Original article

Clinical and genetic differences between heterozygous familial hypercholesterolemia patients with and without type 2 diabetes

Elisenda Climent,[a,b] Sofía Pérez-Calahorra,[c] David Benaiges,[a,b,d] Xavier Pintó,[e] Manuel Suárez-Tembra,[f] Nuria Plana,[g] Rosa M. Sánchez-Hernández,[h] Pedro Valdivielso,[i] Juan F. Ascaso,[j] and Juan Pedro-Botet[a,b,d,*]

[a] Servicio Endocrinología y Nutrición, Hospital del Mar, Barcelona, Spain
[b] Departamento de Medicina, Universitat Autònoma de Barcelona, Campus Universitari Mar, Barcelona, Spain
[c] Unidad Clínica e Investigación en Lípidos y Arteriosclerosis, Hospital Universitario Miguel Servet, IIS Aragón, CIBERCV, Zaragoza, Spain
[d] Institut Hospital del Mar d'Investigacions Mèdiques (IMIM), Barcelona, Spain
[e] Unidad de Lípidos y Riesgo Cardiovascular, Servicio de Medicina Interna, Hospital Universitario Bellvitge, L'Hospitalet de Llobregat, Barcelona, Spain
[f] Unidad de Lípidos y Riesgo Cardiovascular, Hospital San Rafael, A Coruña, Spain
[g] Unidad de Lípidos (UVASMET), Hospital San Joan de Reus, Tarragona, Spain
[h] Unidad de Lípidos, Hospital Universitario Insular, Las Palmas de Gran Canaria, Las Palmas, Spain
[i] Unidad de Lípidos, Hospital Universitario Virgen de la Victoria, Málaga, Spain
[j] Unidad de Lípidos, Hospital Clínico Universitario de Valencia, Valencia, Spain

A B S T R A C T

Introduction and objectives: The lower prevalence of type 2 diabetes mellitus (T2DM) in patients with heterozygous familial hypercholesterolemia (HeFH) could explain why T2DM has not always been identified as an independent predictor of cardiovascular disease (CVD) in different familial hypercholesterolemia cohort studies. The aim of the present study was to evaluate clinical and genetic aspects of HeFH patients with T2DM in the dyslipidemia registry of the Spanish Arteriosclerosis Society.
Methods: HeFH patients were classified according to the presence or absence of T2DM. The clinical, biochemical and genetic characteristics of the 2 groups were compared.
Results: Of the 2301 patients with primary hypercholesterolemia included in the registry, 1724 with a probable or definite diagnosis according to the Dutch Lipid Clinic Network score were finally included. HeFH patients with T2DM had a higher rate of CVD and a less favorable lipid profile, with higher total cholesterol (366.9 ± 86.7 mg/dL vs 342.0 ± 74.7 mg/dL; mean difference 24.894; 95%CI, 5.840-43.949) and non–high-density lipoprotein cholesterol (316.9 ± 87.8 mg/dL vs 286.4 ± 75.4 mg/dL; mean difference 30.500; 95%CI, 11.211-49.790) levels. No significant differences were found between the groups concerning the specific type of HeFH-causing mutation ($P = .720$). After adjustment for major risk factors, logistic regression analysis confirmed a relationship between T2DM and the presence of CVD (OR, 2.01; 95%CI, 1.18-3.43; $P = .010$).
Conclusions: HeFH patients with T2DM have a higher rate of CVD and a less favorable lipid profile, regardless of genetic mutation type. In these patients, T2DM is associated with the presence of CVD.

© 2019 Sociedad Española de Cardiología. Published by Elsevier España, S.L.U. All rights reserved.

Diferencias clínicas y genéticas de los pacientes con hipercolesterolemia familiar heterocigota con y sin diabetes mellitus tipo 2

R E S U M E N

Introducción y objetivos: La menor prevalencia de diabetes mellitus tipo 2 (DM2) en pacientes con hipercolesterolemia familiar heterocigota (HFHe) podría explicar por qué la DM2 no siempre se ha descrito como un predictor de enfermedad cardiovascular (ECV) en estos pacientes. El objetivo del presente estudio fue evaluar los aspectos clínicos y genéticos de pacientes con HFHe y DM2 del registro de dislipemias de la Sociedad Española de Arteriosclerosis.
Métodos: Los pacientes con HFHe se clasificaron según la presencia/ausencia de DM2. Se compararon las características clínicas, bioquímicas y genéticas de ambos grupos.
Resultados: De los 2.301 casos de hipercolesterolemia primaria del registro, se incluyeron 1.724 casos con el diagnóstico cierto o probable según la *Dutch Lipid Clinic Network* para la hipercolesterolemia familiar. Los pacientes con HFHe y DM2 presentaron una tasa más elevada de ECV y un perfil lipídico menos favorable, con niveles más elevados de colesterol total ($366,9 \pm 86,7$ mg/dl frente a $342,0 \pm 74,7$

* Corresponding author: Servicio Endocrinología y Nutrición, Hospital Universitario del Mar, Paseo Marítimo 25-29, 08003 Barcelona, Spain.
  E-mail address: 86620@parcdesalutmar.cat (J. Pedro-Botet).

mg/dl; diferencia media 24,894; IC95%, 5,840-43,949) y colesterol no-unido a lipoproteínas de alta densidad (316,9 ± 87,8 mg/dl frente a 286,4 ± 75,4 mg/dl; diferencia media 30,500; IC95%, 11,211-49,790). No se encontraron diferencias significativas entre los grupos con respecto al tipo de mutación (p = 0,720). Después de ajustar por los principales factores de riesgo, el análisis de regresión logística confirmó una relación entre la DM2 y la ECV (OR = 2,01; IC95%, 1,18-3,43; p = 0,010).

