

**MOLECULAR ANALYSIS OF *APOB*, *SAR1B*, *ANGPTL3*, AND *MTTP* IN PATIENTS WITH
PRIMARY HYPOBETALIPOPROTEINEMIA IN A CLINICAL LABORATORY SETTING:
EVIDENCE SUPPORTING POLYGENICITY IN MUTATION-NEGATIVE PATIENTS**

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Abstract

Background and aims: Primary hypobetalipoproteinemia is generally considered a heterogenic group of monogenic, inherited lipoprotein disorders characterized by low concentrations of LDL cholesterol and apolipoprotein B in plasma. Lipoprotein disorders include abetalipoproteinemia, familial hypobetalipoproteinemia, chylomicron retention disease, and familial combined hypolipidemia. Our aim was to review and analyze the results of the molecular analysis of hypolipidemic patients studied in our laboratory over the last 15 years.

Methods: The study included 44 patients with clinical and biochemical data. Genomic studies were performed and genetic variants were characterized by bioinformatics analysis. A weighted LDL cholesterol gene score was constructed in order to evaluate common variants associated with impaired lipid concentrations and their distribution among patients.

Results: Twenty-three patients were genetically confirmed as affected by primary hypobetalipoproteinemia. In this group of patients, the most prevalent mutated genes were *APOB* (in 17 patients, with eight novel mutations identified), *SAR1B* (in 3 patients, with one novel mutation identified), *ANGPTL3* (in 2 patients), and *MTTP* (in 1 patient). The other 21 patients could not be genetically diagnosed of hypobetalipoproteinemia despite presenting suggestive clinical and biochemical features. In these patients, two *APOB* genetic variants associated with lower LDLc cholesterol were more frequent than in controls. Moreover, the LDL cholesterol gene score, calculated with 11 SNPs, was significantly lower in mutation-negative patients.

Conclusion: Around half of the patients could be genetically diagnosed. The results suggest that in at least some of the patients without an identified mutation, primary hypobetalipoproteinemia may in fact have a polygenic origin.

Keywords: Combined hypolipidemia; Familial hypobetalipoproteinemia; Chylomicron retention disease; Abetalipoproteinemia; Polygenic hypocholesterolemia.

Highlights: Primary hypobetalipoproteinemia has been considered monogenic; however, our findings suggest that in some cases it may also have a polygenic origin.

Abbreviations

HL: hypobetalipoproteinemia

LDLc: LDL cholesterol

ApoB: apolipoprotein B

MTTP: microsomal triglyceride transfer protein

PCSK9: proprotein convertase subtilisin/kexin type 9

SAR1B : secretion associated Ras related GTPase

ANGPTL3 : angiopoietin-like 3

MYLIP: myosin regulatory light chain interacting protein

DGAT1: diacylglycerol O-acyltransferase

NPC1L1: Niemann-Pick C1-like 1 cholesterol transporter 1

FHBL: familial hypobetalipoproteinemia

TG: triglyceride

CMRD: chylomicron retention disease

CM: chylomicron

VLDL: very low density lipoprotein

ABL: abetalipoproteinemia

FHBL1: familial hypobetalipoproteinemia type 1

FHBL2: familial hypobetalipoproteinemia type 2

HDLc: HDL cholesterol

TC: total cholesterol

SNP: single nucleotide polymorphism

HL/M-: hypobetalipoproteinemia mutation-negative patients

HL/M+: hypobetalipoproteinemia mutation-positive patients

INTRODUCTION

Primary hypobetalipoproteinemia (HBL) is a heterogenic group of inherited lipoprotein disorders characterized by low concentrations of low-density lipoprotein (LDL) cholesterol (LDLc) and apolipoprotein B (ApoB) in plasma¹. HBL may manifest as a fat malabsorption syndrome commonly accompanied by growth failure, fat-soluble vitamin deficiency, hepatic steatosis, abdominal distension, diarrhea, vomiting, and neurological and ophtalmological disorders in the context of hypolipidemia¹. Primary HBL can be classified according to various criteria, including clinical and biochemical phenotype, the gene involved, and the mode of inheritance of the condition.

Familial hypobetalipoproteinemia (FHBL) type 1 (FHBL1) (OMIM 615558) is a codominant disorder caused by mutations in the *APOB* gene frequently giving rise to a truncated ApoB protein². Affected heterozygotes are asymptomatic or present with hepatic steatosis, and low levels of LDLc and ApoB³. Homozygotes may be clinically and biochemically indistinguishable from abetalipoproteinemic patients^{2, 3}. FHBL1 can also be caused by loss-of-function mutations in the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) gene which encodes a protein that targets the LDL receptor for lysosomal degradation. *APOB* and *PCSK9* genes are also associated to familial hypercholesterolemia.

Familial combined hypolipidemia (OMIM 605019), also known as familial hypobetalipoproteinemia type 2 (FHBL2), is a rare recessive disorder caused by mutations in the angiopoietin-like 3 (*ANGPTL3*) gene⁴. Loss-of-function mutation increases lipoprotein lipase activity and may decrease high-density lipoprotein (HDL) cholesterol (HDLc) by increasing endothelial lipase, two enzymes that in physiological conditions are catabolized by the action of *ANGPTL3*. Patients typically present with no clear phenotype other than hypolipidemia².

