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Clinical and molecular characteristics of homozygous familial hypercholesterolemia patients: Insights from SAFEHEART registry

Rodrigo Alonso, MD, PhD, Jose Luis Díaz-Díaz, MD, PhD, Francisco Arrieta, MD, Francisco Fuentes-Jiménez, MD, PhD, Raimundo de Andrés, MD, PhD, Pedro Saenz, MD, PhD, Gema Ariceta, MD, José I. Vidal-Pardo, MD, PhD, Fatima Almagro, MD, PhD, Rosa Argueso, MD, Pablo Prieto-Matos, MD, PhD, José P. Miramontes, MD, PhD, Xavier Pintó, MD, PhD, Johana Rodriguez-Urrego, MD, Leopoldo Perez de Isla, MD, PhD, Pedro Mata, MD, PhD***

Fundación Hipercolesterolemia Familiar, Madrid, Spain (Drs Alonso, Rodriguez-Urrego, Perez de Isla and Mata); Department of Nutrition, Clínica Las Condes, Santiago de Chile, Chile (Dr Alonso); Department of Internal Medicine, Hospital Abente y Lago, A Coruña, Spain (Dr Díaz-Díaz); Department of Endocrinology, Hospital Ramón y Cajal, Madrid, Spain (Dr Arrieta); Department of Internal Medicine, IMIBIC, Hospital Universitario Reina Sofía, Universidad de Córdoba, Córdoba, Spain (Dr Fuentes-Jiménez); Department of Internal Medicine, Fundación Jiménez Díaz, Madrid, Spain (Dr de Andrés); Department of Internal Medicine, Hospital de Mérida, Mérida, Spain (Dr Saenz); Department of Pediatric Nephrology, Hospital Vall d'Hebrón, Barcelona, Spain (Dr Ariceta); Department of Endocrinology, Hospital Universitario de Lugo, Lugo, Spain (Drs Vidal-Pardo and Argueso); Department of Internal Medicine, Hospital Donostia, San Sebastian, Spain (Dr Almagro); Department of Pediatrics, Hospital Universitario, Salamanca, Spain (Dr Prieto-Matos); Department of Internal Medicine, Hospital Universitario, Salamanca, Spain (Dr Miramontes); Department of Internal Medicine, Hospital de Bellvitge, Barcelona, Spain (Dr Pintó); and Cardiology Department, Hospital Clínico San Carlos, Madrid, Spain (Dr Perez de Isla)

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BACKGROUND: Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disorder associated with very high levels of cholesterol, accelerated atherosclerosis and very premature death, often secondary to occlusion of the coronary ostia by supravalvular atheroma in untreated individuals.

OBJECTIVE: To describe molecular and clinical characteristics of HoFH enrolled at SAFEHEART registry and to evaluate the role of the type of mutation in clinical expression.

METHODS: SAFEHEART is a registry of molecularly defined familial hypercholesterolemia patients. A standardized phone call is made every year for the follow-up. Patients with confirmed HoFH were selected. Molecular and clinical characteristics were analyzed.

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* Corresponding author. Fundación Hipercolesterolemia Familiar, C/ General Alvarez de Castro 14, Madrid 28010, Spain.

** Corresponding author. Department of Nutrition, Clínica Las Condes, Lo Fontecilla 441, Santiago de Chile, Chile.

E-mail addresses: rodrigoalonsok@gmail.com; pmata@colesterolfamiliar.org

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RESULTS: Thirty-four HoFH patients (27 true HoFH, 4 compound heterozygous familial hypercholesterolemia, and 3 autosomal recessive hypercholesterolemia) have been enrolled in the period 2004–2015. Twenty different mutations in LDLR gene have been detected. Sixteen patients carry defective mutations (DMs), and 15 carry null mutations (NMs). Only patients with NMs met low-density lipoprotein cholesterol (LDL-C) criteria for clinical diagnosis. Patients with NMs had higher untreated LDL-C levels ($P < .0001$), more aortic valve stenosis ($P < .05$), and lower age at first cardiovascular event ($P < .05$) compared to patients with DMs. In the follow-up, 1 liver transplant patient died and 3 cases underwent revascularization procedures. Eight cases started LDL apheresis and 1 case had a liver transplant.

