ApoA-I$_{MALLORCA}$ impairs LCAT activation and induces dominant familial hypoalphalipoproteinemia

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Abstract **Apolipoprotein (apo)A-I is the major protein component of HDL and the cofactor for LCAT. We describe a large Spanish kindred, living in the Mediterranean Island of Mallorca, that presents a dominant form of hypoalphalipoproteinemia. The lipid profile of this family was studied because the proband, a 40-year-old male presenting signs of coronary atherosclerosis, showed severe HDL deficiency. However, none of the other family members had a known history of cardiovascular disease. Sequence analysis of the *apoA-I* gene in affected members identified a 33-base pair deletion, corresponding to residues 165–175 of the mature protein, eliminating the first 11 amino acids of the internal repeat 7. ApoA-I$_{MALLORCA}$ is associated with HDL-cholesterol deficiency (concentration ranging from 8–48% of the value in non-carriers), and a 2- to 3-fold decrease in plasma concentrations of apoA-I and apoA-II and endogenous LCAT activity, concomitant with a slight decrease in serum cholesterol efflux capability. Impairment of LCAT activity in HDL particles containing only mutated forms of apoA-I would not explain a pattern of dominant inheritance. HDL particles containing wild type apoA-I and at least one mutant apoA-I may also present impaired LCAT activity and/or other alterations leading to defective HDL maturation, a situation that would increase HDL lipid catabolism. We conclude that amino acids 165–175 of apoA-I are critical for normal HDL metabolism, at least in part because of their role in LCAT activation. However, apoA-I$_{MALLORCA}$ is not necessarily associated with clinical signs of atherosclerosis.**—Martín-Campos, J. M., J. Julve, J. C. Escolà, J.Ordóñez-Llanos, J. Gómez, J. Binimelis, F. González-Sastre, and F. Blanco-Vaca. **ApoA-I$_{MALLORCA}$ impairs LCAT activation and induces dominant familial hypoalphalipoproteinemia.** ***J. Lipid Res.*** **2002.** 43: **115–123.**

Supplementary key words apolipoprotein • atherosclerosis • coronary heart disease • high density lipoprotein • molecular diagnosis

Many epidemiological and clinical studies have revealed an inverse correlation between plasma levels of HDL cholesterol and the risk of coronary heart disease (CHD) (1, 2). The protective effect of HDL against atherosclerosis is thought to be mediated, at least in part, by its involvement in reverse cholesterol transport from peripheral cells to the liver (3). Apolipoprotein (apo)A-I is the major HDL apolipoprotein. It acts as a cofactor for LCAT (4), which is responsible for the formation of cholesteryl esters in plasma. This activity promotes cellular cholesterol efflux by maintaining an exit gradient that, at the same time, provides substrate for the LCAT reaction (5). In addition, apoA-I is an important ligand for the binding of HDL to cell receptors such as scavenger receptor class B type I (SR-BI) and cholesterol efflux regulatory protein (CERP) (6, 7). These characteristics contribute to the ability of HDL to induce reverse cholesterol transport and, thus, potentially to the protective effect of HDL against atherothrombotic cardiovascular disease.

Levels of plasma HDL cholesterol are partially (around 50%) determined by genetic factors. Approximately 39% of patients with premature CHD present a phenotype of familial hyperlipidemia that include low HDL cholesterol and, of those, around 16% present hypoalphalipoproteinemia with no alteration in apoB-containing lipoproteins (8). Different mutations in the gene encoding apoA-I and LCAT have been identified as the cause of familial hypoalphalipoproteinemia (9, 10). Recently, an autosomal codominant form of hypoalphalipoproteinemia, known as Tangier disease, was found to be caused by mutations in the gene encoding ATP cassette-binding transporter 1 (ABC1) (11) coding for CERP. However, other as yet unknown genes seem to account for an important part of the genetically determined variability of HDL-cholesterol plasma concentrations (12). Recently, the glucocerebrosidase gene (the mutations of which are the cause of Gaucher

Abbreviations: apo, apolipoprotein; CERP, cholesterol efflux regulatory protein; CHD, coronary heart disease; LPA-I, lipoproteins containing apoA-I but not apoA-II; LPA-I:A-II, lipoproteins containing apoA-I and apoA-II; MER, molar esterification rate; SR-BI, scavenger receptor class B type I.

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disease) was found to be an important genetic determinant of HDL cholesterol levels (13).