Chylomicron retention disease (CMRD) or Anderson's disease (OMIM 246700) is a rare recessive disorder caused by mutations in the secretion associated Ras Related GTPase

(SAR1B) gene characterized by the accumulation of lipid droplets within the enterocytes and the selective absence of ApoB-48-containing particles from plasma⁵. Biochemical manifestations include reduced LDLc levels and absence of chylomicrons (CM)⁵.

Abetalipoproteinemia (ABL) (OMIM 200100) is a rare recessive disorder caused by mutations in the microsomal triglyceride transfer protein (*MTTP*) gene. The gene encodes for a molecular chaperone critical for the formation of triglyceride-rich lipoproteins such as very low-density lipoproteins (VLDL) and CM. Patients typically present with an absence of LDLc and CM, low TG levels, and very low vitamin E levels³.

Most of these disorders are very rare.. Estimated prevalence for familial hypobetalipoproteinemia is 1 in 1000-3000 individuals and less than 1 in 1 million individuals for the rest of types of primary HBL^{3, 6}. Studies have reported variants in other genes associated very infrequently with primary HBL, such as the *APOC3* gene⁷, the myosin regulatory light chain interacting protein (*MYLIP*) gene⁸, and the diacylglycerol O-acyltransferase 1 (*DGAT1*) gene⁹.

It is noteworthy that extreme cholesterol phenotypes can result from the accumulation of common small-effect LDLc affecting alleles^{11, 12}. Therefore, the objective of this study was to review, analyze, and evaluate genetic variants in 44 hypolipidemic patients recruited to our laboratory over the last 15 years. This included the search for a potentially polygenic basis for the disorder in patients who were negative for mutations in the well-established genes causing primary HBL.

MATERIALS AND METHODS

Subjects

Subjects were recruited over a 15-year period and were referred to our Metabolism Unit for genetic analysis based on biochemical and/or clinical suspicion of primary HBL, according to the requesting practitioners. Only two patients were followed at our hospital; thus, clinical and/or biochemical data were provided by the medical centers of origin (mainly from Spain) in summarized form, and as a result, the clinical and/or biochemical information obtained was sometimes heterogeneous or incomplete. Primary HBL is usually defined as persistent low total cholesterol with plasma LDLc and ApoB concentrations below the fifth percentile in the Spanish population, 130 mg/dL, 62 mg/dL and 63 mg/dL respectively^{13, 14}. In order to avoid molecular analysis of secondary HBL, the exclusion criteria included severe chronic liver disease, chronic pancreatitis, cystic fibrosis, end-stage renal disease, hyperthyroidism, cachexia, and malabsorption. In some cases, relatives were also submitted for study. Genetic studies were performed to confirm the diagnosis. All blood samples were obtained in accordance with the Helsinki Declaration of 1964, as revised in 2000. The Ethics Committee of the Hospital de la Santa Creu i Sant Pau reviewed and approved the study protocol and all individuals provided written informed consent. To study the polygenic basis, a sample of 503 European subjects from the 1000 Genomes Project (www.1000genomes.org) was used as the control group and gene score distribution compared.

Gene amplification and resequencing

Genomic DNA was isolated from whole blood (usually EDTA or citrate blood) using the QIAamp DNA blood minikit (Qiagen, Hilden, Germany). All the exons and exon-flanking intronic regions of the *MTTP*, *APOB*, *SAR1B*, *PCSK9*, *ANGPTL3*, *APOC3*, *MYLIP*, and *DGAT1* genes were amplified by PCR. The resulting products were purified using GFX PCR DNA and a Gel Band Purification Kit (GE Healthcare, Buckinghamshire, UK) and sequenced using a Big Dye Terminator cycle sequencing kit v.3.1 (Applied Biosystems, Foster, CA,

USA) on an ABI3130XL automated analyzer (Applied Biosystems). The resulting chromatograms were analyzed with the Staden package program¹⁵. The primers used for gene amplification and Sanger sequencing are available upon request. A sequential analysis was performed to optimize the diagnostic process. The *APOB* gene was sequenced first, and if it was negative, the *SAR1B*, *ANGPTL3*, and *MTTP* genes were sequenced. Finally, if no putative pathogenic variants were detected, the rest of the genes were also sequenced.