CONCLUSIONS: HoFH phenotypic expression is highly variable. These patients have high atherosclerotic coronary artery disease risk including aortic valve stenosis and do not achieve the LDL-C treatment goals with standard therapy.

Introduction

Homozygous familial hypercholesterolemia (HoFH) is a rare genetic disease, caused by mutations in both alleles of the low-density lipoprotein receptor (*LDLR*) gene.[1,2] Less frequently, mutations in the apolipoprotein B (*APOB*) and the proprotein convertase subtilisin/kexin type 9 (*PCSK9*) genes are also causative of HoFH. Mutations in the gene-encoding LDL receptor adaptor protein 1 (*LDLRAP1*) produce a very rare recessive condition known as autosomal recessive hypercholesterolemia (ARH) with a similar phenotypic expression. When the patient has the same mutation in both alleles, it is considered a true homozygote, and if both alleles have different mutations, it is considered a compound heterozygous familial hypercholesterolemia (CHeFH) with similar phenotypic expression.[3]

The prevalence of HoFH has been historically estimated in 1 case in 1 million of the general population. Recent data from the Dutch program using molecular diagnosis suggest that the prevalence of HoFH may be as high as 1 case in 300,000.[4,5]

The disease is characterized by severe hypercholesterolemia, cutaneous xanthomas, atheromatous involvement of the valvular and supravalvular region of the aortic root, and atherosclerotic coronary artery disease (ASCAD) that can be clinically evident before 20 years of age, and death can occur before the age of 30 years.[6–8] Owing to the very high risk of premature ASCAD, these patients must be treated from the second year of life with statins and ezetimibe, and most of them will require invasive procedures like LDL apheresis or in exceptional cases, a liver transplant.[4,9] However, with the availability of new drugs approved for HoFH patients like lomitapide and mipomersen affecting the production and secretion of apoB-containing lipoproteins, or PCSK9 inhibitors affecting the regulation of LDLR, some of these patients can significantly reduce their cholesterol levels, reducing the need for these invasive procedures.[10–12]

The SAFEHEART study (Spanish Familial Hypercholesterolemia Cohort Study) was designed to improve insight into the prognostic factors, treatments, and mechanisms that influence the development of ASCVD and mortality in a well-defined FH population.[13] Our aim was to describe the genetic, clinical characteristics, and treatment over time of a HoFH population.

Methods

Study design and subjects recruitment

The Spanish HoFH registry is a subset of the SAFEHEART registry (ClinicalTrials.gov number NCT02693548). It is a nationwide registry that includes FH patients living in Spain regardless of their original nationality. The design and follow-up of SAFEHEART study has been previously described.[13] Briefly, patients with genetic diagnosis of FH are registered and followed-up every year through a standardized phone call to record relevant changes in lipid-lowering treatment (LLT) including LDL apheresis and development of cardiovascular events.

In this study, patients with molecular diagnosis of HoFH enrolled in SAFEHEART from 2004 until 2015 were analyzed, including true HoFH, CHeFH, and ARH patients. Baseline clinical and biochemical data were obtained from clinical reports and contacting physicians responsible for patient care. Cardiovascular disease was defined as any event of ASCAD, aortic valve stenosis and replacement, carotid disease, ischemic stroke, or peripheral vascular disease documented in the medical report.[13]

Cardiovascular event for survival analysis was defined as the occurrence of the first one of the following: Fatal or nonfatal myocardial infarction, fatal or nonfatal ischemic stroke, coronary or carotid revascularization, aortic valve replacement, peripheral artery revascularization, and cardiovascular death (any death related to cardiovascular

disease or derived from cardiovascular therapeutic procedures not described in the previous definitions).

Cases with clinical diagnosis and without genetic confirmation were also included if clinical data were available and the molecular diagnosis of heterozygous FH was confirmed in both parents.

Local ethics committees approved this study, and all eligible subjects or legal representatives gave written informed consent.

