Original Research

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The island of Gran Canaria: A genetic isolate for familial hypercholesterolemia

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Background

Genetic diagnosis of familial hypercholesterolemia (FH) has not been universally performed in the Canary Islands (Spain).

Objectives

This study aimed to genetically characterize a cohort of patients with FH in the island of Gran Canaria.

Methods

Study subjects were 70 unrelated index cases attending a tertiary hospital in Gran Canaria, with a clinical diagnosis of FH, according to the criteria of the Dutch Lipid Clinic Network. Given that 7 of the first 10 cases with positive genetic study were carriers of a single mutation in the *LDLR* gene [p.(Tyr400_Phe402del)], a specific PCR-based assay was developed for the detection of this variant as a first screening step on the remaining subjects. In those without this mutation, molecular diagnosis was completed using a next-generation sequencing panel including *LDLR, APOB, PCSK9, LDLRAP1, APOE, STAP1,* and *LIPA* genes and incorporating copy number variation detection in *LDLR*.

Results

On the whole, 44 subjects (62%) had a positive genetic study, of whom 30 (68%) were heterozygous carriers of the p.(Tyr400_Phe402del) variant. Eleven subjects carried other mutations in *LDLR*, including the novel mutation NM_000527.4: c.877dupG; NP_000518.1: p.(Asp293Glyfs*8). An unclassified *PCSK9* gene variant was found in one subject [(NM_174936.3:c.1496G>A; NP_777596.2: p.(Arg499His)]. Other single patients had mutations in *APOB* (heterozygous) and in *LIPA* (homozygous). All identified variants co-segregated with the disease phenotype.

Conclusions

These findings suggest a founder effect for the p.(Tyr400_Phe402del) *LDLR* mutation in Gran Canaria. A cost-effective local screening strategy for genetic diagnosis of FH could be implemented in this region.

Keywords: Founder effect; Genetic isolation; Familial hypercholesterolemia; Canary Islands

Introduction

Familial hypercholesterolemia (FH, OMIM 144400) is the most common monogenic disorder. It is transmitted in an autosomal dominant pattern with a penetrance above 90%. Phenotypically, it is characterized by very high serum low-density lipoprotein cholesterol (LDL-C), premature atherosclerotic cardiovascular disease (ASCVD), and cholesterol deposits in the skin, tendons, and cornea, in the form of xanthelasmas, xanthomas, and corneal arcus, respectively.[1]

FH is produced by mutations in different genes that regulate cholesterol metabolism.[2] The LDL receptor gene (*LDLR*), with more than 1700 known pathogenic mutations, accounts for 80% of the cases of FH. The remaining are caused by mutations in *APOB* (the gene encoding apolipoprotein B100) and *PCSK9* (subtilisin-convertase proprotein/kexin type 9) and, exceptionally, mutations in *APOE* (apolipoprotein E), *STAP1* (signal transducing adaptor family member 1), and *LDLRAP1* (low-density lipoprotein receptor adaptor protein 1) genes, the latter producing a recessive form of the disease. The 20 to 40% of cases, where no responsible mutation is detected, are believed to be severe forms of polygenic hypercholesterolemia.[3]

Approximately 34 million people in the world suffer from FH, although it is likely to be underdiagnosed and undertreated.[4] The heterozygous form of FH (HeFH) is the most common, with a prevalence of 1:200 to 250 people,[5] whereas the more severe, homozygous form (HoFH), occurs with a frequency of 1:300,000 to 450,000.[6] In some genetically isolated populations, owing to the transmission of one or more mutations with a founder effect, the prevalence is higher. So far, this phenomenon has been reported in Lebanese,[7] French Canadians,[8] Afrikaners,[9] Finns,[10] and Ashkenazi Jews.[11]

In Spain, there is wide genetic heterogeneity in FH, and most of the reported *LDLR* gene mutations have also been observed in other countries. There are no known areas with genetic isolation, and the highest regional prevalence of a single *LDLR* mutation does not exceed 7%.[12,13] The Canary Islands (Spain) are located in the Atlantic Ocean, about 100 miles off the West African coast. With over 845,000 inhabitants, Gran Canaria is the most populous island in the archipelago. Although the Canary Islands have the highest prevalence of hypercholesterolemia in Spain,[14] they have not been subject of any specific epidemiological study on FH.