Characterization of variants and bioinformatics analysis

The nomenclature of the allelic variants follows the recommendations of the Human Molecular Genome Variation Society (<http://www.hgvs.org>). All variants were checked against the Human Gene Mutation Database (HGMD, www.hgmd.cf.ac.uk), 1000 Genome Project (phase3browser.1000genomes.org), and ClinVar (www.ncbi.nlm.nih.gov/clinvar) databases in order to characterize them. No functional studies were performed hence bioinformatics functional analysis was used. The impact of point mutations on the structure and function of the protein was assessed with the following software: SIFT (sift.bii.a-star.edu.sg)¹⁶, PolyPhen2 (genetics.bwh.harvard.edu/pph2/index.shtml)¹⁷, Panther (www.pantherdb.org)¹⁸, Provean (provean.jcvi.org)¹⁹, i-Mutant (gpcr.biocomp.unibo.it/cgi/predictors/I-Mutant3.0)²⁰, SNPs3D (www.snps3d.org)²¹, PMut (mmb.pcb.ub.es/pmut2017)²², Mutation Taster (www.mutationtaster.org)²³, and Mutation Assessor (mutationassessor.org)²⁴. Variants affecting introns were analyzed using Human Splicing Finder v.3.0 software (www.umd.be/HSF3/HSF.html)²⁵. According to the American College of Medical Genetics and Genomics guidelines²⁶, point mutations causing premature stop codons, small insertions, or deletions causing a frameshift and a premature stop codon, large rearrangements, and mutations affecting intron donor or acceptor splice sites (positions +1, +2, -2, or -1) were considered directly pathogenic. The remaining variants (synonymous mutations, missense mutations, small in-frame insertions and deletions, mutations affecting a promoter, 5'UTR or 3'UTR, and intronic variants) were considered pathogenic depending on the existence of functional analysis previously reported in the literature, identification as

pathogenic or likely pathogenic in databases such as ClinVar, or in the absence of previous information, when the programs used in the bioinformatics analysis gave as a result a probable alteration in protein function, protein expression, or gene regulation.

Frequency distribution of variants and LDLc gene score

We used contingency tables to evaluate the frequency distribution of the observed nonpathogenic variants in patients that tested negative for a pathogenic mutation versus 503 European controls from the 1000 Genomes Project. We also calculated a weighted LDLc gene score¹¹ (the weighted sum of the estimated per-allele effect in LDLc changes, where weights are the effect sizes calculated from genome-wide association studies, Supplementary Table 1) and compared its distribution among patients with HBL with no confirmed pathogenic variant, patients with an identified pathogenic mutation, and 503 European controls from the 1000 Genomes Project. Using TaqMan probes (Thermo Fisher Scientific, USA), 11 SNPs influencing LDLc were genotyped: *PCSK9* (rs2479409), *CELSR2*-*SORT1* (rs629301), *APOB* (rs1367117), *ABCG8* (rs4299376), *SLC22A1* (rs1564348), *HFE* (rs1800562), *MYLIP* (rs3757354), *NYNRIN* (rs8017377), *LDLR* (rs6511720), and *APOE* (rs429358 and rs7412)¹¹. This LDLc-specific gene score was calculated using the per-allele beta coefficients reported by the Global Lipid Genetics Consortium²⁷.

Statistical analysis

A nonparametric sample *t*-test and Fisher's exact test were performed with SPSS v13.0 (IBM).

RESULTS

The study included 44 patients, 27 males and 17 females aged 0.5 to 76 years (mean age 15.4 ± 14.0 years). All the patients were Spanish, except for two Italians and one German. All available demographic, clinical, and biochemical information is presented in Supplementary Table 2. Among the 44 patients studied, 23 (52.3%) were considered genetically diagnosed, 17 presented with mutations in the *APOB* gene (73.9%), three in the *SAR1B* gene (13.0%), two in the *ANGPTL3* gene (8.7%), and one in the *MTTP* gene (4.4%). The results for all genetically diagnosed patients are presented in Table 1. Twenty-one patients (47.7%) did not present with a pathogenic mutation; however, most of them presented with genetic variants potentially associated with changes in lipid metabolism.

***APOB* mutations:** Of the 17 patients with *APOB* mutation, total cholesterol (TC) was provided in 12, with values ranging from 25 to 111 mg/dL, average \pm SD 72.8 ± 30.9 . LDLc was provided in 10 patients, and the values ranged from 1 to 46 mg/dl, average \pm SD 19.9 ± 13.7 . ApoB values were referred in nine patients and ranged from 4 to 35 mg/dl, average 24.1 ± 9.3 . HDLc was provided in eight patients and ranged from 21 to 89 mg/dl, average \pm SD 44.8 ± 18.5 . TG was provided in 10 patients, nine of whom presented with decreased values and one of whom presented with values that fell within the reference interval. TG-concentration values ranged from 7 to 96 mg/dL, average \pm SD 29.2 ± 25.5 .

Notably, patient 4 was homozygote for the novel *APOB* mutation c.2726C>A (p.Ser909*). All the *in silico* prediction programs classified this mutation as damaging. The mutation generates an estimated 80% size reduction of the ApoB protein. The parents of the proband were studied and in both cases presented with the mutation c.2726C>A (p.Ser909*) in heterozygosity. No clinical data were provided. The mother presented with decreased TC (93 mg/dL), LDLc (25 mg/dL), apoB (<22 mg/dL), TG (23.7 mg/dL), and vitamin A (0.23 mg/L) and a normal HDLc (63.3 mg/dL). The father also presented with decreased TC (87 mg/dL),

LDLc (34 mg/dL), ApoB (30.9 mg/dL), and HDLc (25.9 mg/dL) but with a normal TG concentration (133.3 mg/dL).

