Arteriosclerosis, Thrombosis, and Vascular Biology

CLINICAL AND POPULATION STUDIES

Phenotypical, Clinical, and Molecular Aspects of Adults and Children With Homozygous Familial Hypercholesterolemia in Iberoamerica

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OBJECTIVE: Characterize homozygous familial hypercholesterolemia (HoFH) individuals from Iberoamerica.

APPROACH AND RESULTS: In a cross-sectional retrospective evaluation 134 individuals with a HoFH phenotype, 71 adults (age 39.3±15.8 years, 38.0% males), and 63 children (age 8.8±4.0 years, 50.8% males) were studied. Genetic characterization was available in 129 (96%). The majority (91%) were true homozygotes (true HoFH, n=79, 43.0% children, 46.8% males) or compound heterozygotes (compound heterozygous familial hypercholesterolemia, n=39, 51.3% children, 46.2% males) with putative pathogenic variants in the *LDLR*. True HoFH due to *LDLR* variants had higher total (P=0.015) and LDL (low-density lipoprotein)-cholesterol (P=0.008) compared with compound heterozygous familial hypercholesterolemia. Children with true HoFH (n=34) tended to be diagnosed earlier (P=0.051) and had a greater frequency of xanthomas (P=0.016) than those with compound heterozygous familial hypercholesterolemia (n=20). Previous major cardiovascular events were present in 25 (48%) of 52 children (missing information in 2 cases), and in 43 (67%) of 64 adults with *LDLR* variants. Children who are true HoFH had higher frequency of major cardiovascular events (P=0.02), coronary heart (P=0.013), and aortic/supra-aortic valve diseases (P=0.022) than compound heterozygous familial hypercholesterolemia. In adults, no differences were observed in major cardiovascular events according to type of *LDLR* variant. From 118 subjects with *LDLR* variants, 76 (64%) had 2 likely pathogenic or pathogenic variants. In 89 subjects with 2 *LDLR* variants, those with at least one null allele were younger (P=0.003) and had a greater frequency of major cardiovascular events (P=0.038) occurring at an earlier age (P=0.001).

CONCLUSIONS: There was a high frequency of cardiovascular disease even in children. Phenotype and cardiovascular complications were heterogeneous and associated with the type of molecular defect.

GRAPHIC ABSTRACT: A graphic abstract is available for this article.

Key Words: atherosclerosis ■ cardiovascular disease ■ cholesterol ■ hypercholesterolemia ■ phenotype

Homozygous familial hypercholesterolemia (HoFH) is a rare disorder, affecting 1 in 300 000 to 1 000 000 people in the general population.[1,2] In >90% of cases, FH is caused by variants in both alleles of the LDL (low-density lipoprotein)-receptor gene (*LDLR*). Other less frequent affected genes are *APOB*, proprotein convertase subtilisin/kexin type 9 (*PCSK9*), or *LDLRAP1*.[1] Clinically, it is characterized by extremely high LDL-C (LDL-cholesterol) levels, in general, >500 mg/dL or >300 mg/dL under standard lipid-lowering treatment

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Nonstandard Abbreviations and Acronyms

CHD	coronary heart disease
CHeFH	compound heterozygous familial hypercholesterolemia
FH	familial hypercholesterolemia
HoFH	homozygous familial hypercholesterolemia
LDL	low-density lipoprotein
LDL-C	low-density lipoprotein cholesterol
LDLR	LDL receptor
MACE	major adverse cardiovascular events
PCSK9	proprotein convertase subtilisin/kexin type 9
THoFH	true homozygous familial hypercholesterolemia
VUS	variant of unknown significance

(statins, ezetimibe, bile acid bind resins, and niacin), cutaneous xanthomas of early appearance, abnormalities of aortic valve and supravalvar region of aortic root, and multivessel atherosclerotic cardiovascular disease.[3–5] Usually, if affected individuals are not treated, they may not survive beyond the third decade of life.[6,7] However, recent evidence suggests that HoFH present a more heterogenous phenotype than previously thought, and this has been ascribed to the severity of the molecular defects that cause the disease.[2,8–10]