In this study, we assessed the genetic determinants underlying FH in a population of Gran Canaria and found a highly prevalent mutation in the *LDLR* gene.

Methods

Subjects

The study population included all index cases of families attending the Lipids Unit of the Complejo Hospitalario Universitario Insular Materno-Infantil (CHUIMI) with a clinical diagnosis of definite FH, according to the criteria of the Dutch Lipid Clinic Network (scores ≥ 8 points),[15] untreated LDL-C concentrations >220 mg/dL, and with both parents born in Gran Canaria. Patients with the following causes of secondary hypercholesterolemia were excluded: hypothyroidism (serum TSH ≥6 mU/l), cholestasis (direct bilirubin ≥1.0 mg/dL), nephrotic syndrome (proteinuria ≥3.5 g/d), advanced chronic kidney disease (estimated glomerular filtration rate <30 mL/min/1.73 m^2), or current use of drugs with significant effects on lipid metabolism (glucocorticoids, retinoids, cyclosporine, antiretrovirals).

Previously, since September 2015, a screening campaign was carried out in the primary care centers covering the influence area of the CHUIMI, to identify patients with undiagnosed FH. Subjects with LDL-C >220 mg/dL and one or more of the following criteria were to be referred to the Lipids Unit: (1) personal or family history of premature coronary heart disease (CHD) (<55 years in men and <60 years in women) and (2) first-degree relatives older than 18 years with total cholesterol >310 mg/dL and/or LDL-C >190 mg/dL or younger than 18 years with total cholesterol >230 mg/dL and/or LDL-C >160 mg/dL. Patients were also referred if they had an LDL-C >100 mg/dL despite high-dose treatment with potent statins (atorvastatin 40-80 mg or rosuvastatin 20–40 mg).

The study followed the ethical standards of the 1975 Declaration of Helsinki and was approved by the local ethics committee. All patients were informed of the study procedure and signed an informed consent form.

Study protocol

Clinical and demographic information was recorded for each participant, including age, sex, birth municipalities of the patients and their parents, previous diagnosis of hypertension and diabetes, smoking, age at diagnosis of FH, history of ASCVD as well as age at diagnosis and clinical form of presentation, and family history of hypercholesterolemia and ASCVD in first-degree relatives. Serum concentrations of total cholesterol, high-density lipoprotein cholesterol (HDL-C), LDL-C, and triglycerides, measured without any lipid-lowering medication, were also collected.

Physical examination included measurement of weight, height, waist circumference and blood pressure, and assessment of the presence of corneal arcus, xanthelasmas, and tendon xanthomas.

Genetic analysis

Genomic DNA was isolated from blood samples collected in EDTA-containing tubes, using a salt precipitation protocol.[16] The first 10 probands were genotyped using next-generation sequencing (NGS) of the *LDLR*, *PCSK9*, and *LDLRAP1* promoter, exons, and intron-exon boundaries and the LDLR-binding domain of *APOB*[17] in Progenika Biopharma SA (Derio, Vizcaya, Spain). Given its prevalence in these subjects, the p.(Tyr400_Phe402del) mutation was set for detection as a first screening test on all remaining subjects, before any further investigation. As the wild-type allele for this variant is 9 bp larger than the mutant allele, the assay was based on this difference. The Primer BLAST application[18] was used to design specific oligonucleotide primers: LDLR_e9_1F (5′-AGGCACTCTTGGTTCCATCG-3′), labeled with 6-carboxyfluorescein (FAM) and LDLR_e9_1R (GAGGAGAGAAGGGCATCAGC). Amplification was performed with 35 cycles (95°C, 1 min; 55°C, 1 min; 72°C, 1 min) on 50 ng genomic DNA, with Taq polymerase, following the manufacturer's instructions (Promega Biotech, Madison, WI, EEUU). PCR products were denatured by adding one volume of deionized formamide, heated at 95°C for five minutes and run through 4% acrylamide:bis acrylamide (19:1), 50% urea denaturing gels in 1xTBE (1xTBE = 89 mM Tris borate, 2 mM EDTA, pH 8.2). After electrophoresis, 6-FAM fluorescence was detected by using an FUJI FLA 9000 Starion scanner (Fujifilm Corporation, Tokyo, Japan), following the manufacturer's instructions.