LDLR gene mutations classification

Deoxyribonucleic acid analysis was performed as previously described.[14] All mutations that have been proven by in vitro functional assays or computed simulated analysis that lead to <2% LDLR activity were considered to be null mutations (NMs). All other mutations were classified as defective mutations (DMs). LDLR status was classified according to the LOVD database (http://www.LOVD.nl/LDLR) as negative and defective on the predicted functional effects of mutations according to their site and severity. In CHeFH cases in which one of the mutations was considered to be null, the case was classified as LDLR negative. Patients with ARH were defined if a mutation in both *LDLRAP1* alleles was detected.[15]

Statistical analysis

An initial descriptive analysis was carried out using a number of cases and percentages for qualitative variables and mean and standard deviation for quantitative variables with a normal distribution. For those quantitative variables lacking a normal distribution, median and interquartile range were estimated. Comparisons of frequencies between qualitative variables were carried out using the chi-squared test, and comparisons of proportions were done by Fisher exact test. Mean values of quantitative variables were compared with the student *t* test for independent data, whereas median values were compared with the nonparametric Mann–Whitney *U* test. Follow-up survival curves, starting from birth, were constructed for null and defective groups using the Kaplan–Meier method, and comparisons were made using the Breslow test. The relationship between variables was considered statistically significant if the *P* value $< .05$. All statistical analyses were performed using the Statistical Package for the Social Sciences (SPSS v 18.0, Chicago, IL).

Results

Molecular characteristics in HoFH

Thirty-one HoFH patients have been diagnosed through deoxyribonucleic acid testing and enrolled in the SAFEHEART registry. Twenty-eight cases are caused by mutations in *LDLR* gene (24 true HoFH and 4 CHeFH), and 3 cases are caused by mutations in *LDLRAP1* gene. No mutations in *Apo B* or *PCSK9* genes have been identified in the registry. Data from 3 individuals without genotyping were also included because they had very high levels of total cholesterol (1203, 762, and 605 mg/dL), and the parents were molecularly defined as heterozygous FH carrying the same NM in *LDLR* gene. These three cases died before the beginning of the registry; 1 case at 5 years post-liver transplant, and 2 cases at 8 years from myocardial infarction. Therefore, the total cohort is constituted by 34 cases (33 cases from Spain and 1 case from Romania living in Spain) belonging to 23 different families. Most HoFHs included in this registry are the result of consanguineous unions rather than a founder gene effect. Only in 1 case, no relationship between parents was found. Characteristics of each patient registered are shown in supplementary Table A.

Twenty different mutations have been detected, and a DM was found in 16 individuals (52%). Two patients with CHeFH were classified as having NM. Additionally, two different NMs were detected in 3 cases with ARH. Molecular characteristics are shown in Table 1.

Clinical characteristics according the type of *LDLR* gene mutations

Mean age at inclusion was 33.4 ± 20.9 years (range from 2 to 83 years). Xanthomas were present in 24 cases (71%), clinical ASCAD in 12 cases (38.7%, mean age of onset was 36 ± 15.5 years), and 6 patients had aortic valve disease confirmed by echocardiography (mean age at diagnosis 20 ± 12 years), 4 of them with aortic valve replacement. Clinical characteristics of individuals regarding the severity of *LDLR* mutation are shown in Tables 2 and 3. There were no significant differences in ASCAD, history of premature atherosclerotic coronary artery disease in parents, and presence of xanthomas between patients with NMs and DMs. Age of onset of ASCAD was below 30 years in 5 individuals with NMs and in 1 of 7 individuals with DMs. Significant differences were observed in age of onset of LLT, aortic valve stenosis and patients with liver transplant between patients with NMs and DMs ($P < .05$).

Patients were followed-up since birth for a median time of 32 years (interquartile range, 14–49 years). Free-event survival was greater in patients with DMs, although the difference was non significant ($P = .054$; Fig. 1).

