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Preliminary Spectrum of Genetic Variants in Familial Hypercholesterolemia in Argentina.

Virginia G. Bañares[a], Pablo Corral[b], Ana Margarida Medeiros[cd], María Beatriz Araujo[e], Alfredo Lozada[f], Juan Bustamante[gh], Roxana Cerretini[a], Graciela López[ij], Mafalda Bourbon[cd] and Laura E. Schreier[ij]

[a] Centro Nacional de Genética Médica, Administración Nacional de Laboratorios e Institutos de Salud –ANLIS- *¨Dr Carlos Malbrán¨*, Ciudad Autónoma de Buenos Aires, Argentina.

[b] Departamento Investigación, Facultad de Medicina, Universidad FASTA, Mar del Plata, Buenos Aires, Argentina

[c] Grupo de Investigação Cardiovascular, Departamento de Promoção da Saúde e Prevenção de Doenças Não Transmissíveis, Instituto Nacional de Saúde *Dr Ricardo Jorge*, Lisboa, Portugal.

[d] University of Lisbon, Faculty of Sciences, BioISI – Biosystems & Integrative Sciences Institute, Campo Grande, Lisboa, Portugal.

[e] Servicio de Nutrición, Hospital Nacional de Pediatría ¨*Dr Juan P. Garraham*¨, Ciudad Autónoma de Buenos Aires, Argentina.

[f] Clínica de Lípidos, Universidad Austral, Buenos Aires, Argentina.

[g] Departamento de Química Biológica, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, IQUIBICEN-CONICET, Buenos Aires, Argentina

[h] Facultad de Ingeniería, Universidad Nacional de Entre Ríos, Entre Ríos, Argentina.

[i] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis. Buenos Aires, Argentina.

[j] Universidad de Buenos Aires, Instituto de Fisiopatologia y Bioquímica Clinica, INFIBIOC. Buenos Aires, Argentina.

Corresponding author: Virginia G. Bañares, Departamento de Genética Experimental, Centro Nacional de Genética Médica, Av. Las Heras 2670 4to piso, 1425, Ciudad Autónoma de Buenos Aires, Argentina.

e-mail: vgbaniares@hotmail.com, vbaniares@anlis.gov.ar

Abstract

Background

Familial hypercholesterolemia (FH) is a genetic disorder characterized by elevated low density lipoprotein cholesterol (LDL-c) and early cardiovascular disease (CVD). As CVD is a leading cause of mortality in Argentina, early identification of patients with FH is of great public health importance.

Objective

The aim of our study was to identify families with FH and, to approximate to the characterization of the genetic spectrum mutations of FH in Argentina.

Methods

Thirty-three not related index cases were selected with clinical diagnosis of FH. Genetic analysis was performed by sequencing, MLPA and bioinformatics tools.

Results

Twenty genetic variants were identified among 24 cases (73%), 95% on the *LDLR* gene. The only variant on APOB was the R3527Q. Four were novel variants: c.-135C>A; c.170A>C, p.(Asp57Ala); c.684G>C, p.(Glu228Asp) and c.1895A>T, p.(Asn632Ile), the bioinformatics' analysis revealed clear destabilizing effects for two of them. The exon 14 presented the highest number of variants (32%). Four variants were observed in more than one case and the c.2043C>A p.(Cys681*) was carried by 18% of index cases. Two true homozygotes, three compound heterozygotes and one double heterozygote were identified.

Conclusion

This study characterizes for the first time in Argentina genetic variants associated to FH and suggest that the allelic heterogeneity of the FH in the country could have one relative common LDLR mutation. This knowledge is important for the genotype-phenotype correlation and for optimizing both cholesterol lowering therapies and mutational analysis protocols. In addition, these data contribute to the understanding of the molecular basis of FH in Argentina.

Author contributions

All authors contributed to the conception and design of this study. VB, AMM and MB were responsible for the genetic data; VB, JB and RC for the bioinformatics tools; GL for the biochemical

data; PC, MBA and AL for the clinical data analysis; VB, PC and LS drafted the work. Critical revisions were made by all authors. All authors have given their final approval of the submitted version, and agree to be accountable for all aspects of the work.

Keywords

Familial Hypercholesterolemia; LDLR gene; cholesterol; genetic variants; mutations; APOB; cardiovascular disease prevention; Argentina; cardiovascular disease; public health.

