo $AI_{Marburg}$ in the German population is about 1/750.

Lipoproteins in Probands with Apo $AI_{Marburg}$

The qualitative lipoprotein profile as determined by density gradient ultracentrifugation was normal in probands with the mutant AI (Fig. 2). Notably, however, all three probands presented with a similar type of dyslipoproteinemia characterized by hypertriglyceridemia and subnormal HDL-cholesterol (Table 1). The distribution of apo AI in the proband's plasma was normal. As in controls, apo AI was found associated with the $alpha_1$-lipoprotein fraction upon electrophoresis (Fig. 3) and with high density lipoproteins upon ultracentrifugation (Fig. 2). SDS-PAGE of lipoprotein fractions demonstrated a normal apoprotein composition of all lipoprotein classes with apo AI occurring in the HDL (Fig. 4). Hence apo $AI_{Marburg}$ is associated with HDL of $alpha_1$-mobility and presumably has normal lipid-binding properties. The finding of elevated triglycerides and decreased HDL-cholesterol in all three probands prompted family studies that were performed to prove the hypothesis of an association of the mutant with dyslipoproteinemia and to establish the genetic nature of the AI-variant.

Genetics of Apo $AI_{Marburg}$

Apolipoprotein AI phenotypes were determined in family members of probands K.M. and K.So. by electrofocusing of apolipoproteins prepurified by agarose gel electrophoresis (method 1) and by ultracentrifugation (method 2). Both techniques yielded identical results. Thirteen blood relatives with apo $AI_{Marburg}$ were detected in three generations of the M-kindred and two individuals with the mutant phenotype were found in the So-kindred (Fig. 5). It is clear from inspection of the pedigrees, that apo $AI_{Marburg}$ is inherited as an autosomal codominant trait. All individuals with apo $AI_{Marburg}$ were heterozygotes, having normal apo AI in addition to the mutant protein. From the eight matings where one parent had the variant there were 10 offspring with a normal AI phenotype and 13 with the Marburg-variant. Assuming, that the deceased spouses from three of these matings (MI-6, I-11, SoI-1) were of normal AI phenotype and excluding the probands (MII-3, SoII-6), the segregation is close to 1:1 (10 normals versus 11 variants).

Apo $AI_{Marburg}$ and Lipid Levels

Analysis of lipid and lipoprotein levels in family members in relation to the apo AI abnormality did not reveal a consistent relationship between the AI variant and lipid parameters. There were several individuals with the AI variant that had normal HDL-cholesterol and no hypertriglyceridemia (MI-10, I-12, III-1, III-2, III-4, III-5, III-6, III-14). On the other hand there were blood relatives with the normal AI phenotype but with hypertriglyceridemia and low HDL-cholesterol (MI-4, I-5, II-7). The situation in the sibship of proband K.M. (MII-3) is especially informative. Here the two non-carriers of the AI^{Ma} -gene (MII-5, II-7) had a similar type of lipid abnormality to affected

Table 1. Lipid concentrations in probands with apo $AI_{Marburg}$

Proband	Age/sex (years)	TG	Ch	VLDL-Ch[a]	LDL-Ch[a]	HDL-Ch[a]	HDL-Ch[b]
		mg/dl serum					
K.M.	45/M	352	236	131	92	13	21
K.So.	38/M	267	244	48	164	31	28
B.B.	70/F	364	255	35[c]	196[c]	24[c]	22

[a] Determined by density gradient ultracentrifugation
[b] Determined after phosphotungstate precipitation
[c] Determined by quantitative electrophoresis (Neubeck et al. 1977)

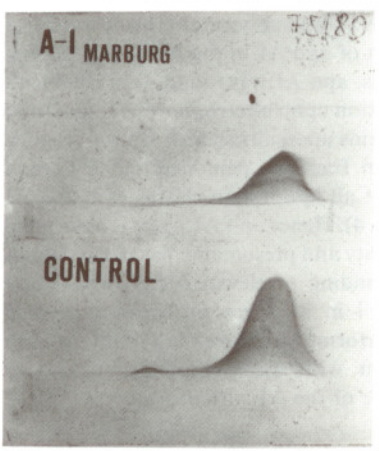

Fig. 3. Two-dimensional immunoelectrophoresis of sera from a proband with apo $AI_{Marburg}$ *(upper panel)* and a control *(lower panel)*. Five microliters serum from each individual were applied. The second dimension gel contains anti-apo AI. Anode for the first dimension is at the right and for the second at the top

