

## Differences in Clinical Presentation Between Subjects With a Phenotype of Familial Hypercholesterolemia Determined by Defects in the LDL-Receptor and Defects in Apo B-100

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**Introduction and objectives.** Familial hypercholesterolemia and familial defective Apo B-100 are phenotypically indistinguishable. At present they can be distinguished by genetic analysis.

**Patientes and method.** We compared the clinical features of 13 subjects with familial defective Apo B-100 and 39 subjects with familial hypercholesterolemia. We used data from first degree relatives to compare morbidity and mortality between the two groups.

**Results.** We found statistically significant differences in total cholesterol and LDL cholesterol, which were lower in the familial defective Apo B-100 group (TC =  $357 \pm 37.3$  mg/dl vs  $415 \pm 79.7$  mg/dl and LDLc =  $270 \pm 34.2$  mg/dl vs  $355 \pm 72.4$  mg/dl). We found no differences in xanthomas, corneal arcus, smoking status, vascular events, blood pressure, BMI or waist/hip ratio. There were no differences between the two groups in the proportions of patients with cardiovascular disease or patients who died. We found statistically significant differences between the groups ( $p = 0.023$ ) in the mean age at first vascular event (familial hypercholesterolemia and first degree relatives:  $45.3 \pm 19.9$  years; familial defective Apo B-100 and first degree relatives:  $51.5 \pm 20.8$  years)

**Conclusions.** We conclude that familial defective Apo B-100 results in clinically milder hypercholesterolemia than familial hypercholesterolemia, and that discerning between them could be helpful to stratify the risk in persons with hereditary hypercholesterolemia.

**Key words:** *Hypercholesterolemia. Low density lipoproteins. Genetics.*

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### Diferencias en la presentación clínica en sujetos con fenotipo de hipercolesterolemia familiar por defectos en el receptor LDL y por defectos de la apo B-100

**Introducción y objetivos.** La hipercolesterolemia familiar y la apo B-100 defectuosa familiar resultan fenotípicamente indistinguibles. Hoy día es posible diferenciarlas mediante la realización de un análisis genético.

**Pacientes y método.** Comparamos las características clínicas de 13 sujetos con apo B-100 defectuosa familiar y 39 sujetos con hipercolesterolemia familiar. Para comparar la morbimortalidad de ambos grupos utilizamos datos de sus familiares de primer grado.

**Resultados.** Observamos diferencias significativas en los valores de colesterol total (CT) y colesterol unido a lipoproteínas de baja densidad (cLDL) de los sujetos afectados, que fueron menores en el grupo de apo B 100 defectuosa familiar (CT  $357 \pm 37,3$  frente a  $415 \pm 79,7$  mg/dl; y cLDL  $270 \pm 34,2$  frente a  $355 \pm 72,4$  mg/dl). No observamos diferencias en cuanto a la presencia de xanthomas, arco corneal, hábito tabáquico, episodio vascular, presión arterial, índice de masa corporal (IMC) e índice cintura/cadera.

No hubo diferencias significativas en cuanto a las proporciones de fallecidos y afectados de enfermedad cardiovascular de uno y otro grupo.

La diferencia de medias de edad alcanzada sin enfermedad cardiovascular ( $45,3 \pm 19,9$  años en la hipercolesterolemia familiar y familiares, y  $51,5 \pm 20,8$  años en la apo B100 defectuosa familiar y familiares) resultó significativa ( $p = 0,023$ ).

**Conclusiones.** La apo B-100 defectuosa familiar produce una hipercolesterolemia clínicamente más benigna que la hipercolesterolemia familiar, por lo que su diferenciación puede ayudar a estratificar el riesgo en los sujetos con estas hipercolesterolemias hereditarias.

**Palabras clave:** *Hipercolesterolemia. Lipoproteínas de baja densidad. Genética.*

## ABBREVIATIONS

FDB: familiar defective Apo B-100.  
 HDL: high density lipoproteins.  
 FH: familial hypercholesterolemia.  
 LDL: low density lipoproteins.  
 PCR: polymerase chain reaction.

