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Familial hypercholesterolemia in Brazil: Cascade screening program, clinical and genetic aspects

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ABSTRACT

Background: There is little knowledge about familial hypercholesterolemia in Brazil. This study presents the first results of genetic cascade screening performed in the city of Sao Paulo. **Material and methods**: Two-hundred and forty-eight suspected index cases were initially included. DNA was extracted from peripheral blood and the complete coding sequence of low-density lipoprotein receptor, exon 7 of proprotein convertase subtilisin/kexin type 9 gene and part of exon 26 of apolipoprotein B genes were sequenced. Multiplex Ligation-dependent Probe Amplification was performed on cases where a causal mutation was not identified through sequencing. After the identification of a causal mutation screening in first-degree relatives was pursued. **Results**: From 248 index cases, a mutation was found in 125 individuals (50.4%). 394 relatives were included in the cascade screening program and a mutation was identified in 59.4%. Seventy different causal mutations in the low-density lipoprotein receptor gene (97.2%) and 2 in the apolipoprotein B gene (2.8%) were found. No mutations were encountered in the proprotein convertase subtilisin/kexin type 9 gene. Mutations in exons 14 and 4 were the most prevalent and, 10 cases of true homozygotes (8 index cases and 2 relatives) and 1 compound heterozygote were identified. The most frequent mutation found was of Lebanese origin, the p.(Cys681*) mutation in exon 14 (8.5%). **Conclusion**: Genetic familial hypercholesterolemia cascade screening is feasible in Brazil and leads to identification of a mutation in approximately half of the index cases with higher rates of success in their relatives.

1. Introduction

Familial hypercholesterolemia (FH) is an autosomal dominant disease [1], characterized by total cholesterol (TC) and low density lipoprotein-cholesterol (LDL-C) elevation, caused by mutations in the low density lipoprotein receptor (*LDLR*) [2] gene, apolipoprotein B (*APOB*) gene or proprotein convertase subtilisin/kexin type 9 gene (*PCSK9*) [3]. It was the first lipid metabolism genetic disease clinically and molecularly characterized [4]. There are over 1600 *LDLR* gene mutations related as a cause of FH so far [5].

FH is one of the most frequent inherited monogenic diseases in the general population. The disease's frequency in European populations in its heterozygotic form varies from 1:200 to 1:500 individuals [6], being very rare in the homozygotic form, where a 1:300,000 to 1:1,000,000 frequency in the general population is estimated, [7,8].

Mutations in the *LDLR* gene represent 85–90% of disease causing mutations in FH patients [9], depending on the inclusion criteria and chosen screening method's sensitivity. The most cost-effective strategy for FH diagnosis is the mutation screening in first-degree relatives of individuals molecularly identified with FH [10,11]. Initially, the first-degree relatives are genotyped. The positive cases are then treated as new index cases (IC) and their first-degree relatives are then tested successively. This is referred as cascade genetic testing screening [12,13]. The cascade screening (CS) system has been used in several countries (e.g. Netherlands, Norway, Iceland, Switzerland, UK and Spain) as a cost-effective way to identify FH patients. However, in most countries, FH is still underdiagnosed and undertreated; with less than 1% FH patients identified [14].

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The importance of early diagnosis and institution of adequate lipid-lowering treatment is based on the knowledge of natural history of this disease. In the heterozygous form, it is estimated that men until 50 years old present an approximately 50% risk of coronary disease onset. Before the age of 60 years, without lipid-lowering treatment, that risk could attain 84% in men and 56% in women [10]. The molecular diagnosis within the family allows for both genetic counseling and immediate treatment establishment, which can lead to significant morbidity and mortality reduction [15].

In Brazil, there are few reports about the molecular basis of FH. The first report was about the Lebanese allele, which was initially suspected as the most common cause of disease in the country [16], although the study group was quite small, with only 18 FH Brazilian patients from 10 unrelated families. The second study, published by the same group, expanded their study and concluded that the Lebanese mutation represented one of the most important causes of FH in Brazil [17].

This study aimed to describe the clinical and genetic data obtained from the CS applied in a large FH Brazilian cohort in the city of Sao Paulo (Hipercol Brasil program).