Patient 8 was a compound heterozygote for the variants c.3G>C (p.Met1Ile) and c.6627_6628delAA (p.Leu2212*). We described both mutations for the first time, and their pathogenicity was predicted by all the *in silico* prediction programs. We estimated that the second mutation generates a 50% size reduction of the ApoB protein. Data and DNA only from the father were sent, testing positive for the c.3G>C mutation (p.Met1Ile) and negative for c.6627_6628delAA (p.Leu2212*) and presenting with decreased LDLc (39 mg/dl) and ApoB (21 mg/dL). One case reported in the literature exhibited an interaction between a heterozygous ApoB mutation and an ApoE2/E2 genotype²⁸. In this case, both father and proband presented with an ApoE3/E3 genotype.

Other novel mutations described in the *APOB* gene in this study were c.133C>T (p.Arg45*); c.1407C>A (p.Tyr469*); c.11728G>T (p.Glu3910*); c.2781delT (p.Ser928Profs*25); and c.10430_10440delTTGACCACAAG (p.Val3477Alafs*2), all of which cause a truncated protein. All the consulted *in silico* programs predicted their pathogenicity. Some previously reported mutations were also found in our cohort and have been included in Table 1 together with a designation of the ApoB truncated forms. Of the submitted relatives, five of them presented with the familial mutation in the *APOB* gene and therefore were genetically diagnosed with FHBL1.

In addition, some variants previously reported to be associated with more subtle changes in lipid or lipoprotein plasma levels were also identified, see Table 2. These reported variants were found in both genetically diagnosed and non-genetically-confirmed patients; of these variants, only three were found exclusively in negative patients: c.2188G>A (p.Val730Ile), c.3383G>A (p.Arg1128His), and c.3427C>T (p.Pro1143Ser).

***SAR1B* mutations:** Three patients were genetically diagnosed for *SAR1B* pathogenic variants, all in homozygous form. We found one novel variant, c.83_84delTG

(p.Leu28Argfs*7), and two previously reported. One of the patients, a 26-year-old male, was referred with only biochemical data and was found to be homozygous for p.Asp137Asn. Two relatives were also diagnosed, one presented the variant c.409G>A (p.Asp137Asn), and the other one presented with c.1-4482_58+1406del5946insGCATGATGGCGGGT, both of them in homozygosity.

ANGPTL3 mutations: As previously reported by our group, two patients were genetically confirmed as homozygous

MTTP mutations: Only one patient was found to be homozygous for a previously described pathogenic variant

Moreover, variants associated with subtle changes in lipid profile were found, see Table 2.

Results of PCSK9, MYLIP, APOC3 and DGAT1 sequencing: Some variants potentially associated with plasma lipid level variability were found for *PCSK9*, *MYLIP* and *APOC3* genes, see Table 2. No variants previously associated with primary HBL were detected for the *DGAT1* gene.

Contingency tables to evaluate the frequency distribution of three common genetic variants of the APOB gene: Several common single nucleotide polymorphisms (SNP) associated with impaired lipid concentrations²⁷ were identified in those patients that tested negative for primary HBL. After performing a Fisher's exact test, three SNP presented with a statistically significantly different frequency of distribution when compared to 503 European subjects from the 1000 Genomes Project (Table 3). The *APOB* variants with statistical significance were the following: rs1367117, allele T, associated with high LDLc ($p=0.00052$); rs1042031, allele A, associated with low LDLc ($p=0.021$); and rs1042034, allele A, associated with high LDLc ($p=0.0014$).

Concerning the 21 patients who could not be genetically diagnosed with primary HBL, TC was provided in 12 of the patients, with values ranging from 57 to 114 mg/dL, average \pm SD of 85.7 ± 19.9 . LDLc was provided in 12 patients, all of whom had decreased values except

for one patient. Values ranged from 13 to 52 mg/dl, average \pm SD 29.9 ± 12.9 . ApoB values were referred in 11 patients and ranged from 16 to 50 mg/dl, average \pm SD 30.3 ± 10.0 . HDLc was provided in 6 patients, two of whom presented with decreased values and four of whom presented with values that fell within the reference interval. The values ranged from 17 to 64 mg/dl, average \pm SD 42.5 ± 16.0 . TG was provided in 9 patients, six of whom presented with decreased values and three of whom were normal. The values ranged from 12 to 75 mg/dL, average \pm SD 38.8 ± 21.1 .

Analysis of the weighted LDLc gene score:

Regarding a possible polygenic basis of the hypolipidemia of these patients, LDLc weighted scores expressed as mean (SD) were as follows: 0.8868 (0.212) for the control population, 503 subjects from the European population in the 1000 Genome Project; 0.8033 (0.217) for 23 primary HBL patients with identified mutation (HBL/M+); 0.7366 (0.327) for 20 primary HBL mutation-negative patients (HBL/M-). The gene score using the 11 SNPs was significantly different between HBL/M- and controls ($p=0.043$), whereas no significant differences were found between HBL/M+ and controls ($p=0.07$) or between HBL/M- and HBL/M+ ($p=0.618$) (Figure 1). Six of the HBL/M- subjects (29%) presented a weighted LDLc gene score below the 10th percentile (0.6088) of the control population. The results for the LDLc scores of all patients are presented in Tables 1 and 2.