## INTRODUCTION

Monogenic hypercholesterolemia is a lipid metabolism disorder characterized by an increase in cholesterol attached to low density lipoproteins (LDL-C), autosomal dominant transmission and a high incidence of premature coronary heart disease.<sup>1</sup> The two best known genes implicated in such disorders are the LDL receptor gene and the apolipoprotein B-100 gene. Mutations in these genes give rise to two types of disease known as familial hypercholesterolemia (FH) and familial defective Apo B-100 (FDB), respectively.

Familial hypercholesterolemia is an autosomal codominant disease caused by defects in the cell surface receptor that recognizes and internalizes the low density lipoproteins (LDL) in plasma. In 1938, Müller first described the disease as a hereditary error in metabolism that leads to the presence of tendinous xanthomas, elevations in plasma cholesterol, and acute myocardial infarction in young patients.<sup>2</sup> Later, Kachadurian defined the clinical and genetic characteristics of the disease and differentiated the heterozygous from the homozygous form in affected Lebanese families.<sup>3</sup> Goldstein and Brown<sup>4</sup> characterized the LDL receptor and related FH to defects in this protein. Finally, in 1983, the DNA of the gene was cloned.<sup>5</sup> The frequency of heterozygotes is estimated to be 1/500 in most populations (European, North American and Japanese) while the frequency of homozygotes is 1/1 000 000.<sup>6</sup>

Familial defective Apo B-100 was first described in 1989 with the identification of a mutation at codon 3500 of the Apo B gene that substituted arginine by glutamine (R3500Q).<sup>7</sup> Mutations around codon 3500 affect the Apo B-100 domain binding to the LDL-R, impeding recognition and binding of Apo B-100 to its receptor in turn causing an increase in the plasma values of LDL.

The phenotype of this disease is similar to that presented by subjects heterozygous for FH, that is, high concentrations of LDL-C, xanthomas, corneal arcus and premature ischemic heart disease, making it hard to differentiate by phenotype between individuals with FH and those with FDB. The prevalence of FDB

is estimated at between 1/300 and 1/700 in central European populations.<sup>8,9</sup> In Spain, the prevalence of FDB has not been established and might vary from region to region, but published data<sup>10,11</sup> suggests a lower prevalence than in other European countries.

Genetic diagnosis has allowed a more accurate comparison between FH and FDB phenotypes. Recently, differences have been found between the two types of hypercholesterolemia, observing a milder phenotype in individuals with FDB,<sup>12,13</sup> both for lipid concentrations and for manifestations of cardiovascular disease.

The objective of the present work is to investigate whether there are differences in the incidence of cardiovascular complications and lipid profile in a sample of subjects with FH and FDB for whom a genetic diagnosis of the defect responsible is available.

## PATIENTS AND METHODS

### Reference population

The Patient Register of the Spanish Familial Hypercholesterolemia Foundation was used to select the sample. This register includes patients from 69 clinical units throughout Spain. To be included, a patient should meet the clinical criteria of certainty of familial hypercholesterolemia proposed by the MED PED (Make Early Diagnosis Prevent Early Death) program of the World Health Organization<sup>14</sup> (Tables 1 and 2). A questionnaire was sent to the central laboratory that included the clinical data from the history and examination of the patient along with blood samples (sent in refrigerated conditions in less

TABLE 1. MED PED score for the diagnosis of familial hypercholesterolemia

Criteria	Points
LDL-C values	
>330 mg/dL	8
250-330 mg/dL	5
190-250 mg/dL	3
150-190 mg/dL	1
Personal history	
Premature coronary heart disease	2
Premature cerebral or peripheral vascular disease	1
Family history	
First degree relation with premature coronary heart disease	1
First degree relation with LDL-C>95 percentile	1
Children <18 years with LDL-C>95 percentile	2
Physical examination	
Presence of xanthomas	6
Presence of corneal arcus (<45 years)	4
Genetic tests	
Mutation of the LDL receptor gene	8

LDL-C indicates cholesterol bound to low density lipoproteins.