2. Methods

The study protocol was approved by the Institutional Ethics Committee (CAPPesq number 3757/12/013) and written informed consent was obtained from all participants or their parents in the case of children and adolescents prior to entering the study. The study population consisted of: 1-subjects previously referred to the Lipid Clinic at the Heart Institute (InCor), University of São Paulo Medical School Hospital, São Paulo, Brazil, with a clinical suspicion of FH; 2- subjects not from the Lipid Clinic but who had performed a cholesterol test for other reasons and presented or referred previous LDL-C concentrations respectively $\geq$ 210 mg/dL (5.4 mmol/L) for adults and $\geq$170 mg/dL (4.3 mmol/L) for children and teenagers obtained from the central laboratory dataset at InCor; 3-subjects referred directly to the CS program due to elevated cholesterol levels. All study subjects were evaluated between January 2011 and June 2013.

2.1. Study design

The criteria for molecular screening of possible IC were any previously routine measured or referred LDL-C $\geq$ 210 mg/dL (5.4 mmol/L) and $\geq$170 mg/dL (4.3 mmol/L) respectively for adults and for children and teenagers. This was considered independently of the results of Simon Broome Register Group (SB) [18] and the Dutch Lipid Clinic Network (DLCN) [19] FH diagnostic scores. The inclusion criteria were chosen due to lack of previous information about the performance of SB and DLCN for the FH diagnosis in the Brazilian population.

After an FH causing mutation identification and characterization of an index case the CS followed the described flowchart shown in Fig. 1 as recommended by Brazilian and International guidelines [12,20]. Initially, first-degree relatives of the IC were invited. If the mutation was found in that individual, his or her own first-degree relatives (second-degree relatives to the IC) were evaluated. If there were any deceased individuals their offspring was tested. The relatives were included in the screening cascade regardless of their TC and LDL-C levels. The cascade screening was performed by nurses. The program approached the family members directly, with permission of the IC. If the IC did not want the program to contact the family members, we waited for them to contact us.

2.1.1. Clinical and laboratory evaluation

A trained nurse applied a questionnaire based clinical anamnesis and performed a standardized physical examination. The former consisted in evaluating the presence of the usual risk factors for coronary heart disease like smoking, hypertension and diabetes mellitus as well as the previous use of lipid lowering medications. The presence of early coronary disease history in both patient and family, and if there was knowledge about the existence of first-degree relatives with high cholesterol were also evaluated. Any evidence about other relevant diseases was also collected. The information about previous plasma cholesterol values of study subjects, with and without lipid lowering treatment, was obtained from patient charts when available.

Of the 248 possible index cases, 175 (70.6%) and 190 (76.6%) answered respectively a survey that contemplated SB [18] or DLCN [19] FH diagnostic criteria. In these questionnaires the possible presence of an FH causing mutation was not considered as a diagnostic criterion. No FH diagnostic criteria were applied to relatives. Clinical examination consisted of weight (kg), height (m), waist, and hip circumferences (cm) and blood pressure determinations. All patients were also objectively examined for the presence of tendinous xanthomas, corneal arcus, and xanthelasmas. All relatives with an identified FH causing mutation were referred to InCor's Lipid Clinic outpatient unit.

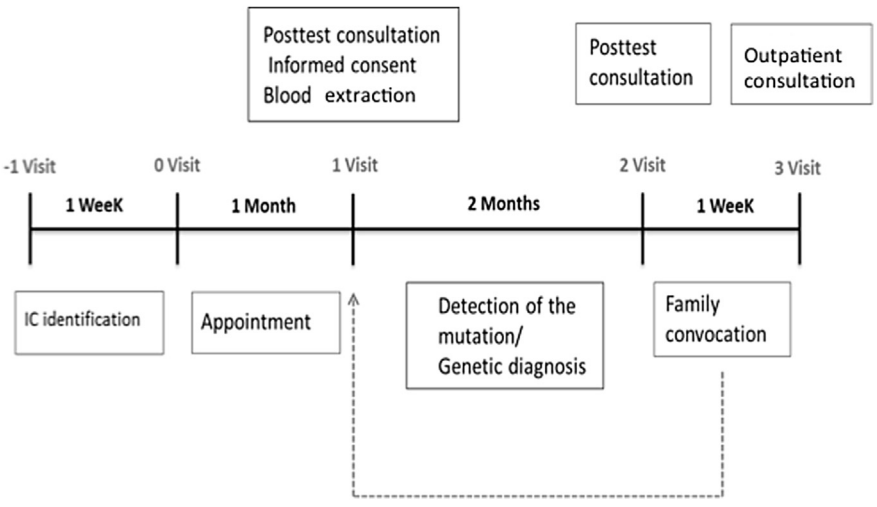

Fig. 1. Cascade screening protocol.