The Iberoamerican community has an estimated population of 640 million individuals. The Iberoamerican FH network was constituted in 2013, with the main objectives of promoting awareness and education on FH and improving early diagnosis and treatment of the disorder in the network countries. Currently, there are 8 countries (Argentina, Brazil, Chile, Colombia, Mexico, Portugal, Spain, and Uruguay) belonging to the network representing 75% of the region's population. It is estimated that there are between 600 and 1,800 HoFH in Iberoamerica, most of them undiagnosed or not treated adequately. In most countries, treatment of HoFH has been limited to the use of statins and ezetimibe, treatments that have been shown to modestly reduce LDL-C levels. Unfortunately, LDL-apheresis, the current gold standard treatment for HoFH, is not available in most countries of the region.

With the exception of Spain, there is very little information about clinical and molecular characteristics of HoFH individuals in Iberoamerica.[10–13] Recently, molecular and clinical characteristics of FH from countries belonging to the Iberoamerican FH network have been described.[14] The objective of this study is to expand this to the identified HoFH adults and children in Iberoamerica, as well as to describe the association of the genetic variant types with phenotypic expression of the disorder.

Highlights

- Clinical and molecular data from a large number of adults and children with HoFH from Iberoamerica are reported.
- Phenotypic expression is highly variable according to the molecular defect.
- True homozygous FH children had higher frequency of MACE than compound heterozygous FH children.
- Subjects with at least one null allele variant had a greater frequency of MACE, occurring at an early age compared with those with defective variants.
- Hypercholesterolemia due to HoFH in Iberoamerica is highly uncontrolled.

MATERIALS AND METHODS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

Study Population

This is a cross-sectional, retrospective study that describes clinical, laboratory, and molecular characteristics of identified adults and children (<18 years old) with HoFH from centers belonging to the Iberoamerican FH network. The inclusion criteria for the study were (1) a positive molecular diagnosis in individuals with a compatible HoFH phenotype; (2) in the absence of molecular testing, a phenotype compatible with HoFH according to international statements.[3,4] The majority of cases reported in this study underwent genetic testing in specialized molecular laboratories in Argentina, Brazil, Mexico, Portugal, Spain, and Uruguay. Subjects with HoFH due to *LDLR, APOB*, or *PCSK9* variants and those with autosomal recessive hypercholesterolemia (*LDLRAP1* gene) were included either if they were true (true HoFH [THoFH]) homozygotes, compound heterozygotes (compound heterozygous FH [CHeFH]), or double heterozygotes. Only individuals with 2 putative variants in trans were accepted for this study; subjects with synonymous or putative splicing point substitutions variants outside the ±1 to 6 region were excluded.

Clinical Data and Laboratory Assessments

Clinical and laboratory data at diagnosis and follow-up when available were obtained from patient medical files or contacting physicians responsible for patient care and included: demographics (age at diagnosis and follow-up, sex, and living status), history of cardiovascular disease, physical examination (xanthomas and corneal arcus), and type of lipid-lowering treatment (including LDL-apheresis) used. Total cholesterol at screening, untreated or highest on treatment (available for all subjects), or complete lipid profile (available for 101 subjects, 75%) were considered for the diagnosis. Lipid profile was also assessed during follow-up if available. LDL-C was calculated by Friedewald equation if triglyceride levels were <400 mg/dL in all centers. Lp(a) (lipoprotein[a]) values (mg/dL) when available were also collected.

Major adverse cardiovascular events (MACE) at the moment of inclusion were defined as any clinical manifestation of coronary

heart disease (CHD; myocardial infarction, angina pectoris, surgical or percutaneous coronary revascularization); atherothrombotic stroke; carotid artery disease defined as obstructive plaques > 50% on doppler ultrasound; peripheral artery disease (claudication, amputation or limb revascularization); and presence of aortic or supra-aortic valve disease defined as presence of heart murmurs either of stenosis or regurgitation in the aortic auscultation area confirmed by Doppler ultrasound or previous valve replacement.

Molecular Analysis

DNA analysis was performed in different laboratories as previously described.[15–19] References used for *LDLR*, *APOB*, *PCSK9*, *LDLRAP1* genes were NM_000527.4, NM_000384.2, NM_174936.3, and NM_015627.2, respectively. cDNA numbering was considered with nucleotide c.1 being the A of the ATG initiation codon p.1.[20] All variants were annotated and checked using Mutalyzer v2.0 and converted to the nomenclature recommended by the Human Genome Variation Society.