**TABLE 2. MED PED classification criteria for the diagnosis of familial hypercholesterolemia**

Total score obtained	Heterozygous FH
>8 points	Definite
6-8 points	Probable
3-5 points	Possible

than 24 hours). Before starting the study, three meetings were held with participant physicians in order to standardize the data, including family history, obtained from the different clinical units. The lipid units and clinical characteristics of all patients with FH referred to these units, as well as manifestations of cardiovascular disease have been published previously.<sup>15</sup>

### Lipid analysis

The serum concentrations of total cholesterol and triglycerides were determined by enzymatic methods with colorimetric detection. Analysis of HDL was performed on the supernatant after precipitation of lipoproteins that contained Apo B with dextran sulfate. We used the Friedewald formula to calculate LDL-C.

### DNA extraction

Genomic DNA was extracted from 10 mL blood samples using commercially available Puregene equipment (Isolation kit, Gentra Systems, MN, US). The DNA was diluted in a solution of Tris-HCl 10 mmol/L, EDTA 0.1 mmol/L buffer at pH 8.0 to a final concentration of 100 µg/mL.

### Analysis of the R3500Q mutation

A fragment of 143 base pairs from exon 26 of the apolipoprotein B gene, which included codon 3500, was amplified by polymerase chain reaction (PCR) using the following primers: APOB3500D:5'CTTACTTTTCCATTGAGTACTCTACC-3' and APOB3500R:5'AGTGCCCTGCAGCTTCACTGAGTTAC-3'. Each primer had two unpaired bases (underlined). We could thus introduce a new recognition site for the ScaI restriction enzyme at the 5'-end and to create a new recognition site at the 3'-end only where the mutant allele was present. The restriction site created by the ApoB3500D primer was used as a control for the restriction enzyme.

The PCR was performed in a final volume of 50 µL containing 500 ng of DNA, 20 mmol/L of Tris-HCl (pH 8.4), 50 mmol/L of KCl and 1.5 mmol/L of MgCl<sub>2</sub>. The remaining components were added: 0.4 µmol/L of each primer, 0.2 mmol/L of each dNTP and

1.5 units of Taq DNA polymerase (Gibco BRL). An initial cycle was performed with a two minute denaturing phase at 94 °C, a one minute hybridization phase at 55 °C, and a one minute extension phase at 72 °C. Next, a further 29 cycles like the one described were performed: denaturing (one minute at 94 °C), hybridization for one minute at 55 °C and extension (one minute at 72 °C). At the end, an extension step of five minutes was performed at 72 °C.

A total of 15 µL of each sample amplified by PCR was digested with 15 units of ScaI (Amersham Pharmacia Biotech) in a volume of 30 µL in accordance with the instructions from the manufacturer. Then, the fragments obtained were submitted to electrophoresis on NuSieve agarose gel (NuSieve GTG, Molecular applications, Rockland, ME, US) for 75 minutes at a constant voltage of 90 V. Normal alleles produce fragments of 125 and 18 base pairs whereas heterozygous mutant alleles produce fragments of 125, 102 and 18 base pairs.

### Sample selection

The R3500Q mutation was found in 967 unrelated subjects with clinical diagnosis of definite monogenic hypercholesterolemia according to the clinical criteria of the MED PED program. Written informed consent was obtained from all subjects participating in the study.

To compare FDB subjects with FH subjects, three FH subjects were selected for every FDB subject through application of the following criteria. Subjects were to be of the same sex and within an age range of five years. Whenever possible, a subject from the same region of Spain was chosen. When more than one subject was available, those from the same province were chosen, and if there were still several subjects to choose from, the one closest in age was selected. When no subject met any of these three conditions, the age difference was extended to six years. When several subjects met the same conditions, the choice was made at random.

### Data on first degree relatives

Participants completed a questionnaire on morbidity and mortality of relatives in a clinical interview to extend the information available for the study of morbidity and mortality of the groups. The questionnaire covered all first-degree relatives, asking whether they were alive or dead, their age and whether they had a history of acute myocardial infarction, aortocoronary bypass, coronary angioplasty and stroke (including the age at which it occurred, if known). The 13 patients with FDB had a total of 67 first-degree relatives and the 39 patients with FH had a total of 181 first-degree relatives. In all cases, we could determine