Introduction

Familial Hypercholesterolemia (FH) is the most common inherited disease associated with the development of premature coronary heart disease (CHD) and it is under-diagnosed and under-treated in most countries. FH prevalence in Caucasian general population is known to be 1/500; however recent studies suggest that it might be higher, 1/300-1/200[1]. Consequently, we expect at least more than 80,000 cases in Argentina.

FH is characterized by elevated serum low density lipoprotein cholesterol (LDL-c) and a family history of premature cardiovascular disease (CVD). Functionally, clearance of plasma LDL is reduced leading to an increase in LDL-c levels favoring the development of premature atherosclerosis and CVD. Life expectancy is shortened by 20 to 30 years in FH patients (WHO Human Genetic Program 1997). Thus, it is important to identify, individuals with FH as early as possible to improve their prognosis by the administration of appropriate therapeutic interventions. The genetic study allows the early identification of these patients. The study of FH in a family context starts with the identification of the genetic variant in the index case within a family and then, the cascade-genetic-screening of this genetic variant among the relatives[234].

FH is caused by mutations in three main genes: the majority of them (90-95%) are in the Low Density Lipoprotein Receptor gene (LDLR), others (~5%) in Apolipoprotein B gene (APOB) and few (~1%) in Proprotein Convertase Subtilisin/kexintype 9 gene (PCSK9)[1]. More than 2900 public variants have been described in the LDLR gene (Leiden Open Variation Database). In general there are not founder effects, only a few populations have specific mutations with a higher frequency, the Afrikaner of South-Africa, French-Canadian, Finnish populations and the Lebanese allele in Middle-East and Brazilian populations[56]. In contrast, there is only one common mutation in the APOB gene among Caucasian populations, the R3527Q[7]. For PCSK9 gene, there are many variants

all over the gene, though their prevalence is very low[8]. The population of Argentina has been genetically influenced by three major differentiated continental contributors; Native American, European, and African[9].

Unlike to the rest of the world, in Latin America there are very few published genetic studies on FH, only Mexico and Brazil conducted genetic characterizations of their populations[1011], and Uruguay has a national plan for early identification of FH. The aim of this report was to identify families with FH, to facilitate, later, early detection of the disease in relatives. Characterize the genetics variants associated to FH detected in the first groups of probands studied in our country and, approximate to the characterization of the genetic spectrum mutations of FH in Argentina.

Patients and methods

Patient selection criteria

This study includes 33 consecutive FH index cases evaluated in Argentina, nine of them were children (5-17 year old) and 24 adults (20-66 years old). The later were categorized as definite or probable according to Dutch Lipid Clinic Network (DLCN) criteria (WHO 1997), when presenting a score value 8 or higher, or 6-7 points, respectively. Highest LDL-c levels, obtained from the clinical history or referred by the patients, were applied for the DLCN score. For children, values of LDL-c above the 95th percentile, according to age and gender, and family history of high cholesterol and/or premature familial cardiovascular disease were considered. Participants were referred to the laboratory by several specialized physicians, from different hospitals throughout the country. The study protocol was approved by the local ethic committee and written informed consents were obtained from the participants before their inclusion in the study.

Biochemical analysis

Total-cholesterol (TC), triglycerides (TG), high density lipoprotein (HDL) and LDL-cholesterol concentrations were determined in fasting serum samples, by standardized enzymatic methods with kits (Roche Diagnostic/Mannheim/Germany) in a Cobas C-501 autoanalyzer. Serum lipid measurements were under good quality control, average coefficient of variation values for these parameters were: CV intra-assay <2.3 %, CV inter-assay <3.0 %.

Molecular genetic analysis

DNA was isolated from whole blood samples by the Salting Out protocol[12]. Samples were analyzed during the years 2013-2015 in stages; first, the 18 exons of the *LDLR* gene, flanking areas and

promoter, were sequenced by the Sanger method; then, segments of exons 26 and 29 of APOB gene (active site of the protein); and finally a large rearrangements study was conducted by multiplex ligation-dependent probe amplification (MLPA) technique[13], on those samples without genetic variant identified. All of the observed variants were confirmed by a new sequencing. In the 9 samples analyzed during 2015, all exons and their flanking regions of Apolipoprotein E (APOE), LDLR, Low Density Lipoprotein Receptor Adaptor Protein 1(LDLRAP1), PCSK9 and APOB genes, with ability to detect indels up to 15bp, were analyzed with Ampliseq™/Post-Light™/Ion-Semicondutor/Sequencing (Next Generation Sequencing), Ion Personal Genome Machine®System.