In Silico Classification

In silico analysis was performed for every variant using open-access software and included the following programs: Protein Variation Effect Analyzer Sorting Tolerant From Intolerant, PolyPhen-2, and Mutation Taster for prediction of protein structure/function changes and evolutionary conservation; Neural Network Splice Site Prediction Tool, MAXENTSCAN, FSPLICE, and Human splicing finder for prediction of splicing defects, as previously described.[21]

Variant Classification

Variant classification was performed according to the American College of Medical Genetics and Genomics 2015 guideline[22] as pathogenic, likely pathogenic, variant of unknown significance (VUS), likely benign and benign, with FH specifications described in Chora et al.[21] Only individuals with pathogenic, likely pathogenic and VUS were included in the genetic characterization study.

Allele Classification

All nonsense, large deletions, frameshift variants, and all variants that have been proven by in vitro functional assays that lead to <2% LDLR activity were considered to be null variants. All other variants were classified as defective. For all cases where no functional assays were performed, an estimated (*) allele classification was given (null* or defective*), according to the predicted functional effects of the variants considering their type (structural and functional) and in silico analysis (3 concordant programs). Variants where the in silico programs mentioned above were not possible to be performed (as for in-frame deletions-insertions) or variants that did not have a concordant in silico classification in at least 3 programs could not be classified concerning their allele type. Also, variants where a prediction of null or defective alleles could not be made, as for splicing variants without transcript quantification, classification according to allele type was also not performed. Subjects with variants fulfilling the 2 latter situations were not included for allele type-based analysis. In CHeFH cases in which at least one of the variants was considered as null (or null*), the case was included in the null category for data analysis.

Statistical Analysis

Continuous data are expressed as means±SD, except for Lp(a) that was expressed as medians (%25; %75). Categorical data are expressed as frequency (%). Subjects were analyzed according to their gene variants, lipid profile (highest values available), risk factors for cardiovascular disease, as well as presence of MACE and type of lipid-lowering medication. Statistical analysis was performed using SPSS software (version 25.0 for Windows; SPSS, Chicago, IL). Comparison of frequencies between qualitative variables was carried out using the χ^2 or Fisher exact tests. Mean values of quantitative variables were compared with the Student *t* test or ANOVA for independent data, whereas median values were compared with the nonparametric median tests Mann-Whitney or Kruskal-Wallis. A $P<0.05$ was considered statistically significant. To reduce uncertainty, *P* values were considered as nominal when data from all participants were not available for analysis.

Ethics

Local ethics committees approved each country study, and eligible subjects, or legal representatives in the case of children and teenagers, gave written informed consent for study participation. Data analysis was totally anonymized.

RESULTS

The study population consisted of 134 individuals with a HoFH phenotype: 71 adults (mean age, 39.3±15.8 years, 38.0% males) and 63 children (mean age 8.8±4.0 years, 50.8% males). Molecular testing was available for 129 out of 134 subjects (96%). The great majority (91%) were THoFH (n=79, 43.0 % children [n=34], 46.8% males) or CHeFH (n=39, 51.3% children [n=20], 46.2% males) with variants in the *LDLR* gene (Figure I in the Data Supplement). Only one true homozygote for *APOB* variant was found in Chile. No THoFH for gain of function *PCSK9* variants were encountered in the region, but a compound heterozygote for *PCSK9* had been described in Portugal.[23] Five THoFH for *LDLRAP1* variants were encountered: 1 in Brazil, 1 in Colombia, and 3 in Spain. Table I in the Data Supplement shows the distribution of subjects according to country of origin and genotype.