Conclusiones: Los pacientes con HFHe y DM2 presentan una tasa más elevada de ECV y un perfil lipídico menos favorable, independientemente del tipo de mutación. La diabetes mellitus es un factor asociado a la presencia de ECV en estos pacientes.

© 2019 Sociedad Española de Cardiología. Publicado por Elsevier España, S.L.U. Todos los derechos reservados.

Abbreviations

apo: apolipoprotein
CVD: cardiovascular disease
FH: familial hypercholesterolemia
HeFH: heterozygous familial hypercholesterolemia
LDLR: low-density lipoprotein receptor
T2DM: type 2 diabetes mellitus

INTRODUCTION

Heterozygous familial hypercholesterolemia (HeFH), caused by mutations in genes encoding for the low-density lipoprotein (LDL) receptor, apolipoprotein (apo) B, proprotein convertase subtilisin/kexin type-9 (PCSK9) or apo E,[1] leads to elevated LDL cholesterol levels, producing a higher incidence of cardiovascular events.[2] These patients are considered at high cardiovascular risk according to the European and American clinical guidelines.[3,4] Moreover, the recent guidelines of the American Association of Clinical Endocrinologists and the American College of Endocrinology consider established cardiovascular disease (CVD) and familial hypercholesterolemia (FH) or diabetes to constitute an extreme cardiovascular risk category and recommend an LDL cholesterol level of < 55 mg/dL as the therapeutic target.[5]

However, CVD seems to be highly variable in HeFH patients, even in those sharing the same pathogenic mutation.[6] In this respect, the International Atherosclerosis Society proposed certain criteria to define a phenotype of patients with severe HeFH[7] with a probable increased cardiovascular risk. Risk stratification in HeFH is an important issue; however, these proposed criteria do not seem to solve this problem.[8,9] Therefore, it is essential to identify FH patients at higher risk of CVD to allow clinical decision-making on the indication for aggressive lipid-lowering therapies such as PCSK9 inhibitors.

On the other hand, available evidence suggests a decreased risk of type 2 diabetes mellitus (T2DM) in patients with HeFH compared with the general population, although the exact mechanism of this possible protective effect is still not fully understood.[10,11] Although T2DM is an independent predictor of CVD in the general population,[12,13] this may not be the case in the HeFH population. Hence, while T2DM was an independent cardiovascular risk factor in British Columbia (Canada),[14] the Dutch FH cohort[15] and the CASCADE-FH registry of the United States[16], this finding could not be replicated in other FH cohorts such as those from Quebec (Canada),[17] Utah (United States),[18] Brazil,[19] Australia,[20] and Greece.[21] In a Spanish registry,[22] although T2DM was considered a prognostic factor in the univariate analysis, it was not included in the final analysis, probably due to the low prevalence of impaired glucose metabolism in patients with HeFH.

This may be explained, at least in part, by the low incidence of impaired glucose metabolism in this specific population. By contrast, in a recent meta-analysis and systematic review assessing the association of risk factors and CVD in HeFH patients, smoking, hypertension, and T2DM accounted for more than a fourth of CVD risk in HeFH patients.[23]

The present study aimed to evaluate the clinical, biochemical and genetic characteristics of HeFH patients with T2DM compared with those without T2DM in the large dyslipidemia registry of the Spanish Arteriosclerosis Society.

METHODS

Study protocol

The dyslipidemia registry of the Spanish Arteriosclerosis Society was created in 2013 as an online registry in which 50 certified lipid units distributed throughout Spain enter cases with different forms of primary hyperlipidemias. These lipid units are the centers in the Spanish National Health Service where most cases of primary hyperlipidemias are referred for clinical management. The registry was approved by a central ethics committee to include anonymous clinical data (*Comité Ético de Investigación Clínica de Aragón*, Zaragoza, Spain) in accordance with the ethical guidelines of the Declaration of Helsinki. The patients did not have to sign an informed consent form as the data were obtained from an official national registry; however, since September 2018, participants' written consent has been obtained in accordance with the instructions of the Ethics Committee. Minimum data for the inclusion of cases in the registry are the following: age, sex, smoking status, personal history of hypertension, diabetes and CVD with age at the first event, body mass index, waist circumference, complete lipid profile including total cholesterol, LDL cholesterol, triglycerides and high-density lipoprotein (HDL) cholesterol levels without lipid-lowering treatment at diagnosis, and current lipid and biochemical parameters at the moment of inclusion in the registry. CVD in the registry is defined as coronary heart disease (myocardial infarction, acute coronary syndrome with stenosis > 50% of a main coronary artery and coronary revascularization), stroke (ischemic and hemorrhagic), aortic aneurysm and lower limb ischemia (intermittent claudication with ankle/brachial index < 0.90 or revascularization of lower limb arteries). T2DM is defined as fasting blood glucose > 125 mg/dL or taking blood-glucose-lowering drug therapy.

In the present study, inclusion criteria were patients aged ≥ 18 years with probable (6-8 points) or definite (> 8 points) HeFH according to the Dutch Lipid Clinic Network criteria,[24] and complete minimum data for the registry such as information on family history of hypercholesterolemia and premature CVD, personal history of tendon xanthomas and corneal arch before the age of 45 years, genetic study of *LDLR*, *APOB* or *PCSK9*, age at statin onset and age at T2DM diagnosis.