DISCUSSION

The present study presents the outcomes of our molecular diagnosis of hypolipidemic patients in a clinical laboratory setting. Although the study actually resulted from an unprogrammed clinical practice setting, it constitutes the largest experience published in relation to the genetic diagnosis of primary HBL in Spain. The most recent genetic studies in Spanish patients presenting with low LDLc described the clinical and genetic heterogeneity of a total of five patients with FHBL^{33, 34}. The benefits of knowing the patient genotype include identification of additional family members as well as appropriate clinical follow-up and treatment, which includes adherence to a low-fat diet and supplementation with essential fatty acids and fat soluble vitamins. Out of 44 referred patients, 23 were genetically confirmed for the studied genes as affected by monogenic causes of primary HBL phenotype. Among them, the most prevalent mutated genes found in our study were *APOB* (17 of 23 patients), *SAR1B*, *ANGPTL3*, and *MTTP*, with substantially fewer mutations found in these other genes. Indeed, this should be the recommended sequence of actions with which to screen for primary HBL in our media in case that Sanger sequencing method is used. Despite their important role in lipid metabolism, Sanger sequencing did not allow identifying pathogenic variants in *PCSK9*, *APOC3*, *MYLIP*, or *DGAT1*. The inclusion of relatives in the study revealed seven further cases that were genetically confirmed. Interestingly, two of the relatives with *APOB* mutations did not present any sign of disease, demonstrating that screening for mutations in relatives should be strongly recommended because some cases might be underdiagnosed. Thus, early diagnosis is important for preventing or minimizing clinical sequelae.

There was a clear overlap between clinical and biochemical data and the affected genes involved in primary HBL. The latter further revealed that it may not be a feasible strategy to predict whether or not a patient will present with a monogenic detectable mutation by means of clinical and biochemical phenotype. However, steatosis or liposoluble vitamin deficiency would rule out mutations in *ANGPTL3* as a monogenic cause of HBL². Additionally, the

pattern of Mendelian inheritance in a family may direct the analysis towards *APOB* (co-dominant) or *ANGPTL3*, *SAR1B* and *MTTP* (recessive).

Because most of our HBL/M+ patients presented with *APOB* mutations, it is worth recalling that the clinical symptoms could include hepatic steatosis, liposoluble vitamin deficiency, diarrhea, growth failure, cognitive impairment, and multineuritis. Previously published data described *APOB* heterozygous mutation carriers individuals as asymptomatic, or have increased susceptibility to diseases that may go underdiagnosed for producing few symptoms, such as liver steatosis¹. The symptomatology observed in some of our patients may be a consequence of a decreased intestinal capacity for chylomicron synthesis/secretion (INTRODUIR REFERÈNCIA: Hooper AJ et al. J Clin Endocrinol Metab 2007; 92: 1474-1478) which is largely dependent on the conservation of the intestinal ApoB-48 sequence,. Protein truncation will always affect the complete liver-specific ApoB-100, indicating that the *APOB* gene will not form the full protein needed for VLDL, intermediate-density lipoprotein (IDL), LDL and Lipoprotein (a) structure and function³⁵.

There were 21 index patients which could not be genetically diagnosed. It is well known that up to 50% of interindividual variation in plasma LDLc is due to genetic variation by an accumulation of small-effect LDLc-impairing alleles^{11, 12}. In that sense, we observed a statistically significantly different frequency distribution of three *APOB* common genetic variants associated with LDLc concentration when comparing 503 subjects from the 1000 Genome Project with negative patients (HBL/M-) (Table 3). Two of the *APOB* genetic variants could be contributing to the observed phenotype, because the allele associated with decreased levels of LDLc was more frequent in HBL/M- than in the control population. These findings led us to test the recently proposed LDLc gene score to distinguish polygenic and monogenic forms of familial hypercholesterolemia^{11, 12} and primary HBL³⁶ through a panel of SNPs that are known to influence LDLc levels. The primary HBL group without known mutation (HBL/M-) had a significantly lower mean weighted LDLc gene score when compared to the European 1000 Genome Project participants suggests that some HBL/M-

patients may present a polygenic inheritance pattern. It is noteworthy that a recent work carried out with 121 women with LDLc below the first percentile, around 50% of the cases with primary HBL could be explained by polygenic predisposition³⁶. Recent studies showed a polygenic basis for some cases previously identified as monogenic familial hypercholesterolemia^{11, 12}, we did not find differences in the weighted LDLc gene of HBL/M+ and HBL/M-, suggesting that polygenicity influences phenotype expression in both forms of the disease.

This study has several limitations mainly due to the retrospective manner in which it was conducted from a clinical laboratory. One is the heterogeneous degree of clinical information available according to that supplied by the clinician who requested the molecular analysis. The requests for molecular analyses were accepted although the information received was sometimes incomplete. Despite this, the results (identification of pathogenic gene variants in 52% of the cases) are similar or better to that obtained by us and others in the case of Familial Hypercholesterolemia (Martín-Campos JM et al. [Autosomal dominant hypercholesterolemia in Catalonia: Correspondence between clinical-biochemical and genetic diagnostics in 967 patients studied in a multicenter clinical setting](#). J Clin Lipidol 2018; in press), which argues in favor of a solid suspicion of primary hypobetalipoproteinemia by the practitioners that requested the genetic study. Another limitation is that large rearrangements in the studied genes were not evaluated. According to HGMD, large rearrangements in these genes are scarce. Lastly, the idea of finding negative patients harboring these or other pathogenic variants only detectable through whole exome or genome sequencing should not be ruled out since this study does not exclude the possibility that other pathogenic and low-frequency mutations in other genes which could also cause primary HBL.