In subjects without p.(Tyr400_Phe402del) mutation, molecular diagnosis was completed at the Gendiag Laboratory (Barcelona, Spain) using an NGS panel including *LDLR, APOB, PCSK9, LDLRAP1, APOE, STAP1,* and *LIPA* genes. Multiplex ligation primer amplification was also performed to detect large-scale copy number variations in *LDLR*.[19] Sequencing included *LIPA* (encoding lysosomal acid lipase) because some *LIPA* mutations, known to be linked to Wolman disease and cholesterol ester storage disease, have also been found in subjects with a clinical phenotype overlapping HeFH.[20]

The potential pathogenicity of all genetic variants was evaluated with *in silico* prediction tools (PolyPhen2, SIFT, and Mutation Taster)[21-23] and classified, according to the Association for Clinical Genetic Science, into five categories: (1) clearly not pathogenic; (2) unlikely to be pathogenic; (3) unknown significance; (4) likely to be pathogenic; and (5) clearly pathogenic.[24] Potentially pathogenic variants found in probands were ascertained in family studies to verify co-segregation with hypercholesterolemia.

Statistical analyses

Results were summarized as means ± SD or medians (interquartile range) for continuous variables and frequencies (%) for categorical variables. Percentages were compared using the chi-square test (χ^2), means with Student's *t*-test, and medians by Wilcoxon's test for independent data. Statistical significance was established at a two-tailed *P* value < .05. Data were analyzed using the statistical package SPSS, version 20.0 (IBM Corporation, Armonk, NY).

Results

In total, 70 unrelated probands (50 women and 20 men; mean age 52.2 ± 14.4 years) met the inclusion criteria (Table 1). An initial approach involved the selection of 10 unrelated index cases, chosen from different geographic origins within the island of Gran Canaria, who underwent NGS screening as described in the Methods section. Seven of them were carriers of same p.(Tyr400_Phe402del) in frame deletion (SNP Id: rs879254826), already described in a previous Spanish study involving patients from Gran Canaria.[25] This predominant mutation was then screened in the rest of the index cases, revealing that a total of 30 (43%) were heterozygous carriers of this deletion. Among the subjects who did not have p.(Tyr400_Phe402del) mutation, 11 carried one of six other variants in *LDLR* (see Table 2). One of them [c.877dupG; p.(Asp293Glyfs*8)], not described before, was detected in a 45-year-old man with CHD since the age of 33 years and with untreated total and LDL-C concentrations of 346 and 258 mg/dL, respectively. The frameshift caused by this mutation generates a truncated polypeptide lacking the C-terminal end of LDLR in the ligand-binding domain and, therefore, may be considered as a null allele, classified as category 5 (clearly pathogenic). The other five variants, all of them classified as categories 4 (likely to be pathogenic) or 5 were c.-135C>G, in the promoter region,[25,26] also identified in three probands; c.760C>T [p.(Gln254*)][27] in one subject; c.1091G>A [p.(Cys364Tyr)],[19] identified in three subjects; c.1609G>T [p.(Gly537*)][25] in two; and c.185C>T [p.(Thr62Met)][28] in another one (Table 2). Two of the patients with *LDLR* mutations, one heterozygous for p.(Gln254*) and one for p.(Cys364Tyr), were found to be double heterozygotes as they also carried the c.60_65dupGCTGCTGCT [p.(Leu22_Leu23dup)] variant in the *PCSK9* gene (ClinVar: 265916).[29] The first case was a 61-year-old woman with myocardial infarction at age 43, total cholesterol of 395 mg/dL, and LDL-C of 337 mg/dL; the second was a 59-year-old male without ASCVD, with total cholesterol of 397 mg/dL and LDL-C of 334.4 mg/dL. Both subjects had tendon xanthomas. In addition to the described *LDLR* mutations, variants were also found in other genes. These included one unclassified variant in *PCSK9* [NM_174936.3:c.1496G>A; NP_777596.2: p.(Arg499His)] and another one in *APOB* [NM_000384.2:c.8882A>G; NP_000375.2: p.Asn2961Ser; ClinVar: 477822], both of uncertain clinical significance. All variants in *LDLR* and p.Asn2961Ser in *APOB* were predicted *in silico* to be deleterious.