Patients with HeFH were classified according to the presence or absence of T2DM at the moment of inclusion in the registry. The clinical, biochemical and genetic characteristics of the 2 groups were compared.

Exclusion criteria were diagnosis of homozygous FH and lack of data on family or personal history of CVD, lipid-lowering therapy, age at T2DM onset or absence of genetic analysis.

Biochemical measurements

Blood samples were collected after an overnight fast (> 10 hours) and were processed for laboratory analyses the same day. Serum total cholesterol, triglycerides and HDL cholesterol levels were measured locally using enzymatic methods. Serum LDL cholesterol concentration was calculated using the Friedewald formula.

Genetic analysis

DNA was isolated from whole blood using standard methods and screening for LDLR, APOB and PCSK9 mutations was carried out using the Lipochip Platform (Progenika Biopharma S. A., Bilbao, Spain). This specific platform consists of 2 consecutive steps: the first is LIPOchip microarray analysis for the detection of the most frequent Spanish point mutations in the LDLR gene and in the APOB exon 26, as well as CNVs in LDLR. When the LIPOchip microarray yielded a negative result (no mutation was found), the LDLR, APOB (binding domain) and PCSK9 gene coding sequences, exon-intron boundaries, and short proximal intronic sequences were sequenced with a GS Junior system (Roche Diagnostics Corporation, Basel, Switzerland).[25]

Statistical analysis

Data are expressed as mean ± standard deviation for continuous variables. Categorical variables are expressed as percentages and frequencies. The Student t test was performed to assess differences between 2 means. The chi-square test or the Fisher exact test wase used to evaluate the degree of association among categorical variables. Logistic regression analysis was applied and odds ratio (OR) and 95% confidence intervals (95%CI) were calculated to assess whether, after adjustment for the remaining variables influencing the presence of CVD, the existence of an impaired glucose metabolism

(including T2DM diagnosis, fasting blood glucose and HbA1c) was associated with the presence of CVD in patients with HeFH. The initial model was unadjusted; the second model was adjusted for age and male sex; the third model for age, male sex, smoking, and HDL cholesterol concentrations and finally, the last model was adjusted for age, male sex, smoking, HDL cholesterol concentrations, hypertension, and body mass index. A 2-sided P value < .05 was considered statistically significant. Analyses were performed with SPSS (version 19.0 for Windows; SPSS, Chicago, IL, United States).

RESULTS

Of the 2301 patients with primary hypercholesterolemia included in the registry, 1724 with a probable or definite diagnosis according to the Dutch Lipid Clinic Network score were finally included (figure 1). The mean age of the study cohort was 52.2 ± 15.4 years, and 822 (47.7%) were male, with a mean body mass index of 25.9 ± 4.5 kg/m². Eighty-seven of the 1724 (5%) included in the final analysis met the criteria for T2DM (table 1).

Clinical characteristics

Paternal and maternal hypercholesterolemia rates were higher in patients with a normal glucose profile compared with those with T2DM. However, rates of premature CVD disease and DM in a first-degree relative were higher in the HeFH group with T2DM.

Compared with HeFH without T2DM, those with T2DM were more frequently male (60.9% vs 47.0%; P = .004), were older (64.2 ± 11.2 years vs 51.5 ± 15.4 years; P < .001), and had a higher body mass index (29.5 ± 4.4 kg/m² vs 25.7 ± 4.4 kg/m²; P < .001).

These patients also had higher rates of CVD (42.5% vs 11.5%; P < .001) regardless of the involved area (coronary, cerebrovascular or lower extremities) and higher hypertension rates (58.6% vs 14.4%; P < .001). T2DM patients also more frequently had xanthomas (42.5% vs 30.4%; P < .001) and corneal arch (40.2% vs 26.6%; P < .001) compared with those without DM. The rate of abdominal aortic aneurysms was lower in HeFH patients with T2DM than in those with a normal glucose profile (0.0% vs 0.1%; P < .001). The remaining clinical characteristics are shown in table 1.
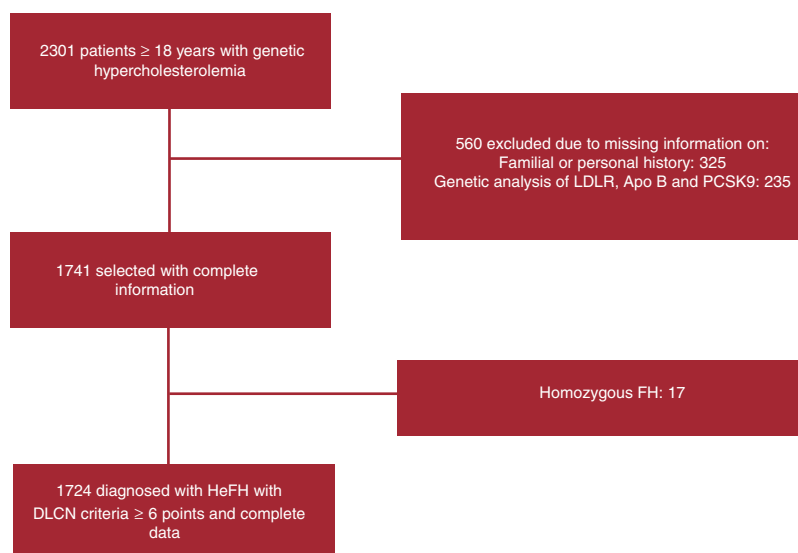
Figure 1. Study flowchart of the patients finally included in the analysis. apo, apolipoprotein; DLCN, Dutch Lipid Clinic Network; FH, familial hypercholesterolemia; HeFH, heterozygous familial hypercholesterolemia; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type-9.

