

## Familial hypobetalipoproteinemia in a hospital survey: genetics, metabolism and non-alcoholic fatty liver disease

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### ABSTRACT

**Introduction.** Familial hypobetalipoproteinemia (FHBL) is an autosomal dominant disease characterized by abnormally low levels of apolipoprotein-B (apoB) containing lipoproteins. FHBL is caused by *APOB*, *PCSK9* or *ANGPTL3* mutations or is associated with loci located in chromosomes 10 and 3p21. However, other genes should be involved. This study describes the kinetic parameters of the apoB containing lipoproteins and sequence abnormalities of the *APOB* and *PCSK9* genes of FHBL patients identified in a large hospital based survey. **Material and methods.** Cases with primary or secondary causes of hypobetalipoproteinemia were identified. ApoB kinetics were measured in cases with primary forms in whom truncated forms of apoB were not present in VLDL (n = 4). A primed constant infusion of [<sup>13</sup>C] leucine was administered, VLDL and LDL apoB production and catabolic rates measured by a multicompartmental model and compared to normolipemic controls. In addition, these subjects had an abdominal ultrasound and direct sequencing was carried out for the *PCSK9* and apoB genes. **Results.** Three individuals had normal apoB production with increased catabolic rate; the remaining had reduced synthetic and catabolic rates. Various polymorphisms, some of them previously unreported (\*), in the *PCSK9* gene (R46L, A53V, I474V, D480N\*, E498K\*) and in the apoB gene (N441D\*, Y1395C, P2712L, D2285E\*, I2286V, T3540S\*, T3799M\*) were found in the FHBL patients. We found hepatic ultrasound changes of hepatic steatosis in only one of the four probands. **Conclusion.** FHBL without truncated apoB is a heterogeneous disease from a metabolic and a genetic perspective. Hypobetalipoproteinemia is a risk factor but not an obligate cause of steatosis.

**Key words.** Hypocholesterolemia. Proprotein convertase subtilisin/kexin type 9. ApoB-100 kinetics and metabolism. Hepatic steatosis.

### INTRODUCTION

Familial heterozygous hypobetalipoproteinemia (FHBL) is an autosomal dominant disease, characterized by low serum levels of total cholesterol, LDL cholesterol and apoB.<sup>1</sup> Its presence is suspected when plasma cholesterol is below 3.88 mmol/L (150 mg/dL) in an otherwise healthy individual.<sup>2</sup> Long-term follow-up of a number of cohorts, such as the

Framingham population, revealed that individuals with FHBL are less susceptible to develop cardiovascular disease<sup>3</sup> and run a greater risk of nonalcoholic fatty liver disease caused by the imbalance in the hepatic synthesis and export of lipids.<sup>4</sup> There are several causes of FHBL. ApoB mutations resulting in truncations are best characterized, with more than 30 truncated forms reported.<sup>5,6</sup> Hypobetalipoproteinemia may result from a decreased apoB secretion (of both normal and truncated apoB) or from an increased plasma depuration of the apoB variant.<sup>6-9</sup> However, this represents less than 2% of the FHBL.<sup>10</sup> Latour described the kinetic abnormalities of the apoB-containing lipoproteins (using endogenous labeling with [<sup>13</sup>C] leucine) in four FHBL subjects without truncated apoB.<sup>11</sup> They found a normal VLDL- and LDL-apoB production rate and a two to three fold increase in its catabolic rate.<sup>12</sup> Yuan des-