ApoA-I is a single polypeptide chain with 243 amino acid residues and two regions: a globular amino-terminal domain (residues 1–43) and a lipid-binding carboxyl-terminal domain (residues 44–243) (14). Analysis of the secondary structure of the latter permitted identification of eight 22 mer and two 11 mer tandem amino acid sequence repeats that have been associated with amphipathic α-helices. These are, in most cases, punctuated by prolines that induce turns. The 22 mer repeats could form tandem antiparallel helices that are perpendicular to the plane of the phospholipid bilayer (the picket-fence model), at least in the discoidal HDL particles (14, 15). These amphipathic α-helices act as protein detergents that stabilize the structure of lipoprotein particles (15). Computer analysis indicated that significant differences in hydrophobic and amphipathic character does exist between different repeats, suggesting that they could have different lipid-binding properties. Strong lipid-associating properties have been demonstrated to be located in repeat 1 (residues 44–65) and repeat 10 (residues 220–241) (16). Repeat 4 (residues 99–120), in the central region of apoA-I, is very important for stabilization of the lipid-apolipoprotein complex, while repeats 5–7 (residues 121–186) contribute to the initial rates of lipid-apolipoprotein association (17). Deletion of repeat 6 (residues 143–164) or repeat 7 (residues 165–186) resulted in a 98–99% reduction in LCAT activation compared with wild-type apoA-I (18), thus demonstrating the important role of these domains in LCAT action.

Several naturally occurring apoA-I variants associated with low levels of HDL cholesterol have been identified (9); thus, they are very likely to present impaired functional properties. In general, the affected phenotype has a codominant inheritance pattern. Hence, only the family members that are homozygous for the mutation present very low concentrations of HDL cholesterol. However, cases also exist of dominantly inherited familial hypoalphalipoproteinemia due to mutations in the apoA-I gene (19–24), all located in repeats 6 and 7 (residues 143–186). In the present study, we describe a novel deletion mutation in the human apoA-I gene that induces dominantly inherited hypoalphalipoproteinemia characterized by HDL-cholesterol deficiency concomitant with a milder decrease in plasma apoA-I and apoA-II concentrations and LCAT activity.

MATERIALS AND METHODS

Subjects

The study group consisted of a family with severe HDL-cholesterol deficiency. The proband (subject III-12 in the pedigree, **Fig. 1**), a Caucasian male born in 1957 on the Mediterranean island of Mallorca, Spain, was referred to a local hospital in 1996 because of progressively worsening effort-dependent angina. At that time, an abnormally low level of HDL cholesterol in plasma (0.10 mmol/l) was detected. He had been a smoker and presented severe lesions in coronary arteries: 90% narrowing in the proximal portion of the left anterior descending artery, 80% narrowing in the mid portion of the circumflex and complete occlusion in the mid right artery, with depressed ventricular function

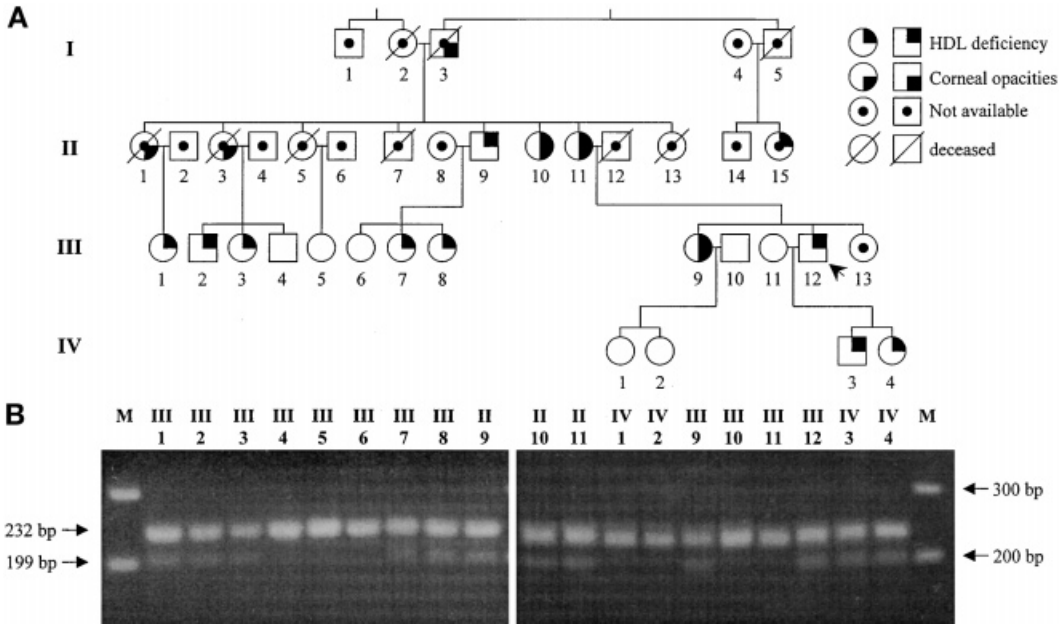

Fig. 1. A: Pedigree of the studied family constructed according to clinical, biochemical and genetic data. An arrow indicates the proband. HDL-cholesterol, with no other biochemical or genetic data, was available from three individuals: I-1, II-14, and II-15. These HDL-cholesterol concentrations were 0.89, 1.42, and 0.12 mmol/l, respectively. On this basis, and even though it is very likely that II-15 is a carrier and I-1 and II-14 non-carriers, the three were considered as not available because of incomplete information. B: Agarose (3%) gel electrophoresis of the PCR product of part of exon 4 of the apoA-I gene.