Lipid profile according to the severity of *LDLR* gene mutations

Untreated total cholesterol (TC) level was available in all cases, and untreated LDL-C level was available in 22 subjects (10 with NMs and in 12 with DMs). On the other

Table 1 Molecular characteristics of HoFH at SAFEHEART

Nucleotide change	Protein effect	Functional classification	Exon	N
Mutation *LDLR* gene				
c.2397_2405del; c.1690 A > C	p.(Val800_Leu802del); p.(Asn564His)	Defective	17, 11	1
c.1199_1207del	p.(Tyr400_Phe402del)	Defective	9	1
c.953 G > T	p.Cys318Phe)	Defective	7	1
c.800 A > C	p.(Glu267Ala)	Defective	5	1
c.1027 G > A	p.(Gly343Ser)	Defective	7	1
c.2475 C > A	p.(Asn825Lys)	Defective	17	1
c.97 C > T	p.(Gln33*)	Null	2	1
c.1646 G > A	p.(Gly549Asp)	Null	11	1
c.1048 C > T	p.(Arg350*)	Null	7	1
c.313+5G > A	p.Leu64_Pro105delinsSer	Null	3i	1
c.898 A > G	p.(Arg300Gly)	Defective	6	1
c.1706-?_1845+?del	p.(asp569Glyfs*29)	Null	11i_12i	1
c.1897 C > T	p.(Arg633Cys)	Defective	13	2
c.1898 G > A; 2390-?_2583+?del	p.(Arg633His); p.(?)	Defective/null	13, 16i_18i	2
c.916_919dup; c.185 C > T	p.(Asp307Alafs*3); p.(Thr62Met)	Null/defective	6, 2	2
c.1618 G > A; c.451_453del	p.Ala540Thr); p.(Ala151del)	Defective/defective	11, 4	2
c.1775 G > A	p.(Gly592Glu)	Defective	12	2
c.313+2dupT	p.Leu64_Pro105delinsSer	Null	3i	3
c.1342 C > T	p.(Gln448*)	Null	9	3
c.1783 C > T	p.(Arg595Trp)	Defective	12	3
Mutation *LDLRAP* gene				
c.344-?_694+?del	p.(?)	Null*	3i_4i	2
c.207delC	p.(Ala70ProfsX19)	Null	1	1

hand, TC and LDL-C levels with different LLT were available in 12 and 15 subjects with NMs and DMs, respectively. Ranges for untreated and treated LDL-C levels in patients with NMs and DMs are shown in Figure 2. Patients with NMs have significantly higher untreated TC and LDL-C levels compared with individuals with DMs ($P < .0001$); however, there were no differences in lipid levels at inclusion (Table 2).

Untreated TC levels were significantly lower in patients with CHeFH compared with true HoFH with NMs (615 mg/dL vs 910 mg/dL, respectively; $P < .05$). No significant differences were observed between CHeFH subjects and individuals with DMs.

All patients with NMs and two patients with DMs had untreated LDL-C >500 mg/dL. On the other hand, excluding those individuals on LDL apheresis or with liver

Table 2 Clinical and biochemical characteristics of HoFH patients

Variables	Total (31)	Null mutations (n = 15)	Defective mutations (n = 16)	*P*
Age (y)	33.4 ± 20.9	23 ± 16.5	37.6 ± 21.4	.071
Age range (y)	2–83	5–46	2–83	
Age onset treatment (years)	22.7 ± 19.3	7.2 ± 4.9	34.3 ± 17.8	.0001
Length of treatment (years)	10.1 ± 10.0	9.9 ± 13.5	10.3 ± 7.2	.921
Xanthomas (%)	22 (71)	13 (86.7)	9 (56.3)	.11
Untreated TC (mg/dL)	683.5 ± 275.4	903 ± 187.7	477.8 ± 159.4	.0001
Treated TC (mg/dL)	251.6 ± 113.1	275.9 ± 164.9	233.4 ± 47.4	.334
Untreated LDL-C (mg/dL)	590.1 ± 274.5	820 ± 166.5	398.5 ± 181.9	.0001
Treated LDL-C (mg/dL)	189.9 ± 113.3	218 ± 162.9	167.1 ± 42.0	.252
Untreated HDL-C (mg/dL)	43.9 ± 9.5	40.8 ± 8.6	48.7 ± 9.4	.117
Treated HDL-C (mg/dL)	44.9 ± 11.6	40.3 ± 9.6	48.5 ± 12.0	.066
High-intensity statin (%)	16 (51.6)	5 (33.3)	11 (68.8)	.049
LDL apheresis (%)	2 (6.5)	2 (13.3)	0 (0)	.46
Transplantation (%)	4 (16.12)	4 (26.6)	0 (0)	.042

TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol.