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cribed one FHBL kindred with a chromosome 3p21 loci linkage;<sup>13</sup> in this case there was a reduced apoB production and an increased LDL-apoB catabolic rate.<sup>14</sup> Some apoB mutations that affect its kinetic behavior without truncated variants have been described in FHBL. The mutations L343V and R463W<sup>15</sup> enhance the affinity of the apoB domain ba1 with the microsomal triglyceride transfer protein (MTP), increasing the retention of nascent VLDL particles in the endoplasmic reticulum, and lowering their secretion. The R3480P mutation increases the affinity of apoB for the LDL receptor leading to a lower conversion (up to 70%) of VLDL to LDL.<sup>16</sup> Finally, SNPs like P2712L alters the structure and function of apoB by modifying the alpha helices.<sup>17-19</sup> The functional characteristics of P2712L have not been described, even though it has been identified among subjects with hypocholesterolemia. Loss-of-function mutations and SNPs of the PCSK9 gene are linked with hypocholesterolemia<sup>20-24</sup> and are a possible cause of FHBL and include: L82X, G106R, Y142X, W428X and C679X.<sup>21</sup> As well as in the *apoB-100* gene, numerous SNPs can be related with low cholesterol levels.<sup>25</sup> Recently, Musunuru and coworkers found two *ANGPTL3* mutations in FHLB patients.<sup>26</sup> Thus, various pathogenic mechanisms and genes participate in FHBL. The heterogeneity of this condition was identified primarily by studying the kinetics of the apoB containing lipoproteins in probands identified in lipid clinics. As a consequence, the association with various phenotypes linked with FHLB (i.e. hepatic steatosis and type 2 diabetes) has been difficult to be assessed due to the referral bias. Special interest exists in cases without apoB truncations, since information about the co-existing conditions of these cases is scant.

This study describes the kinetic parameters of the apoB containing lipoproteins, the sequence abnormalities of the apoB and PCSK9 genes and the hepatic ultrasonography from FHBL patients without apoB truncations identified in a hospital based survey.

## MATERIALS AND METHODS

### Selection of subjects

The protocol was approved by the Ethics Committee of the Instituto Nacional de Ciencias Médicas y Nutrición and all patients gave informed consent. FHBL was defined by total serum cholesterol levels below 3.36 mmol/L (130 mg/dL) on two separate occasions, affecting at least one first degree family member (ascendant, descendent or sibling), and

without secondary causes for hypocholesterolemia. The cholesterol threshold used for the definition of FHBL is beneath the 5<sup>th</sup> percentile for the adult Mexican population.<sup>27</sup> Cases were recruited at a university hospital. All plasma lipid analyses performed during a one year period at the laboratory of our Institute were recorded. The sample was composed of 22270 cases, 80% of them were attending the out-patient clinics. For cases with hypocholesterolemia, first degree relatives were invited to have a plasma lipid profile and were considered affected if their cholesterol levels were 3.36 mmol/L (130 mg/dL) or less.

### Analytical methods

Blood was collected in tubes with EDTA and plasma was separated from blood cells by low-speed centrifugation. Subsequently, VLDL ( $\delta < 1.006$  g/mL), IDL ( $\delta = 1.006$  to  $1.035$  g/mL) and LDL ( $\delta = 1.036$  to  $1.064$  g/mL) were isolated from 4 mL of plasma by sequential ultracentrifugation. We looked for truncated forms of apoB using 3 to 6% gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels stained with Coomassie blue.

### Metabolic study

Patients were admitted at the Metabolic Unit of our Institute to assess the kinetics of the apoB containing lipoproteins. Patients were asked to keep their diet unaltered during the two previous weeks. They attend the clinic after a 12 hour-fasting period. A bolus of [<sup>13</sup>C] leucine (0.85 mg/kg) was administered through an intravenous catheter in one arm followed by a constant infusion of  $0.85 \text{ mg} \cdot \text{kg}^{-1} \cdot \text{h}^{-1}$  for 8 hours. After the infusion of [<sup>13</sup>C] leucine stopped, patients remained fasting for 8 hours more and then resumed their usual diets. A total of 37 samples were drawn through a second intravenous catheter in the other arm. Blood samples were taken at the following time periods (min): -30 (prior to the infusion), 0 (beginning of the infusion), 5, 10, 15, 20, 30, 40, 50, 60, 75, 90, 105, 120, 150, 180, 210, 240, 300, 360, 420, 480 (end of the infusion), 495, 510, 525, 540, 570, 600, 630, 660, 720, 780, 840, 960, 1440 and in days 3 and 5. All samples were used for determination of plasma leucine enrichment, and 31 for VLDL and LDL apoB leucine enrichment. ApoB and lipid concentrations were measured in five samples during each kinetic study to evaluate the presence of a steady state, at 1, 4, 8, 12, and 16 hours.<sup>28-30</sup>

**Isolation of plasma amino acids**

Plasma amino acids were separated from 0.3 mL of plasma by cation exchange chromatography.<sup>31</sup>