TABLE 3. . Comparison of quantitative variables studied in FH and FDB subjects

	P	FDB			FH		
		No.	Mean	Standard deviation	No.	Mean	Standard deviation
SBP, mm Hg	.722	13	126	14.8	39	128	17.4
DBP, mm Hg	.488	13	75	9.96	39	78	12.0
BMI, kg/m <sup>2</sup>	.673	13	25.9	3.18	39	26.4	4.48
Hip/waist ratio	.599	12	0.830	0.108	37	0.846	0.084
Total cholesterol, mg/dL	.020	12	357	37.3	39	415	79.7
Triglycerides, mg/dL	.174	11	101	33.0	36	130	66.1
HDL-C, mg/dL	.066	10	63.7	22.5	34	51.9	15.5
LDL-C, mg/dL	.001	10	270	34.2	33	355	72.4

SBP indicates systolic blood pressure; DBP, diastolic blood pressure; BMI, body mass index or Quetelet index (weight in kg/height in m<sup>2</sup>); P, probability that the differences found are due to chance in comparisons of quantitative variables with Student's *t* test and qualitative variables with the  $\chi^2$  test.

TABLE 4. Qualitative variables: relative frequencies and P value in the  $\chi^2$  test to compare FDB and FH

	FDB	FH	P
Xanthomas	2/13	11/39	.35
Corneal arcus	4/12	16/39	.55
Tobacco	5/13	13/39	.66
Vascular events	1/13	3/39	>.99

FDB indicates familial defective Apo B-100; FH, familial hypercholesterolemia. Vascular events: all individuals whose questionnaire showed a history of acute myocardial infarction, coronary angina, coronary revascularization surgery, coronary angioplasty, intermittent claudication or stroke were considered to have had a vascular event.

P indicates probability that the differences found are due to chance, in comparisons of quantitative variables with Student's *t* test and qualitative variables with the  $\chi^2$  test.

whether the first-degree relation was alive or not. The age was recorded for 60 of the 67 first-degree relatives in the FDB group and for 172 of the 181 first-degree relatives in the FH group. Cardiovascular events were reported in seven of the 67 first-degree relatives of the FDB group and in 25 of the 181 first-degree relatives in the FH group.

### Statistical analysis

For the statistical analysis of the data, the Stat View 5.0 computer program was used. The Student *t* test was used to compare unpaired quantitative variables, and for dichotomized qualitative variables, the Fisher and  $\chi^2$  tests were used. The lipid concentrations used in the statistical analysis correspond to measurements with no lipid-lowering treatment. A vascular event was defined as the presence of acute myocardial infarction, coronary angina, coronary revascularization surgery, coronary angioplasty, intermittent claudication or stroke.

## RESULTS

A total of 13 heterozygous carriers of the R3500Q mutation were found. We selected 39 subjects with FH according to the concordance criteria described above.

The main clinical characteristics of the subjects with FH and FDB are described in Tables 3 and 4. There were significantly lower concentrations of total cholesterol and LDL-c in subjects with FDB compared to those with FH. Higher concentrations of HDL-C were observed in subjects with FDB, though the difference did not reach statistical significance. Prevalence of other phenotypic characteristics and other cardiovascular risk factors showed no differences between the two populations.

Data from first-degree relatives were used, 67 from FDB and 181 from FH, comparing the morbidity/mortality of both groups.

For the familial data, the mean age was greater in relatives of subjects in the FDB group (51.5 years) than in the FH group (46.1 years), but this difference was not significant ( $P=.051$ ). However, the difference in mean age until a vascular event was significant ( $P=.023$ ), with subjects with FH and relatives reaching 45.3 years before a vascular event compared to 51.5 years in FDB subjects and relatives. The proportions of deaths (17/67 FDB and 39/181 FH) and those affected by cardiovascular disease (7/67 BDF and 25/181 HF) did not differ significantly ( $P=.52$  and  $P=.48$ , respectively) between the two groups.

## DISCUSSION

Our results show a milder hypercholesterolemia in the group with FDB than in the group with mutation of the LDL-R gene. Given the study design, we should limit our conclusions to the comparison between FH and FDB without extrapolating to the description of cardiovascular risk of these two types of hypercholesterolemia in Spain. However, the lipid

profile is similar to the one published in other studies of FH in Spain.<sup>16,17</sup>

The observations reflected in the literature undoubtedly show that the FDB mutation gives rise to a clinical syndrome indistinguishable from classical heterozygous FH in the presence of suitable environmental and genetic conditions.<sup>18</sup> But its lower prevalence in the general population prevents it from being clearly associated with coronary events, as illustrated by the work of Brousseau et al<sup>19</sup>, who screened for FDB in 622 cases of myocardial infarction and 639 control subjects, without finding any differences between the two groups.