Table 1 Clinical and biochemical characteristics of family probands

Sex (% women)	71.4
Age (y)	52.2 ± 14.4
Smokers (%)	
Current	22.8
Former	11.4
Nonsmokers	64.3
Age of FH diagnosis (y)	30.8 ± 16.6
Family history of ASCVD (%)	61.4
Age of familial ASCVD (y)	52.4 ± 11.4
Personal history of ASCVD (%)	17.1
Age of personal ASCVD (y)	47.8 ± 11.5
Type of ASCVD (%)	
Coronary heart disease	91.7
Stroke	8.3
Hypertension (%)	45.7
Diabetes (%)	24.3
Body mass index (kg/m^2)	28.0 ± 5.4
Systolic blood pressure (mmHg)	130 (90 to 190)
Diastolic blood pressure (mmHg)	80 (53 to 110)
Waist circumference (cm)	92.3 ± 16.1

Tendon xanthomas (%)	34.3
Total cholesterol (mg/dL)	363 (291 to –634)
HDL cholesterol (mg/dL)	57 ± 15
LDL cholesterol (mg/dL)	268 (221 to –575)
Triglycerides (mg/dL)	142 (50 to –380)
Lipoprotein(a) (mg/dL)	48 (2 to –322)

ASCVD, atherosclerotic cardiovascular disease; FH, familial hypercholesterolemia; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 2 *LDLR* mutations found in patients from Gran Canaria

Variant	Proband's lipid levels (mg/dL) Number of families Number of affected subjects	Region Domain	Mutation type Functional consequence ClinVar classification	ClinVar ID
c.-135C>G Chr19:11089414 rs879254375	Total-C 320.7 and LDL-C 266.9 3 6	Promoter	Promoter variant No or low transcript levels Pathogenic/likely pathogenic	250956
c.185C>T p.(Thr62Met) Chr19:11100340 rs376207800	Total-C 330 and LDL-C 230.4 1 1	LDLR A1 L1 domain pfam00057	Missense substitution Functional change Pathogenic	161273
c.760C>T p.Gln254Ter Chr19: 11106630 rs759109699	Total-C 389 and LDL-C 293.2 1 5	LDLR A6 L6 domain pfam00057	Nonsense substitution Truncated polypeptide lacking Ct end from LDLR A6 domain Pathogenic	251436
c.877dupG p.Asp293Glyfs*8 Chr19:11107451	Total-C 346 and LDL-C 258.4 1 2	LDLR A7 L7 domain pfam00057	Frameshift Truncated polypeptide lacking Ct end from LDLR A7 domain	Novel
c.1091G>A p.Cys364Tyr Chr19: 11111544 rs879254788	Total-C 391 and LDL-C 304.7 3 7	EGF_CA Calcium-binding EGF-like domain smart00179	Missense substitution Disulfide bridge between Cys364 and Cys377 is disrupted: Calcium binding compromised Likely pathogenic	369852
c.1199_1207delACCTCTTCT p.Tyr400_Phe402del Chr19:11113290-11113298 rs879254826	Total-C 377 LDL-C 292.2 30 97	LDLR B1 W1/YWTD domain smart00135	In frame deletion First residues of first LDLRB/YWTD motif are deleted Uncertain significance	251727
c.1609G>T p.Gly537Ter Chr19: 11116116 rs879254958	Total-C 367.5 and LDL-C 285.4 2 6	LDL B4 W14/YWTD domain smart00135	Nonsense substitution; Truncated polypeptide lacking Ct end from LDLR B4 domain Pathogenic	251933

