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Mini Review

Familial Hypercholesterolaemia in Portugal: A Need for More Diagnostic Screening

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Keywords

Familial hypercholesterolaemia; Lipoprotein metabolism; Coronary artery disease; LDLR; APOB; PSCK9

Abbreviations

FH: Familial Hypercholesterolaemia; LDL: Low Density Lipoprotein; CHD: Coronary Heart Disease; TC: Total Cholesterol; LDL-C: LDL Cholesterol; HeFH: Heterozygous FH; HoFH: Homozygous FH; ARH: Autosomal Recessive Hypercholesterolemia; LDLR: Low Density Lipoprotein Receptor; APOB: apolipoprotein B; PCSK9: Proprotein Convertase Subtilisin/Kenin Type 9; OMIM: Online Mendelian Inheritance In Man; RT-PCR: Reverse Transcriptase Polymerase Chain Reaction; DHPLC: Denaturing High Pressure Liquid Chromatography; MLPA: Multiplex Ligation-Dependent Probe Amplification; RNA: Ribonucleic Acid

Introduction

Familial Hypercholesterolaemia (FH) (MIM 144010) is an autosomal dominant disorder of lipoprotein metabolism, which is characterized by the increased level of circulating Low Density Lipoprotein (LDL) cholesterol that leads to lipid accumulations in arteries, and sometimes in tendons (xanthomas) and around the eyes (xanthelasmas), resulting in premature atherosclerosis and increased risk of Coronary Heart Disease (CHD [1]. If remains untreated, FH can increase the cumulative risk for CHD by up to 20-fold (men are at 20% higher risk than women), the relative risk to develop premature CHD for men and women with FH aged between 20 and 39 years old is about 100-fold increased compared to the normal population [1,2]. These data demonstrate a serious need for diagnostic screening for the disease. The treatment with cholesterol-lowering medications will promote the arrest of atherosclerosis progression and prevention of CHD.

Currently, FH remains to be under diagnosed and undertreated in the general population. Based on prevalences between 1/500 and 1/200, 14-34 million individuals worldwide have FH [3]. Recent evaluation of the extent to which FH is under diagnosed and undertreated worldwide demonstrated that <1% of FH cases are diagnosed in most countries, except the Netherlands (71% of diagnosed FH cases), Norway (43%), Iceland (19%), Switzerland (13%), the UK (12%), and Spain (6%) [3]. Although Portugal should have about 20,000 cases, this disease is severely under diagnosed in the country, it is estimated that 80% of FH patients are not even clinically identified [4].

Nowadays, FH can be diagnosed either clinically or genetically. Both of these methods have their advantages and disadvantages. Clinical diagnosis can be useful in diagnosing relatives of known FH patients and avoiding the assessment of patients, who are probably not FH cases. Moreover, the cost of clinical diagnosis is low. However, clinical diagnosis cannot distinguish between FH cases due to mutations in different genes, between Heterozygous FH (HeFH) and homorozygous FH (HoFH), Autosomal Recessive Hypercholesterolemia(ARH)[5]ornon-familialhypercholesterolemia (secondary hypercholesterolemia, sitosterolemia, etc.), it can miss asymptomatic FH cases (e.g. in the pediatric population) [3,6]. While genetic testing can provide not only the early diagnosis, but also a strategy to prevent the risk of cardiovascular complications. It can diagnose asymptomatic FH cases, distinguish between all types of FH cases and have a prognostic significance for succeeding generations. The high cost, the polygenetic nature of the disease and affection of the phenotype by many non-genetic factors are among limitations of genetic testing [3,6]. Most probably, the optimal and most effective diagnosis should be based both on clinical and genetic tests. For example cascade screening is a strategy that already proved to be cost-effective in the FH diagnosis. It is characterized by the combination of initial clinical diagnosis with subsequent genetic testing by the next scheme: genetic test confirms the mutation in the index patient, then first degree relatives are screened for the same mutation and new confirmed cases are treated as new index cases and their first degree relatives are screened [6].

So far, there are no single internationally accepted criteria for the clinical diagnosis of FH [6]. Three main criteria are currently used for FH diagnostics: Dutch Criteria (the Netherlands), MEDPED Criteria (USA), and Simon Broome Criteria (UK). The US criteria are based on lipid levels and age, while the UK and Dutch criteria classify FH cases as definite, probable, and possible using not only lipid levels, but also physical signs, family and personal history [6]. According to Simon Broome Criteria, which is the most widely used by physicians, plasma Total Cholesterol (TC) and LDL-C levels in FH cases range from 7.5 mmol/L (290 mg/dl) and 4.9 mmol/L (190 mg/dl), respectively, in adults, and from 6.7 mmol/L (260 mg/dl) and 4 mmol/L (155 mg/dl), respectively, in children [6].