Table 3 Cardiovascular disease in HoFH

Type of CVD	Total	Null mutations (n = 15)	Defective mutations (n = 16)	*P*
ASCAD, n (%)	12 (39)	5 (33.3)	7 (43.8)	.55
PASCAD in parents, n (%)	9 (29)	6 (40.0)	3 (18.8)	.25
Aortic valve stenosis, n (%)	6 (19)	5 (33.3)*	1 (6.3)†	.014
Age at first ASCAD event, y	36.6 ± 21.7	18.0 ± 9.6	45.8 ± 20.1	.03

ASCAD, atherosclerotic coronary artery disease; CVD, cardiovascular disease; PASCAD, premature atherosclerotic coronary artery disease.
*3 cases with valve replacement.
†1 case with valve replacement.

transplantation, two subject with NMs and none of the subjects with DMs had LDL-C levels >300 mg/dL with maximum LLT.

Lipid-lowering treatments

At inclusion, 69% of patients with DMs and 50% (5 cases of 10 receiving LLT) of subjects with NMs were receiving high-intensity statin treatment in combination with ezetimibe and/or resins. Patients with NMs started LLT at younger age compared to cases with DMs (7.2 vs 34.3 years, respectively, $P < .0001$). At inclusion, 2 cases were on LDL apheresis that started 4 and 7 years before, and 4 cases with NM had received a liver transplant (mean age, 13 years). Two of these severe cases withdrew LLT after the liver transplant, and another 2 cases still require moderate doses of statins.

Follow-up

The mean follow-up until 2015 was 6.9 years (range, 3–11 years). In this period, one patient (subject 7 in the supplementary Table A) started lomitapide added on maximum-tolerated LLT achieving an additional LDL-C reduction of 52% without significant adverse events.[16]

Another patient (subject 3 in the supplementary Table A) with DM was enrolled in a phase 3 multicenter randomized, double blind, placebo-controlled trial with anti-PCSK9 antibodies (Amgen 20110271) achieving a total LDL-C reduction around 70% and LDL-C levels around 170 mg/dL.

Three patients with previous ASCVD developed cardiovascular events during the follow-up. One had carotid ACTP at 46 years, and the other two subjects had coronary revascularization procedures at ages 13 and 52 years. The

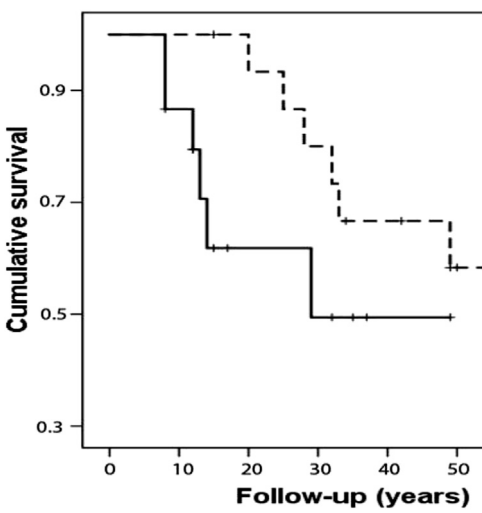

Figure 1 Kaplan-Meier curves for CVD-free survival in HoHF patients according to the type of mutation (dashed line, defective mutations; solid line, null mutations); $P = .054$.