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

Reference mRNA is NM_000527.4; reference genome is Assembly GRCh38; and reference peptide is NP_000518.1. ClinVar classification is available at https://www.ncbi.nlm.nih.gov/clinvar/.

One subject was homozygous for the rs116928232 variant in *LIPA* (NM_000235.3:c.894G>A; NP_000226.2:p.Gln298His, ClinVar: 203361),[30] likely to affect splicing and result in complete deletion of exon 8. This was a 47-year-

old woman with total cholesterol of 590 mg/dL, HDL-C of 31 mg/dL, LDL-C of 519 mg/dL, and triglycerides of 200 mg/dL, subclinical atherosclerosis detected by carotid ultrasound, and minimally increased liver enzymes, but no established ASCVD or hepatosplenomegaly. Three siblings were genotyped for the mutation, two of whom were carriers, but none was homozygous.

Finally, in two probands, one variant of uncertain clinical significance was identified in heterozygosis in *LDLRAP1*. The variant (NM_015627.2:c.604_605TC>CA; NP_056442.2:p.Ser202His), coincident with ClinVar: 4776, but leading to a different missense substitution, was found in isolation in a 52-year-old woman with total cholesterol of 352 mg/dL and LDL-C of 243 mg/dL and a paternal history of CHD at age 54. In the second case, the same mutation was associated with p.(Cys364Tyr) mutation in *LDLR*.

To summarize, of 70 unrelated probands, 44 had a positive genetic study (62%). Among these, 30 subjects were heterozygous carriers of the p.(Tyr400_Phe402del) variant of *LDLR*, representing 43% of the selected probands and 68% of those with a positive genetic diagnosis. Eleven subjects carried other mutations in *LDLR*, one in *PCSK9*, one in *APOB*, and one was homozygous for a *LIPA* mutation. In addition, two of them had an *LDLRAP* variant in heterozygosis (one associated to an *LDLR* mutation).

Family studies were performed on all subjects with *LDLR* mutations, except one with p.(Thr62Met) mutation, as well as on the subject carrying the p.(Arg499His) variant in *PCSK9* (data not shown). A total of 83 relatives of the probands with *LDLR* mutations were studied, and co-segregation of the mutations with hypercholesterolemia was confirmed. A son of two heterozygous probands for p.(Tyr400_Phe402del) had HoFH and has been reported before.[31]

Table 3 shows the clinical and biochemical characteristics of all carriers of the p.(Tyr400_Phe402del) mutation, compared with those of individuals carrying other *LDLR* mutations. There were no differences in concentrations of total or LDL-C or in frequencies of xanthomas or ASCVD. However, carriers of the p.(Tyr400_Phe402del) mutation had a higher frequency of diabetes (17.8% vs 0%, $P = .021$), in spite of similar age and other risk factors for diabetes, such as body mass index or waist circumference. Nevertheless, they did have higher triglyceride and lower HDL-C concentrations.