Genetic diagnosis of FH is currently based on the testing of defects in the low density lipoprotein receptor gene (*LDLR,* OMIM 606945), the gene for apolipoprotein B (*APOB,* OMIM 107730), and the proprotein convertase subtilisin/kenin type 9 (*PCSK9,* OMIM

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607786) gene. The frequency of FH is about 1/200-500 for HeFH and 1/160000-1000000 for HoFH [1,7]. The LDLR is responsible for the binding and subsequent cellular uptake of apolipoprotein B- and E-containing lipoproteins [8], APOB is the major protein component of triglyceride-rich and low density lipoproteins, it is responsible for carrying cholesterol to tissues [9], and PCSK9 is a serine protease that reduces both hepatic and extra hepatic LDLR levels and increases plasma LDL cholesterol [10]. Mutations in any of these genes can cause impairment of normal uptake and clearance of LDL by the liver. More than 1300 mutations (account for 60-80% of FH) are known so far in the *LDLR* gene [11], they include single-nucleotide mutations, copy number variations, and splicing mutations and can be divided into five distinct classes according to how they affect LDLR expression and function (null (produce no detectable protein), transport-defective, biding-defective, internalization-defective and recycling-defective); more than 20 mutations are related to FH in the *PCSK9* gene (account for 0-3% of FH), and several - in the *APOB* gene (e.g., rs5742904; account for 1-5% of FH) [1,3,12,13].

In Portugal many patients have TC above 350 mg/dl and triglycerides below 200 mg/dl, which suggests the presence of FH in this population [14-16]. The Portuguese population has much low percentage of patients with tendon xanthomas (present in only 8.5% FH cases) than other populations where the presence of tendon xanthomas is generally considered diagnostic of FH (present in 22.5% of Spanish FH patients and 78.8% of English FH patients) [14,15]. It contributes to higher level of undiagnosed FH and makes molecular diagnostics of great importance for this population. In the Portuguese population three common genes have been analyzed for the association with FH, namely *LDLR*, *APOB*, and *PCSK9* [4,11,13-15,17]. Individuals form Azores, Madeira, and Mainland have been studied in family-based and case-based studies. Known mutations have been analyzed as well as mutation screening has been performed in these studies using such methods as sequencing, DHPLC, MLPA, RT-PCR and fragment analysis, site-directed mutagenesis, protein expression *in vitro*, etc. In total, 113 genetic variants (point, nonsense, splice site and missense mutations, mutations in the promoter region, deletions and insertions, polymorphisms) were described for the Portuguese population [4,11,13-15,17]. 93 genetic variants have been described for the *LDLR* gene, 51 of them were associated with higher risk for FH, 39 genetic variants were reported as novel [4,13-15,17], two risk variants of seven described (among them 5 novel) have been reported for the *PCSK9* gene [4,14,15,17], and five of 13 (12 of them were novel) – for the *APOB* gene (Table 1) [4,11,14,15,17]. Most of these data were obtained from the Portuguese Familial Hypercholesterolemia Study that was initiated at the National Institute of Health in 1999 year, aiming to perform an epidemiological study to determine the prevalence and distribution of FH in Portugal and to better understand the pathophysiology of CHD in these patients [14]. The clinical criteria for FH used in this study were adapted from the UK Simon Broome Criteria. The genetic diagnosis of FH was performed in three phases and an optional fourth phase: I - screening for the common mutations in the *APOB* gene and analysis of the *LDLR* gene, II - identification of large rearrangements in the *LDLR* gene using MLPA technique, III - screening of the *PCSK9* gene (if no mutation was found in phases I and II), and IV (only performed when putative splicing mutations, not described before or without functional studies, are found). Analysis of relatives was performed as the cascade screening [4]. Index cases and relatives with or without a clinical diagnosis of FH were enrolled in the Portuguese Familial Hypercholesterolemia Study. In total, genetic analysis was performed in 684 index cases and 1210 relatives. Only in 52.8% of index cases (340/49.7% in the *LDLR* gene, including eight homozygotes and seven compound heterozygotes, 9/1.32% in the *APOB* and 12/1.75% in the *PCSK9*) and 45.2% of relatives (529/43.72% in the *LDLR* gene, 13/1.07% in the *APOB* and 5/0.41% in the *PCSK9*) studied, a mutation causing disease was identified [4,14,15]. This fact can be explained by several reasons, e.g. some patients do not meet inclusion criteria, there are genetic changes in another gene(s) involved in LDLR function, regulation or LDL metabolism, the underlying gene defect causing FH in these patients is in a non-coding region of the *LDLR* gene that may affect its expression or RNA processing, or non-familial hypercholesterolemia is mistakenly taken for FH [4].

Table 1: Genes and the number of allelic variants studied for the association with FH in the Portuguese population.

Gene	Number of studied allelic variants	Reference
APOB	13^{N}	[3,11,14,15]
LDLR	93^{N}	[3,13-15,17]
PCSK9	7^{N}	[3,14,15]

N: Novel mutation(s).

More detailed information on mutations soon will be available in online PorMUTa database (the Portuguese population database of genetic variants studied for the association with genetic diseases and genetic variants modulating drug response, http://www.pormuta.uac.pt).

The Portuguese population, with severely under diagnosed FH and much lower percentage of patients with such diagnostic symptom as tendon xanthomas helping to diagnose FH clinically, has a serious need for diagnostic screening for this disease. For many years the diagnosis of FH was based on the analysis of lipid levels and family history. However, recent developments in molecular genetics allow rapid and robust genetic testing that can provide not only the early diagnosis, even in silent cases, but also a strategy to prevent the risk of cardiovascular complications.

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