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Mutational analysis and genotype-phenotype relation in familial hypercholesterolemia: The SAFEHEART registry

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ABSTRACT

Background and aims: Familial hypercholesterolemia (FH) is an autosomal dominant disease of cholesterol metabolism that confers an increased risk of premature atherosclerotic cardiovascular disease (ASCVD). Therefore, early identification and treatment of these patients can improve prognosis and reduce the burden of cardiovascular mortality. The aim of this work was to perform the mutational analysis of the SAFEHEART (Spanish Familial Hypercholesterolaemia Cohort Study) registry.
Methods: The study recruited 2938 individuals with genetic diagnosis of FH belonging to 775 families. Statistical analysis was performed using SPSS v23.
Results: A total of 194 variants have been detected in this study, 24 of them were never described before. About 88% of the patients have a pathogenic or likely pathogenic variant. Patients with null variants have a more severe phenotype than patients with defective variants, presenting with significantly higher levels of atherogenic particles (total cholesterol, LDL-cholesterol and apolipoprotein B).
Conclusions: This study shows the molecular characteristics of the FH patients included in the SAFEHEART registry and the relationship with the phenotypic expression. The majority of the genetic variants are considered to be pathogenic or likely pathogenic, which confers a high level of confidence to the entry and follow-up data analysis performed with this registry concerning FH patients' prognosis, treatment and survival.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disease of cholesterol metabolism that confers an increased risk of premature atherosclerotic cardiovascular disease (ASCVD) [1,2]. The prevalence of the heterozygous FH form ranges from 1/250 individuals in some north European countries [3] to 1/500 [4]. Patients with FH have in average a three- to thirteen-fold greater risk of premature ASCVD compared with normolipidemic non-FH individuals [5]. Sudden death and acute ischemic heart disease are the main causes of death among these subjects [5,6]. Early diagnosis and lowering of low-density lipoprotein-cholesterol (LDL-C) significantly reduce ASCVD risk and improve quality of life in individuals with FH [7].

The relationship between phenotype and genotype for this disorder has been explored [8,9], but the absence of large well-characterized cohorts has limited the potential of these studies. The SAFEHEART registry (Spanish Familial Hypercholesterolemia Cohort Study) provides a unique opportunity to improve the knowledge about the prognostic factors, treatment and mechanisms that influence the development of premature ASCVD risk

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and mortality in an FH population. To our knowledge, this is the largest longitudinal study of a molecularly characterized heterozygous FH population that reflects real-life clinical care of patients [10–12].

The aims of this work was to perform a mutational analysis of the SAFEHEART registry to improve the understanding of the relation phenotype/genotype in the clinical expression in FH patients.

2. Materials and methods

The SAFEHEART study is an open, multicentre, nationwide, long-term prospective cohort study of molecularly defined patients with heterozygous FH in Spain that was initiated in 2004 [10–12]. Inclusion criteria were index cases with genetic diagnosis of FH and their relatives. This study was approved by the local ethics committees and all eligible subjects gave written informed consent.

Demographic and clinical characteristics were obtained and recorded at inclusion through a medical interview. Data include age, classic cardiovascular risk factors, cardiovascular disease, presence of xanthomas, physical examination, and current treatment for hypercholesterolemia. Cardiovascular disease was defined as the presence of any of the following: 1) Myocardial infarction: proved by at least two of the following: classic symptoms (>15 min), specific electrocardiographic changes and increased levels of cardiac biomarkers (>2 × upper limit of normal); 2) angina pectoris: diagnosed as classic symptoms in combination with at least one unequivocal result of one of the following: exercise test, nuclear scintigraphy, dobutamine stress ultrasound scan or > 70% stenosis on a coronary angiogram; 3) percutaneous coronary intervention or other invasive coronary procedures; 4) coronary artery bypass grafting; 5) ischemic stroke demonstrated by computed tomography or magnetic resonance imaging or documented transitory ischemic attack. Premature ASCVD was defined as the occurrence of the first event before 55 years of age in men and before 65 years of age in women. When a patient reported at inclusion that she/he had history of cardiovascular disease (CVD) (or cardiovascular heart disease (CHD)), a medical report from her/his physician was requested to confirm the diagnosis.