Table 1
Clinical characteristics of patients with heterozygous familial hypercholesterolemia included in the study

	Non-T2DM	T2DM	P
Variable			
Number of patients	1637	87	
Male sex	769 (47.0)	53 (60.9)	.004*
Age, y	51.5 ± 15.4	64.2 ± 11.2	<.001*
BMI, kg/m²	25.7 ± 4.4	29.5 ± 4.4	<.001*
Family history			
Paternal hypercholesterolemia	655 (40.0)	30 (34.5)	<.001*
Maternal hypercholesterolemia	763 (46.6)	26 (29.9)	<.001*
Paternal CVD	232 (14.2)	14 (16.1)	<.001*
Maternal CVD	119 (7.3)	6 (6.9)	.001*
Premature CVD in a first-degree relative	492 (30.1)	37 (42.5)	<.001*
Diabetes mellitus in a first-degree relative	152 (9.3)	24 (27.6)	<.001*
Hypertriglyceridemia in a first-degree relative	72 (4.4)	5 (5.7)	.029*
Personal history			
CVD	188 (11.5)	37 (42.5)	<.001*
Ischemic heart disease	160 (9.8)	32 (36.8)	<.001*
Stroke	25 (1.5)	3 (3.4)	<.001*
Peripheral vascular disease	21 (1.3)	6 (6.9)	<.001*
Abdominal aortic aneurysm	2 (0.1)	0 (0.0)	<.001*
Hypertension	236 (14.4)	51 (58.6)	<.001*
Systolic blood pressure, mmHg	123.6 ± 22.2	131.3 ± 29.7	.002*
Diastolic blood pressure, mmHg	76.1 ± 30.1	76.5 ± 21.5	.895
Xanthoma	498 (30.4)	37 (42.5)	<.001*
Corneal arch	435 (26.6)	35 (40.2)	<.001*
Liver steatosis	26 (1.6)	5 (5.7)	.001*
Smoking	367 (22.4)	13 (14.9)	<.001*
Total DLCN score	15.2 ± 5.2	16.5 ± 6.2	.050
Lipid-lowering treatment	1451 (88.6)	78 (89.7)	.795
Type of lipid-lowering treatment	Statins 656 (40.1) Combined 783 (47.8) Other 12 (0.7)	Statins 18 (20.7) Combined 60 (69.0) Other 0 (0.0)	.006*

BMI, body mass index; CVD, cardiovascular disease; DLCN, Dutch Lipid Clinic Network; T2DM, type 2 diabetes mellitus.
Data are expressed as No. (%) or mean ± standard deviation.
  * Statistically significant results.

Biochemical parameters

The cardiovascular event was prior to the start of lipid-lowering treatment in 140 cases; of these, 21 (15%) had T2DM. The biochemical parameters of HeFH patients with and without lipid-lowering treatment were also analyzed.

Based on biochemical results without lipid-lowering treatment, patients with T2DM and HeFH had a less favorable lipid profile, with higher total (366.9 ± 86.7 mg/dL vs 342.0 ± 74.7 mg/dL; P = .011) and non-HDL (316.9 ± 87.8 mg/dL vs 286.4 ± 75.4 mg/dL; P = .002) cholesterol levels. Furthermore, HDL cholesterol concentrations were lower (50.0 ± 20.8 mg/dL vs 55.6 ± 15.9 mg/dL; P = .002) and triglycerides were higher (173.5 ± 107.0 mg/dL vs 119.6 ± 81.7 mg/dL; P < .001) than in the group with normoglycemia. No differences were found in LDL cholesterol levels between groups (table 2).

Blood examination results of patients on lipid-lowering treatment were also evaluated (table 3). In this scenario, total cholesterol (217.4 ± 55.9 mg/dL vs 199.6 ± 50.3 mg/dL; P = .004) and LDL cholesterol (130.0 ± 61.0 mg/dL vs 75.0 ± 56.8 mg/dL; P = .033) levels were significantly higher in the non-T2DM group than in patients with T2DM.

Genetic analysis

Seventy-four (85.1%) and 1410 (86.1%) patients with and without T2DM, respectively, had a pathogenic mutation related to HeFH (P = .890). The *LDLR* mutation was the most frequent, being present in 81.6% and 82.6% of patients with and without T2DM,

Table 2
Lipid profile without lipid-lowering treatment in patients with heterozygous familial hypercholesterolemia included in the study

Variable	Non-T2DM	T2DM	P
Total cholesterol, mg/dL	342.0 ± 74.7	366.9 ± 86.7	.011*
HDL-C, mg/dL	55.6 ± 15.9	50.0 ± 20.8	.002*
Non-HDL-C, mg/dL	286.4 ± 75.4	316.9 ± 87.8	.002*
LDL-C, mg/dL	239.3 ± 72.3	284.0 ± 81.3	.227
Triglycerides, mg/dL	119.6 ± 81.7	173.5 ± 107.0	<.001*

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus.
Data are expressed as mean ± standard deviation.
  * Statistically significant results.