The reported variants were considered "novel" when they were not listed in the University College London LDLR-FH database, Jojo Genetics database or in the Human Gene Mutation Database, accessed in July 2016. The reference sequences used for LDLR and APOB were, respectively, NM_000527.4 and NM_000384.2 and cDNA numbering was considered following the Human Genome Variation Society nomenclature. Variants were classified according to the American College of Medical Genetics and Genomics Practice 2015 Guidelines (ACMG)[14].

Bioinformatics analysis

Variants without functional studies were evaluated with bioinformatics programs: PolyPhen-2, Sorting-Tolerant-From-Intolerant (SIFT), Mutation-Taster and Protein-Variation-Effect-Analyzer (PROVEAN) for prediction of amino-acid substitutions and indels; and Splice-Site-Predictor (Splice-Port) and Neural Network Predictions of Splice Sites in Human (NetGen2) for prediction of splicing defects. The structure of the protein domain at the positions of novels variants was visualized with the programs VMD (Visual Molecular Dynamics) and Protter[15], the protein reference sequence was the UniProtKB-P01130 and the protein structures were selected from the PDB (protein-data-base). For variant classification, it was considered that a variant had a deleterious effect when all of the software tools had a prediction of being pathogenic. If this was observed, it was considered as supportive evidence for variant classification according to the ACMG Guidelines.

Statistical analysis

The statistical analysis was performed using SPSS 15.0, differences between groups were assessed by chi-squared test, considering significant a $p<0.05$.

Results

The clinical and biochemical characteristics of the 33 index cases with clinical diagnosis of FH are

shown in table 1. Nine children and twenty four adults were included, among which five homozygous -four children and one adult- and one double-heterozygous adult were detected. No differences in gender proportions were observed between groups (p=0.626).

All children were on diet and under medication and, the majority of adults (n=22) were on medication, and on diet (n=18). Xanthomas or xanthelasmas were seen on the homozygous and double-heterozygous adults, on 2 of the homozygous children and on 16 of the heterozygous adult patients. Measured lipid profiles showed a wide variation depending on the treatments that patients received. Table 1B showed the highest lipid values referred by the clinicians.

Genetic variants were identified in 24/33 (73%). All the individuals with severe clinical FH were genetically confirmed as true-homozygous (n=2), compound-heterozygous (n=3) or double-heterozygous (n=1). In the heterozygous state, a genetic variant was identified in 4/5 children and in 14/22 adults. In 90% of the individuals who present DLCN score value of 8 or higher (n=19), and 45% of those with score values of 6-7 (n=5), genetic variants were detected.

Twenty different mutations were found, 95% (n=19) were on the LDLR gene, and 5% (n=1) on the APOB gene. No variants were observed on PCSK9, LDLRAP1 or APOE genes in the nine samples with full genetic study performed. The genetic variants identified, associated with FH, are listed in table 2. The exon 14 presented the highest number of mutations, with 4 different variants in 8 index cases, which represent 32% of the total variants identified in this sample (figure 1A). It is follow by intron 3 and exons 4, 7 and 13, with 2 variants each of them. The most common mutation type was a missense, in 16/20 variants; the rest were three insertions and one deletion. Four variants were observed in more than one index case: three on the LDLR gene, c.2043C>A p.(Cys681Stop), “Lebanese mutation“, was present in 6 index cases, c.920A>G p.(Asp307Gly) and c.1783C>T p.(Arg595Trp), and the variant c.10580G>A p.(Arg3527Gln) in the APOB gene. The genetic composition of individuals who carry two genetic variants is shown in table 3. Variants not previously described in other populations, and thus considered as novel variants, are presented in Table 2B. All of them were located on the LDLR gene and were of the missense type. The four applied bioinformatics tools predicted defective alleles in all cases, their results and the classification according to the ACMG guidelines are shown on the table 2B. These four variants and the c.2054delC p.(685fs*24) are the five ones described only in the argentine population.

The protein structure at positions 57, 228, and 632 were visualized on structures selected from the PDB: 1LDR and 1F5Y, 2LGP and 3P5B respectively. There are two calcium ions that interact with the protein, and the asp 57 seems to be one of the amino acids interacting with one of the ions

and with Glu51 and Cys52, stabilizing the structural region (figure 1B). Multiple sequence alignment showed that the asparagine 57, glutamic-acid 228 and asparagine 632 of the ldlr protein are highly conserved amino acids positions.