Venous blood samples were taken after 12 h of fasting. Serum, plasma, and DNA samples were aliquoted and preserved at −80 °C. The molecular studies have been described elsewhere [13]. Large deletions or insertions were analysed by Multiplex Ligation-dependent Probe Amplification (MLPA) [13]. Cascade screening has been promoted by the Spanish FH Foundation and a Coordinating Centre was responsible for managing the follow-up.

For the objectives of this study, only cases with molecular diagnosis of FH (IC and relatives carrying a mutation) were included in the analysis.

2.1. Biochemical characterization

Biochemical parameters were analysed in a central laboratory. Values for total cholesterol (TC), LDL-C, HDL cholesterol (HDL-C), triglycerides (TG), lipoprotein (a) (Lp(a)), apolipoprotein A (apoAI) and apolipoprotein B (apoB) were available for the majority of the individuals. Values used for the analysis were preferably values without medication at the time of inclusion in the SAFEHEART registry (n = 581), and when these were not available, TC, LDL-C and apoB values under medication were corrected for medication using correction factors of 1.4, 1.6 and 1.48, respectively, as described before [14,15]. Previous published LDL percentiles for the Spanish population were used for data analysis [16].

2.2. Genetic annotation

All variants were checked using Mutalyzer v2.0 and converted to the nomenclature recommended by the Human Genome Variation Society (HGVS). The reference sequences used for *LDLR*, *APOB* and *PCSK9* were respectively NM_000527.4, NM_000384.2 and NM_174936.3, and cDNA numbering was considered following the HGVS nomenclature, with nucleotide c.1 being A of the ATG initiation codon p.1 [17].

2.3. In silico analysis

The following software tools were applied to all variants: PolyPhen-2 [18], Sorting Tolerant From Intolerant (SIFT) [19] and Mutation taster [20] for prediction of amino acid substitutions; Splice-Site Predictor (Splice Port) [21], Neural Network Splice Site Prediction Tool (NNSSP) [22] and Neural Network Predictions of Splice Sites in Human (NetGen2) [23] for prediction of splicing defects. When all programs were concordant, a final *in silico* classification of pathogenic or benign was achieved accordingly; when concordance did not exist a classification of "?" was attributed.

2.4. Allele type

Patients with nonsense, frameshift, large rearrangements or other variants shown *in vitro* to have less than 2% LDLR activity were considered to be carriers of a null allele variant (including missense, small indels and splicing variants). Patients with an alteration where LDLR activity has been determined to be less than 80% or when a functional study has not been performed, but the overall *in silico* analysis indicated pathogenicity, were considered to be carriers of a defective allele variant. Patients were considered to be carriers of a non-determine allele (NDA) variant when: 1) a splicing variant was found and there are no functional studies with transcript quantification or LDLR activity determination, not allowing the identification of an allele as null or defective; 2) the *in silico* classification of the 3 programs used was not concordant.

2.5. Mutation functional classification

Functional classification was performed according to the American College of Medical Genetics and genomics (ACMG) 2015 guideline [24] that classify variants as: pathogenic, likely pathogenic, variant of unknown significance (VUS) and likely benign. Variants with a classification of benign were not included in this study.

2.6. Allele frequency analysis

If a variant was described in more than 5% of the studied population (Minor Allele Frequency (MAF) > 5%) in the 1000 Genomes database (1 KG) [25], the Exome Sequencing Project (ESP) (URL: http://evs.gs.washington.edu/EVS/) database [26], or ExaC [27], the alteration was considered to be a common variant or a polymorphism, and therefore a neutral alteration. These variants were not included in the present work.

2.7. Statistical analysis

Statistical analysis was performed using SPSS software (version 23.0 for Windows; SPSS, Chicago, Illinois). Comparison of frequencies between qualitative variables was carried out using the Chi-squared/Fisher exact test. Median values were compared with the non-parametric tests Mann-Whitney. Pearson correlation was conducted to determine associations between quantitative

variables. Comparison of variances was carried out using the Levene test. A p-value <0.05 was considered statistically significant.