Table 3 Clinical and biochemical characteristics of *LDLR* mutation carriers: index cases and their affected relatives

	p.(Tyr400_Phe402del)	Other *LDLR* mutations	*P*
Number of cases	97	27	
Sex (% women)	56	69.2	.223
Age (y)	44.3 ± 17.9	46.1 ± 17.3	.662
Hypertension (%)	36.7	23.1	.196
Diabetes (%)	17.8	0	.021
ASCVD	23.1	11.5	.99
Age of ASCVD (y)	45.8 ± 10.4	45.3 ± 13.7	.945
BMI (kg/m^2)	27.3 ± 5.1	27.2 ± 7.1	.906
Waist (cm)	88 (57–121)	82 (62–130)	.559
Tendon xanthomas (%)	31.6	34.6	.334
Total cholesterol (mg/dL)	351 (213–634)	345 (239–465)	.489
HDL-cholesterol (mg/dL)	52 ± 15	60 ± 15	.015
LDL-cholesterol (mg/dL)	274 (143–575)	262 (165–385)	.446
Triglycerides (mg/dL)	119 (40–343)	86 (47–380)	.009
Lipoprotein(a) (mg/dL)	40 (2–300)	30 (2–322)	.287

ASCVD, atherosclerotic cardiovascular disease; BMI, body mass index; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Figure 1 shows the pedigree of a large family affected by the p.(Tyr400_Phe402del) mutation. LDL-C was very high in all carriers, with expression of the disease from very early ages, as demonstrated by the case of a girl with serum LDL-C values of 207 mg/dL at the age of one. Furthermore, several of the family members had premature CHD (Table 4).

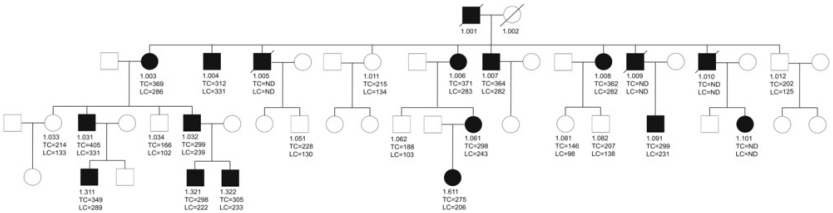

Figure 1 Family pedigree with p.(Tyr400_Phe402del) mutation in *LDLR* gene. Blackened symbols indicate carriers of the p.(Tyr400_Phe402del) and affected members (>95th percentile adjusted for sex and age). White symbols indicate nonaffected members The crossed line indicates deceased patients. Lipid values and presence of atherosclerotic cardiovascular disease are shown in Table 4.

Table 4 Lipid values and atherosclerotic cardiovascular disease in a family pedigree with p.(Tyr400_Phe402del) mutation in the *LDLR* gene

Subject	Age (y)	Total-C (mg/dL)	HDL-C (mg/dL)	LDL-C (mg/dL)	Triglycerides (mg/dL)	ASCVD
1003	70	369	45	286	192	CHD (46 y)
1004	68	312	22	331	262	CHD (68 y)
1005						SD (28 y)
1011	65	215	54	134	136	
1012	52	202	58	125	93	
1006	64	371	55	283	165	
1007	61	364	42	282	200	CHD (33 y)
1008	59	362	54	282	131	
1009						SD (30 y)
1010						SD (32 y)
1031	46	405	22	331	262	CHD (41 y)
1032	40	299	45	239.2	74	
1033	50	214	66	133	75	
1034	48	166	46	102	91	
1051	37	228	87	130	55	
1061	31	298	33	243.4	108	
1062	39	188	70	103	74	
1081	39	146	39	98	46	
1082	37	207	46	138	114	
1091	26	299	42	231	133	
1311	24	349	20	289	200	
1321	19	298	55	222	105	
1322	15	305	54	233.4	88	
1611	1	275	46.2	206.8	110	

ASCVD, atherosclerotic cardiovascular disease; CHD, coronary heart disease (age at diagnosis); HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; SD, sudden death (age at diagnosis); Total-C, total cholesterol.