2.2. DNA extraction

Non-fasting blood samples were drawn (10 mL) in an EDTA tube from all patients. DNA extraction was made according the a salting out method, as described by Miller et al. [21].

2.3. LDLR, APOB and PCSK9 genes sequencing and Multiplex Ligation-dependent Probe Amplification (MLPA)

The mutation detection was initially made by *LDLR* gene sequencing. If a disease causing mutation was not found, the *PCSK9* (Exon 7) and *APOB* (part of exon 26) genes both considered as hotspots were studied (primers available upon request). In the case of a negative result in all three genes, the MLPA technique [22] was used to search for deletions or insertions in the *LDLR* gene (*LDLR* P062-C2 kit MRC-Holland). The genomic DNA was amplified with the polymerase chain reaction (PCR) and the amplification confirmation was made with electrophoresis in 1% agarose gel. The samples were purified with ExoSAP-IT (USB Corporation), according to the manufacturer's instructions. Sanger sequencing was performed in the ABI3500xl sequencer (Applied Biosystems) and the sequences were analyzed with the software SeqMan (DNASTAR Lasergene 9).

2.4. Characterization of pathogenic mutations

To confirm if a detected mutation was pathogenic or not, first it was verified if it had been previously associated with FH in the literature. Then, two online prediction databanks were consulted: the British Heart Foundation [23,24] and the JoJo Genetics DNA Diagnostiek [25]. The protein damage prediction was verified in three sites: Polyphen [26], Sift [27] and Mutation Taster [28]. If there was contradictory information, both familial segregation and the existence of the alteration were checked in non-FH populations.

2.5. Statistical analysis

Continuous variables are presented as mean ± standard deviations. Categorical variables are presented as number (%). Data normality was tested by the Kolmogorov–Smirnov test. Clinical and laboratory variables were compared within IC and relative groups respectively presenting or not mutations by Student *t* test or by Wilcoxon's rank test if necessary. Categorical variables were evaluated by chi-square test. Significance was considered at a p value <0.05. Sensitivity, specificity, positive (PPV) and negative predictive values (NPV) for the presence of a FH causing mutation were calculated for DLCN and SB scores. All calculations were performed using the SPSS software (version 13.0).

3. Results

Table 1 shows the clinical and laboratory characteristics of the 642 individuals screened in the program according or not to the presence of a FH causing mutation. The first 248 IC are included in the present analysis. From these, a mutation was found in 125 individuals (50.4%). The families from those IC with an identified mutation were contacted and 394 relatives were also included in the program. In them, a mutation was detected in 234 (59.4%) individuals. Therefore for every index case 1.8 affected relatives were encountered.

3.1. Index cases

Table 1 shows that IC presenting mutations were younger, smoked less, had a greater prevalence of xanthomas, xanthelasmas and corneal arcus than those without identified mutations (all p values < 0.05). On the other hand, there was a greater prevalence of lipid lowering medication use in those not presenting an identified mutation ($p = 0.023$). There were no differences between the groups regarding gender, BMI, previous history of hypertension, diabetes mellitus or history of early onset coronary heart disease.

Table 1
Clinical and biochemical features from index cases and relatives with and without an identified FH causing mutation.