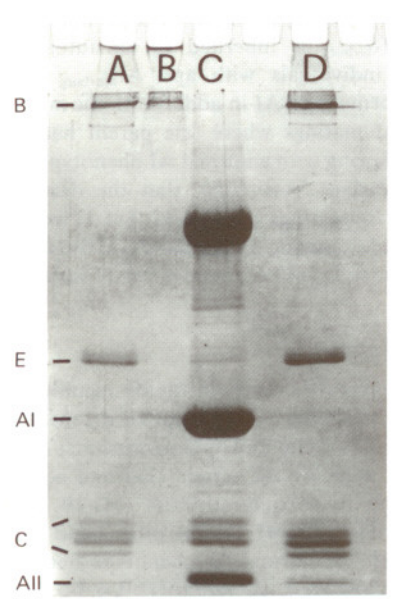

Fig. 4a–d. SDS-PAGE of lipoprotein fractions VLDL (**a**), LDL (**b**), HDL (**c**) from proband K.M. with apo $AI_{Marburg}$ and VLDL from a control (**d**)

sibs (MII-1, II-3, II-9; compare Table 2). This strongly suggests that the dyslipoproteinemia co-exists independently from the AI variant in this family. The analysis of the M-kindred was complicated and interpretation is biased by the high prevalence of diabetes mellitus, obesity, and thyroid disease in this family (Table 2). These conditions are known to affect plasma lipid levels and moreover many of the family members were taking drugs that reportedly also have an influence on plasma lipids. Therefore a rigorous statistical comparison of kindred members with and without the variant could not be performed since this analysis necessarily would be biased.

The high prevalence of diabetes mellitus in this family suggests that a strong genetic component operates in the kindred. All individuals with diabetes that were available for analysis of plasma lipids had hypertriglyceridemia or at least borderline triglyceride levels (MI-10, I-12). However, hypertriglyceridemia was also present in several non-diabetic blood relatives suggesting that there is an independent cause for hypertriglyceridemia and that hypertriglyceridemia is not secondary to diabetes in all family members.

Of particular interest were those blood relatives that were carriers of the AI^{Ma} gene but had neither diabetes mellitus nor hypertriglyceridemia. Seven such individuals were identified (MIII-1, III-2, III-4, III-5, III-6, III-14; SoII-5) and six of these had HDL-cholesterol in the normal range (39–53 mg/dl). However, all except the one with the subnormal HDL-cholesterol (SoII-5, age 40 years) were young (age range 13–23 years), and dyslipoproteinemia might not be expressed in young individuals with the mutant. Since even females with the AI variant and diabetes mellitus that ranged in age from 40–72 years had HDL-cholesterol in the normal range (43–52 mg/dl) this does not seem a likely explanation.

Apo E Phenotypes and Dysbetalipoproteinemia in the M-Kindred

An unexpected finding of this study was the detection of three individuals with the homozygous apo E-2/2 phenotype (MIII-11, III-12, III-13). We have shown in previous studies that this phenotype is associated with a specific form of dyslipoproteinemia (Utermann et al. 1977, 1979a, b). The three sibs with phenotype apo E-2/2 detected here showed the typical lipoprotein abnormality characterizing this phenotype. Plasma cholesterol concentrations were subnormal. Two of the sibs (MIII-11, III-12) clearly had hypobetalipoproteinemia (LDL- and IDL-cholesterol 27 mg/dl; see Table 2). There was no distinct LDL peak upon density gradient ultracentrifugation. Rather there was a continuous particle distribution from LDL