3. Results

A total of 775 unrelated families with 2938 FH individuals are included in the SAFEHEART registry: 206 children and adolescents under 18 years (6 index cases and 200 relatives) and 2732 adults (769 index cases and 1963 relatives) (Supplementary Fig. 1). These individuals are distributed within all Spanish regions (Supplementary Table1). Demographic and clinic data are shown in Table 1. Comparing adult index cases (IC) and their relatives, statistically significant differences were observed in age ($p < 0.005$), Lp(a) ($p = 0.037$), ApoAI ($p = 0.003$), premature ASCVD ($p < 0.001$), tendon xanthomas ($p < 0.005$), and patients on medication ($p < 0.005$). When the same analysis was conducted separately for male and female genders, the results were very similar, except for premature ASCVD that was double in males than in females (Supplementary Tables 2 and 3). For the paediatric group, the low number of IC does not allow an accurate comparison although by mean comparison it can be observed that IC children are more affected than their relatives (Table 1).

3.1. Mutational analysis

A total of 194 variants have been detected in the SAFEHEART cohort (189 in LDLR and 5 in APOB), 65 (33%) of these have been functionally proved to be the cause of the disease. Following the ACMG classification, 88% (170) are classified as pathogenic or likely pathogenic: 111 are considered pathogenic (1940 individuals, 502 families), 59 likely pathogenic (752 individuals, 215 families); 11% [21] are classified as VUS (221 individuals, 49 families); 1% [3] are classified as probably benign (25 individuals, 9 families) (see Materials and Methods for variant classification). There are 10 double heterozygotes (9 in LDLR and 1 in APOB), but all in the same gene. The description of the alterations by functional class and gene is presented in Supplementary Table 4.

From the 194 variants, 24 have not been reported previously. The main characteristics of these novel variants are shown in Table 2.

The distribution of the 3 most common FH mutations by region is presented in Supplementary Fig. 2; the most common mutation in the top 3 per region is c.1342C > T, p.(Gln488*) present in 7 different regions. The 10 most common variants in this cohort represent 40% of all enrolled cases (Fig. 1) and include 3 nonsense mutations (p.(Gln33*), p.(Gln154*) and p.(Gln488*)), 4 splicing variants (2 functional confirmed, c.1358+1G > A and c.1845+1G > C and 2 putative variants, c.313+1G > C and c.2389+4A > G), 1 regulatory, c.-135C > G, the APOB3527 mutation and 1 missense (p.(Asn564His)) and a small deletion (p.(Val800_Leu802del)) in the same allele, both proven to be pathogenic [28].

3.2. Phenotype vs. genotype

Patients were classified according to allele type in null allele (N = 911), defective allele (N = 1259) and non-determined allele (NDA, N = 473) as described in Materials and methods. Adults patients with null allele variants presented with a more severe phenotype, with significantly higher values of atherogenic markers (TC, LDL-C, ApoB and ApoB/*ApoI*) than patients with defective allele variants ($p < 0.05$ for all) except for Lp(a) and TG values. No statistically significant differences were observed in HDL-C and ApoA1 between patients with null and defective allele variants (Table 3). Comparison of the biochemical profile of patients with null and defective allele variants with that of NDA carriers showed that the phenotype of the latter is more similar to those with null mutations than those with defective mutations, in terms of LDL-C levels and frequency of xanthomas.

The percentage of patients on lipid-lowering medication was higher in those patients with null allele variants and lower in those cases with NDA allele variants. Regarding premature ASCVD, a significant difference was observed only between patients with null and NDA mutations. It was also seen that patients with null variants have a lower dispersion of LDL values than carriers of defective mutations (Supplementary Fig. 3).

When the phenotype and genotype of both genders were compared separately, similar results were obtained. However, it is worth noting that ASCVD rates in males are approximately double than in women (15.5% *vs* 5.8%, respectively) (Supplementary Tables 5 and 6).

4. Discussion

This study shows the molecular characteristics of the FH

Table 1
Demographic and clinic data of the 2938 patients (with molecular diagnosis of FH) at inclusion in the SAFEHEART registry.