Table 3
Current laboratory parameters of patients with heterozygous familial hypercholesterolemia included in the study

Variable	Non-T2DM	T2DM	P
Total cholesterol, mg/dL	217.4 ± 55.9	199.6 ± 50.3	.004*
HDL-C, mg/dL	57.0 ± 16.0	50.4 ± 12.6	<.001*
Non-HDL-C, mg/dL	160.4 ± 55.0	149.2 ± 49.8	.064
LDL-C, mg/dL	130.0 ± 61.0	75.0 ± 56.8	.033*
Triglycerides, mg/dL	103.4 ± 91.9	136.1 ± 72.0	.001*
Glucose, mg/dL	91.4 ± 12.4	132.0 ± 37.0	<.001*
TSH, U/mL,	2.5 ± 2.8	2.0 ± 1.1	.313
HbA1c, %	5.6 ± 0.4	6.9 ± 0.9	<.001*

HDL-C, high-density lipoprotein cholesterol; HbA1c, glycosylated hemoglobin; LDL-C, low-density lipoprotein cholesterol; T2DM, type 2 diabetes mellitus; TSH, thyroid-stimulating hormone.
Data are expressed as mean ± standard deviation.
* Statistically significant results.

Table 4
Genetic analysis of included patients

Variable	Non-T2DM	T2DM	P
Positive genetic mutation	1410 (86.1)	74 (85.1)	.890
Causative gene	APOB 48 (2.9)	APOB 1 (1.1)	.720
	APOE 1 (0.1)	APOE 0 (0.0)	
	LDLR 1352 (82.6)	LDLR 71 (81.6)	
	PSCK9 9 (0.5)	PSCK9 2 (2.3)	

APO, apolipoprotein; LDLR, low-density lipoprotein receptor; PCSK9, proprotein convertase subtilisin/kexin type-9; T2DM, type 2 diabetes mellitus.
Data are expressed as No. (%)

respectively. The remaining results of the genetic study of the included patients are detailed in table 4 and figure 2.

Logistic regression analysis

The association between impaired glucose metabolism and CVD in patients with HeFH was also evaluated (table 5). After adjustment for several risk factors such as age, male sex, smoking, HDL cholesterol levels, hypertension, and body mass index, a significant association was found between T2DM and CVD (OR, 2.01; 95%CI, 1.18-3.43; P = .010).

DISCUSSION

Recent clinical evidence has demonstrated that CVD among patients with HeFH has substantially improved with current management, with the prevalence of CVD being one third of that reported before the statin era.[26] However, CVD in HeFH is extremely heterogeneous. Although specific HeFH risk factors such as the type of mutation and the presence of tendon xanthomas have been previously described,[27] classic cardiovascular risk factors also appear to play a role in explaining differences in the presentation of CVD in the FH population.[7]

In the present study, HeFH patients with T2DM had higher rates of hypertension and CVD, including ischemic heart disease, stroke, and peripheral vascular disease, but a lower rate of abdominal aortic aneurysm. Moreover, they also had a less favorable lipid profile, with higher total cholesterol, non-HDL cholesterol, and triglyceride levels and lower HDL cholesterol levels. Finally, the frequency and type of genetic mutation was similar in both groups, with mutations in LDLR being the most frequently encountered mutation in this cohort.

T2DM is an established cardiovascular risk factor associated with an increased risk of coronary, cerebral and peripheral vascular disease.[28] Thus, it is not surprising that, in the present cohort, the CVD rate was higher in patients with impaired glucose metabolism than in those without T2DM. Concurring with these results, Yanagi et al.[29] also found an 87% and 59% increase in the prevalence of coronary artery disease in patients with FH with impaired glucose tolerance and T2DM compared with the 43% in patients with a normal glucose profile.

Furthermore, in agreement with the present study, recent data from the Canadian FH cohort suggested that diabetic FH patients represent a high cardiovascular risk population owing to the presence of concomitant cardio-metabolic risk factors.[17] In agreement with previous studies,[30,31] other factors associated with insulin resistance such as older age, increased body mass index and high blood pressure were also more frequent in the T2DM group in the present study, probably also playing a role in the higher cardiovascular risk in this specific population.

Since atherogenic dyslipidemia is the characteristic lipid abnormality in T2DM, it seems reasonable that, in the present cohort, HDL cholesterol levels were lower and triglyceride levels were higher in the group of patients with T2DM. Nevertheless, LDL cholesterol levels were comparable between groups, also in agreement with other reports.[29,32] Moreover, concurring with the present results, a previous study published by our group, which included 354 patients with probable HeFH and 1378 with definite
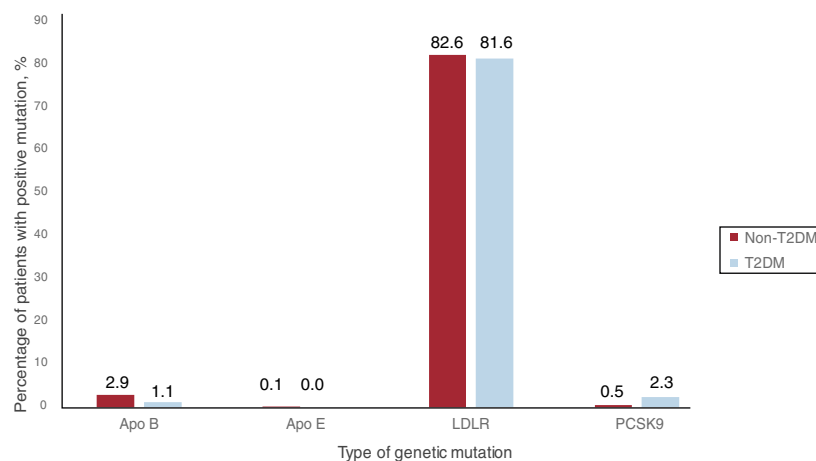
Figure 2. Percentage of patients with the different heterozygous familial hypercholesterolemia causative mutations. apo, apolipoprotein; LDLR, low-density liporotein receptor; PCSK9, proprotein convertase subtilisin/kexin type-9; T2DM, type 2 diabetes mellitus.