Discussion

The most striking result in this study is the high prevalence of a single mutation in *LDLR* [p.(Tyr400_Phe402del)], which is responsible for 43% of clinical FH and 68% of those with a positive genetic diagnosis. In most regions worldwide, FH displays broad genetic heterogeneity,[32] and Spain is no exception: no predominant *LDLR* mutation had been reported before and most of those described were present in several regions.[12,13,25] Indeed, in a study of 2246 unrelated patients with FH, the most prevalent variants in Spain added up to 30.6% and the most common mutation (c.313+1G>C+c.274C>G; NA+p.Gln71Glu) represented only 6.5%.[13]

To put our findings in perspective, in a previous study performed in Mallorca, the largest Balearic Island, whose population size is similar to that in Gran Canaria, the authors found 24 different, nonrecurring FH mutations.[33] In contrast to the Balearic Islands, the Canaries have remained poorly communicated until the middle of the last century. Although several investigations have pointed out that the Canarian people hold genetic traits from the aboriginal islanders, their genetic origin is mainly Caucasian, contributed by descendants of the Spanish conquerors who arrived to the islands mostly during the 15th and 16th centuries.[34] Therefore, it is reasonable to believe that some inheritable disorders have spread within the islands through the transmission of certain mutations with a founder effect. This has been shown to be the case for specific mutations causing rare, recessive disorders, such as Wilson's disease[35] and type 2 tyrosinemia.[36]

Indeed, the prevalence of p.(Tyr400_Phe402del) mutation is very high in this population, but not as high as that described for other founding mutations in other genetically isolated regions, where the founding group could have been smaller, or endogamy more pronounced. This is the case for Lebanon, where p.(Cys681X) mutation represents 81.5% of the cases of FH[7] or for Ashkenazi Jews, where the Lithuanian mutation (G197del) is the cause of 80% of FH.[11] Other genetically isolated regions include the French Canadian community, who have an FH prevalence of 1:154 and three founding mutations accounting for 80% of the cases,[8] Finland, where five mutations account for a bit more than 75% of FH,[10] and Afrikaners, in whom 90% of cases are caused by one of the three mutations.[9] In genetically isolated regions, the prevalence of FH and the percentage of cases with homozygous forms of the disease are especially high. Nevertheless, in our population, the prevalence of HoFH is similar to that usually reported for FH.[6]

The frequency of the p.(Tyr400_Phe402del) mutation is not reported in dbSNP, Ensembl, ExAc, gnomAD, or Exome Variant Server databases. The finding of such a large cohort of probands carrying this variant, which co-segregated with the FH phenotype, provides an excellent framework to approach the issue of the functional significance of this variant. This in-frame deletion removes a tyrosine residue from a highly conserved motif in the first YWTD domain (smart00135) of the LDLR polypeptide, which, in turn, is part of a group of six repeats forming the beta propeller, a functional domain also present in other receptors, kinases, and extracellular matrix components.[37] Functional analyses have revealed that this domain is required for the acid-dependent ligand dissociation at the endosome and the recycling of the receptor back to the cell surface.[38] Interestingly, this deletion is also close to the region interacting with PCSK9,[39] where structural changes could be expected to occur after the three amino-acid deletion. In any case, the expected functional consequence would be reduced availability of the recycled receptor at the cell surface, compromising the rate of LDL uptake. Indeed, our findings demonstrate that this mutation leads to a typical FH phenotype, characterized by severe hypercholesterolemia and increased cardiovascular risk. In fact, the patient who is homozygous for this mutation had LDL-C concentrations above 900 mg/dL and very poor response to lipid-lowering treatments,[31] very similar to what would be expected of a null allele. Therefore, we propose that this variant, currently still classified as of unknown significance (ClinVar: 251727), is pathogenic.

An unexpected finding in this study was the high prevalence of diabetes (17.8%) in the carriers of p.(Tyr400_Phe402del) mutation, above what has been previously described in the background population of the island.[40] None of the carriers of any other *LDLR* mutations had diabetes, which, acknowledging small sample size, is in agreement with recent studies suggesting that FH could protect against the disease.[41,42] Therefore, the association of p.(Tyr400_Phe402del) mutation with diabetes is especially surprising and prompts us to perform confirmatory studies and accurately assess if there is co-segregation of diabetes with the mutation.