Discussion

This study describes for the first time the genetic variants associated to FH in a sample of definite/probable index cases in Argentina. Considering that the current population in Argentina is about 41 million inhabitants and that the estimated prevalence of heterozygous FH, varies from 1/200 to 1/500 in the general population[1], it is expected that Argentina presents between 82,000 to 205,000 FH cases. Up to date, unfortunately less than 1% of these cases have been identified.

A high detection rate was observed since 24 out of the 33 index cases (72%) presented variants – either pathogenic, like pathogenic or of uncertain significance- in genes associated to FH. We hope that this rate decrease in the future, when more physicians of our country ask for these analyses. All patients were under hypocholesterolemic treatment at time of genetic study. The frequency of other risk factors such as hypertension, obesity or diabetes were similar than other populations[11116]**.** Lipid data correspond to the highest levels registered before medication, which endorse the FH condition. As expected, LDL-c was outstandingly higher in homozygous patients respect to heterozygous cases. In the double heterozygote patient, LDL-c value was lower than in the true homozygous (493 vs. 690 mg/dl), and the phenotype characteristics were less marked, being both of the same gender, in concordance with their genotype constitution as previously described[17].

The spectrum of mutations in the Argentine population is presents quite heterogeneous. It must be highlighted that the Lebanese genetic variant was carried by 6 out of 33 (18%) of the probands suggesting that it would have a high proportion in our population, the same as in neighboring countries, as Brazil. By the 19th century an important immigration from the Middle East, mainly Syrian and Lebanese came to Argentina[18]. It is probable that this proportion declines when more cases are studied, as happened in Brazil, where this alteration initially was observed in near 20% of the cases, but after improving the number of evaluated families, the value actually decreased to around the 10%[1011]. Another interesting result is the fact that 32% of all observed variations were on exon 14 representing the 27% (9/33) of the cases. The study of exon 14 as a first step prior to the full genetic test -all genes and deletions by MLPA- could be an alternative to implement the genetic study of FH in the population of Argentina.

Regarding the true-homozygous patients, c.[920A>G];[920A>G] and c.[2054delC];[2054delC], family histories of inbreeding have been confirmed in both cases. The others four cases carried at least one of the variants observed in more than one index case. The Lebanese allele, was observed in two compound heterozygotes; the APOB R3527Q, the most frequent mutation among Caucasians, was observed in the double-heterozygote; the variant c.920A>G p.(Asp307Gly) was observed in two families, unrelated, both residents in the northern region of the country, this variant has also been described in population from Spain, with whom we share ancestors; and c. 1783C>T p.(Arg595Trp) also present in the Spanish population. Two mutations were identified in all patients with severe clinical phenotype.

Up to the moment relatives were not included in this study. However, we started to evaluate some relatives to confirm the segregation and correlation genotype/phenotype of novel variants when was possible. Thus, another true-homozygous was also identified, who was brother of one of the true-homozygous index case (data not shown).

As regards to the possible functional effect of the novel variants, the c.-135C>A, is located into a C-repetitive sequence of 5 cysteine residues of the repeat 3 of the sterol response element (SRE) sequence on the LDLR gene promoter, mainly regulated at the transcriptional level by sterol through the SRE residing within the promoter. This variant is place in a CT-rich stretch sequence into this regulatory element, involved in LDLR gene expression at the transcriptional level as a transactivator. In addition, at this same position, another variant was previously described, c.-135C>G. Its functional study showed 5-15% residual ldlr activity in homozygous[5]. Variants at positions -136, -137 and -138, also in the CT-rich sequence have also showed decreased functional activity[19,20,21].

The three mutations impacting over the protein structure will be discussed in terms of molecular implications. The c.170A>C p.(Asp57Ala) produces a change from an aspartic-acid (polar) by an alanine (hydrophobic). In concordance with the high conservation of this position through nature, this structural position seems to be crucial for two molecular reasons: i) local stabilization of the tertiary structure, through two hydrogen bonds formed with Glu51 and Cys52 backbone sites observed at PDB 1F5Y (Figure 1B); ii) hydrogen bond network stabilizing interactions between ion and accessible water molecules from solvent. The mutation Asp57Ala disrupts these above mentioned stabilizations, weakening this local structure. Another genetic variant at position 169, Asp57Ala, producing the same destabilization effect was reported[22].