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(44% ejection fraction). In January 1997 he underwent a successful three-vessel coronary bypass surgery. He agreed to participate in a study designed to investigate the origin of the hypoalphalipoproteinemia, together with his son and daughter, mother and aunt, sister and two nieces, wife and brother-in-law. The study was later extended to other members of the family (an uncle and eight cousins), reaching a total of 19 subjects belonging to three generations. All subjects gave informed consent to the study protocol, which was approved by the ethics committee of the hospital.

Lipid assays

Blood was collected from the proband and family members after an overnight fast and shipped to Barcelona at 4°C. Plasma lipid, apo, and lipoprotein concentrations were determined by standard commercially-available kits adapted to a BM/HITACHI 911 autoanalyzer (Roche Diagnostics GmbH, Mannheim, Germany), with the exception of plasma apoA-II concentration, which was determined using a commercial radial diffusion immunoassay (Immuno AG, Vienna, Austria). Quantification of lipoproteins containing apoA-I but not apoA-II (LPA-I particles) was performed by electroimmunodiffusion using the HIDRAGEL LPA-I kit (Sebia, Issy-les-Moulineaux, France). Lipoprotein particles containing apoA-I and apoA-II (LPAI:A-II) were calculated as the difference between the concentrations of apoA-I and LPA-I (25).

DNA isolation, amplification, and sequence analysis of the *apoA-I* gene

Genomic DNA was extracted from 200 μl blood samples with a QIAamp DNA Blood Mini kit (QUIAGEN GmbH, Hilden, Germany). Six pairs of primers were used to amplify the four exons of the human *apoA-I* gene, as previously described (26). Conditions for PCR amplification were as follows: 100–200 ng genomic DNA, 1× PCR buffer (Promega, Madison, WI), 1.5 mmol/l $MgCl_2$ (except 1 mmol/l for exon 1), 200 μmol/l deoxynucleotide triphosphate (dNTP), 0.4 μmol/l of each primer and 2.5 units/50 μl of Taq DNA polymerase (Promega). After incubation at 95°C for 5 min, the mixture was subjected to 35 amplification cycles of 30 s at 94°C, 30 s at 55°C to 65°C, depending on the primer pair used, and 1 min at 72°C, ending with a final extension for 7 min at 72°C. After verifying amplification by 1% agarose gel electrophoresis, aliquots of the PCR reactions were purified from remains of primers and nucleotides (dNTPs) following a method previously described (27) and subjected to automatic cycle sequencing of both strands (ABI PRISM Model 377 DNA Sequencer, Perkin Elmer, Branchburg, NJ).

Enzyme activities and cholesterol efflux

Plasma LCAT activity was determined using reassembled HDL containing 1-palmitoyl-2-oleyl-sn-3-phosphocholine, [^{3}H]-cholesterol and apoA-I (28). Endogenous LCAT activity was measured using the lipoproteins of whole and apoB-depleted plasma as substrate (29) and expressed as molar esterification rates (MER). Cholesteryl ester transfer protein activity was measured as the transfer of labeled cholesteryl oleate from HDL to LDL, using lipoproteins isolated from pools of human normolipidemic individuals and plasma of the subjects as the source of CETP (30). Paraoxonase/arylesterase activity was assayed using 1.0 mM phenylacetate as substrate (31) in plasma obtained from blood collected in lithium-heparin tubes. The capacity of serum to promote cholesterol efflux from Fu5AH rat hepatoma cells in culture was measured as previously described (32).

Electrophoresis and immunoblot analyses

Sudan Black prestained plasma samples were subjected to electrophoresis on 4–20% non-denaturing polyacrylamide gradient gels (BioRad, Hercules, CA). The electrophoresis was performed at 125 V for 20 h, after which the gel was first scanned and then subjected to immunoblot analysis. The separated proteins were electrophoretically transferred from the polyacrylamide gel to nitrocellulose membranes (Amersham Pharmacia Biotech, Buckinghamshire, UK) as previously described (33). The membrane was then incubated with rabbit antiserum to human apoA-I (Roche Diagnostics GmbH) and afterwards with sheep anti-rabbit IgG conjugated to horseradish peroxidase (Pierce, Rockford, IL). Human apoA-I was detected using chemiluminescence (Pierce).

Statistical analysis

All values are expressed as mean ± SEM. Comparison of data from the two groups was performed by Student's *t*-test or Mann-Whitney U test, depending on whether the distribution of data was Gaussian or not. A value of $P < 0.05$ was considered statistically significant.