	Children						Adults					
	IC	n	Rel	n	Total	n	IC	n	Rel	n	Total	n
Age (years)	16.0 ± 1.1	6	14.9 ± 1.9	200	14.9 ± 1.9	206	49.3 ± 13.6*	769	44.4 ± 16.3*	1963	45.8 ± 15.7	2732
Male (%)	50% (3)	3	51.5%	103	51.5%	106	46.2%	356	45.9%	902	45.9%	1258
BMI	22.3 ± 2.4	6	21.5 ± 3.7	200	21.5 ± 3.4	206	26.8 ± 4.7	767	26.2 ± 4.9	1932	26.4 ± 4.8	2699
TC (mg/dL)	295.7 ± 30.5	6	272.7 ± 60.8	191	273.4 ± 60.2	197	325.1 ± 73.2	748	322.2 ± 71.8	1932	322.9 ± 72.2	2680
LDL -C (mg/dL)	236.1 ± 32.4	6	212.2 ± 63.0	191	212.9 ± 62.4	197	259.5 ± 75.8	748	256.9 ± 73.7	1932	257.6 ± 74.3	2680
HDL-C (mg/dL)	48.0 ± 6.9	6	49.3 ± 9.8	191	49.3 ± 9.7	197	50.1 ± 13.0	748	50.0 ± 12.7	1932	50.1 ± 12.8	2680
TG (mg/dL)**	73.0 (49.5)	6	62.0 (29.8)	191	62.0 (30.3)	197	86.0 (51.7)	748	81.0 (52.0)	1932	82.4 (52.0)	2680
Lp(a) (mg/dL)**	21.4 (81.1)	6	18.0 (40.4)	186	18.0 (41.3)	192	24.8 (51.3)*	693	21.4 (44.4)*	1805	22.1 (46.3)	2498
apoAI (mg/dL)	126.3 ± 22.5	6	124.6 ± 22.7	186	124.6 ± 22.6	192	139.1 ± 29.3*	703	135.5 ± 27.8*	1818	136.5 ± 28.3	2521
apoB (mg/dL)	140.9 ± 24.2	6	124.7 ± 35.8	186	125.2 ± 35.6	192	161.7 ± 46.8	703	157.3 ± 44.8	1818	158.5 ± 45.4	2521
apoB/apoAI (mg/dL)	1.13 ± 0.25	6	1.02 ± 0.31	186	1.03 ± 0.31	192	1.19 ± 0.39	703	1.22 ± 0.66	1818	1.22 ± 0.59	2521
LDL-C ≥ P75th (%)	100%	6	96.3%	183	91.7%	189	97.8%	524	96.4%	1369	96.8%	1893
LDL-C ≥ P95th (%)	100%	6	90.5%	172	90.8%	178	89.2%	478	86.7%	1231	87.4%	1709
Tendon Xant (%)	0%	0	0.5%	1	0.5%	1	19.3%*	149	11.8%*	233	14%	382
pASCVD (%)	0%	0	0%	0	0%	0	15.7%*	121	6.9%*	135	9.4%	256
Medication (%)	66.7%	4	47.5%	95	48.1%	99	94.0%*	725	78.2%*	1536	82.6%	2261

*$p < 0.05$ IC *versus* relatives. Values are expressed as mean ± standard deviation (SD), except in "**" where values are expressed as median and interquartile range.
IC, index Case; Rel, relatives; TC, total cholesterol, LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; Lp(a), lipoprotein(a); apoA1, apolipoprotein A1; apoB, apolipoprotein B; LDL-C percentile; P75th, 75th percentile; P95th, 95th percentile. Xant, xanthomas; pASCVD, premature atherosclerotic cardiovascular disease.