A second mutation worth noticing is the pCys364Tyr missense substitution, which is the second most prevalent *LDLR* mutation, affecting seven members of three apparently unrelated families. This mutation affects a highly conserved cysteine residue in all EGF-CA domains (smart00179), which is essential for the formation of three critical disulphide bonds within this domain. In fact, analogous variations in other EGF-CA domains are also the cause of dominant mutations,[43] reinforcing the view that this variant is pathogenic.

It is interesting to note that two subjects presenting particularly severe phenotypes were double combined heterozygotes for *LDLR* mutations [one heterozygous for p.(Gln254*) and one for p.(Cys364Tyr)] in conjunction with the c.60_65dupGCTGCTGCT [p.(Leu22_Leu23dup)] variant in the *PCSK9* gene (ClinVar: 265916: 42). Although this *PCSK9* mutation is classified as of uncertain clinical significance, a recent report showed that it leads to a reduced LDLR expression at the plasma membrane and to a 20% reduction in LDL uptake.[44]

Regarding other genes, among the subjects with clinical FH, a case of lysosomal acid lipase deficiency, an autosomal recessive disease, leading to cholesterol ester deposits in adults, was found. The mutation identified in this patient [p.(Ser202His)] is the most frequent one in *LIPA*[45] and has been detected in other subjects with an FH phenotype in the absence of mutations in other causative genes,[46] which highlights the relevance of assessing *LIPA* in this

context.[20]

In addition, an *LDLRAP1* variant of uncertain clinical significance was identified in heterozygosis. Homozygous forms of *LDLRAP1* mutations are the cause of a recessive form of FH. According to a recent Spanish study, 0.5% of subjects with a clinical diagnosis of FH who are genotyped for *LDLRAP1* are heterozygous carriers of pathogenic or probably pathogenic variants.[47] These mutations could play a role in polygenic forms of hypercholesterolemia. It seems rather improbable that these variants are the only cause of the FH phenotype in the carrier identified in our cohort.

This study highlights that it is possible to implement a cost-effective strategy in the genetic diagnosis of FH in Gran Canaria, prioritizing the identification of the most prevalent mutation in all cases with a clinical suspicion of the disease. Furthermore, affected subjects could receive adequate genetic counseling, to avoid new cases of HoFH.

We acknowledge that this study has some limitations, the main one being the small size of the studied population, and the fact that it represents the reference area of the CHUIMI (Southern and Eastern parts of the island) but might not be representative of the rest of Gran Canaria. Furthermore, we restricted genetic studies to subjects with LDL-C > 220 mg/dL and Dutch Lipid Clinic Network score ≥ 8, so FH cases with lower scores could have been excluded. This LDL-C cutoff point has shown a high specificity for positive genetic testing in Spanish population.[48] However, it would be important to determine the prevalence of p.(Tyr400_Phe402del) mutation in the entire population with probable FH. Finally, we did not perform the NGS and multiplex ligation primer amplification analysis in carriers of p.(Tyr400_Phe402del) mutation, so we cannot rule out additional variants in other candidate genes, especially among people with severe phenotypes. However, the study includes a broad number of families and is the first epidemiological study on FH performed in the Canary Islands.

Conclusions

The p.(Tyr400_Phe402del) mutation in the *LDLR* gene represents 68% of the genetic diagnoses of FH in the studied population and is responsible for almost half of the clinical cases of the disease. The island of Gran Canaria seems to be a genetically isolated region for FH and is the perfect setting for a population-based diagnostic strategy for FH.

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Highlights

- Familial hypercholesterolemia (FH) usually has a very heterogeneous genetic basis.
- In genetic isolates, most cases of FH are caused by certain *LDLR* founder mutations.
- In Gran Canaria, 43% of probands share the p.(Tyr400_Phe402del) variant in *LDLR*.
- This mutation co-segregates with the disease phenotype.
- Gran Canaria could be a new genetic isolate for FH.

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