RESULTS

Biochemical study of plasma from the proband and his relatives showed that twelve members of the family (available for the complete study) had HDL-cholesterol deficiency, with a plasma concentration ranging from 0.10–0.61 mmol/l **(Table 1)**. These subjects had HDL-cholesterol concentrations on average 24% of those of non-carriers and also presented a significant increase in the percentage of free cholesterol, together with an almost ($P = 0.06$) significant rise in plasma triglycerides. The pedigree of the affected family is shown in Fig. 1. In this Figure, the dominant inheritance of the HDL-cholesterol deficiency and, also, its segregation with a double DNA band obtained from the product of a PCR of part of exon 4 of the apoA-I gene, can be observed. This double band was not detected during verification of PCR amplification in routine 1% agarose gel electrophoresis. It was only observed when analyzed by 3% agarose gel electrophoresis.

Sequencing of this part of exon 4 of the proband showed a mixture of two sequences (**Fig. 2**), beginning in nucleotide 565 (of the coding region) using the forward primer and from nucleotide 592 using the reverse primer. Since the sequences immediately prior to the anomalous region are the same in both strands (TGGCC), it is concluded that the mutation consists of a deletion of 33 base pairs (bp) (from 565–597), since one of these sequences was found deleted. No other mutation was found in the rest of the sequence of the apoA-I gene (exons and exon-intron boundaries) analyzed. In consequence, and following the nomenclature proposed by the Nomenclature Working Group (34), the proband was heterozygous for the in-frame mutation P165-A175del referred to mature apoA-I. The alteration was called apoA-$I_{MALLORCA}$ after the island on which it was found. The lower band (199 bp, see Fig. 1B) was excised from 3% agarose gel and sequenced to verify the presence and nature of the deletion, which were confirmed (Fig. 2).

In order to ascertain the functional effect of the deletion found in apoA-I, several types of studies were performed. Analysis of HDL isolated by ultracentrifugation

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TABLE 1. Plasma lipid profiles of carrier and non-carrier family members

Subjects	Gender	Age	Cholesterol				TG
			Total	HDL	LDL	Free	
			mmol/l	*mmol/l*	*mmol/l*	*%*	*mmol/l*
Carriers							
II-9	M	77	3.41	0.56	ND	35.19	1.08
II-10	F	66	5.95	0.17	4.52	32.44	2.74
II-11	F	72	4.06	0.61	3.00	32.27	0.99
III-1	F	65	4.76	0.33	ND	40.97	2.16
III-2	M	67	3.71	0.39	ND	28.84	1.67
III-3	F	66	2.95	0.46	ND	46.78	1.51
III-7	F	36	2.90	0.35	ND	41.38	1.02
III-8	F	34	2.74	0.28	ND	39.42	1.10
III-9	F	38	2.58	0.11	1.90	33.72	1.24
III-12 (i)	M	41	4.13	0.10	3.54	30.02	1.08
IV-3	M	18	2.48	0.15	1.87	33.47	1.01
IV-4	F	11	5.01	0.13	4.31	27.74	1.24
Mean ± SE		49.3 ± 6.4	3.72 ± 0.32	0.30 ± 0.05	3.19 ± 0.47	35.19 ± 1.67	1.40 ± 0.16
Non-carriers							
III-4	M	55	7.20	1.17	ND	24.31	1.88
III-5	F	57	6.36	1.22	ND	30.19	0.90
III-6	F	39	4.62	2.02	ND	29.44	0.75
III-10	M	39	5.65	1.06	4.14	26.90	0.98
III-11	F	39	3.42	1.11	1.99	28.65	0.70
IV-1	F	19	2.85	1.04	1.52	25.61	0.64
IV-2	F	14	3.08	1.30	1.57	26.95	0.46
Mean ± SE		37.4 ± 6.2	4.74 ± 0.65	1.27 ± 0.13	2.31 ± 0.62	27.44 ± 0.80	0.90 ± 0.18
Carriers versus non-carriers			ns ($P = 0.13$)	$P < 0.001$	ns ($P = 0.28$)	$P < 0.01$	ns ($P = 0.06$)

(i) Indicates the proband; F, female; M, male; ND, not determined.

demonstrated that affected family members exhibited a 3.5-fold decrease in mass and a marked alteration in their chemical composition (**Fig. 3**). Carriers of apoA-I$_{MALLORCA}$ presented a decreased percentage of proteins and cholesteryl esters and an increased proportion of triglycerides and phospholipids.

Plasma apoA-I and apoA-II concentrations were approximately 49% and 41%, respectively, in carriers of the mu-