Table 5

Logistic regression analysis evaluating the association between impaired glucose metabolism and cardiovascular disease in patients with heterozygous familial hypercholesterolemia

	Model 1	Model 2	Model 3	Model 4
T2DM OR (95%CI)	5.72 (3.64-8.99) $P < .001^*$	2.97 (1.82-4.87) $P < .001^*$	2.65 (1.59-4.44) $P < .001^*$	2.01 (1.18-3.43) $P = .010^*$
Fasting blood glucose OR (95%CI)	1.03 (1.02-1.04) $P < .001^*$	1.01 (1.00-1.03) $P = .025^*$	1.01 (1.00-1.03) $P = .027^*$	1.01 (0.99-1.02) $P = .105$
HbA1c OR (95%CI)	2.30 (1.45-3.64) $P < .001^*$	1.78 (1.10-2.88) $P = .018^*$	1.83 (1.11-3.01) $P = .018^*$	1.52 (0.92-2.53) $P = .104$

95%CI, 95% confidence interval; HbA1c, glycosylated hemoglobin; HDL, high-density lipoprotein; OR, odds ratio; T2DM, type 2 diabetes mellitus.
Model 1: Unadjusted.
Model 2: Adjusted for age and male sex.
Model 3: Adjusted for age, male sex, smoking, and HDL cholesterol concentrations.
Model 4: Adjusted for age, male sex, smoking, HDL cholesterol concentrations, hypertension and body mass index.
  * Statistically significant results.

HeFH, found LDL cholesterol concentration not to be a risk factor associated with the prevalence of T2DM.[33] Similarly, other prospective studies also failed to find a direct relationship between LDL cholesterol levels and increased diabetes risk.[34,35]

As for the lipid profile after initiation of lipid-lowering treatment, as mentioned previously in the results section, total and LDL cholesterol levels were significantly higher in the non-T2DM group than in patients with T2DM. This can be probably explained by the greater percentage of lipid-lowering treatment use in the group of patients with an impaired glucose metabolism.

Around 85% of patients both with and without T2DM had a causative genetic mutation of HeFH; however, no differences between groups were observed in the frequency or distribution of the type of affected gene. These results agree with those of a study conducted with the same registry of patients that found no differences in the prevalence of T2DM according to the presence of certain gene mutations causing HeFH, including *LDLR*, *APOB* and *PCSK9*.[33]

However, unlike the previously described results, other studies have found a possible association between HeFH causative mutations and modifications in the risk of showing alterations in glycemic metabolism. For instance, Besseling et al.[10] found a lower prevalence of T2DM in patients with an *LDLR*-negative mutation with a dose-dependent association compared with those with a defective *LDLR* or *APOB* mutation, thus suggesting a possible relationship between the severity of the genetic alteration and T2DM protection. Similarly, Saavedra et al.[36] concluded that the InsLEU variant of the *PCSK9* gene was associated not only with a lower risk of coronary events but also with an increased occurrence of pre-DM and DM. In summary, HeFH causative mutations have been related to both an increase and a decrease in altered glycemic metabolism, although the present study found no association between the presence of T2DM and certain genetic mutations. Hence, owing to the conflicting results in this field, further studies will be needed to confirm a possible relationship between the type of HeFH causative mutation and an altered glycemic profile.

Finally, and in line with the previously mentioned results, a positive association was found between impaired glucose metabolism (including T2DM, fasting blood glucose levels and HbA1c) and CVD in HeFH patients, thus confirming once again the close relationship between them.

The present study has some limitations. First, because it was an observational study with a cross-sectional design, no causal relationship could be inferred from the results. The number of patients with T2DM and HeFH was relatively small in comparison with the general Spanish population (5% vs 13.8% in > 18 years and 29.8% in the age range 65-71 years),[37] thus concurring with the possible protective effect of HeFH on diabetes risk. As for the population included, participants were extracted from the national registry of a Mediterranean country where lower coronary heart disease rates are expected compared with other populations worldwide, albeit with a high prevalence of T2DM.[37] Furthermore, all participants included in this study were extracted from the dyslipidemia registry of the Spanish Arteriosclerosis Society. These patients are therefore treated and followed up at specialized lipid units following a standardized protocol and, consequently, the present results cannot be extrapolated to the whole Spanish population.

CONCLUSIONS

In the present study, HeFH patients with T2DM had higher rates of hypertension and CVD, together with a less favorable lipid profile. A positive association was confirmed between impaired glucose metabolism and CVD in HeFH patients. No differences were found between groups in the presence or distribution of the different causative genetic mutations of HeFH. Future studies in this field will help to identify possible differences between patients diagnosed with HeFH with and without impaired glucose metabolism.