**Isolation and hydrolysis of apoB**

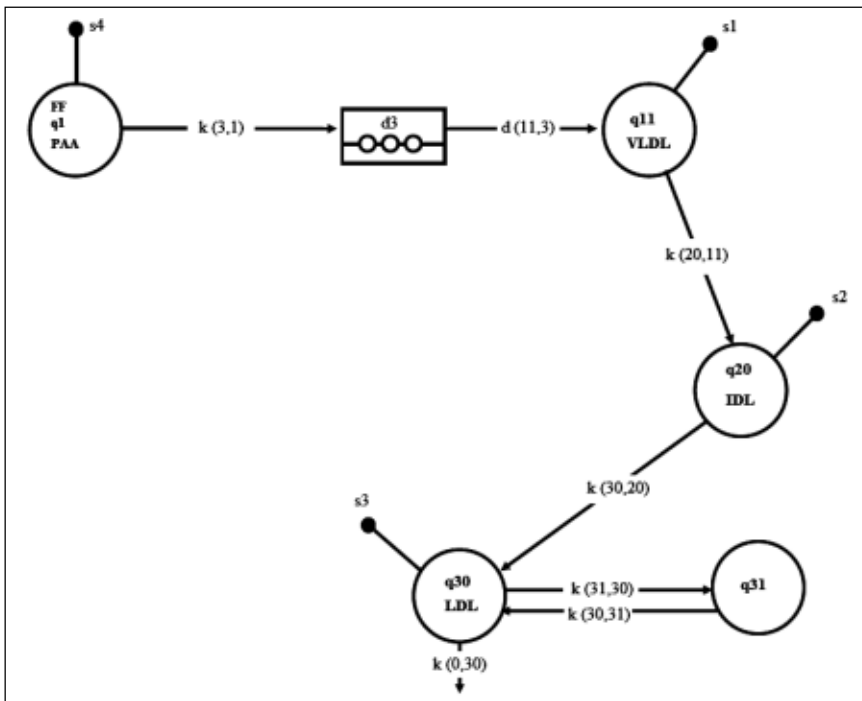
ApoB was separated by precipitation with butanol-isopropyl ether. Precipitated apoB was dried under nitrogen and hydrolyzed in 6N HCl for 16 hours at 110°C; HCl was subsequently evaporated.<sup>32</sup>

**Determination of enrichment and calculation of tracer: tracee ratio**

Amino acids obtained from plasma samples by cation exchange chromatography or from the hydrolyzed apoB were derivatized to N-heptafluorobutyryl n-propyl esters. The enrichment of the isotope [<sup>13</sup>C] leucine, and the tracer: tracee ratios were calculated by gas chromatography-mass spectroscopy using electron impact ionization (Agilent Technologies/HP 6890 Series GC System and 5973 Mass Selective Detector, Hewlett-Packard). The enrichment of the [<sup>13</sup>C] leucine administered was 99% (Cambridge Isotope Laboratories).

**Model showing the metabolism of apoB and calculation of kinetic parameters**

A multicompartmental model was used (Figure 1) to describe the tracer-tracee ratios of leucine in VLDL, and LDL apoB using the SAAM II program (University of Washington, St. Louis MO). Each compartment or pool represents a group of kinetically homogenous particles. The model represents the minimum structural complexity in order to explain the data for [<sup>13</sup>C] leucine enrichment and the relative mass of apoB throughout each fraction of lipoproteins. We have used this model previously to describe apoB kinetics in VLDL and LDL.<sup>11</sup> Compartment q1 is a plasma leucine forcing function that describes the enrichment of plasma leucine both during and following the 8 hour tracer infusion. A delay compartment (d3) accounts for isotopic dilution with unlabeled intracellular leucine and a time delay for VLDL apoB synthesis, packaging and secretion. ApoB appears in plasma in VLDL (q11), and converted to IDL (q20) and LDL (q30) through subsequent delipidations. The catabolic rate of each compartment is the sum of the individual constant rates at the exit of each compartment. It may be assumed that all the apoB enters the plasma as part of the VLDL, and apoB production rate is determined as the product of VLDL apoB pool size and the catabolic