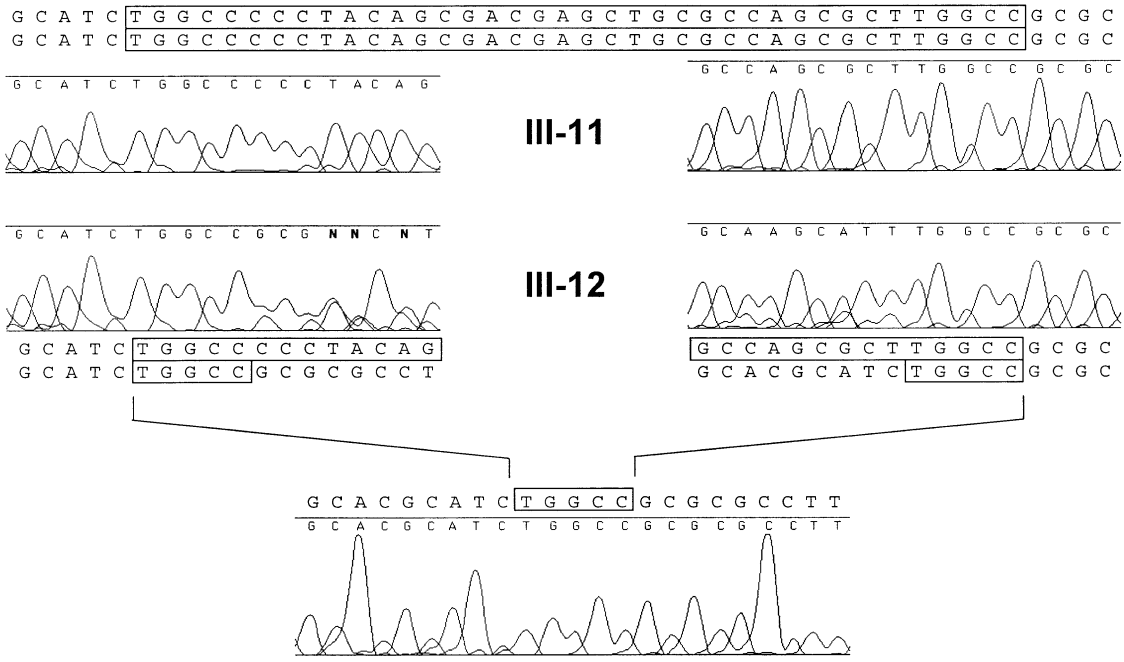

Fig. 2. Results from sequencing a fragment of exon 4 of the apoA-I gene amplified from the DNA of a control member of the family (III-11) and the proband (III-12), using a forward primer (left) and a reverse primer (right). Upper part: a fragment of the sequence of exon 4 of the wild type apoA-I (III-11) and proband apoA-I (III-12). Lower part: a fragment of the sequence of the purified 199 bp band, shown in Figure 1B, obtained from the proband.

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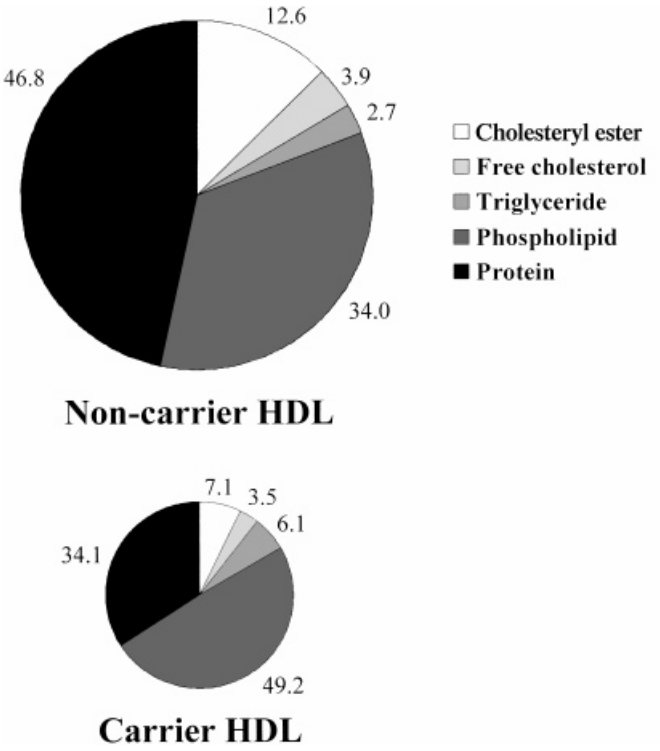

Fig. 3. Mass (mg/l) and composition (percentage) of plasma HDL, isolated by sequential ultracentrifugation, from apoA-$I_{MALLORCA}$ carriers and non-carriers. Surface is proportional to HDL mass (which was 599 mg/l and 2112 mg/l, respectively, for carriers and non-carriers).

tated apoA-I compared with non-affected family members (**Table 2**). Concentrations of LPA-I and LPA-I:A-II particles were around 44% and 51% in affected members compared with non-affected members, respectively, which would be consistent with a redistribution of apoA-I from LPA-I to LPA-I:A-II particles.

LCAT activity toward endogenous substrates (using either total plasma or apoB-depleted plasma) was significantly diminished in affected members compared with non-affected members, whereas no change was found in LCAT activity toward an exogenous substrate between the two groups (**Table 3**). Plasma cholesterol MER in affected individuals was less diminished (44% of those of non-affected individuals) than the MER of apoB-depleted plasma (28% of non-affected individuals). Serum cholesterol efflux was significantly diminished in carriers of apoA-$I_{MALLORCA}$, being 73% of that of non-affected members (Table 3). CETP activity and paraoxonase-arylesterase activity, both measured with an exogenous artificial substrate, were not significantly different between carriers and non-carriers (76.3 ± 5.75 vs. 115 ± 16.51 mmol/l/h, $P = 0.07$ and 147.9 ± 8.56 vs. 136.3 ± 12.51 mmol/l/h, $P = 0.46$, respectively), although there was a trend toward lower CETP in carriers.