Several studies have been performed that show an apparently milder phenotype in FDB, but the results were not statistically significant because of the small sample sizes.<sup>12,13,20,21</sup> To increase the sample size, our study included first-degree relatives of the index cases. We must remember that patients in this sample are relatively young (mean age 45 years) and that the data requested relate to immediate kin (parents, sons and daughters and brothers and sisters). Therefore we would expect the participants would know a great deal about the outcomes and variables used in this analysis (death, age, vascular events), though we may underestimate the true proportions, particularly for morbidity. Furthermore, only half these relatives would be carriers of the mutation, thus the effects of the disease would be diluted by the other half who are non-carriers. Even so, other studies have resorted to a similar way of increasing the sample size. Kotze et al<sup>22</sup> screened for the Apo B 3500 mutation in type IIa and IIb hyperlipemic subjects, recruiting 21 first- and second-degree relatives. They concluded that cholesterol values may be lower in FDB compared to FH. The differences between the two types of hypercholesterolemia seem to have a certain dependence on age. Thus, Tybjaerg-Hansen and Humphries<sup>23</sup> produced curves of cumulative frequency of coronary disease according to age in subjects with FDB and FH, finding that, for both men and women, there is no difference in the risk of coronary disease until the age of 60 years. The results from the study by Maher et al in England are somewhat more similar to ours. These authors compared the clinical expression and coronary angiography of a group with FDB and another with FH, finding that patients with FDB developed symptoms of coronary heart disease later (50.3±8.8 years vs 44.9±11 years).<sup>24</sup> The age of presentation of coronary heart disease and the difference between FH and FDB is very similar to that observed in the present study (45.3 years in the FH group and 51.5 years in the FDB group) and to results from other authors in different countries.<sup>24,21</sup>

The type of FH can vary according to whether the

LDL-R mutation leads to a defective receptor or no functional receptor (receptor negative). A study conducted in Belgium compared patients with FDB and both types of mutation of FH, finding similar total cholesterol and LDL values for FDB and FH of the defective receptor type, and both had lower values of total cholesterol and LDL than patients with FH of the receptor-negative type.<sup>22</sup>

The incidence of coronary heart disease is mostly determined by the extent and duration of elevated cholesterol values, even when angiography of the coronary arteries is normal. The reduction in lipid concentrations improves endothelial dysfunction,<sup>25</sup> thus a lower concentration of LDL-C could explain the lower cardiovascular risk, though qualitative differences in LDL may also play a role.<sup>24,26</sup>

The milder phenotype is thought to cause compensating changes that would lead to an increase in capture of very low density lipoprotein remnants by the LDL receptor, but mediated by Apo E.<sup>13,27,28</sup> For example, there may be an upregulation of the activity of the LDL-R, particularly in younger subjects with BDF,<sup>29</sup> but these mechanisms would depend on age and older FDB subjects would lose their ability to compensate. Homozygotes with FDB retain 20% of their normal LDL binding capacity, reinforcing this hypothesis.<sup>28</sup> This binding capacity occurs in the large low density particle subfraction because Apo E is associated with these particles. These mechanisms would not operate in subjects with FH, who lack the active LDL receptor.

Our results, taken in the context of the international literature discussed above, suggest that genetic diagnosis of different types of hypercholesterolemia is important when evaluating the risk of vascular events because such a diagnosis identifies genetic defects of different seriousness. This could be useful in the management of the patients.

## CONCLUSIONS

Though subjects with FH and FDB are clinically indistinguishable on an individual level, and genetic analysis is required to tell them apart, these two conditions do have different lipid profiles and different ages for presentation of vascular events. The phenotype is more benign in FDB, particular in those under 70 years.

Our results support the idea that genetic analysis of the cause of hypercholesterolemia may help to stratify risk in subjects with these hereditary forms of hypercholesterolemia.

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