*Figure 1. The multi-compartmental model used to calculate kinetic parameters. See "methods" for details.*

rate of compartment 11. A plasma volume of 0.45 dL/kg body weight was assumed. The constant rates from the model were adjusted in order to minimize the sum of the squared predictions against the observed enrichment of [<sup>13</sup>C] leucine for VLDL and LDL. For comparison, the previously reported kinetic values of healthy normolipemic controls were used.<sup>6</sup>

### Genotyping

Genomic DNA was obtained from peripheral blood leucocytes, following the phenol chloroform method for extraction. Direct sequencing was carried out for the PCSK9 and apoB genes using the ABI Prism 3100® sequencer. The primers for PCR amplification and sequencing of the PCSK9 gene, previously described<sup>20</sup> are shown in table 1 with temperature alignments (°C). Exons 5, 9, 10, 11,

21, 22, 23, 24, 25 and 26 of the apoB gene were analyzed, in order to eliminate the possibility of nonsecreted truncated forms present (apoB-29 or shorter), and for searching mutations not related to truncated forms of apoB.<sup>1</sup> ApoB gene primers and temperature alignments (°C) are shown in table 2. ApoE genotypes were determined by gene amplification and restriction endonuclease cleavage with *HhaI*.<sup>33</sup>

### Abdominal ultrasonography

Abdominal ultrasonography was performed in all four patients. NAFLD was suspected if any of the following abnormalities was found in the ultrasound: diffuse hypoechoic texture (bright liver), increased liver echo texture in comparison with kidney and vascular blurring or deep attenuation.

**Table 1.** Oligonucleotides used to examine the *PCSK9* gene (ref. 20). Alignment temperature is shown in °C.

<i>PCSK9</i>	Temp	Sense 5' → 3'	Antisense 3' → 5'
Promoter	58	AAGCAATCTCTCAAGGAGCA	GACTGTGCAGGAGCTGAAGTT
Exon 1	58	CAGCTCCAGCCAGGATTC	GATCGTGCCAAGCGAAGAG
Exon 2	60	CCTGAATGGCACATTTGAAAG	TGCTCAATACATACTTGCTGTCC
Exon 3	62	CTCTATGCCAGACCGTGTG	GTGCTGAGTCCCAAAGCC
Exon 4	60	GACTTGGGTCTCTTGGC	TGGCTGGATGGATGAACG
Exon 5	64	GCTTCCACAGACAGGTAAGCAC	GGGTTTCTTCATCTGCACTCG
Exon 6	60	TCGCAGCAGCATTTCCAC	TCCAAAGCCAGAAGGGTTC
Exon 7	64	AGTCAGATTTTCTTAGGAGGG	ACTGAGTGTCTTGAAGGCAC
Exon 8	62	TGAGAGGAGGCTGTCTTACCTC	GAGGAGGGCTTAAAGAACAATCTC
Exon 9	64	GTAAGGAGGATGACGCCACC	TTACAGAAGAGCTGGAGTCTGG
Exon 10	58	AGCTCCTTGTCGCCAGAAG	GAGTATGGAAGTGAAGTCTGG
Exon 11	60	GGCTCAGAGAGGTTGAATGG	GCATCTACCTGGCAAACCG
Exon 12	60	TGGTAGGCATCTGTCTATCTCC	GAAGCATCCCCATCCCC

**Table 2.** Oligonucleotides used to examine the exons of the *apoB* gene. Exon 26 was revised in 6 segments. Alignment temperature is shown in °C.