Using gradient gel electrophoresis probed with antibodies to apoA-I, HDL from non-affected individuals (III-10 and III-11) migrated as a highly heterogeneous population of particles that were strongly stained and ranged from >7.1 nm to <10.4 nm (**Fig. 4**). In contrast, HDL par-

TABLE 2. Plasma apolipoprotein and specific HDL subpopulation concentrations in the family studied

Subjects	ApoA-I	ApoA-II	LPA-I	LPA-I:A-II	ApoB	Lp[a]
Carriers						
II-9	90	14.2	28.4	61.6	ND	12.7
II-10	57	10.9	20.3	36.7	147.8	9.6
II-11	102	20.9	35.2	66.8	92.5	<8.0
III-1	66	10.9	21.3	44.7	ND	<8.0
III-2	75	27.5	17.6	57.4	ND	<8.0
III-3	89	22.5	29.5	59.5	ND	<8.0
III-7	67	12.6	14.6	52.4	ND	9.8
III-8	63	12.6	15.5	47.5	ND	<8.0
III-9	45	4.3	9.8	35.2	81.3	<8.0
III-12 (i)	35	10.9	9.8	25.2	110.4	<8.0
IV-3	47	7.3	19.4	27.6	56.7	36.7
IV-4	41	6.0	4.9	36.1	116.3	<8.0
Mean ± SE	64.8 ± 6.1	13.4 ± 2.0	18.9 ± 2.6	45.9 ± 4.0	100.8 ± 12.8	7.6 ± 2.9
Non-carriers						
III-4	141	44.0	40.2	100.8	ND	17.1
III-5	131	35.8	47.5	83.5	ND	91.6
III-6	171	35.8	65.7	105.3	ND	17.8
III-10	125	29.2	41.5	83.5	117.8	35.6
III-11	119	27.5	41.0	78.0	62.9	44.4
IV-1	115	27.5	37.7	77.3	49.1	14.7
IV-2	123	29.2	26.1	96.9	52.9	11.0
Mean ± SE	132.1 ± 7.2	32.7 ± 2.3	42.8 ± 4.5	89.3 ± 4.3	70.7 ± 16.0	33.2 ± 10.8
Carriers versus non-carriers	$P < 0.01$	$P < 0.01$	$P < 0.01$	$P < 0.01$	ns ($P = 0.18$)	$P < 0.001$

All values are in mg/dl. (i) Indicates the proband; ND, not determined.

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TABLE 3. LCAT activity towards exogenous and endogenous substrates, and measurement of cholesterol efflux

Subjects	LCAT			
	Endogenous Substrate		Exogenous Substrate	Cholesterol Efflux
	MER-Plasma	MER-HDL		
		μmol/l/h		
Carriers				
II-9	69.16	12.75	85.10	0.33
II-10	45.30	3.95	59.15	0.20
II-11	65.51	23.20	64.95	0.40
III-1	50.70	4.80	91.05	0.26
III-2	55.70	6.36	68.00	0.27
III-3	93.85	2.70	91.05	0.26
III-7	60.00	11.16	47.30	0.22
III-8	60.50	7.66	83.05	0.23
III-9	38.30	4.35	92.00	0.22
III-12 (i)	34.70	2.45	78.30	0.18
IV-3	51.45	2.85	76.05	0.19
IV-4	65.06	3.45	79.50	0.14
Mean ± SE	57.52 ± 4.54	7.14 ± 1.75	76.29 ± 4.04	0.24 ± 0.02
Non-carriers				
III-4	213.55	23.80	123.90	0.32
III-5	180.50	24.85	70.50	0.32
III-6	104.05	34.20	97.70	0.39
III-10	126.20	25.65	46.00	0.34
III-11	129.40	24.70	79.00	0.33
IV-1	77.95	22.70	95.40	0.29
IV-2	73.60	25.95	103.10	0.30
Mean ± SE	129.32 ± 19.58	25.98 ± 1.43	87.94 ± 9.53	0.33 ± 0.01
Carriers versus non-carriers	$P < 0.001$	$P < 0.001$	ns ($P = 0.21$)	$P < 0.01$

(i) indicates the proband; MER, molar esterification rate of cholesterol.

ticles of apoA-$I_{MALLORCA}$ carriers showed different size patterns. Only two types of HDL particles were detected in the proband (III-12). Another carrier (II-10) presented a similar band pattern to that of the proband. A predominance of larger HDL particles (II-9, III-2, and III-3) and a more heterogeneous size particle profile (III-9) are also represented (Fig. 4).