The c.684G>C, p.(Glu228Asp), is on a highly conserved position due to is located in a calcium ion stabilization area. Both amino-acids are polar and able to establish hydrogen bonds, but Asp is shorter, so this kind of interactions can be lost. In concordance, predictions of the energetic impact of this mutation on the closest region stability suggest a clear destabilizing effect. There are other cases reported with this same amino acid being replaced by amino-acids with similar properties and with functional studies showed reduced receptor activities[1623]. The predictive bioinformatics tools estimate a deleterious effect. It remains to be performed a segregation family study, not availably in this case, to contribute to establish the association with FH.

The c.1895A>T, p.(Asn632Ile), introduces the change of a polar amino-acid by a non polar amino-acid. Regarding this mutation, according to PDBs 1IJQ and 3M0C, this position is located at the protein surface, in a highly polar region. Possibly, the mutation by an isoleucine disrupts key interactions that can be achieved with other putative molecules. However, there is no evidence of interacting structures through this exposed structural region. Changes at the neighboring positions, 631 and 633, on the same domain have been reported, and bioinformatics tests predict deleterious results in all the cases[242526].

The homozygous who presented the c.2054delC p.(685fs*24) has previously been described[27]. The patient received a liver transplantation before her 30s with favorable evolution, and the correlation between genotype/phenotype has been confirmed through the family.

It is important to highlight those cases (n=9) presenting FH clinical features without detected genetic variant. It must be considered that some gene areas (deep-intronic regions, distal promoter) were not covered. Moreover, there are still remaining to completely evaluate APOB, PCSK9, LDLRAP1 and APOE genes in the majority of the samples. The genetic defect is the most important factor in the clinical expression of FH; however, other genetic, environmental and/or metabolic factors could play an important role in modulating the atherosclerotic burden in this population[2829]. In addition, the comparison in clinical and biochemical characteristics, between cases with and without mutations, will be important to be assessed when the number of the study sample increase, given that the presence and the type of mutation can influence lipid profile and treatment efficacy[30].

As a limitation of this study we recognize that the sample number is small, however it showed a high detection rate[1031]. It is worth to stress that this is a randomly selected sample, meeting the criteria for a genetic study of FH, constituting a heterogeneous group, coming from different parts of our country. On the one side, this could be seen as an advantage because results can be better

transfer to our general population. Another limiting point is that functional studies of the new variants were not carried out, which would complete the knowledge of these variants.

Finally, the most relevant findings in this study were the detection of the Lebanese variant as the most common one in the argentine FH population (18%), four novel variants in the LDLR gene, c.-135C>A, c.170A>C p.(Asp57Ala), c.684G>C p.(Glu228Asp) and c.1895A>T p.(Asn632Ile), and that in 27% of the cases, the genetic variant was found in LDLR gene exon 14. This suggests that the assessment of exon 14 in the argentine population could be a low cost alternative approach in a first step for the study of FH, prior to do the complete genetic test. The present study with a high detection rate, 72% contributes to the knowledge of genetic variants spectrum causing FH in our country.

Conflict of interest

Authors declare not conflict interest.

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Table 1: Clinical and biochemical characteristics of index cases according to the FH phenotype

(A) Clinical features

	Children		Adults	
	Heterozygous	Homozygous	Heterozygous	Homozygous [a]
Number of cases (n) =33	5	4	22	2
Age (years) Range	10,0 ± 4,6 5 - 17	12,5 ± 3,9 8 - 17	49,2 ± 10,7 20 - 66	33,5 ± 5,5 28 – 39
Gender, Female, *n* (%) Male, *n* (%)	4 (80,0) 1 (20,0)	2 (50,0) 2 (50,0)	13 (59,1) 9 (40,9)	2 (100) 0 (0)
Detection rate, %	80	100	64	100
Body mass index, (kg/m^2 Overweight, *n* (%) Obese, *n* (%)	 0 (0) 1 (20,0)	 1 (25,0) 0 (0)	 10 (58,9) 1 (5,9)	 0 (0) 0 (0)
On diet, *n* (%)	5 (100)	4 (100)	16 (72,7)	2 (100)
On medication, *n* (%)	5 (100)	4 (100)	20 (95,2)	2 (100)
Physical activity, *n* (%)	5 (100)	3 (75,0)	10 (45,5)	1 (50,0)
Smoking, *n* (%)	0 (0)	0 (0)	2 (9,0)	0 (0)
Cardio Vascular Disease, *n* (%)	1 (17,0)	2 (50,0)	7 (31,8)	1 (50,0)
Tendon Xanthoma, *n* (%)	0 (0)	2 (50,0)	6 (28,6)	2 (100)
Corneal arcus, *n* (%)	0 (0)	0 (0)	2 (9,0)	1 (50,0)
Parent CVD, *n* (%)	2 (40,0)	2 (50,0)	17 (77,3)	2 (100)
Hypertension, *n* (%)	0 (0)	0 (0)	4 (18,2)	0 (0)
Diabetes, *n* (%)	0 (0)	0 (0)	1 (4,5)	0 (0)