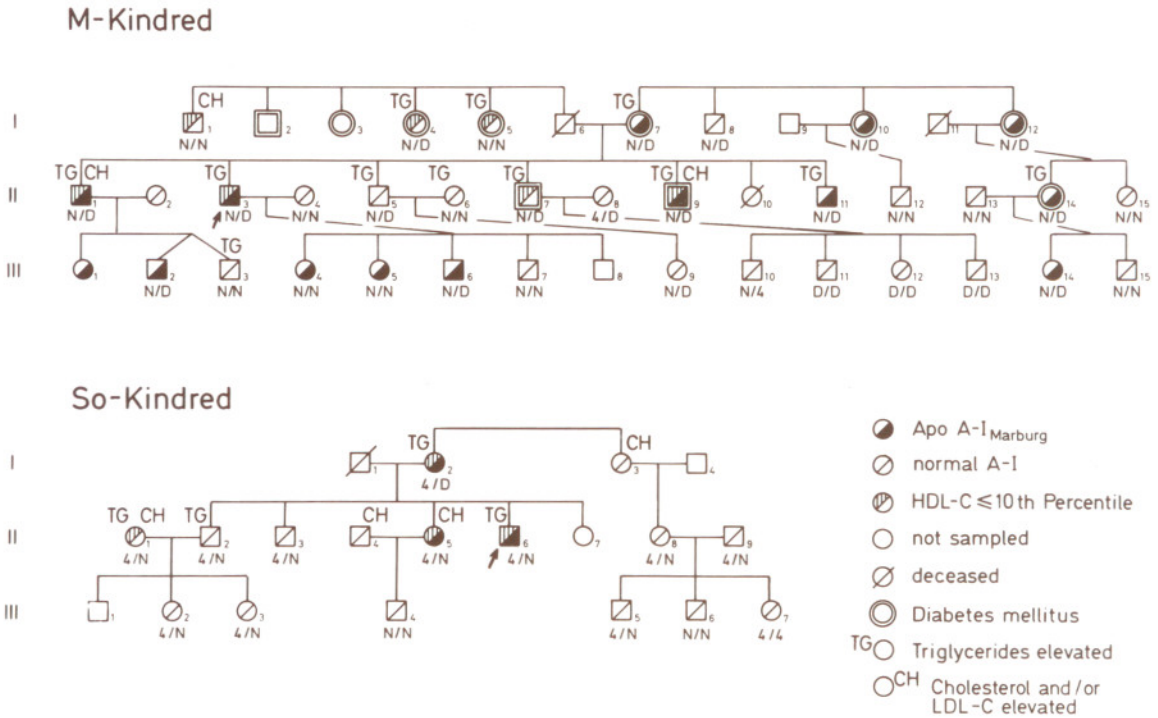

Fig. 5. Pedigrees of the M- and So-kindreds. *Arrows* denote the propositi. Apo E phenotypes (Utermann et al. 1982b) are indicated under the symbols. These correspond to the new nomenclature (Zannis et al., to be published) as follows: 4/4 = apo E-4/4; N/N = apo E-3/3; D/D = apo E-2/2; 4/N = apo E-4/3; 4/D = apo E-4/2; N/D = apo E-3/2

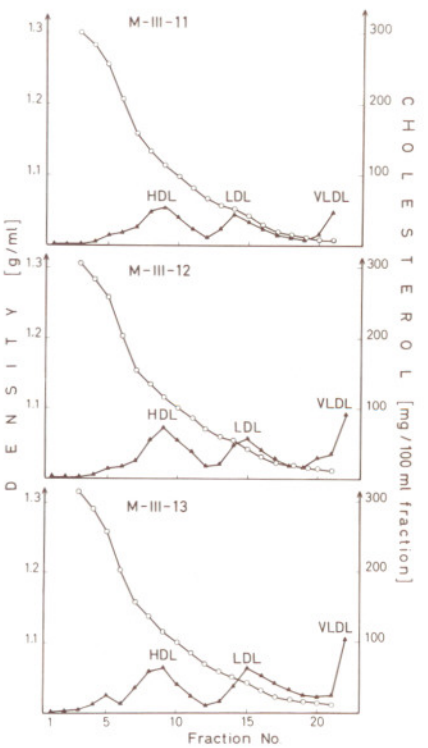

Fig. 6. Density gradient ultracentrifugation of the sera from the three sibs with apo E-2/2 phenotype from the M-kindred. ○——○ density; ▲——▲ cholesterol. Compare Fig. 2 for normal lipoprotein-cholesterol distribution in a control subject

towards, and into the IDL density range (Fig. 6). VLDL-cholesterol was not (MIII-11) or only moderately elevated (MIII-12, III-13), but the VLDL exhibited a beta-VLDL subfraction upon agarose gel electrophoresis (not shown), and hence was of abnormal composition. Notably the youngest sib (MIII-13) had the highest total cholesterol and LDL-cholesterol levels, a finding in agreement with previous observations on children and young adults with phenotype apo E-2/2 (Utermann et al. 1979a, b).

Independent Segregation of Apo AI and Apo E Phenotypes

There is one informative mating in the M-kindred that provides evidence for an independent segregation of the structural genes for apo AI and apo E. MII-3, the proband of the M-kindred is heterozygous at the AI locus (genotype AI^N/AI^{Ma}) and heterozygous at the apo E locus (genotype $\varepsilon^3/\varepsilon^2$), whereas his spouse (II-4) is homozygous at both loci (AI^N/AI^N; $\varepsilon^3/\varepsilon^3$). From the children of this couple two (III-4, III-5) have inherited the ε^3 allele and the AI^{Ma} gene from their double heterozygous father. One child (III-7) has inherited ε^3 allele but the normal AI^N gene, and one (III-6) has inherited the ε^2 allele and the mutant AI^{Ma} gene. This situation is incompatible with a close linkage of the two gene loci.