Figure 5 shows a hypothetical representation of the secondary structure of apoA-$I_{MALLORCA}$, which can be compared with the proposed structure of the wild type apoA-I.

DISCUSSION

Molecular diagnosis

A 40-year-old male patient was studied at a local hospital because of CHD. Plasma analyses revealed severe HDL cholesterol, apoA-I, and apoA-II deficiency in plasma (less than 8%, 30%, and 33% of normal values, respectively), with no other major alteration in the lipid profile. Family study demonstrated that the proband, like other members of the family, had an inherited dominant form of hypoalphalipoproteinemia. The dominant effect of the mutation was established on the basis of a reduction of more than 50% in the HDL mass of carriers compared with non-carriers (Fig. 3). Percentage of plasma free cholesterol was moderately increased in affected members of the family; however, inheritance of LCAT deficiency is recessive rather than dominant (35, 36). Therefore, we looked at the apoA-I gene in which a minority of mutations have been found to produce a dominant HDL deficiency. These mutations are located in repeats 6 or 7 (amino acids 143–186) and are known as apoA-I_{MILANO}, apoA-$I_{SEATTLE}$, apoA-I_{PARIS}, apoA-I_{OSLO}, apoA-$I_{ZAVALLA}$, apoA-$I_{FINLAND}$, and apoA-$I_{ZARAGOZA}$ (19–24, 37, 38). Concomitantly, and because apoA-I deficiency was less dramatic than that of HDL cholesterol, the possibility of an SR-BI mutation was considered and ruled out (data not shown). DNA analysis showed the proband and the other affected members to be heterozygous carriers of an apoA-I mutation consisting of an in-frame deletion of 33 base pairs, which encode from amino acids 165–186. Thus, the patient and at least 11 other members of the family appeared to have two classes of apoA-I species, the normal species and a mutant species, which we named apoA-$I_{MALLORCA}$. The latter lacks the first 11 amino acids (P165-A175del) of one (repeat 7) of the 22-amino-acid amphipathic alpha helical regions. The presence of a proline residue at the beginning of most of the apolipoprotein repeats is essential since it induces the formation of kinks that permit the antiparallel position of the α-helices. ApoA-$I_{MALLORCA}$ lacks a proline residue between repeats 6 and 7, thus breaking the plasticity of the central domain of the apoA-I and, presumably, rendering an α-helix formed by 29 residues (Fig. 5). This region (repeat 7) has previously been demonstrated to be vital for LCAT activation (18).

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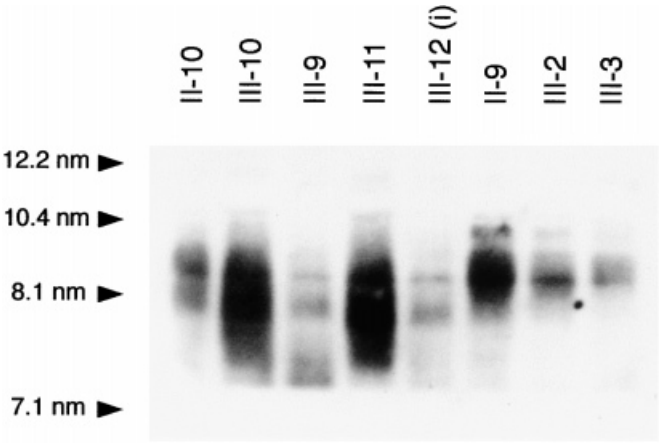

Fig. 4. Immunoblot probed with antibodies to apoA-I of a non-denaturing gradient gel electrophoresis of 2 μl of plasma taken from carriers and non-carriers (III-10 and III-11) and carriers of the apoA-$I_{MALLORCA}$ mutation [rest of lanes: II-10, III-9, III-12(i) which is the proband, II-9, III-2 and III-3] (see Materials and Methods for details). Stoke diameters of standards are indicated (in nm) on the left.

Effects of apoA-$I_{MALLORCA}$ in HDL metabolism

All carriers of apoA-$I_{MALLORCA}$ presented low but variable HDL cholesterol (ranging from 8–48% of control) and apoA-I (ranging from 31–71% of normal value), as well as other HDL parameters, reflecting the influence of other environmental and genetic factors in addition to the presence of apoA-$I_{MALLORCA}$. HDL isolated from affected members including the proband was (expressed in percentages) rich in triglycerides and phospholipids and poor in cholesteryl esters and proteins compared with non-carriers. The decrease in the HDL proportion of esterified cholesterol probably resulted from decreased endogenous LCAT activity in HDL. However, as LCAT assays (using exogenous substrates) of apoA-$I_{MALLORCA}$ carriers did not differ from those of non-affected controls, it is likely that reduced LCAT activity was due to impaired LCAT activation rather than a defect in enzyme structure or mass. The fact that the preβ-HDL/α-HDL ratio was not increased (data not shown) with the presence of a functional LCAT deficiency could be due to the well-known increased catabolism of small lipid-poor preβ-HDL particles (39). Also, hepatic lipase inhibition by increased preβ-HDL could limit a further increase in preβ-HDL and could be implicated in the increased percentage of HDL triglyceride seen in the carriers (40).