[a]The double-heterozygote was included in this group.

(B) Lipid profile

Lipids levels correspond to the highest referred by the clinicians.

	Children				Adults			
	Heterozygous (n=5)		Homozygous (n=4)		Heterozygous (n=22)		Homozygous (n=2)	
mg/dl	Media	SD	Media	SD	Media	SD	Media	SD
LDL-C	281,25	16,153	537,75	95,056	303,53	66,634	581,50	125,158
TC	381,50	18,046	587,00	93,696	391,25	87,251	811,50	263,751
TG	134,25	58,982	110,00	29,451	151,50	64,760	155,50	74,246
HDL-C	44,50	3,873	35,50	7,141	49,36	15,565	39,00	16,971
Apob°	203,00	39,00	314,00	36,592	115,70	27,023	345,00 *	-

°Determinate under-treatment. SD, standard desviation. *Value available only for one of the two cases.

Table 2: Genetic variants identified among index cases.

A) Previously reported in other populations

Gene	Location	Genetic variant	Protein variant	Allele type - validated by FS or predicted (*)	ACMG Classificattion	Number of carriers
LDLR	Exon 3	c.284G>A	p.(Cys95Tyr)	Defective *	VUS	1
LDLR	Intron 3	c.313+1G>A	p.Leu64_Pro105delinsSer / p.Pro105_Ala860delinsArgLysCysGlyProAlaPheAlaIleGluProIle	Defective	Pathogenic	1
LDLR	Intron 3	c.313+2dupT	p.Leu64_Pro105delinsSer	Defective/null	Like pathogenic	1
LDLR	Exon 4	c.337G>T	p.(Glu113*)	Null	Pathogenic	1
LDLR	Exon 5	c.727T>C	p.(Cys243Arg)	Defective *	VUS	1
LDLR	Exon 6	c.920A>G	p.(Asp307Gly)	Defective *	VUS	2
LDLR	Exon 7	c.980dupA	p.(His327*fs*5)	Null *	Pathogenic	1
LDLR	Exon 7	c.1003G>A	p.(Gly335Ser)	Defective	Like pathogenic	1
LDLR	Exon 8	c.1069G>A	p.(Glu357Lys)	Defective *	VUS	1
LDLR	Exon 12	c.1783C>T	p.(Arg595Trp)	Defective *	VUS	2
LDLR	Exon 13	c.1879G>A	p.(Ala627Thr)	Defective *	Like pathogenic	1
LDLR	Exon 14	c.1916T>A	p.(Val639Asp)	NA	VUS	1
LDLR	Exon 14	c.2043C>A	p.(Cys681*)	Defective	Pathogenic	6
LDLR	Exon 14	c.2054C>T	p,(Pro685Leu)	Defective	Pathogenic	1
LDLR	Exon 14	c.2054delC	p.(Pro685*fs*24)	Null *	Pathogenic	1
APOB	Exon 26	c.10580G>A	p.(Arg3527Gln)	Defective	Pathogenic	2

B) Novel genetic variants in the LDLR gene, in the Argentine population

Gene	Gene position	Genetic variant	Genomic position *	Protein variant	Bioinformatic analysis				ACMG Clasification
					Polyphen, score	SIFT, score	Mutation Taster, score	PROVEAN, score	
LDLR	promoter	c.-135C>A	g.5034C>A	----	NA	NA	Disease causing, 0.999	NA	VUS
LDLR	Exon 2	c.170A>C	g.15945C>A	p.(Asp57Ala)	probably damaging, 1 (sensitivity 0.00; specificity 1)	affect function protein, 0.001	Disease causing, 0.999	Deleterius, -7.32	VUS
LDLR	Exon 4	c.684G>C	g.21210G>C	p.(Glu228Asp)	Possibly damaging, 0.561 (sensitivity 0.88; specificity 0.91)	damaging, 0.006	Disease causing, 0.999	Deleterius, -2.76	Like pathogenic
LDLR	Exon 13	c.1895A>T	g.35761A>T	p.(Asn632Ile)	probably damaging, 0,995 (sensitivity 0.68; specificity 0.97)	affect function protein, 0.000	Disease causing, 0.999	Deleterius, -8.41	VUS