Table 1 shows subjects' lipid profile at diagnosis (baseline values or highest on treatment) according to genotype with total cholesterol values for the whole cohort (n=134) and a complete lipid profile for 101 (75%) subjects. Of importance, 17% (n=23) of study subjects were not in use of lipid-lowering treatment at screening. THoFH due to *LDLR* variants had higher total cholesterol ($P=0.015$) and LDL-C ($P=0.008$) as well as lower HDL-C (high-density lipoprotein cholesterol; $P=0.012$) compared with CHeFH. No differences in total cholesterol and LDL-C were found when compared THoFH to *LDLRAP1* subjects, although the latter tended to have higher LDL-C than CHeFH. However, these analyses

Table 1. Complete Lipid Profile According to Genotype (n=101) and TC for the Whole Cohort (n=134) of Patients With HoFH

	n*	TC*	LDL-C*	HDL-C*	TG*	TC†
LDLR true	56/79	692±182	628±181	38±12	128±48	656±224
LDLR compound	30/39	589±147	518±149	45±14	131±80	569±142
LDLRAP1 true	4/5	715±155	649±141	40±7	129±44	648±201
APOB true	1/1	405	330	36	183	405
PCSK9 compound	1/1	305‡	220‡	50‡	58‡	305
LDLR+*APOB* double	3/3	570±108	487±99	51±14	160±58	570±108
LDLR+*PCSK9* double	1/1	454	325	73	390	454
Without genetic test	5/5	626±196	546±192	34±10	208±81	626±196
P value; *LDLR* true vs *LDLR* compound		0.015	0.008	0.012	0.520	0.039
P value; *LDLR* true vs *LDLRAP1*		0.658	0.568	0.577	0.830	0.987
P value; *LDLR* compound vs *LDLRAP1*		0.117	0.064	0.738	0.699	0.329

At screening 17% of study subjects were not receiving lipid-lowering treatment. TC, LDL-C, HDL-C, and TG in mg/dL. HDL-C indicates high-density lipoprotein cholesterol; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; and TG, triglycerides.

*Baseline or highest on-treatment values at screening for complete lipid profile (available for 101 subjects).

†Baseline or highest on treatment for total cholesterol values at screening for all 134 homozygous FH patients.

‡On-treatment values were considered for diagnosis.

were compromised by the small number of subjects on the *LDLRAP1* group.

Table 2 shows clinical and laboratory characteristics of children (n=54) and adults (n=64) with THoFH or CHeFH due to *LDLR* variants (n=118). THoFH children (n=34) tended to be diagnosed at a younger age (*P*=0.051) and had a greater frequency of xanthomas (*P*=0.016) than those with CHeFH (n=20). Previous MACE was present in 25 (48%) of 52 children, with missing information in 2 cases. Mean age for the first MACE was 11 years. Children who are THoFH had higher occurrence of MACE (*P*=0.02), CHD (*P*=0.013), and aortic valve disease (*P*=0.022) than children with CHeFH. In Table II in the Data Supplement clinical and laboratory characteristics of all children according to clinical (n=4) and molecular diagnoses (n=59) are shown.

However, in adults, MACE was present in 43 (67%) out of 64 individuals with *LDLR* variants, and no significant differences were observed in each individual component of MACE between true or compound heterozygotes. However, differently from children, the most frequent clinical manifestation in adults was CHD in either THoFH or CHeFH.

Regarding plasma lipids, a complete lipid profile was available for 28 (84%) THoFH and 16 (80%) CHeFH children (Table 2). True homozygous had higher total (*P*=0.023), LDL-C (*P*=0.018), and lower HDL-C (*P*=0.026) than CHeFH children. In adults, a complete lipid profile was available in 28 (62%) THoFH and 14 (74%) CHeFH, and only LDL-C was significantly higher in THoFH (*P*=0.045). Lp(a) was available in 17 children and 27 adults and no differences were observed between THoFH and CHeFH: children 26 (5; 52) mg/dL versus 29 (16; 82) mg/dL, (nominal *P*=0.841) and in adults 31 (10; 65) mg/dL versus 34 (19; 121) mg/dL (nominal *P*=0.581).

Children with THoFH had a significantly higher frequency of at least one null allele than those with CHeFH (*P*=0.016). In children with aortic/supra-aortic valve disease, 75% had *LDLR* null variants, compared with only 25% of the adults. All children developed aortic/supra-aortic valve disease before CHD manifestations. In adults, the frequency of null/null individuals was significantly higher in THoFH (*P*=0.035).