ACKNOWLEDGMENTS

FUNDING

CONFLICTS OF INTEREST

J.F. Ascaso reports personal fees from Sanofi, Mylan, Ferrer, and Novo Nordisk, outside the submitted work; J. Pedro-Botet reports nonfinancial support from Astra-Zeneca, Esteve, Merck, Mylan, and Sanofi-Aventis, outside the submitted work; X. Pintó reports grants from FIS-ISCIII and CIBER-ISCIII, personal fees from Amgen, Abbott, Sanofi, Lacer, Rubió, and Esteve, outside the submitted work; P. Valdivielso reports personal fees from Amgen, Sanofi, and MSD, grants and personal fees from Ferrer, and personal fees from Esteve, outside the submitted work; other authors report no conflicts of interest related to this work.

WHAT IS KNOWN ABOUT THE TOPIC?

- Patients with HeFH are at increased cardiovascular risk.
- Patients with HeFH have a lower T2DM rate.
- T2DM has not always been described as a cardiovascular risk factor in these patients.

WHAT DOES THIS STUDY ADD?

- Patients with HeFH and T2DM have a higher CVD risk.
- Patients with HeFH and T2DM have a less favorable lipid profile.
- No differences were observed regarding specific genetic mutations in HeFH patients with or without T2DM.
- T2DM is associated with the presence of CVD in these patients.