Discussion

Apo AI is a prominent protein constituent of human plasma HDL. Several genetic disorders affecting primarily the metabolism of HDL have been described over the past two decades.

Table 2. Laboratory and clinical data on members of the Mu- and So-kindred with apo $AI_{Marburg}$

Case no.	Age/sex (years)	Ch	TG	VLDL-Ch[a]	LDL-Ch[a]	HDL-Ch[a]	HDL-Ch[b]	Apo E type	Apo AI type	Clinical features	Medications
		mg/dl									
Mu-kindred											
I-1	64/M	245	99	28	170	47	31	3/3	N	Obesity	
I-2	M									Diabetes mellitus	Diet
I-3	F									Diabetes mellitus	Euglucon
I-4	66/F	198	260	37	132	30	34	3/2	N	Diabetes mellitus, obesity, retinopathia diabetica, stroke	Insulin
I-5	71/F	196	230	38	127	31	33	3/3	N	Diabetes mellitus, hypothyroidism, peripheral vascular disease	Diet L-Thyroxine
I-6	M										
I-7	71/F	224	200	42	136	46	44	3/2	Ma	Diabetes mellitus, obesity	Diet Lipostabil
I-8	75/M	171	126	9	108	54	52	3/2	N		
I-9	81/M										
I-10	72/F	138	164	23	76	39	44	3/2	Ma	Diabetes mellitus (hypothyroidism)	Euglucon L-Thyroxine
I-11	M										
I-12	69/F	162	183	7	119	36	52	3/2	Ma	Diabetes mellitus	Euglucon L-Thyroxine
II-1	47/M	266	200	47	188	31	32	3/2	Ma	Obesity	
II-2	44/F	165	85	3	101	61	53	—	N	Strumectomy	L-Thyroxine
II-3	45/M	236	352	131	92	13	21	3/2	Ma	Obesity, duodenitis, hepatomegaly, alcohol abuses, glaucoma, stenocardia	
II-4	46/F	213	82	12	112	89	67	3/3	N		
II-5	44/M	257	299	112	112	33	34	3/2	N	Obesity	
II-6	46/F	221	246	17	148	56	62	3/3	N	Obesity	
II-7	43/M	193	273	36	136	21	28	3/2	N	Diabetes mellitus, obesity	Euglucon
II-8	40/F	175	79	11	112	52	52	4/2	N		
II-9	41/M	246	213	41	177	28	26	3/2	Ma	Diabetes mellitus, obesity	Diet
II-10	F										
II-11	33/M	187	268	36	102	49	45	3/2	Ma		
II-12	40/M	145	56	2	85	58	79	3/3	N		
II-13	43/M	126	104	6	64	56	59	3/3	N		
II-14	40/F	214	502	62	118	34	43	3/2	Ma	Diabetes mellitus, obesity, varicosis, ulcus cruris	Diet
II-15	38/F	179	107	10	127	42	48	3/3	N	Strumectomy	L-Thyroxine
III-1	19/F	104	60	2	75	27	42	3/2	Ma	Obesity	
III-2	13/M	141	133	19	87	35	42	3/2	Ma		
III-3	13/M	174	200	37	88	49	40	3/3	N	Obesity	
III-4	23/F	185	116	10	123	52	53	3/3	Ma		
III-5	22/F	188	76	11	120	57	53	3/3	Ma	Struma, anemia	L-Thyroxine Fe
III-6	20/M	180	103	9	133	38	40	3/2	Ma		
III-7	16/M	167	78	22	88	57	45	3/3	N		

III-8	5/M									
III-9	17/F	130	118	13	73	44	48	3/2	N	
III-10	22/M	152	76	5	102	45	56	4/3	N	
III-11	20/M	82	121	13	27[c]	42	45	2/2	N	Dyslipoproteinemia
III-12	16/F	90	105	25	27[c]	38	47	2/2	N	Dyslipoproteinemia
III-13	11/M	142	108	30	56[c]	56	53	2/2	N	Dyslipoproteinemia
III-14	19/F	135	130	20	73	42	42	3/2	Ma	
III-15	15/M	95	89	8	47	40	47	3/3	N	Mental retardation
So-kindred										
I-2	67/F	228	205	21	154	52	38	4/2	Ma	Obesity
I-3	62/F	312	150	25	225	N.D.	62	—	N	
II-1	41/F	303	211	42	222	37	29	4/3	N	
II-2	45/M	220	260	39	139	41	36	4/3	N	
II-3	43/M	159	111	7	84	68	65	4/3	N	
II-4	39/M	306	138	—	—	—	—	—	N	
II-5	41/F	234	80	7	174	54	39	4/3	Ma	
II-6	38/M	244	267	48	165	31	28	4/3	Ma	
II-8	39/F	259	193	24	165	70	46	4/3	N	
II-9	45/M	189	176	25	97	69	58	4/3	N	Hypertension
III-2	19/F	192	93	11	127	55	39	4/3	N	
III-3	15/F	171	87	12	99	60	51	4/3	N	
III-4	20/M	176	86	12	91	73	65	3/3	N	
III-5	20/M	196	93	11	103	82	67	4/4	N	
III-6	18/M	197	100	10	112	75	60	3/3	N	
III-7	14/F	184	88	5	110	69	59	—	N	