A total of 45 different *LDLR* variants were found in THoFH and are described in Table III in the Data Supplement as well as the LDL-C levels presented by their carriers, when available. Twenty-three THoFH subjects are carriers of 12 VUS variants. Due to the classical HoFH phenotype, LDL-C >500 mg/dL, or LDL-C >300 mg/dL on treatment, presented by their carriers, at least 6 could be considered disease causing (highlighted in Table III in the Data Supplement). However, functional studies will be necessary to assess all these variants to establish their pathogenicity.

From 118 subjects with *LDLR* variants, 76 (64%) had 2 likely pathogenic or pathogenic variants. The remaining 42 subjects carried at least one VUS meaning that there was not enough evidence for these variants to be considered at least likely pathogenic according to the American College of Medical Genetics and Genomics classification.[21,22]

Table IV in the Data Supplement shows clinical and laboratory data for 89 subjects with 2 *LDLR* variants in which it was possible to classify these as null or defective alleles. Those with at least one null allele were diagnosed at a younger age (*P*=0.032), had a greater frequency of MACE (*P*=0.038), especially aortic/supra-aortic valve disease (*P*=0.026), occurring at an earlier age (*P*=0.001), and a trend to higher frequency of xanthomas (*P*=0.051) than those subjects with defective variants. Also, those with null variants tended to have a greater mortality

Table 2. Clinical and Laboratory Characteristics of 118 HoFH Children and Adults With Identified *LDLR* Variants

	Children (n=54)			Adults (n=64)		
	THoFH (n=34)	CHeFH (n=20)	*P* Value	THoFH (n=45)	CHeFH (n=19)	*P* Value
Age at diagnosis, y	7.9±4.0	10.1±4.0	0.051	37.8±16.5	43.7±13.2	0.140
Age at inclusion, y	12.9±6.7	13.3±5.1	0.424	45.6±17.8	49.6±13.7	0.410
Male sex, n (%)	15 (55.9%)	11 (55.0%)	0.999	18 (40.0%)	7 (36.8%)	0.999
Living status (dead), n (%)	5 (14.7%)	2 (10.0%)	0.999*	5 (11.2%)	0 (0%)	0.309*
Xanthomas, n (%)	31 (91.2%)	12 (60.0%)	0.016	27 (60.0%)	12 (63.2%)	0.267
Corneal arcus, n (%)	15 (48.4%)	6 (30.0%)	0.377	28 (62.2%)	9 (47.4%)	0.408
Hypertension, n (%)	0 (0%)	0 (0%)	…	13/33 (39.4%)	3/13 (23.1%)	0.070*
Diabetes mellitus, n (%)	0 (0%)	0 (0%)	…	3/33 (9.1%)	0/13 (0%)	0.001*
Current smokers, n (%)	0 (0%)	0 (0%)	…	2/33 (6.1%)	2/13 (15.4%)	0.001*
Allele type						
Null/Null	13 (38.2%)	6 (30.0%)	0.108	9 (20.0%)	2 (10.5%)	0.035
At least one Null	21 (61.7%)	8 (40.0%)	0.016	16 (35.6%)	9 (47.4%)	0.162
Defective/Defective	6 (17.6%)	6 (30.0%)	0.045	20 (44.4%)	4 (21.1 %)	0.001
Cardiovascular disease						
MACE, n (%)	19/33 (55.9%)	6/19 (31.6%)	0.040*	29/45 (45.3%)	14/19 (73.7%)	0.007
CHD, n (%)	5/28 (17.9%)	1/19 (8.3%)	0.001*	23/43 (53.5%)	12/19 (63.2%)	0.583*
Stroke, n (%)	0/19	0/12	…	0/45 (0%)	1/19 (5.3%)	0.227
PVD, n (%)	1/19 (5.3%)	1/12 (8.3%)	0.005*	1/40 (2.5%)	0/19 (0%)	0.688*
Aortic/supra aortic valve disease, n (%)	13/31 (41.9%)	4/17 (23.5%)	0.009*	4/43 (9.3%)	1/17 (5.9%)	0.267*
Age first event, y (n)	11.1±4.6	11.0±2.0	0.947*	30.3±15.6	38.0±12.7	0.440
Lipid levels						
TC, mg/dL†	767±178	645±149	0.014	630±202	541±122	0.024
Lipid profile‡	N=28/34	N=16/20	…	N=28/45	N=14/19	…
TC, mg/dL	753±171	636±150	0.023*	630±173	536±127	0.088*
LDL-C, mg/dL	691±170	568±146	0.018*	564±171	461±135	0.045*
HDL-C, mg/dL	37±14	45±16	0.026*	39±11	44±12	0.189*
TG, mg/dL	122±37	109±50	0.184*	133±57	155±102	0.626*