FS functional study, NA not-available

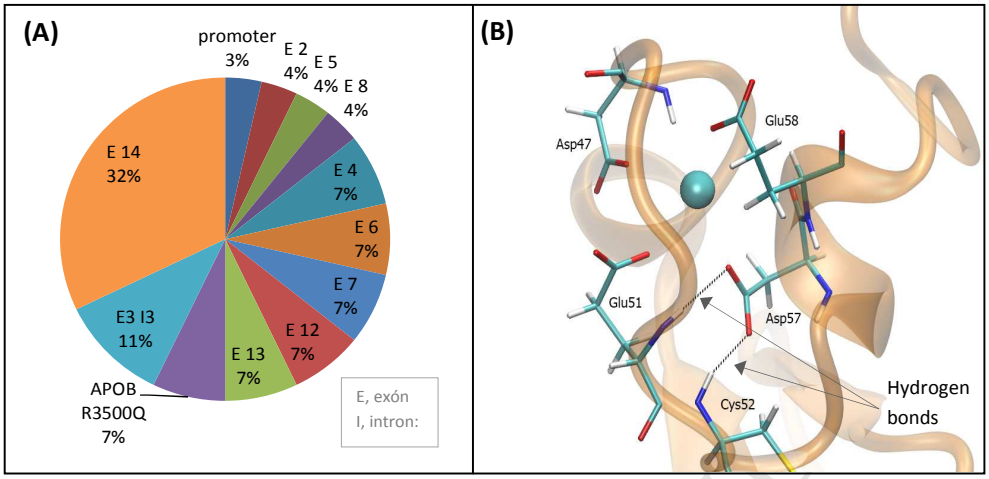
Table 3: Genetic composition of carriers of more than one genetic variant associated with FH.

Genetic variants in more than one index case are in bold.

Phenotype	Age	LDL-C	Xn	CA	CVD	Genotype	Proteins
Homozygous	17	581	si	no	si	LDLR **c.[920A>G];[920 A>G]**	Ldlr p.**[(Asp307Gly)];[(Asp307Gly)]**
Homozygous	28	670	si	si	si	LDLR c.[2054delC];[2054delC]	Ldlr p.[(Met685fs*24)];[(Met685fs*24)]
Comp heterozygous	8	482	si	no	no	LDLR **c.[920A>G];**[1879G>A]	Ldlr p.**[(Ala627Thr)]**;[(Asp307Gly)]
Comp heterozygous	11	439	no	no	no	LDLR **c.[1783C>T];[2043C>A]**	Ldlr p.**[(Arg595Trp)];[(Cys681*)]**
Comp heterozygous	14	649	no	no	si	LDLR c.[1895A>T];**[2043C>A]**	Ldlr p.[(Asn632Ile)];**[(Cys681*)]**
Doble heterozygous	39	493	si	no	no	LDLR c.[284G>A]; APOB **c.[10580G>A]**	Ldlr p.[(Cys95Tyr)]; Apob p.**[(Arg3527Trp)]**

Xn: xantomas; CA: corneal arcus; CVD: cardiovascular disease.

Figure 1: Genetics variants: (A) Distribution by genes region, (B) Visualization of the calcium ion region, where the affected amino acid by the genetic variant c.170A>C, p.(Asp57Ala) take place.



(B) Visualization of the structure PDB 1F5Y of the LDLR protein showing an aspartic acid (Asp) at position 57 close to the calcium ion, in cyan sphere. Other Asp and Glu amino acids at the same structural region are also shown.

Highlights

First description of Familial Hypercholesterolemia mutations in Argentina

Identification of seven patients with severe Familial Hypercholesterolemia

Wide genetic heterogeneity with one relatively common allele, the Lebanese mutation

Description and deep bioinformatics characterization of four novel genetic variants

Studying the exon 14 in a first step could be a low cost approach for this population