In conclusion, our report expands the existing molecular information relating to primary HBL, confirming the heterogenic nature and presentation of this syndrome. Although this condition has been considered a monogenic disorder, our findings support the idea that some cases may also have a polygenic origin as recently suggested³⁶. The concept of polygenicity,

demonstrated previously in other dyslipidemias^{11, 12, 37, 38}, should be further examined in patients with clinical and biochemical data of primary HBL without monogenic disorders.

Conflicts of interest

None

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Author contributions

FBV, JMMC, JJ and MT devised the study. JMMC and MT did the data analysis. ABV, MC, SM, RR, and NF contributed to the genotyping. FBV, JMMC and MT interpreted the data and wrote the manuscript. JJ also discussed the results and contributed to the final version of the manuscript.

Figure 1.

Weighted LDLc score as mean (SD) was 0.8868 (0.212) for EUR controls, 0.8033 (0.217) for HBL/M+ patients, and 0.7366 (0.327) for HBL/M- patients. The significance of gene score between HBL/M- and controls (**A**), HBL/M+ and controls (**B**), and HBL/M- and HBL/M+ (**C**) was $p = 0.043$, $p = 0.07$, and $p = 0.618$, respectively.

Table 1. Genetic data from patients affected with one or two pathogenic variants potentially causing primary HBL.

Patient	Gene	DNA (Pathogenic Variants) ^a	Protein ^b	ApoB %	ID ^c	Proband ^d	LDLc score
8	<i>APOB</i>	c.3G>C	p.Met1Ile		new	Hz	0.972
		c.6630_6631delAA	p.Leu2212*	ApoB-48	new	Hz	
7	<i>APOB</i>	c.133C>T	p.Arg45*	ApoB-0	new	Hz	1.007
13	<i>APOB</i>	c.1124G>A	p.Ser375Lysfs*93	ApoB-10	CS072142 ³⁹	Hz	0.700
12	<i>APOB</i>	c.1315C>T	p.Arg439*	ApoB-9	CM021487 ⁴⁰	Hz	0.783
1	<i>APOB</i>	c.1407C>A	p.Tyr469*	ApoB-10	new	Hz	0.813
9	<i>APOB</i>	c.1407C>A	p.Tyr469*	ApoB-10	new	Hz	1.044
2	<i>APOB</i>	c.2726C>A	p.Ser909*	ApoB-19	new	Hz	0.576
4	<i>APOB</i>	c.2726C>A	p.Ser909*	ApoB-19	new	Hm	0.707
14	<i>APOB</i>	c.2726C>A	p.Ser909*	ApoB-19	new	Hz	0.130
11	<i>APOB</i>	c.2781delT	p.Ser928Profs*25	ApoB-20	new	Hz	0.881
5	<i>APOB</i>	c.3843-2A>G	NA		CS072139 ⁴¹	Hz	0.958
15	<i>APOB</i>	c.3997C>T	p.Arg1333*	ApoB-29	rs121918383/CM880006 ⁴²	Hz	0.946
10	<i>APOB</i>	c.6034C>T	p.Arg2012*	ApoB-44	rs147863759/CM962634 ⁴³	Hz	0.655

3	<i>APOB</i>	c.7600C>T	p.Arg2534*	ApoB-55	CM051005 ⁴⁴	Hz	0.915
17	<i>APOB</i>	c.7600C>T	P.Arg2534*	ApoB-55	CM051005 ⁴⁴	Hz	0.655
16	<i>APOB</i>	c.10430_10440delTTGACCACAAG	p.val3477Alafs*2	ApoB-76	new	Hz	0.835
6	<i>APOB</i>	c.11728G>T	p.Glu3910*	ApoB-86	new	Hz	0.546
20	<i>SAR1B</i>	c.1-4482_58+1406del5946InsGCATGATGGCGGGT	NA	-	CG081079 ²⁹	Hm	0.965
18	<i>SAR1B</i>	c.83_84delTG	p.Leu28Argfs*7	-	new	Hm	0.566
19	<i>SAR1B</i>	c.409G>A	p.Asp137Asn	-	rs28942109/CM035597 ³⁰	Hm	0.903
21	<i>ANGPTL3</i>	c.363_367delCTCAA	p.Asn121Leufs*3	-	rs398122988/CD1110133 ³¹	Hm	1.070
22	<i>ANGPTL3</i>	c.363_367delCTCAA	p.Asn121Leufs*3	-	rs398122988/CD1110133 ³¹	Hm	1.018
23	<i>MTTP</i>	c.1691T>C	p.Ile564Thr	-	CM068327 ³²	Hm	0.835

HL, hypobetalipoproteinemia. NA: not applicable; ^a, positions are given according to the reference sequences: NM_000384 (*APOB*), NM_016103 (*SAR1B*), NM_014495 (*ANGPTL3*), and NM_000253 (*MTTP*). ^b, positions are given according to the UniProt sequences: P04114 (*APOB*), Q9Y6B6 (*SAR1B*), Q9Y5C1 (*ANGPTL3*), and P55157 (*MTTP*). ^c, in dbSNP (rs) and/or Human Gene Mutation Database (HGMD). ^d, Hm, homozygous; Hz, heterozygous.