Only subjects with complete lipid profile are included in the analysis, 81.5% of the children and 65.6% of the adults. CHD indicates coronary heart disease; CheFH, compound heterozygous familial hypercholesterolemia; HDL-C, high-density lipoprotein cholesterol; HoFH, homozygous familial hypercholesterolemia; LDL-C, low-density lipoprotein cholesterol; MACE, major cardiovascular events; PVD, peripheral vascular disease; TC, total cholesterol; TG, triglycerides; and THoFH, true HoFH

*Nominal *P* values when data not available for all study subjects.

†Baseline or highest on treatment for total cholesterol values at screening for 118 homozygous FH patients.

‡Baseline or highest on-treatment lipid levels from the same sample.

frequency (*P*=0.062). The baseline untreated or highest on-treatment values of lipid profiles were available for 63 studied subjects (71%) and showed higher concentrations of total cholesterol (*P*=0.035), LDL-C (*P*=0.024), and lower HDL-C (*P*=0.005) in those presenting at least one null variant. No differences in Lp(a) values were observed between these 2 groups 31 (22; 57) mg/dL and 29 (9; 47) mg/dL), nominal *P*=0.131, respectively, for null (n=16) and defective (n=19).

Figure II in the Data Supplement shows that THoFH and CHeFH children with either null or defective alleles had higher LDL-C compared with adults (679±170 mg/dL versus 561±97 mg/dL, both null alleles; *P*=0.03, and 654±136 mg/dL versus 503±153 mg/dL, both defective, *P*=0.03). This in part may be related to the fact that 22% of the children were untreated at the time of clinical diagnosis when compared with 10.9% of adults.

There was a marked difference among countries concerning available lipid-lowering treatments (Table V in the Data Supplement), and this was reflected in the frequency of use of different therapies. Most patients (70%) were using statins in monotherapy or combined with ezetimibe. However, there was a greater use of either PCSK9 inhibitors or the microsomal triglyceride transfer protein inhibitor, lomitapide, in those with null variants than in those with defective ones (28.3% versus 5.6% *P*=0.012) in countries where these medications were available. Overall, there were very low rates of LDL-apheresis (n=11, 8.2% of all studied subjects). All subjects on combined therapy with PCSK9 inhibitors (n=7), lomitapide (n=10), on LDL-apheresis (n=11), or that

were submitted to liver transplantation (n=7) had LDL-C reductions over 50%, the minimum recommended target to treat these individuals[2,4] (data not shown).

DISCUSSION

In this study, clinical and molecular data from a large number of adults and especially children with HoFH from Iberoamerica are reported. Molecular diagnosis was performed in 96% of studied subjects; therefore, it was possible to show genotype/phenotype associations. The main findings of this study are (1) that most HoFH in Iberoamerica are caused by *LDLR* defects; (2) this population is characterized by high frequency of early-onset MACE, even in children; (3) phenotype and cardiovascular complications are heterogeneous and associated with the type of molecular defect, and those bearing a null *LDLR* variant had a more severe phenotype; and (4) cholesterol levels are mostly uncontrolled and not on target independently of the encountered genotype.