REFERENCES

1. Hovingh GK, Davidson MH, Kastelein JJ, O'Connor AM. Diagnosis and treatment of familial hypercholesterolaemia. *Eur Heart J.* 2013;34:962–971.
2. Cenarro A, Etxebarria A, de Castro-Orós I, et al. The p.Leu167del mutation in APOE gene causes autosomal dominant hypercholesterolemia by down-regulation of LDL receptor expression in hepatocytes. *J Clin Endocrinol Metab.* 2016;101:2113–2121.
3. Catapano AL, Graham I, De Backer G, et al. 2016 ESC/EAS guidelines for the management of dyslipidaemias. *Eur Heart J.* 2016;37:2999–3058.
4. Grundy SM, Stone NJ, Bailey AL, et al. 2018 AHA/ACC/AACVPR/AAPA/ABC/ACPM/ADA/AGS/APhA/ASPC/NLA/PCNA guideline on the management of blood cholesterol: executive summary: a report of the American College of Cardiology/American Heart Association Task Force on Clinical Practice Guidelines. *J Am Coll Cardiol.* 2019;73:3168–3209.
5. Jellinger PS, Handelsman Y, Rosenblit PD, et al. American Association of Clinical Endocrinologists and American College of Endocrinology guidelines for management of dyslipidemia and prevention of cardiovascular disease. *Endocr Pract.* 2017;23(Suppl 2):1–87.
6. Ferrieres J, Lambert J, Lussier-Cacan S, Davignon J. Coronary artery disease in heterozygous familial hypercholesterolemia patients with the same LDL receptor gene mutation. *Circulation.* 1995;92:290–295.
7. Santos RD, Gidding SS, Hegele RA, et al. International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. Defining severe familial hypercholesterolaemia and the implications for clinical management: a consensus statement from the International Atherosclerosis Society Severe Familial Hypercholesterolemia Panel. *Lancet.* 2016;4:850–861.
8. Pérez-Calahorra S, Sánchez-Hernández RM, Plana N, et al. Dyslipidemia Registry of Spanish Arteriosclerosis Society. Value of the definition of severe familial hypercholesterolemia for stratification of heterozygous patients. *Am J Cardiol.* 2017;119:742–748.
9. Humphries SE, Cooper JA, Capps N, et al. Coronary heart disease mortality in severe vs. non-severe familial hypercholesterolaemia in the Simon Broome Register. *Atherosclerosis.* 2019;281:207–212.
10. Besseling J, Kastelein JJ, Defesche JC, Hutten BA, Hovingh GK. Association between familial hypercholesterolemia and prevalence of type 2 diabetes mellitus. *JAMA.* 2015;313:1029–1036.
11. Vohl MC, Gaudet D, Moorjani S, et al. Comparison of the effect of two low-density lipoprotein receptor class mutations on coronary heart disease among French-Canadian patients heterozygous for familial hypercholesterolaemia. *Eur J Clin Invest.* 1997;27:366–373.
12. Sarwar N, Gao P, Seshasai SR, et al. Emerging Risk Factors Collaboration. Diabetes mellitus, fasting blood glucose concentration, and risk of vascular disease: a collaborative meta-analysis of 102 prospective studies. *Lancet.* 2010;375:2215–2222.
13. Singh GM, Danaei G, Farzadfar F, et al. The age-specific quantitative effects of metabolic risk factors on cardiovascular diseases and diabetes: a pooled analysis. *PLoS ONE.* 2013;8:e65174.
14. Allard MD, Saeedi R, Yousefi M, Frohlich J. Risk stratification of patients with familial hypercholesterolemia in a multi-ethnic cohort. *Lipids Health Dis.* 2014;13:65.
15. Besseling J, Kindt I, Hof M, Kastelein JJ, Hutten BA, Hovingh GK. Severe heterozygous familial hypercholesterolemia and risk for cardiovascular disease: a study of a cohort of 14,000 mutation carriers. *Atherosclerosis.* 2014;233:219–223.
16. De Goma EM, Ahmad ZS, O'Brien EC, et al. Treatment gaps in adults with heterozygous familial hypercholesterolemia in the United States: data from the CASCADE-FH Registry. *Circ Cardiovasc Genet.* 2016;9:240–249.
17. Paquette M, Brisson D, Dufour R, Khoury Éaue, Gaudet D, Baass A. Cardiovascular disease in familial hypercholesterolemia: validation and refinement of the Montreal-FH-SCORE. *J Clin Lipidol.* 2017;11:1161–1167.
18. Hopkins PN, Stephenson S, Wu LL, Riley WA, Xin Y, Hunt SC. Evaluation of coronary risk factors in patients with heterozygous familial hypercholesterolemia. *Am J Cardiol.* 2001;87:547–553.
19. Pereira C, Miname M, Makdisse M, Kalil Filho R, Santos RD. Association of peripheral arterial and cardiovascular diseases in familial hypercholesterolemia. *Arq Bras Cardiol.* 2014;103:118–123.
20. Chan DC, Pang J, Hooper AJ, et al. Elevated lipoprotein(a), hypertension and renal insufficiency as predictors of coronary artery disease in patients with genetically confirmed heterozygous familial hypercholesterolemia. *Int J Cardiol.* 2015;201:633–638.
21. Pitsavos CH, Chrysohoou C, Panagiotakos DB, et al. Exercise capacity and heart rate recovery as predictors of coronary heart disease events, in patients with heterozygous familial hypercholesterolemia. *Atherosclerosis.* 2004;173:347–352.
22. Perez de Isla L, Alonso R, Mata N, et al. Predicting cardiovascular events in familial hypercholesterolemia: the SAFEHEART Registry (Spanish Familial Hypercholesterolemia Cohort Study). *Circulation.* 2017;135:2133–2144.
23. Akioyamen LE, Genest J, Chu A, Inibhunu H, Ko DT, Tu JV. Risk factors for cardiovascular disease in heterozygous familial hypercholesterolemia: a systematic review and meta-analysis. *J Clin Lipidol.* 2019;13:15–30.
24. Nordestgaard BG, Chapman MJ, Humphries SE, et al. Familial hypercholesterolaemia is underdiagnosed and undertreated in the general population: guidance for clinicians to prevent coronary heart disease: consensus statement of the European Atherosclerosis Society. *Eur Heart J.* 2013;34:3478–3490.
25. Stef MA, Palacios L, Olano-Martín E, et al. A DNA microarray for the detection of point mutations and copy number variation causing familial hypercholesterolemia in Europe. *J Mol Diagn.* 2013;15:362–372.
26. Pérez-calahorra S, Laclaustra M, Marco-Benedi V, et al. Effect of lipid-lowering treatment in cardiovascular disease prevalence in familial hypercholesterolemia. *Atherosclerosis.* 2019;284:245–252.
27. Civeira F, Castillo S, Alonso R, et al. Tendon xanthomas in familial hypercholesterolemia are associated with cardiovascular risk independently of the low-density lipoprotein receptor gene mutation. *Arterioscler Thromb Vasc Biol.* 2005;25:1960–1965.
28. Grundy SM, Howard B, Smith Jr S, Eckel R, Redberg R, Bonow RO. Prevention conference VI: diabetes and cardiovascular disease: executive summary: conference proceeding for healthcare professionals from a special writing group of the American Heart Association. *Circulation.* 2002;105:2231–2239.
29. Yanagi K, Yamashita S, Kihara S, et al. Characteristics of coronary artery disease and lipoprotein abnormalities in patients with heterozygous familial hypercholesterolemia associated with diabetes mellitus or impaired glucose tolerance. *Atherosclerosis.* 1997;132:43–51.
30. Reaven GM. Statins, familial hypercholesterolemia and type 2 diabetes. *J Intern Med.* 2016;280:421–422.
31. Fuentes F, Alcala-Diaz JF, Watts GF, et al. SAFEHEART Investigators. Statins do not increase the risk of developing type 2 diabetes in familial hypercholesterolemia: The SAFEHEART study. *Int J Cardiol.* 2015;201:79–84.
32. Sun D, Cao YX, You XD, et al. Clinical and genetic characteristics of familial hypercholesterolemia patients with type 2 diabetes. *J Endocrinol Invest.* 2019;42:591–598.
33. Climent E, Pérez-Calahorra S, Marco-Benedí V, et al. Effect of LDL cholesterol, statins and presence of mutations on the prevalence of type 2 diabetes in heterozygous familial hypercholesterolemia. *Sci Rep.* 2017;7:5596.
34. Hanley AJ, Wagenknecht LE, Norris JM, et al. Insulin resistance, beta cell dysfunction and visceral adiposity as predictors of incident diabetes: the Insulin Resistance Atherosclerosis Study (IRAS) Family study. *Diabetologia.* 2009;52:2079–2086.
35. Von Eckardstein A, Schulte H, Assmann G. Risk for diabetes mellitus in middle-aged Caucasian male participants of the PROCAM study: implications for the definition of impaired fasting glucose by the American Diabetes Association. *Prospective Cardiovascular Munster J Clin Endocrinol Metab.* 2000;85:3101–3108.
36. Saavedra YG, Dufour R, Baass A. Familial hypercholesterolemia: PCSK9 InsLEU genetic variant and prediabetes/diabetes risk. *J Clin Lipidol.* 2015;9:786–793.
37. Soriguer F, Goday A, Bosch-Comas A, et al. Prevalence of diabetes mellitus and impaired glucose regulation in Spain: the Di@bet.es Study. *Diabetologia.* 2012;55:88–93.