	Index cases ($n = 248$)					Relatives ($n = 394$)				
	Mutation + ($n = 125$)		Mutation − ($n = 123$)		p value*	Mutation+ ($n = 234$)		Mutation − ($n = 160$)		p value*
		n		n			n		n	
Age (years)	50.4 ± 17.7	125	55.0 ± 12.4	123	0.841	43.3 ± 17.9	234	44.6 ± 17.1	160	0.816
Males (%)	40.8	51	30.9	38	0.104	40.2	94	45.6	73	0.282
Females (%)	59.2	74	69.1	85		59.8	140	54.4	87	
Hypertension (%)	32.8	41	40.7	50	0.499	28.6	67	20.0	32	0.027
Diabetes (%)	7.2	9	11.4	14	0.375	9.8	23	10.0	16	0.570
Early coronary disease(%)	12.0	15	23.6	29	0.126	11.1	26	3.8	6	0.001
Acute myocardial infarction (%)	11.2	14	17.1	21	0.347	9.8	23	1.9	3	0.001
Angina (%)	20.8	26	15.4	19	0.033	11.1	26	5.6	9	0.039
Pharmacological treatment (%)	74.4	93	84.6	104	0.023	62.4	146	19.4	31	0.000
BMI (kg/m^2)	27.2 ± 5.1	95	27.4 ± 4.3	120	0.837	26.1 ± 4.9	229	27.6 ± 14.1	160	0.161
Smoking (%)	4.0	5	15.40	19	0.032	12.0	28	13.1	21	0.694
Tendon xanthoma (%)	6.4	8	0	0	0.001	2.6	6	0	0	0.026
Xantehlasmas (%)	11.2	14	4.9	6	0.011	5.6	13	1.3	2	0.015
Corneal Arcus (%)	27.2	34	10.6	13	0.000	13.7	32	4.4	7	0.001
Baseline TC (mg/dL)	403 ± 84	60	309 ± 55	36	0.000	362 ± 77	63	247 ± 127	3	0.017
On treatment TC (mg/dL)	259 ± 113	78	224 ± 68	74	0.022	222 ± 55	102	215 ± 46	11	0.689
Baseline LDL-C (mg/dL)	323 ± 85	59	224 ± 45	36	0.000	288 ± 79	59	155 ± 98	3	0.006
On treatment LDL-C (mg/dL)	191 ± 109	78	147 ± 64	72	0.003	152 ± 49	100	136 ± 41	11	0.298
Baseline HDL-C (mg/dL)	46 ± 12	58	49 ± 14	36	0.201	46 ± 12	58	52 ± 27	3	0.436
On treatment HDL-C(mg/dL)	45 ± 11	78	48 ± 13	73	0.089	46 ± 12	101	51 ± 14	11	0.278
Baseline TG (mg/dL)	144 ± 59	58	185 ± 135	36	0.047	130 ± 55	60	205 ± 57	3	0.027
On treatment TG (mg/dL)	121 ± 68	76	159 ± 114	73	0.014	107 ± 68	101	142 ± 66	11	0.104

Baseline exams: patients without medication; Current exams: exams when the patient was included in the program. Early coronary disease: Male <55 and female <60 years-old. Medications used for cholesterol treatment were statins, ezetimibe, resins, fibrates and niacin *$\rho > 0.05$; TC: total cholesterol; TG: triglycerides.

Those presenting mutations had higher baseline and on-treatment total (TC) and LDL-C values (all p values <0.05).

3.2. Relative patients

Table 1 shows that there were no differences between relative cases presenting or not mutations regarding age, gender, smoking, and BMI. However, those with mutations had a greater prevalence of hypertension ($p = 0.027$) and early coronary heart disease presence ($p = 0.001$). Similarly to IC those with mutations had a greater prevalence of xanthomas and corneal arcus (all p values < 0.05). There was also a greater prevalence of lipid-lowering medication use ($p < 0.001$) and higher baseline and on treatment TC and LDL-C values (all values $p < 0.05$).

Table 2 shows the distribution of patients according to both the DLCN and SB scores in those presenting or not identified mutations. In those 175 IC patients in whom the DLCN was applied a definitive and a probable FH diagnosis was made in respectively 27.7% ($n = 49$) and 32.2% ($n = 57$) of study subjects, summing up 59.9% of studied participants with at least a probable diagnosis. In individuals with, at least, probable FH a causal mutation was identified in 65 out of 105 participants (61.9%). Definitive and probable FH diagnoses were made respectively in 4.1% ($n = 8$) and 58.5% ($n = 111$) of 190 study subjects where the SB criteria was applied. A pathogenic mutation was found in 56 patients with probable diagnosis and 8 with definitive diagnosis (53.8%). The specificity, sensitivity, positive and negative predictive values of both DLCN and SB scores to detect a FH causing mutation are also shown in Table 2.