<i>ApoB</i>	Temp	Sense 5' → 3'	Antisense 3' → 5'
5	62	AGTGCCACCCAGCTTACTTC	CTTCCTATGTAAGTATGATGGAG
9	58	AGAGCCAGATCTAGCAGGCAT	CAAAGTCTGCTCCTTACTCTTG
10	58	ATGGTTCTGAGCTCCAAGTTG	CAAGAGTAAGGAGCAGAGTTTG
11	54	CAGGTAATGTGATGCCTCCAGC	AGGGAAGTAAAAGGTGTCTGTC
21	58	CTGGGATTACAGGTGTGAGC	TCTGCCACTCTGATTGTAGAC
22-23	64	TGTGGCTGTTTCTCTGAACC	ACATTCAGCTTTGTGTAAGTGG
24	58	AGTGACTGGCAACGAAGATTC	CTAATGCAAAGATGC CACAGTG
25	62	GCTTTGTGAGGATTTGAGTGTGTTG	GCTTTGTGAGGATTTGAGTGTGTTG
26-1	60	CAGATGGAGGAGTCTATTGCAC	CTTATACTTCCCATTGGTGTGAC
26-2	64	TGCTTATCAGGCCATGATTCTG	TCCTTGGCATGTGAAACTTGTGTC
26-3	62	GACAAGTTTACATGCCAAGGA	ACTTTGGCTCTGAAGGCATTG
26-4	58	GAAACCAAGGCCACAGTTGC	CTTCGTTTGTGAGGTGGTTC
26-5	58	CGGATTCATTCTGGGTCTTTC	TCTACAGTTTGGTTTTTACGTG
26-6	57	CCTCCACTGAAAGATTTCTCTATGGG	GATCCTTAGAAGGACACCTAAGGTCC

**Statistical analysis**

Results are presented as absolute values and as medians for the group with FHBL as well as the normolipemic controls along with their demographic characteristics and values for production (mg/kg of body weight/day) or catabolic rates (pools/day). In order to compare groups, non parametric statistics were used (Mann-Whitney U test). A two-tailed value of  $P < 0.05$  was considered statistically significant.

**RESULTS**

**Clinical characteristics of the participants**

Hypocholesterolemia was found in 3,712 subjects. Secondary causes of hypocholesterolemia were demonstrated in 3,667, leaving us with 45 individuals with potentially primary hypobetalipoproteinemia. Of the 45 subjects, an autosomal dominant inheritance was found in seven. All of them were invited to participate. However, only four subjects agreed to participate in the metabolic and genetic study. The clinical characteristics of the four study subjects are shown in table 3. The family structure of the four

pedigrees is illustrated in figure 2. We looked for truncated forms of apoB using 3 to 6% gradient sodium dodecyl sulfate (SDS)-polyacrylamide gels stained with Coomassie blue. No abnormal bands were detected in the cases.

**ApoB kinetics**

Concentrations of apoB and lipids had variations lower than 5% during the primed constant [<sup>13</sup>C] leucine infusion. These observations assured us that the required steady state occurred during the course of kinetic study. The kinetic parameters are presented in table 4. Results are compared against data obtained in ten normolipemic healthy controls. In cases A, B and C, VLDL and LDL apoB production was similar to controls and the catabolic rate was increased in 2 to 3 fold. In contrast, case D had very diminished production and catabolic rates.

**Liver function tests and abdominal ultrasonography**

Abdominal ultrasonography was performed in the four probands (A, B, C, and D). Case A had hepatic