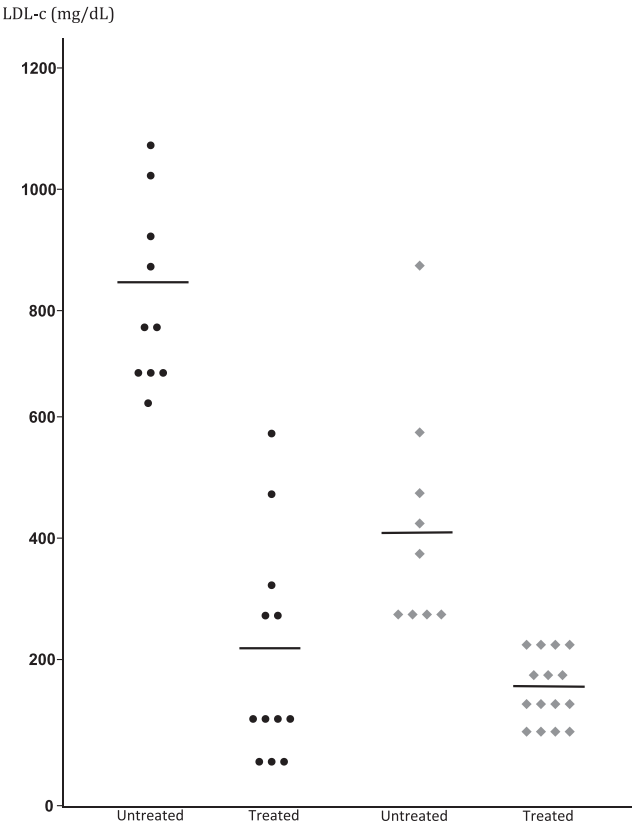

Figure 2 Untreated and treated low-density lipoprotein cholesterol levels in homozygous FH. Horizontal lines indicate mean LDL-C levels. Untreated LDL-C levels: 820 ± 166.5 (NMs) and 398.5 ± 181.9 (DMs) mg/dL, $P < .0001$. Treated LDL-C levels: 218 ± 162.9 (NMs) and 167.1 ± 42 (DMs), $P = .252$.

younger patient underwent a liver transplant at 15 years. Another patient who had heart and liver transplants before his enrollment died during the follow-up as a consequence of septicemia due to chronic immunosuppression. Eight cases (6 with NMs and 2 with DMs) started LDL apheresis achieving 4 of them LDL-C levels around 100 mg/dL (supplementary Table A).

Autosomal recessive hypercholesterolemia patients

Three cases with ARH were diagnosed at ages 4, 14, and 24 years. Mean untreated TC and LDL-C were 496 and 431 mg/dL respectively. There was no history of ASCAD. Xanthomas were present in 2 subjects (supplementary Table A). Patients started LLT at 5 years and were receiving combined therapy with a high statin dose and ezetimibe.

Discussion

We report the molecular and clinical characteristics in a longitudinal cohort of molecularly defined HoFH patients. This study shows a high molecular and phenotypic variability of the disorder, and that homozygotes with NMs express a more severe phenotype than do homozygotes with DMs. Moreover, only 50% of individuals met LDL-C criteria used for the clinical diagnosis.

In SAFEHEART, only HoFH individuals with genetic diagnosis are enrolled, regardless of their cholesterol levels. The most accepted clinical criteria for the diagnosis of HoFH are untreated LDL-C >500 mg/dL, or an LDL-C >300 mg/dL in patients on maximum-tolerated LLT.[4,8] In this study, only patients with NM met these biochemical criteria. Most patients with DMs had LDL-C levels below these cut-off points, overlapping with those levels usually observed in heterozygous FH patients.[4] Sjouke et al have shown that half of their molecularly defined HoFH population in The Netherlands had untreated LDL-C levels >500 mg/d. However, no information according to the severity of the molecular defect is provided.[5] Recently, a study comparing 3 different HoFH cohorts, suggested that molecular diagnosis is more sensitive than clinical criteria for the detection of the disorder.[17]

As similar reported in other HoFH cohorts with molecular confirmation, we observed a high variability in patient's age, cholesterol levels, and ASCAD regarding functionality of LDLR.[5,18,19] A high percentage of HoFH cases are not diagnosed in childhood. In this cohort, 81% of individuals with DMs and 17% of patients with NMs were older than 40 years of age at the moment of genetic diagnosis. The eldest patient enrolled in SAFEHEART is a woman with DM, currently aged 86 years, who started statin treatment at age 63 years and had her first ASCAD at age 79 years. This case shows that in some HoFH patients, other factors besides LDL-C levels and the molecular defect could play a role in the phenotypic expression.