As previously described mostly in European and North American populations,[9,10,24–26] the comparison of clinical and biochemical data by allele type and distribution showed significant differences in plasma lipids between studied groups. Both THoFH children and adults showed more severe hypercholesterolemia than their CHeFH counterparts. In children, almost 40% of studied individuals in which information was available presented some manifestation of cardiovascular disease, mostly aortic/supra-aortic valve disease, and there was a higher frequency of MACE in the THoFH individuals. However, because the latter may carry 2 defective alleles, whereas subjects that are CHeFH can bear 2 null alleles, the comparison between true and compound HoFH individuals may not reflect the phenotypic differences and consequent cardiovascular complications. Indeed, subjects with null alleles are expected to have a more severe phenotype than subjects with defective allele variants because the former will have virtually no functional LDL receptors on hepatocytes.[10,25,27] However, subjects with 2 defective allele variants will have some residual LDL receptor activity, a higher plasma LDL clearance, and therefore, cardiovascular disorders may show up later in life. Indeed, in this study, those presenting at least one null allele were diagnosed earlier, had a more severe lipid profile, a greater frequency, and earlier MACE, and possibly early death onset than those with defective alleles. This was apparent when children were compared with adults, the lower frequency of null variants in the latter probably indicates a survival bias especially when the frequency of aortic/supra-aortic valve disease is concerned. Of course, a greater time exposure to high LDL-C in adults certainly is responsible for the greater frequencies of CHD when compared with children.

The information gathered on THoFH subjects can be useful to classify the variants carried by these subjects. If a patient is a true homozygote for a certain variant and presents a severe phenotype (untreated LDL-C >500 mg/dL or LDL-C >300 mg/dL on treatment), the variant found must be the one causing disease if it produces such a severe phenotype; however, functional assays should always be performed to confirm pathogenicity because there is the rare chance that the patient could have another undetected variant that defines the phenotype.[28]

From the list of 45 variants found in true homozygous subjects, 12 are VUS but 6 were found in subjects with a classical HoFH phenotype. These variants are likely to be causal and, therefore, pathogenic. However, there are 3 variants shown in homozygosity p.(Arg300Gly), p.(Ser326Cys), and p.(Arg595Trp) where the subjects who carry them had a milder phenotype (LDL-C, 300–400 mg/dL). This could have been explained by the fact that the variant presented was benign and a heterozygous pathogenic variant was missed or the variant retained a fairly high LDL receptor activity (60-80%) as happens for example with recycling variants.[29] Indeed this might help explain many cases of genetically defined HoFH individuals who presented LDL-C levels below the thresholds accepted by scientific societies to define HoFH as previously described for either adults and children.[8,26] Functional studies are even more crucial in these cases to attribute a correct and definite diagnosis for each patient. This might have implications for patient management and also for genetic counseling and cascade screening.

Independent of its cause, the HoFH phenotype is associated with a stern prognosis when MACE are concerned.[30] In this population, an elevated frequency of either CHD or aortic/supra-aortic valve events of early-onset were encountered in adults and especially children. One concerning finding is that most individuals were not adequately treated and that there was an elevated number of individuals not receiving lipid-lowering therapies. This study was not designed to identify the causes of these discrepancies, but lack of availably of more effective therapies like PCSK9 inhibitors, lomitapide, and LDL-apheresis in countries outside Europe might have influenced on the findings. Indeed, recent evidence shows the influence of socioeconomic development on adequacy of care and availability of lipid-lowering medications for FH individuals in developed and developing nations.[31] At any rate, results presented here are important to show the inadequate situation and severity of disease in the region, and these data should be taken to governments to improve models of care, especially availability of LDL-apheresis[4] that is the gold standard for treatment of these individuals.

This study has several limitations: (1) its retrospective design and absence of some clinical information reduces

the accuracy of the findings, however, this was the case with many other descriptions of HoFH populations[8–10]; (2) despite the elevated number of cases included in the study, this may represent only 1/5 of the at least predicted 680 homozygotes in the region; (3) the lack of a common evaluation and treatment protocol for the different centers from the different countries involved may explain heterogeneity of some findings; (4) a complete lipid profile and Lp(a) values were not available in all cases; and finally (5) the highest reported lipid values were not necessary untreated. However, there are some strengths including the elevated number of subjects, especially children in comparison with previous observations,[26] the high rate of genetic diagnosis and careful molecular characterization of studied subjects, and information on homozygous FH coming especially from Latin America countries, where FH is severely underdiagnosed.[14]

In conclusion, HoFH in Iberoamerica is characterized by a high frequency of early-onset cardiovascular disease, even in children. Phenotype and cardiovascular complications are heterogeneous and associated with the type of molecular defect. Finally, LDL-C levels in HoFH are highly uncontrolled.

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