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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.

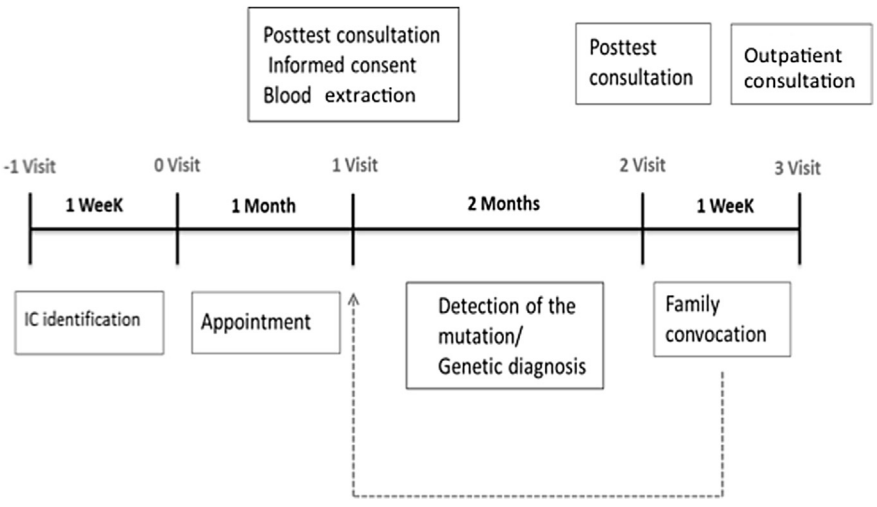

Fig. 1. Cascade screening protocol.