Table 3 shows the mutations found in the 125 IC and their distribution according to the affected exons in the *LDLR* and *APOB* genes. No mutations were found in the *LDLR* gene promoter region or in exons 15 and 18. Mutations in exons 14 and 4 were the most prevalent and the p.(Cys681*) mutation in the exon 14 was found in 11 subjects (8.5% of all 129 IC with identified mutations).

Table 4 shows the deletions and duplications found in the *LDLR* gene with MLPA technique; 10 different deletions in 11 IC and 2 different duplications in 3 IC were detected. Putting together the small mutations, large deletions and duplications, 71 different mutations were found in *LDLR* gene and 2 in the *APOB* gene. From all screened patients, 10 cases of true homozygotes (8 IC and 2 relatives), and 1 compound heterozygote (p.(Ser177Leu) and c.941-?_1186+?del) were identified (Tables 3 and 4). No mutations were found on the tested region of the *PCSK9* gene.

4. Discussion

This study described for the first time the population included in a FH genetic CS program performed in a tertiary university hospital in the city of Sao Paulo, Brazil (Hiperol Brasil program). Considering both the current Brazilian population of 201 million inhabitants and the estimated prevalence of heterozygous FH, varying from 1/200 to 1/500 individuals in the general population [14], it is expected that Brazil has from 402,000 to 670,000 FH cases. Unfortunately it is also estimated that <1% of these cases are diagnosed and treated [14].

From the 125 IC with an identified mutation, 394 relatives were invited to participate in the genetic screening project. Those individuals with identified mutations were admitted for treatment at InCor's lipid clinic if they had not been originally referred from that clinic for molecular diagnosis. Patients with hypercholesterolemia but without an identified mutation were oriented to keep their treatment, when it had already been prescribed, or were referred to other physicians for treatment. All patients were oriented about the implications of a possible FH diagnosis and about the importance of screening cholesterol levels within their families.

Recent studies show that CS has the best cost-effectiveness and acceptability both for FH patients and their doctors [20,29]. However this information was not yet available from previous small investigations performed in the Brazilian population [16,17]. Despite its greater size in comparison with the previous investigations this study also cannot answer if genetic CS is cost effective in Brazil. This occurs consequently to the descriptive nature of this study and also to the heterogeneity of models of health care in Brazil. However, the fact that for every index case the CS program was able to find almost 2 affected individuals suggests that early detection and treatment of FH patients with high potency statins, that are all generic medications in Brazil, can indeed be cost effective.

The finding of a FH causing mutation in approximately half of the IC was similar to the 45% positivity found by Bourbon et al. in a Portuguese population where FH was diagnosed by the SB criteria [30]. Similar results to this study, a 52.3% prevalence of *LDLR* mutations were encountered in 2,400 Dutch patients from different lipid clinics with clinical FH diagnosis according to SB and DLCN or US MEDPED criteria [31]. Notwithstanding that this screening program was not designed to test the performance of clinical criteria to diagnose FH in the Brazilian population, the current study suggests that using LDL-C thresholds is a valid approach to do genetic CS in suspected FH patients.

Table 2
Distribution and performance of Dutch Lipid Clinic Network (DLCN, $n = 175$) and Simon Broome (SB, $n = 190$) criteria categories in possible index cases presenting or not an identified FH causing mutation.

DLCN	Mutation +		Mutation −		Sensitivity	Specificity	PPV	NPV
	%	*n*	%	*n*	%[a](CI)	% (CI)	% (CI)	%(CI)
0 to 2 points	3.6	3	19.8	18				
3 to 5 points − possible	19.0	16	36.3	33	96.4 (89.1–99.0)	19.7 (12.4–29.7)	52.5 (44.4–60.6)	85.7 (62.6–96.2)
6 to 8 points − probable	32.1	27	31.9	29	77.3 (66.7–85.5)	56.0 (45.2–66.3)	61.9 (51.8–71.0)	72.8 (60.7–82.4)
>8 points −definitive	45.2	38	12.1	11	45.2 (34.4–56.4)	87.9 (78.9–93.5)	77.5 (63.0–87.7)	63.4 (54.3–71.7)
Total	100	84	100	91				
SB								
Definitive	10.5	8	0	0	10.5 (4.9–20.2)	100 (95.9–100)	100 (59.7–100)	62.6 (55.1–69.5)
Probable	73.7	56	48.2	55	84.2 (73.6–91.2)	51.7 (42.2–61.1)	53.7 (44.4–62.8)	83.0 (71.9–90.5)
No	15.8	12	51.8	59				
Total	100	76	100	114				

[a] 95% Confidence Interval (CI). For calculations in DLCN, possible classification included all individuals with 3 or higher points; probable classification included all individuals with 6 or higher points; definitive classification included all individuals with higher than 8 points. For calculations using SB criteria, probable classification included all individuals with probable and definitive criteria; PPV − postive predictive value; NPV − negative predictive value.