Table 2
Description and overall *in silico* classification of the 24 new alterations found in this study.

cDNA	Protein	Gene	Alteration type	Overall *in silico*	Allele type - predicted	ACMG
c.2853G > A	p.(=)	APOB	PS (synonymous)	?	NPD	VUS
c.8148C > T	p.(=)	APOB	PS (synonymous)	?	NPD	VUS
c.10588G > A	p.(Val3530Met)	APOB	PS (missense)	?	Defective	VUS
c.-228G > C	p.(?)	LDLR	PS (regulatory)	NA	NPD	VUS
c.91_104del	p.(Glu31Argfs*16)	LDLR	Del (14 nucleotides)	?	Null	Pathogenic
c.131G > T	p.(Trp44Leu)	LDLR	PS (missense)	Pathogenic	Defective	VUS
c.191-?_2583+?del	p.(?)	LDLR	Del (large rear)	NA	Null	Likely pathogenic
c.362_376del	p.(Cys121_Gln125del)	LDLR	Del (in frame)	Pathogenic	Defective	Likely pathogenic
c.440_450dup	p.(Ala151Profs*59)	LDLR	Ins (11 nucleotides)	?	Null	Pathogenic
c.479G > T	p.(Cys160Phe)	LDLR	PS (missense)	Pathogenic	Defective	Likely pathogenic
c.706T > A	p.(Cys236Ser)	LDLR	PS (missense)	Pathogenic	Defective	VUS
c.890A > C	p.(Asn297Thr)	LDLR	PS (missense)	Pathogenic	Defective	VUS
c.890A > G	p.(Asn297Ser)	LDLR	PS (missense)	Pathogenic	Defective	VUS
c.910G > C	p.(Asp304His)	LDLR	PS (missense)	Pathogenic	Defective	Likely pathogenic
c.987C > A	p.(Cys329*)	LDLR	PS(missense)	?	Null	Pathogenic
c.1013G > T	p.(Cys338Phe)	LDLR	PS (missense)	Pathogenic	Defective	Likely pathogenic
c.1061-?_1586+?del	p.(?)	LDLR	Del (large rear)	NA	Null	Likely pathogenic
c.1359-?_1586+?del	p.(?)	LDLR	Del (large rear)	NA	Null	Likely pathogenic
c.1359-27T > G	p.(?)	LDLR	PS (splicing)	?	NPD	VUS
c.1749C > G	p.(His583Gln)	LDLR	PS (missense)	Pathogenic	Defective	Likely pathogenic
c.1981C > A	p.(Pro661Thr)	LDLR	PS (missense)	Pathogenic	Defective	VUS
c.2011del	p.(Thr671Profs*2)	LDLR	Del (1 nucleotides)	?	Null	Pathogenic
c.2054C > A	p.(Pro685Gln)	LDLR	PS (missense)	Pathogenic	Defective	Likely pathogenic
c.2270del	p.(Pro757Leufs*8)	LDLR	Del (1 nucleotides)	?	Null	Pathogenic

Allele type - predicted means that the allele type has been predicted by the analysis of the nature of the alteration or using the results of the *in silico* analysis. PS, point mutation; Del, deletion; large rear, large rearrangements; Ins, insertion.
Overall *in silico* as described in the Materials and methods section; ?, the *in silico* analysis was not conclusive; NA, not applicable; NPD, not possible to determine ACMG, classification by the American College of Medical Genetics and genomics.