Table 2. Variants of unknown significance associated to genetically and non-genetically diagnosed patients.

Patient	Gene	DNA ^a	Protein ^b	ID ^c	Associated to ^d	Proband ^e	LDLc score
1	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.813
		c.11248C>T	p.His3750Tyr	new		Hz	
2	<i>APOB</i>	c.293C>T	p.Thr98Ile	rs1367117/CM020623	Cholesterol ^{46, 47}	Hz	0.576
3	<i>APOB</i>	c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	0.915
5	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.958
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
6	<i>APOB</i>	c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	0.546
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
7	<i>APOB</i>	c.293C>T	p.Thr98Ile	rs1367117/CM020623	Cholesterol ^{46, 47}	Hz	1.007
		c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
8	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hm	0.972
		c.1254G>A	p.Glu4181Lys	rs1042031/CM068651	Ischemia and stroke ^{51, 52}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	

9	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	1.044
		c.3740A>G	p.Tyr1247Cys	rs61741164	Possibly Damaging	Hz	
		c.11245C>T	p.His3750Tyr	new		Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	
		c.13451C>T	p.Thr4484Met	rs12713450/CM104784	Hypertriglyceridemia ⁵³	Hz	
15	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.946
16	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ^{45,7}	Hz	0.835
		c.11248C>T	p.His3750Tyr	new		Hz	
17	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.655
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
19	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.903
21	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	1.070
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	
22	<i>APOB</i>	c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	1.018
23	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.835
24	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	1.138
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	
	<i>MYLIP</i>	c.1025A>G	p.Asn342Ser	rs9370867/CM116474	High degrad. of LDLR ⁵⁶	Hz	
					No effect ^{57, 58}		
	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hm	

25	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.450
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338 Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>PCSK9</i>	c.43_44insCTG	p.Leu23_Gly24insLeu	rs35574083/CI102605/CE062370	Low LDLc ^{60, 61}	Hz	
		c.137G>T	p.Arg46Leu	rs11591147/CM061168	Low LDLc, ↓risk CHD ⁶²	Hz	
		c.158C>T	p.Ala53Val	rs115836807CM102603	Hypocholesterolemia ⁶⁰	Hz	
		c.1420G>A	p.Val474Ile	rs562556/CM045990	LDLc ^{54, 55}	Hz	
	<i>MTTP</i>	c-101-478G>T	Not applicable	rs1800591/CR982417	Low LDLc, SNAFLD ^{63, 64}	Hz	0.819
		c.891G>C	p.Gln297His	rs2306985/CM1510847	Low LDLc ⁶³	Hz	
		c.383T>C	p.Ile128Thr	rs3816873		Hz	
	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hm	
26	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hm	0.837
27	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM 984191	ApoB ⁴⁵	Hz	
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338 Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>PCSK9</i>	c.43_44insCTG	p.Leu23_Gly24insLeu	rs35574083/CI102605/CE062370	Low LDLc ^{60, 61}	Hz	
		c.158C>T	p.Ala53Val	rs11583680/CM102603	Hypocholesterolemia ⁶⁰	Hz	

		c.1420G>A	p.Val474Ile	rs562556/CM045990	LDLc ^{54, 55}	Hz	
28	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.281
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	
	<i>MTTP</i>	c.891G>C	p.Gln297His	rs2306985/CM1510847	Low LDLc ⁶³	Hz	
		c.1151A>C	p.Asp384Ala	rs17029215/CM057721		Hz	
29	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.867
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>APOC3</i>	c.102T>C	p.Gly34=	rs4520/CM942309	↓ plasma TG ⁶⁵	Hz	
		c.*40G>C	NA	rs5128/041556	↑TG concentrations ⁵⁹	Hz	
		c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hz	
30	<i>APOB</i>	c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	-
	<i>PCSK9</i>	c.1420G>A	p.Val474Ile	rs562556/CM045990	LDLc ^{54, 55}	Hz	
31	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.935
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338 Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>MTTP</i>	c.891G>C	p.Gln297His	rs2306985/CM1510847	Low LDLc ⁶³	Hm	
32	<i>APOB</i>	c.3383G>A	p.Arg1128His	rs12713843/CM040371	“Recessive” HBL ⁶⁶	Hz	1.136
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	