With regard to lipid levels, a wide variability was observed in untreated and treated LDL-C levels according to the severity of *LDLR* gene mutation. With the exception of one case, individuals with DM were clustered in lower untreated and treated cholesterol levels compared to those individuals with more severe mutations. It has not been possible to compare different allelic combinations in CHeFH patients due to the small number of subjects with this condition. However, their untreated TC levels were similar to those observed in subjects with DM. It has been previously demonstrated that phenotype is similar between true HoFH and CHeFH.[5] Comparing to other studies, mean untreated TC levels in patients from SAFEHEART were higher than in patients from The Netherlands (527 mg/dL),[5] similar to South African patients (670 mg/dL)[20] and lower than patients in the United Kingdom (782 mg/dL in alive patients).[19] These differences could be explained in part by the genetic heterogeneity in each population, and also because 90% of patients in this study are true homozygous FH, whereas in the other studies, almost half of individuals are CHeHF that have variable residual activity in the receptor. It should be highlighted that most mutations have been classified as defective or null by computer simulated analysis, and it has been shown that this method is not always conclusive for most of the alteration, at least in the heterozygous condition.[21]

Forty percent of the Spanish HoFH patients had history of ASCAD, similar to other cohorts.[5,19] No differences were observed in ASCAD between patients with NMs and DMs. However, patients with more severe mutations had ASCAD at a younger age and also had a higher frequency of aortic valve stenosis. Survival curves show that the appearance of cardiovascular events depend on the severity of *LDLR* gene mutation, although the difference does not reach the significance threshold, probably due to the relatively small number of patients. The results of this study are similar to those described by Goldstein and Brown in 1982 showing that ASCAD was similar in homozygous patients considered to be receptor-negative or defective according LDLR activity measured in cultured fibroblasts and also that the onset of ASCAD is earlier in those individuals with no detectable activity.[22]

Patients with ARH had LDL-C levels similar to those observed in defective HoFH, as previously reported.[15] The response to LLT is better than HoFH, and no cases have developed ASCAD during the follow-up.

With the exception of 2 patients with liver transplants, the rest of the subjects were on statin treatment at enrollment. It has been shown by Raal et al that HoFH patients treated with statins with or without LDL apheresis had a significant reduction in mortality and also a decrease in major adverse cardiac events despite a modest LDL-C reduction.[20] In most HoFH patients, standard therapy with statins, ezetimibe, and resins does not sufficiently lower LDL-C levels. Therefore, most patients require LDL apheresis or exceptionally a liver transplant.[4,8,9,23] In this study, 9 patients are currently on LDL apheresis. It has been

associated with a significant reduction in mortality in adults with HoFH and slows the progression of aortic root atheroma in children if it is initiated as early as possible (<6 years), before aortic root lesions develop.[24–27]

Another outstanding option in some patients could be a liver transplant that replaces defective LDL receptors in the patient, producing an improvement of LDL-C levels to near-normal levels.[28,29] In this cohort, 5 patients underwent a liver transplant, and in 2 of them, it was performed after the development of ASCAD and aortic valve stenosis. Two of these patients still require statin therapy. Other two patients died, one as consequence of septicemia and the other in the postoperative period after a liver transplant. Long-term cardiovascular benefits of liver transplant are not clear due to the few cases reported in the literature and the short-term follow-up.[29,30] Liver transplantation has some disadvantages,in particular, the need for life-long immunosuppressive therapy,[4,8] and should be an exception in HoFH treatment.

In the last years, mipomersen, lomitapide, and PCSK9 inhibitors have been approved to treat HoFH because of their efficacy in the reduction of LDL-C levels.[10–12] In this cohort, 2 patients started with some of these drugs achieving significant reductions in LDL-C levels with acceptable tolerance and without significant adverse events. The use of these new drugs can contribute to achieve LDL-C goals and reduce the frequency or even avoid LDL apheresis.[10,16]

Strengths and limitations

SAFEHEART is an observational study of a population with genetic diagnosis of FH that are followed-up every year through a standardized protocol. Considering LDL-C levels for the diagnosis of HoFH, most of the cases with DMs could not be diagnosed. Nevertheless, we recognize that most of the clinical data at entry were obtained from medical records and information provided by patients or their physicians, and there are some incomplete data retrieval.