Table 3
Mutations found in 108 of 125 (86.4%) index cases by *LDLR*, *APOB* and *PCSK9* gene sequencing.

Location	Aminoacid change	Nucleotide change	$N = 108$
LDLR Gene			
exon 1	p.(Gly2Arg)	c.4G > C	2
exon 1	p.(Trp10Arg)	c.28T > C	2
exon 2	p.(Tyr42*)	c.126C > A	2
exon 3	p.(Cys82Ser)	c.245G > C	1
exon 4	p.(Asp224_Ser226dup)	c.670_678dupGACAAATCT	4
exon 4	p.(Asp221Gly)	c.662A > G	5
exon 4	p.(Cys160Tyr)	c.479G > A	1
exon 4	p.(Cys184Tyr)	c.551G > A	1
exon 4	p.(Asp168Ala)	c.503A > C	1
exon 4	p.(Glu228Gln)	c.682G > C	1
exon 4	p.(Glu228Lys)	c.682G > A	1
exon 4	p.(Pro181Leu)	c.542C > T	1
exon 4	p.(Gln163*)	c.487C > T	1
exon 4	p.(Ser177Leu)	c.530C > T	3
exon 5	p.(Arg257Trp)	c.769C > T	1
exon 6	p.(Asp301Gly)	c.902A > G	1
exon 6	p.(Cys276Trp)	c.828C > G	1
exon 6	p.(Glu312Valfs*19)	c.935_936delAG	1
exon 6	p.(His285Tyr)	c.853C > T	1
exon 7	p.(Arg350*)	c.1048C > T	3
exon 7	p.(Gly343Ser)	c.1027G > A	3
exon 7	p.(Glu317Glyfs*15)	c.949dupG	1
exon 7	p.(Ser326Cys)	c.977C > G	3
exon 8	p.(Cys364Arg)	c.1090T > C	1
exon 8	p.(Cys392*)	c.1176C > A	1*
exon 8	p.(Cys368Tyr)	c.1103G > A	1
exon 8	p.(His388Profs*53)	c.1158_1162dupC	1
exon 8	p.(Gly373Asp)	c.1118G > A	4(1*)
exon 9	p.(Ala431Thr)	c.1291G > A	3(2*)
exon 9	p.(Arg406Trp)	c.1216C > T	1
exon 9	p.(Ile451Thr)	c.1352T > C	1
exon 9	p.(Leu401Val)	c.1201C > G	1
exon 9	p.(Gln448*)	c.1342C > T	1
exon 10	p.(Asp492Asn)	c.1474G > A	1
exon 10	p.(Asp492Thrfs*15)	c.1474delG	1
exon 11	p.(Ala540Thr)	c.1618G > A	1*
exon 11	p.(Gly546Asp)	c.1637G > A	1
exon 11	p.(Gly549Asp)	c.1646G > A	2
Exon 11 +exon17	p.(Asn564His) + p.(Val800_Leu802del)	c.1690A > C + 2393_2401delTCCTCGTCT	1
exon 12	p.(Arg595Trp)	c.1783C > T	1
exon 12	p.(Asp601His)	c.1801G > C	3
exon 12	p.(Glu602*)	c.1804G > T	2
exon 12	p.(Gly592Glu)	c.1775G > A	1
exon 12	p.(Arg574His)	c.1721G > A	1
exon 13	p.(Ile624del)	c.1871_1873delTCA	1*
exon 13	p.(Phe629Tyrfs*16)	c.1885_1886insA	3
exon 14	p.(Cys681*)	c.2043C > A	11
exon 14	p.(Pro699Leu)	c.2096C > T	5
exon 14	p.(Trp666*)	c.1997G > A	1
exon 14	p.(Pro685Leu)	c.2054C > T	1
exon 17	p.(His837Thrfs*23)	c.2509delC	2
exon 17	p.(Lys811*)	c.2431A > T	1
exon 17	p.(Tyr828Cys)	c.2483A > G	1
exon 17	p.(Val806Glyfs*11)	c.2416dupG	1
intron 10	_	c.1586 + 1 *G* > A	3
intron 3	_	c.313 + 1 *G* > A	3
intron 6	_	c.941-4 *G* > A	2
APOB gene			$N = 2$
exon 26	p.(Arg3527Gln)	c.10580G > A	1
exon 26	p.(Arg3558Cys)	c.10800C > T	1