**Table 3.** Clinical characteristics of FHBL without apoB truncations and normolipemic controls.

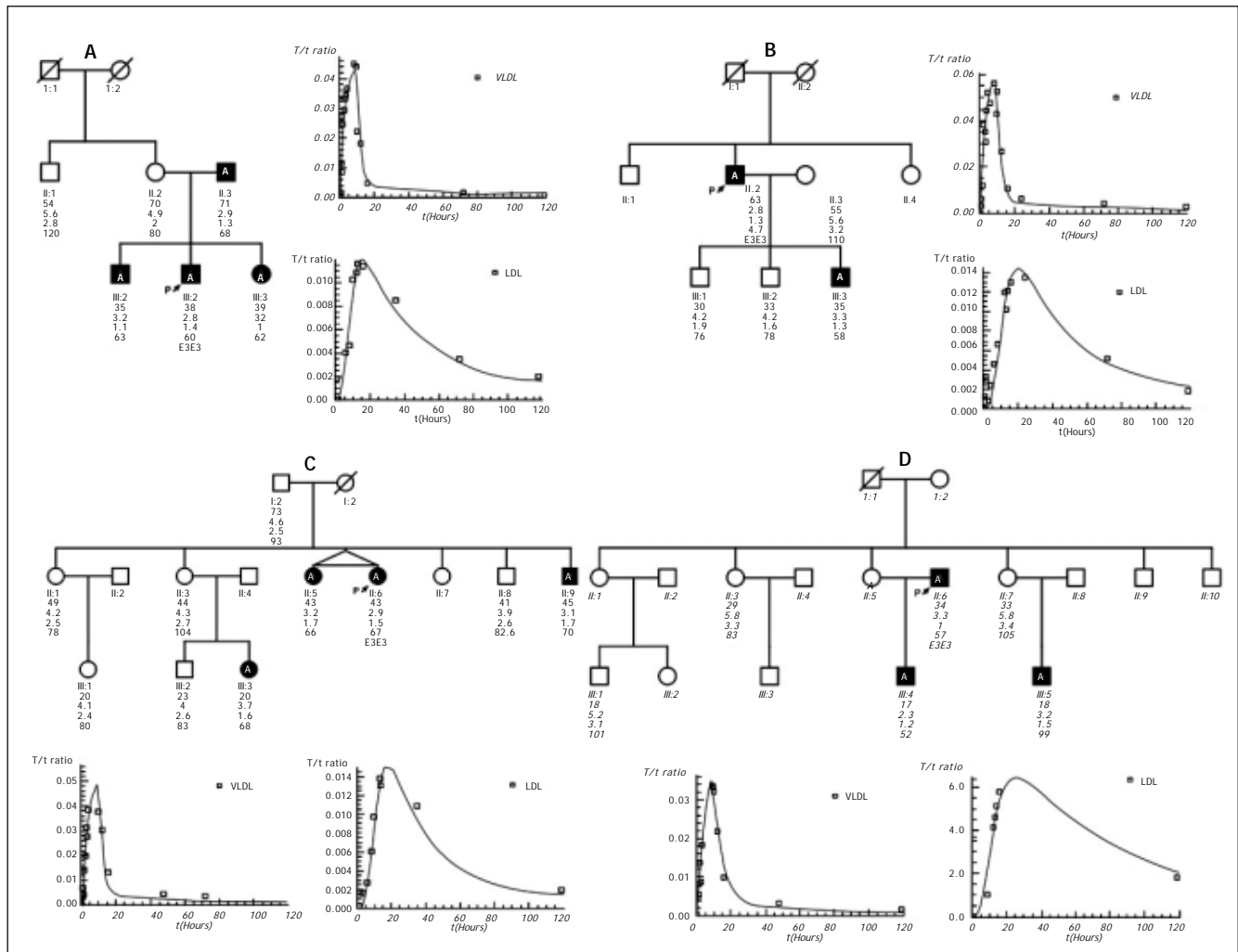
Subject	Sex	Age years	BMI Kg/m <sup>2</sup>	Cholesterol mmol/L	HDL mmol/L	LDL mmol/L	TG mmol/L	ApoB100 g/L
Case A	M	38	22.1	2.82	1.13	1.47	0.46	0.60
Case B	M	63	25	2.8	0.93	1.29	0.67	0.47
Case C	F	43	25.6	2.97	0.77	1.57	1.33	0.67
Case D	M	34	23	3.31	1.5	1.06	1.61	0.57
FHBL group	—	40.5	24.3	2.85	1.03	1.38	1	0.59
Control group	—	30.5	25.6	4.45	1.1	2.8	0.75	0.89
P value*	—	NS	NS	0.006	NS	0.006	NS	0.007

\* Mann-Whitney U test.

**Table 4.** Kinetic parameters of the apoB lipoproteins in the 4 cases and controls.

	Production rate (mg/kg/day)		Catabolic rate (pools/day)	
	VLDL	LDL	VLDL	LDL
Case A	21.1	21.2	16.4	2.6
Case B	15.5	15.8	14.5	1.3
Case C	17.2	17.3	13.4	1
Case D	7.7	7.8	4.6	0.5
Cases	17.2	17.3	14.5	1.3
Controls	20.5	15.1	9.3	0.39
P value*	NS	NS	0.024	0.012

\* Mann-Whitney U test.



**Figure 2. A, B, C, D.** Structure of the four families studied. Autosomal dominant inheritance is confirmed, an arrow indicates the proband. Black boxes represent individuals with hypocholesterolemia. The numbers beneath the boxes correspond to: age (years), total cholesterol, LDL cholesterol, apoB-100 and for the probands, the E3 genotype. Tracer: tracee ratios represented for the incorporation of [<sup>13</sup>C] leucine over time (hours) in the apoB VLDL and apoB LDL among the subjects studied.