	<i>PCSK9</i>	c.43_44insCTG	p.Leu23_Gly24insLeu	rs35574083/C1102605/CE062370	Low LDLc ^{60, 61}	Hz	
		c.137G>T	p.Arg46Leu	rs11591147/CM061168	Low LDLc, ↓risk CHD ⁶²	Hz	
		c.158C>T	p.Ala53Val	rs11583680/CM102603	Hypocholesterolemia ⁶⁰	Hz	
		c.1420G>A	p.Val474Ile	rs562556/CM045990	LDLc ^{54, 55}	Hz	
	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hm	
	<i>MTTP</i>	c.891G>C	p.Gln297His	rs2306985/CM1510847	Low LDLc ⁶³	Hz	
		c.1151A>C	p.Asp384Ala	rs17029215/CM057721		Hz	
33	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	1.018
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338 Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>PCSK9</i>	c.43_44insCTG	p.Leu23_Gly24insLeu	rs35574083/C1102605/CE062370	Low LDLc ^{60, 61}	Hz	
		c.158C>T	p.Ala53Val	rs11583680/CM102603	Hypocholesterolemia ⁶⁰	Hz	
	<i>APOC3</i>	c.102T>C	p.Gly34=	rs4520 /CM942309	↓ plasma TG ⁶⁵	Hz	
		c.*40G>C	NA	rs5128/041556	↑TG concentrations ⁶⁷	Hz	
		c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hz	
34	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hz	0.963
35	<i>APOB</i>	c.2188G>A	p.Val730Ile	rs12691202/CM981492	Impairment of HBL ⁶⁸	Hz	0.573
	<i>APOC3</i>	c.102T>C	p.Gly34=	rs4520/CM942309	↓ plasma TG ⁶⁵	Hm	
36	<i>APOC3</i>	c.102T>C	p.Gly34=	rs4520/CM942309	↓ plasma TG ⁶⁵	Hz	1.023
		c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hz	

37	<i>APOB</i>	c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	0.776
	<i>APOC3</i>	c.*71G>T	NA	rs4225/R1611071	↓TG concentration ⁵⁹	Hz	
38	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	-0.187
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
	<i>APOC3</i>	c.*71G>T	NA	rs4225/CR1611071	↓TG concentration ⁵⁹	Hz	
39	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hz	0.651
	<i>APOC3</i>	c.102T>C	p.Gly34=	rs4520/CM942309	↓ plasma TG ⁶⁵	Hm	
40	<i>APOB</i>	c.3427C>T	p.Pro1143Ser	rs72653077/CM087422	Hypocholesterolemia ³⁵	Hz	0.506
41	<i>APOB</i>	c.1853C>T	p.Ala618Val	rs679899/CM984191	ApoB ⁴⁵	Hm	0.831
		c.8216C>T	p.Pro2739Leu	rs676210/CM980093	Hypocholesterolemia ^{49, 50}	Hz	
		c.1254G>A	p.Glu4181Lys	rs1042031/CM068651	Ischemia and stroke ^{51, 52}	Hz	
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hz	
42	<i>APOB</i>	c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	1.027
		c.293C>T	p.Thr98Ile	rs1367117/CM020623	Cholesterol ^{46, 47}	Hz	
43	<i>APOB</i>	c.293C>T	p.Thr98Ile	rs1367117/CM020623	Cholesterol ^{46, 47}	Hz	0.659
		c.13013G>A	p.Ser4338Asn	rs1042034/CM133911	Total cholesterol ⁴⁸	Hm	
		c.13451C>T	p.Thr4484Met	rs12713450/CM104784	Hypertriglyceridemia ⁵³	Hz	
44	<i>APOB</i>	c.293C>T	p.Thr98Ile	rs1367117/CM020623	Cholesterol ^{46, 47}	Hz	0.427

^a, positions are given according to the reference sequences: NM_000384 (APOB), NM_016103 (SAR1B), NM_014495 (ANGPTL3), NM_000253 (MTTP), NM_174936 (PCSK9), NM_013262 (MYLIP), and NM_000040 (APOC3). ^b, positions are given according to the UniProt sequences: P04114 (APOB), Q9Y6B6 (SAR1B), Q9Y5C1 (ANGPTL3), P55157 (MTTP), Q8NBP7 (PCSK9), Q8WY64 (MYLIP), and P02656 (APOC3). ^c, in dbSNP (rs) and/or Human Gene Mutation Database (HGMD). ^d, CHD: Coronary heart disease; SNAFLD: Susceptibility to non alcoholic fatty liver disease. ^e, Hm, homozygous; Hz, heterozygous.

Table 3. APOB common variants associated with plasma LDLc concentrations.

SNP ID	HGMD ^a	Location ^b	DNA ^c	Protein ^d	<i>p</i> ^e	Freq ^g		Freq ^h	Impact ⁱ	Teslovich et al. (2010) ^j
						MA ^f	EUR	HBL/M-		
rs1367117	CM020623	exon 4	c.293C>T	p.Thr98Ile	0.00052	T	0.298	0.028	moderate	Allele T associated with high LDLc (p=4.5E-18)
rs1042031	CM068651	exon 29	c.12541G>A	p.Glu4181Lys	0.021	A	0.213	0.028	moderate	Allele A associated with low LDLc (p=1.83E-20)
rs1042034	CM133911	exon 29	c.13013G>A	p.Ser4338Asn	0.0014	G	0.218	0.417	moderate	Allele A associated with high LDLc (p=8.32E-22)

^a Identification in Human Gene Mutation Database. ^b According to reference sequence NM_000384. ^c According to UniProt sequence P04114. ^d Affected protein position. ^e Signification in Fisher's exact test. ^f Minor allele (MA). ^g Frequency in the European population of the 1000 Genomes database. ^h Frequency in hypobetalipoproteinemia mutation negative patients (HBL/M-). ⁱ Result of the in silico analysis

regarding the impact on the functionality of the protein. ⁱ Association in the GWAS of Teslovich ²⁷.

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