* Homozygosis.

Mutations were found in 59.4% of relatives, these results however, differ from data originated in the Netherlands where only 37% of relatives were diagnosed according to their carrier status [15]. A possible explanation can be the use of different enrollment processes into the screening programs.

Similarly to investigations in European populations [14] the great majority of mutations in this study were found in the *LDLR* with a small percentage encountered on the *ApoB* gene. No mutations in the *PCSK9* gene were found a fact that could be ascribed by its low frequency in the literature [14] and also to methodological limitations in this study where only its exon *7* was studied.

Table 4
Deletions and duplications found in the *LDLR* gene with MLPA in 17 of 125 (13.6%) index cases.

Name	Nucleotide change	$N = 17$
[Pr_EX18del]	c.-187-?_*2584del	1
[EX13_EX18del]	c.1846-?_*2514del	3
[EX16_EX17del]	c.2312-?_2547+?del	1
[EX16_EX18del]	c.2312-?_*2514del	1*
[EX7_EX8del]	c.941-?_1186+?del	1
[EX7_EX10del]	c.941-?_1586+?del	Compound heterozygote
[EX7_EX14del]	c.941-?_2140+?del	1
[EX3_EX6del]	c.191-?_940+?del	2
[EX17del]	c.2390-?_2547+?del	1
[EX11_EX12del]	c.1587-?_1845+?del	1
[EX15_EX16del]	c.2141-?_2389+?del	1
[Pr_EX6dup]	c.-187-?_940+?dup	3(1*)
[Ex11_Ex12dup]	c.1587-?_1845+?dup	1

* Homozygosis.

As previously described from smaller Brazilian FH populations [16,17] the most frequent mutation found was of Lebanese origin. Countries with more advanced and widespread FH CS projects have detected a greater number of different mutations than the one found in our program respectively 522, 250, 200 and 78 in the Netherlands [32], Spain [33], the United Kingdom [23] and Portugal [34]. It is possible that when a greater number of screened subjects is achieved, and also when subjects from other regions of country are studied, more mutations will be encountered. According to the Brazil's Geography and Statistics Institute the Brazilian [35] population is mainly composed of descendants of immigrants from Portugal, Sub-Saharian Africa, Spain, Italy, Syria and Lebanon, Germany and Japan. Further studies are necessary to compare the mutations found in our population with the ones from those countries.

One interesting finding of our population was the small prevalence of tendinous xanthomas 2.2% in the whole population and 3.9% in those with identified mutations. This is a low value in comparison with the literature [36]. It is possible that limitations of physical examination, the non-use of Achilles tendon ultrasound for diagnosis and the previous use of lipid lowering medications by 2/3 of those patients could explain our results. The small prevalence of xanthomas helps to explain the low sensitivity of the DLCN and SB criteria in our population.

4.1. Study limitations

This study has several limitations. Despite the greatest number of FH patients in one Brazilian study, it is not possible to have a precise representation of this disease in a continental country like Brazil. Therefore a larger number of subjects from different parts of the country will have to be studied. Also, since cholesterol levels without the use of statins were not available from most IC (around 60%) and no correction for the use of those medications was performed, further data will be necessary to test the performance of SB and DLCN FH diagnostic scores in the Brazilian population. Since we did not evaluated the effects of treatment on LDL-C in FH new cases, the overall effectiveness of cascade screening for coronary heart disease prevention remains to be established in Brazil. Finally, a better characterization of tendinous xanthomas, will be necessary to study the prevalence of these abnormalities in our population.

In conclusion our data provide a general view of the FH scenario in the city of Sao Paulo, Brazil and highlights the importance of a CS program establishment in the country.

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