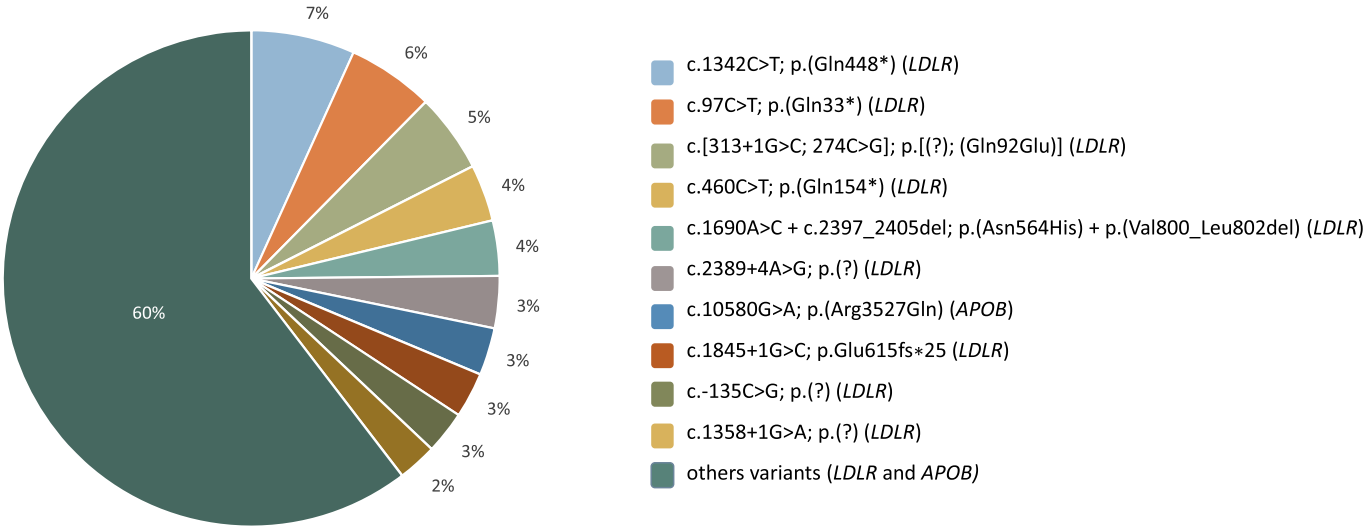

Fig. 1. Schematic representation of the mutational spectrum of the SAFEHEART registry.

patients included in the SAFEHEART registry and the relationship with phenotypic expression. This registry is to our knowledge the only cohort of FH families molecularly characterized with a follow-up of more than 5 years. The genetic characterization of this cohort is very important for a better interpretation of follow-up data. The majority (88%) of the variants presented by patients included in this registry are considered to be pathogenic or likely pathogenic, which strongly supports all data analysis that can be performed with this cohort concerning FH patients' prognosis, treatment and survival. Moreover, the SAFEHEART registry is a nationwide study, which allows the extrapolation of all results to the FH Spanish population.

The genotype/phenotype characterization of a great number of individuals with the same variant has the power to discriminate between different types of variants. In this cohort, it has been shown that patients with null allele variants have a more severe phenotype than carriers of defective variants. This has been referred before [29–31], but this analysis of a large number of FH individuals with the same type of alteration adds solid evidence that determining the allele type can provide additional information for patient prognosis, highlighting which patients need an early initiation or more intensive lipid-lowering treatment. In addition, null allele carriers present a lower LDL values dispersion than defective alteration carriers, indicating the consistency of higher cholesterol levels presented by null allele carriers. However, to determine the allele type, functional assays need to be performed for all putative missense and splicing variants to identify which

Table 3
Clinical and biochemical characteristics of the 2643 adults patients presented in this study regarding the allele type.

	Null	n	Defective	n	NDA	n	*p* null *vs.* NDA	*p* null *vs.* defective	*p* NDA *vs.* defective
Clinical									
Age (yr)	45.9 ± 15.8	911	45.8 ± 15.7	1259	46.7 ± 15.9	473	0.541	0.830	0.453
BMI (kg/m^2)	26.4 ± 4.7	911	26.4 ± 4.9	1257	26.4 ± 4.8	470	0.902	0.626	0.646
Td Xant (%)	16.6%	151	10.7%	150	14.7%	78	0.050	**<0.001**	**0.016**
pASCVD (%)	10.2%	93	9.1%	114	7.3%	39	**0.040**	0.114	0.135
Therapeutic (%)	85.5%	779	79.3%	1112	74.9%	397	**<0.001**	**<0.001**	**0.001**
Lipid profile (mg/dL)									
TC	327.6 ± 69.4	911	320.1 ± 66.9	1259	329.3 ± 69.8	473	0.951	**0.007**	**0.024**
LDL-C	264.1 ± 69.0	911	253.9 ± 68.9	1259	265.4 ± 70.3	473	0.863	**<0.001**	**0.003**
HDL-C	49.2 ± 12.3	911	50.4 ± 12.7	1259	49.9 ± 13.5	473	0.487	0.052	0.422
TG*	81.0 (52.0)	911	84.0 (54.0)	1259	82.7 (47.5)	473	0.369	0.146	0.793
Lp(a)*	24.0 (48.7)	865	21.0 (43.8)	1166	24.2 (46.6)	435	0.976	0.343	0.462
apoA1	136.8 ± 27.9	871	136.6 ± 27.7	1181	135.6 ± 28.8	436	0.466	0.896	0.519
apoB	163.6 ± 43.6	871	153.2 ± 42.6	1181	160.7 ± 43.7	436	0.126	**<0.001**	**0.002**
apoB/apoA1 ratio	1.24 ± 0.39	871	1.17 ± 0.58	1181	1.26 ± 0.83	436	0.622	**<0.001**	**0.001**