Since many HDL particles contain more than one apoA-I molecule, the mutant apoA-I may exert a dominant effect by accelerating catabolism of the lipid component of HDL and/or that of the whole HDL particle. Consistent with this possibility, this increase in catabolism of HDL apolipoproteins has been described previously in several apoA-I mutations that induce dominant hypoalphalipoproteinemia (38, 41, 42). Therefore, apoA-$I_{MALLORCA}$ may induce increased HDL catabolism that would explain its dominant inheritance. Consistent with this assumption, we have not identified apoA-$I_{MALLORCA}$ in the plasma of carriers using immunoblots of either SDS-PAGE or isoelectrofocusing. However, further studies are needed to improve our understanding of the mechanisms involved in this HDL deficiency. We speculate that the new helix formed by the deletion, even though it presents the same charge distribution characteristic of the central α-helices (data not shown), induces a change in HDL particles containing at least one apoA-$I_{MALLORCA}$ molecule which impairs LCAT catalysis and accelerates HDL (especially lipid) catabolism.

Susceptibility to atherothrombotic cardiovascular disease

Despite low HDL-cholesterol levels, most individuals with apoA-I variants (19–24, 38) do not have increased susceptibility to overt clinical manifestations of atherothrombosis. Consistently, there was no clinical evidence of CHD in the members of the family studied, with the exception of the proband. Study of the risk of atherosclerosis in subjects with severe inherited hypoalphalipoproteinemia has been the subject of intense research, and in most cases the increase in risk was much lower than expected (19, 24, 35, 38). This could be due, at least in part, to the fact that the decreased level of HDL is usually associated with low LDL cholesterol (43) and that antioxidant activities of enzymes such as paraoxonase seem to be largely conserved (44). The development of apoA-I-deficient mice has enabled detailed study of this animal model as well as the study of the effects of other genetically induced forms of HDL deficiency (45). In accordance with the results of human studies, and despite the HDL deficiency, mice lacking apoA-I were not prone to diet-induced atherosclerosis (46). However, the susceptibility to atherosclerosis in apoA-I knockout mice crossbred with apoB transgenic mice increased compared with that of apoB transgenic mice (47, 48). Taken together, these observations suggest that HDL deficiency does not lead directly to atheroscle-

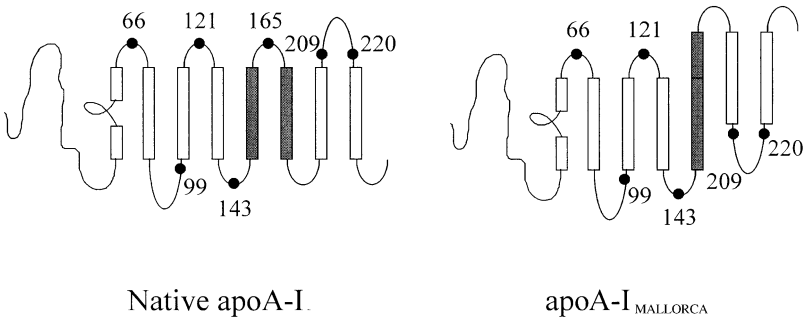

Fig. 5. Schematic representation of the predicted secondary structure of wild type apoA-I and apoA-$I_{MALLORCA}$. Boxes represent amphipathic α-helices. Black circles represent proline residues. Shaded boxes represent internal repeats 6 and 7.

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rosis but, rather, is a permissive factor that would permit atherosclerosis development due to other cardiovascular risk factors. Some of these risk factors were evaluated in the family of this study. Triglyceride and apoB levels were not significantly elevated in the carriers, whereas Lp[a] concentrations were decreased, as in previous reports on patients with LCAT deficiency (49). The LDL:HDL cholesterol ratio, which is a better predictor of risk than either measurement alone (50), was found to be dramatically increased in the proband (35.4) compared with that calculated in non-carriers (<4) and the remaining carriers (<18), except for two of them: II-10 (26.6), a 66-year-old woman and IV-4 (33.1), an 11-year-old girl with very decreased HDL-cholesterol rather than increased LDL-cholesterol. The proband had, furthermore, a history of smoking and a reported history of combined hyperlipidemia. It is well known that when dealing with CHD risk, a combination of environmental factors and multifactorial genetic interactions will determine development (or not) of the disease. However, much remains to be known of both types of factors and their interaction.

In conclusion, a new apoA-I mutation has been identified in a large family in Mallorca, a Spanish island in the Mediterranean. This mutation is a P165-A175 deletion of repeat 7 of the mature apoA-I that impairs the functional properties of the molecule in such a way that dominant hypoalphalipoproteinemia is induced, at least in part, due to their inability to activate LCAT. This mutation, however, does not necessarily induce CHD.

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