**Table 5.** Changes found in the apoB-100 gene.

Exon	Position	Nucleotide Change	Aminoacid change	Case A	Case B	Case C	Case D
11	441	A → G	N441D*	+ -			
26	1395	A → G	C1395Y	++	++	++	
	2285	C → A	D2285E*				+ -
	2286	A → G	V2286I				++
	2712	C → T	P2712L				
	3540	A → T	T3540S*				+ -
	3799	C → T	T3799M*				+ -

\* Previously not reported. ++ homozygote. +- heterozygote.



**Table 6.** Changes found in the PCSK9 gene.

Exon	Position	Nucleotide change	Aminoacid change	Case A	Case B	Case C	Case D
1	leucine stretch	ins CTG	21-22InsL		+ -		
	46	G → T	R46L		+ -		
	53	C → T	A53V	+ -	+ -		
9	474	G → A	V474I	++	++	++	++
	480	G → A	D480N*	+ -			+ -
	498	G → A	E498K*			+ -	

\* Previously not reported. ++ homozygote. +- heterozygote.

steatosis; the other three were normal. The ultrasound results were in agreement with AST, ALT and GGT levels. Case A was the only study subjects that had increased plasma concentrations of liver enzymes.

### Genetic Study

None of the mutations of the apoB gene known to cause FHBL were found. The genotypes found for cases A, B, C and D are presented in table 5 (*APOB*) and in table 6 (*PCSK9*). Previously unreported changes are marked with an asterisk. The effects of the detected variants were then revised with the Polymorphism Phenotyping Program (PolyPhen I, II). The variants were classified as benign (-), possible damaging (+) or probably damaging (++), the last with probable effects on the protein structure or function.

Proband A had the variants N441D\* (-) and C1395Y (++) for the apo B gene and A53V (-), I474V (-) and D480N\* (-) for the PCSK9 gene. Proband B had the variants C1395Y (++) for the apo B gene and 21-22 InsL (-), R46L (-), A53V (-) and I474V (-) for the PCSK9 gene. Proband C had the variants C1395Y (++), P2712L (++), T3540S\* (-) and T3799M\* (++) for the apo B gene and I474V (-) and E498K\* (+) for the PCSK9 gene. Proband D had the variants D2285E\* (+) and V2286I (-) for the apo B gene and I474V (-) and D480N\* (-) for the PCSK9 gene. All four cases were homozygotes for the apoE3 allele (E3/E3).

### DISCUSSION

This report presents the abnormalities of the kinetic behavior of the apoB containing lipoproteins and the sequence changes of *PCSK9* and *APOB* genes found in four FHBL individuals, detected in a teaching hospital-based survey. Hypobetalipoproteinemia was explained by a remarkable increase in

VLDL-, LDL-apoB fractional catabolic rate in probands A, B and C and a very significant decrease in apoB production rate in case D. Hepatic steatosis was found in only one case. Thus, our report provides evidence to support the heterogeneous nature of FHBL. In addition, it shows that hepatic steatosis may not be a constant feature of this condition, if well abdominal ultrasound is not a sensitive method for detecting mild steatosis.<sup>34</sup>

In proband A, two *APOB* variants were found (N441D and C1395Y). The first was not described previously and is located in the same apoB region that contains the FHBL mutations L343V and R463W.<sup>15</sup> These mutations enhance the affinity of the apoB domain  $\beta\alpha 1$  with the microsomal triglyceride transfer protein (MTP) during lipoprotein assembly.<sup>35</sup> Mildly elevated liver serum enzyme concentrations has been reported in the L343V and in the R463W FHBL subjects, similar to that found in proband A. The possible effects of other nonsynonymous nontruncating mutations within *APOB* in human FHBL that occur within the  $\beta\alpha 1$  domain include A31P, G274S, L324M, G912D and G945S.<sup>36</sup> The patient was heterozygous for the SNPs, which may explain the normal lipoprotein production rate found. The second apoB variant (C1395Y) had possible damaging effects on the protein structure, as predicted by the PolyPhen software. This variant is located in the middle of the  $\beta$ -1 sheet, one of the major lipid-associating motifs in apoB. It is far from the receptor-binding domains of apoB. Thus, it is not possible to propose that the C1395Y variant of *APOB* may alter the catabolism of the apoB-containing particles.