Values are expressed as mean ± standard deviation (SD), except in "*" where values are expressed as median and interquartile range. yr, years; Td Xant, tendon xanthomas; pASCVD, premature atherosclerotic cardiovascular disease; TC, total cholesterol, LDL-C, LDL cholesterol; HDL-C, HDL cholesterol; TG, triglycerides; Lp(a), lipoprotein(a); apoA1, apolipoprotein A1; apoB, apolipoprotein B; NDA, non-determined allele; $p < 0.05$ in bold.

produce an LDLR with less than 2% activity (null allele); for stop mutations, large deletions, small deletions and insertions that lead to a frame shift, and consequently the introduction of a premature stop codon, no further evidence is necessary to prove the non-functionality of the LDLR, being classified automatically as null alleles. In this cohort, about 60% of all alterations found are well-established null alleles or have functional characterization, which is higher than the worldwide rate of 50% [32].

Interestingly, in this cohort, the index cases and relatives do not present a different phenotype, in contrast with what has been found in other cohorts [33,34], probably due to an improvement in dietary habits including decreased saturated fat intake of this FH Spanish population consuming a Mediterranean type diet.

Although patients with null variants present significantly higher TC and LDL-C than patients with defective mutations, the difference between them is less than 20 mg/dL. This could explain in part the fact that there is no difference in premature ASCVD in these 2 groups. Another reason is the fact that in this cohort only 19% are index cases, the remaining are relatives so a dilution effect can be seen for null allele carriers. In fact, the role of the type of mutation in ASCVD is controversial. A previous report of this population, analysing only index cases, showed differences in risk in patients with null and defective mutations [35]. However, later reports of the entire population including index cases and relatives have not shown any relationship [5].

A higher percentage of patients on medication was observed in those cases with null allele variants compared with those with defective allele and NDA variants, suggesting that the mutational functional effect is important in determining the severity of the phenotype and consequently the treatment; thus the knowledge of the functional effect of each variant can add evidence for the clinician to start the patient on the right medication.

More than 10% of the variants in the SAFEHEART cohort are novel, showing that the complexity of *LDLR* variants spectrum continues to grow, even though nearly 1700 different alterations have been found worldwide (http://databases.lovd.nl/shared/genes/LDLR) [36]. Nevertheless 40% of all cases in this cohort have one of the 10 most common variants, a picture seen in most countries [3,29,37,38].

We acknowledge some strengths and limitations of our study. To our knowledge, this is the largest longitudinal study of a molecularly characterized heterozygous FH population that reflects real-life clinical care of patients. However, there are some limitations, a pre-treatment lipid profile is lacking for most patients at enrolment, because they were under treatment by the patient's physician. Therefore, a reliable lipid profile in this registry is missing and lipid profile values were estimated based on published corrections. This can introduce some bias for the patient phenotype and can change the data, in contrast to genetic data, which do not change with time, medication or adherence to treatment.

This study shows the molecular characteristics of the FH patients included in the SAFEHEART registry and the relationship with phenotypic expression. The genetic characterization of this registry is relevant for a better interpretation of follow-up data. The majority of the genetic variants are considered to be pathogenic or likely pathogenic, which confers a high level of confidence to the entry and follow-up data analysis performed in the future with this registry concerning FH patients' prognosis, treatment and survival.

Conflict of interest

The authors declared they do not have anything to disclose regarding conflict of interest with respect to this manuscript.

Author contributions

MB performed data analysis and interpretation and drafted the manuscript; ACA performed statistical and data analysis; RA and NM participated in recruitment of patients, database design and data input; TP performed biochemical determinations and DNA extraction; MB, ACA, RA and PM revised the final version of the manuscript; PM conceived, design and supervised the SAFEHEART study. All authors contributed to the final version of the paper.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.atherosclerosis.2017.04.002.

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