In conclusion, our study shows that HoFH has a high variability in phenotypical expression with some overlapping in LDL-C levels with those described in heterozygous FH patients, especially in HoFH patients with DMs. HoFH patients have high ASCAD risk including aortic valve stenosis, and most patients do not achieve the LDL-C treatment goals. These results emphasize that HoFH patients need an early detection and more intensive LLT. The availability of new classes of drugs in combination with a high-intensity LLT including LDL apheresis may help patients to achieve lower LDL-C levels to prevent ASCAD.

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Author contributions: Drs Alonso, Rodriguez-Urrego, and Mata designed the study. Drs Alonso, Díaz-Díaz, Arrieta, Fuentes-Jiménez, de Andrés, Saenz, Ariceta,Vidal-Pardo, Almagro, Argueso, Prieto-Matos,Miramontes, Pintó, and Mata participated in inclusion of patients and data acquisition. Drs Alonso, Perez de Isla, Rodriguez-Urrego, and Mata participated in writing the manuscript. Drs Rodriguez-Urrego and Perez de Isla did the statistic analysis. All authors have revised it critically and approved the final version for submission it critically and approved final version to be submitted.

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Appendix

Supplementary Table A

Patient (family)	Type Mutation	Gender	Age at inclusion	Clinical CAD (age)	Aortic valve stenosis	LDL-C baseline	LDL-C inclusion	Status follow-up
1 (1)	Def	F	83	Yes (79)	No	600 (TC)	220	Alive
2 (2)	Def	F	47	No	No	386	136	Alive
3 (3)	Def	M	40	No	No	555	231	Alive
4 (4)	Null	M	26	No	No	1120 (TC)	83‡	Alive
5 (4)	Null	F	24	No	No	910 (TC)	112‡	Alive
6 (4)*	Null	F	8	Yes (8)	NA	1203 (TC)	NA‡	Died
7 (5)	Null‖	F	42	Yes (29)	Yes	781	119§	Alive
8 (5)	Null‖	F	46	No	Yes	690	303	Alive
9 (6)*	Null	M	5	No	NA	762 (TC)	NA	Died
10 (6)*	Null	F	8	Yes (8)	NA	695 (TC)	NA	Died
11 (7)	Null	M	11	No	No	611	293†	Alive
12 (7)	Null	M	9	No	No	692	465†	Alive
13 (7)	Null	F	5	No	No	892	565	Alive
14 (8)	Def	M	38	Yes (32)	No	284	139	Alive
15 (8)	Def	M	43	No	No	312	149	Alive
16 (9)	Def	M	35	Yes (25)	No	425	216	Alive
17 (10)	Null¶	F	14	No	No	253	NA	Alive
18 (10)	Null¶	M	24	No	No	529	330	Alive
19 (11)	Def	F	48	No	Yes	537 (TC)	157†	Alive
20 (12)	Null¶	F	4	No	No	511	374	Alive
21 (13)	Def	M	23	No	No	498	246†	Alive
22 (14)	Def ‖	M	46	No	No	350 (TC)	175	Alive
23 (14)	Def ‖	M	43	Yes (33)	No	490 (TC)	114†	Alive
24 (15)	Def	F	50	Yes (NA)	No	250	220 (TC)	Alive
25 (16)	Null	F	8	No	No	780	117†	Alive
26 (17)	Def	M	2	No	No	885	163†	Alive
27 (18)	Null	F	7	Yes (13)	Yes	674	66†,‡	Alive
28 (19)	Def	M	36	No	No	341	151	Alive
29 (20)	Null	M	30	Yes (12)	Yes	1103	142‡	Died
30 (21)	Null	F	9	No	Yes	1044	88†	Alive
31 (22)	Null	M	5	No	No	933	266†	Alive
32 (23)	Def	F	61	No	No	255	126	Alive
33 (23)	Def	M	60	Yes (57)	No	250	122	Alive
34 (23)	Def	M	59	Yes (49)	No	341	162	Alive

NA, data not available.
*Died before registered.
†On LDL apheresis.
‡Liver transplantation.
§On lomitapide plus statin and ezetimibe.
¶Autosomal recessive hypercholesterolemia.
‖Compound heterozygous FH.