[a] Determined after ultracentrifugation
[b] Determined after phosphotungstate precipitation
[c] Includes IDL, see Fig. 6

These genetic low-HDL-syndromes recently have gained considerable attention due to the postulated association between low HDL-cholesterol and risk for premature arteriosclerosis. Among these disorders are Tangier disease (Herbert et al. 1978), LCAT deficiency (Gjone et al. 1978), Fish eye disease (Carlson 1982), and apo AI_{Milano} (Franceschini et al. 1980). All of these were detected because of the lipoprotein abnormality and/or clinical features associated with the disorder. The families with a variant of apo AI reported here are the first example where the probands were detected by screening for apoprotein abnormalities. In such studies it is expected that mutants with and without metabolic and clinical consequences will be detected.

The present investigation provides clear evidence for the genetic character of the apo $AI_{Marburg}$ variant that is inherited as an autosomal codominant trait. Of major interest is the question on whether or not this mutant is associated with lipoprotein abnormalities. Notably all three probands with apo $AI_{Marburg}$ detected in this study clearly had hypertriglyceridemia and low HDL-cholesterol. However, this association was not corroborated by the family studies where no association of the mutant with dyslipoproteinemia could be demonstrated. The comparison of unadjusted lipid levels of family members, with and without the mutant, demonstrated a tendency towards higher triglyceride and lower HDL-cholesterol concentrations in the former (data not shown; compare Table 2). However these data could not be subjected to rigorous statistical analysis since there was a high incidence of diabetes mellitus and thyroid disease in one kindred, and since many family members were under therapy with drugs that effect lipid metabolism. The family data moreover provide evidence for a mechanism resulting in hyperlipidemia that is independent from both diabetes and the AI mutant. Individuals with hyperlipidemia were overrepresented in both kindreds but several of the hyperlipidemics had neither the mutant nor a disease associated with secondary hyperlipidemia. There may be several reasons for the failure to demonstrate an association between dyslipoproteinemia and the $AI_{Marburg}$ variant in family members. First, the presence of hypertriglyceridemia and low HDL-cholesterol in the three probands may be due to the selection procedure. Two of the probands were identified in groups with a high incidence of this form of dyslipoproteinemia (hyperlipidemics, patients with coronary heart disease). The finding of the lipoprotein abnormality in a blood donor with the mutant might be due to chance. An alternative hypothesis would be that there is a mild form of, or a tendency towards dyslipoproteinemia in individuals with the mutant, but that this is masked in these kindreds by the coexistence of other forms of hyperlipidemia. The data do not exclude a moderate effect of the mutant AI^{Ma} gene on plasma HDL-cholesterol and/or triglycerides. However this effect, if present, cannot be assessed from the analysis of the kindreds investigated here. Certainly data on more probands and families with the $AI_{Marburg}$ mutant are needed to provide a definitive answer.

The apo $AI_{Marburg}$ variant apparently is different from the recently described apo AI_{Milano}, not only by its isoprotein pattern, but also in it's consequences for HDL-metabolism. All five individuals with apo AI_{Milano} that were identified in an Italian family had markedly decreased HDL-cholesterol, ranging from 6.3–17.2 mg/dl and exhibited significant hypertriglyceridemia (Franceschini et al. 1981). This is in apparent contrast to the findings in apo $AI_{Marburg}$ where HDL-cholesterol ranges from 21–53 mg/dl and where several carriers of the mutant allele had no hypertriglyceridemia.

The systematic screening for apolipoprotein A variants only recently became possible (Utermann et al. 1982a). A further mutant of apo AI has already been identified in our laboratory (apo $AI_{Giessen}$, Utermann et al. 1982a) and several are expected to be found in the future. The analysis of these mutants, hopefully, will result in a better understanding of HDL metabolism and of structure-function relationships in these apoproteins.

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