In proband B, we also found the C1395Y variant of *APOB* and some PCSK9 polymorphisms not associated with changes in protein function (R46L, A53V and I474V). Proband B shares the same apoB kinetic abnormalities with proband A. However, we do not have evidence to propose that the C1395Y va-

riant of *APOB* is responsible for the abnormally high apoB catabolic rate. However, additional studies are required to assess the effect of the C1395Y variant in apoB function and its potential association with FHBL.

Four *APOB* variants were found in patient C; three of them were probably damaging (C1395Y, P2712L and T3799M) and 2 previously unreported (T3799M and T3540S). Of special interest is P2712L. It located near the LDL receptor binding domain, beneath amino acids 3000 to 3500. Although the ApoB-100 binding domain for the LDL receptor is located between amino acids 3359 and 3369, mutations in amino acids 3500 and 4369 also alter the interaction between apoB and the LDL receptor. Thus, the P2712L mutation may be able to modify the exposure of the domain that interacts with the LDL receptor. This is in accordance with the increased LDL-apoB catabolism found in the case reported here. The P2712L variant of *APOB* affects the secondary structure of the protein and its hydrophobicity.<sup>15-17</sup> The amino acid change is found in an area within a small and hydrophobic alpha helix. The P2712L substitution may form a larger helicoidal region and improve the catabolic efficiency of VLDL and LDL, resulting in hypocholesterolemia. Thus, the P2712L substitution could be one of the explanations for the hypocholesterolemia in patient C.

Proband D had a different kinetic profile. This case was the only one that had a very low production rate of apoB-VLDL and -LDL. The previously unreported *APOB* variant D2285E was found in this subject; PolyPhen software predicted that the substitution may damage protein structure or function. The variant is located in the  $\alpha 2$  helix region. Additional studies are required to assess the effect of the D2285E variant in apoB function and its potential association with FHBL.

Hepatic steatosis is a condition frequently associated with FHBL. In humans, the fat content of the liver is three times greater in FHBL cases than in matched controls. However, there is a remarkable variability in the fat content of the liver, even in FHBL cases caused by the same genetic defect (e.g. apoB truncations) (37). A portion of the variation is related to other metabolic variables (i.e. intraperitoneal adipose tissue, obesity and insulin sensitivity). Tanoli and coworkers reported that lean FHBL subjects and lean controls have similar amounts of liver fat. Intraabdominal fat was found to be a major determinant of the amount of liver fat.<sup>4</sup> The regression line between these two variables for FHBL subjects was significantly steeper than the line for

controls. This suggests that FHBL subjects are more susceptible to developing fatty livers at any given amount of abdominal adipose tissue than are controls.<sup>38</sup> However, some groups of FHBL patients (e.g. those associated with the 3p21 locus) had no fat accumulation in the liver.<sup>39</sup> Our study sample well reflects the variability of the association between hepatic steatosis and FHBL. Only one of the study participants had hepatic steatosis. This individual was lean and had a normal fasting glucose concentration. Reasons for the absence of hepatic steatosis in the other three subjects are unclear and remain to be elucidated. A limitation of our study is the absence of measures of insulin sensitivity and intra-abdominal fat.

Our report extends the available information in the FHBL field as it confirms the heterogeneous nature of the disease. In addition, it describes the kinetic abnormalities of the apoB containing particles in subjects with the P2712L variant of apoB. In contrast to previous reports (consisting on a study of randomly detected FHBL patients), our study uses a sampling process designed to be representative of the population that seeks medical care in a teaching hospital. In addition, kinetic, genetic and abdominal ultrasonography assessments were evaluated. These characteristics allow us to generalize our results to a population most likely to be detected in a medical environment with the required resources to look for the etiology of the disease.

## ABBREVIATIONS

- **apoB-100:** Apolipoprotein B-100.
- **FHBL:** Familial hypobetalipoproteinemia.
- **PCSK9:** Proprotein convertase subtilisin/kexin type 9.
- **SNPs:** Single-nucleotide polymorphisms.
- **NAFLD:** Non-alcoholic fatty liver disease.
- **LDLR:** Low density lipoprotein receptor.

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