Atherosclerosis 249 (2016) 52–58

Contents lists available at ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Efficacy of clinical diagnostic criteria for familial hypercholesterolemia genetic testing in Poland

Agnieszka Mickiewicz [a, *], Magdalena Chmara [b], Marta Futema [c], Marcin Fijalkowski [a], Krzysztof Chlebus [a], Rafał Galaska [a], Tomasz Bandurski [e], Marcin Pajkowski [a], Monika Zuk [b], Bartosz Wasag [b], Janusz Limon [b], Andrzej Rynkiewicz [d], Marcin Gruchala [a]

[a] 1st Department of Cardiology, Medical University of Gdansk, Gdansk, Poland
[b] Department of Biology and Genetics, Medical University of Gdansk, Gdansk, Poland
[c] Centre for Cardiovascular Genetics, Institute of Cardiovascular Science, University College London, London, UK
[d] Department of Cardiology and Cardiosurgery, University of Warmia and Mazury, Olsztyn, Poland
[e] Department of Radiology, Informatics and Statistics, Medical University of Gdansk, Gdansk, Poland

ARTICLE INFO

Article history:
Received 17 October 2015
Received in revised form
8 February 2016
Accepted 18 March 2016
Available online 26 March 2016

Keywords:
Familial hypercholesterolemia
Clinical criteria
Coronary artery disease
LDLR
APOB

ABSTRACT

Background and Aims: Familial hypercholesterolemia (FH), which leads to premature cardiovascular events, still remains underrecognized and undertreated in most countries. Untreated FH individuals aged 20–39 years are at 100-fold higher risk of mortality from coronary heart disease compared to those of a general population. Therefore, special efforts should be implemented to diagnose FH patients at early stages of life.
The aim of this study was to evaluate the efficacy of the revised Dutch Lipid Clinic Network (DLCN) criteria proposed by the Polish Lipid Experts Forum to select index FH patients for DNA mutational analysis in Poland.
Methods: The study included 193 unrelated adult patients (mean age 48 ± 13 years) with clinical diagnosis of FH based on the revised DLCN score, tested sequentially for mutations in *LDLR* and *APOB* genes using bidirectional Sanger sequencing and MLPA techniques. The cut-off points of the clinical FH criteria score were assessed by ROC statistics to identify patients with the highest probability of carrying an FH-causing mutation.
Results: The causal heterozygous *LDLR* or *APOB* mutation was identified in 41% (80/193) of probands. Adults aged <40 years were more likely to carry an FH-causing mutation compared to subjects aged ≥40 years (65% vs. 33%; $p < 0.001$). LDL-C thresholds for the molecular diagnosis of FH were 5.79 mmol/l for individuals aged<40 and 6.7 mmol/l for subjects ≥40 years old. The threshold values of the clinical diagnostic score for efficient selection of patients for genetic testing were 5 and 7 points for individuals aged <40 and ≥40 years, respectively.
Conclusions: The study validated the efficacy of proposed clinical FH criteria for the disease diagnosis in Poland. The clinical criteria score thresholds for positive FH molecular diagnosis differ depending on age (<40 and ≥40 years). We propose that in the healthcare systems with limited genetic testing resources individuals younger than 40 years, who fulfill the clinical criteria for possible, probable or definite FH should qualify for the FH mutation testing. The index patients aged ≥40 years with clinical diagnosis of probable or definite FH should also qualify for the genetic testing.

1. Introduction

Individuals with familial hypercholesterolemia (FH) are at increased risk of premature cardiovascular events and death [1]. If untreated, heterozygous FH (HeFH) patients will present symptoms of coronary heart disease (CHD) during or after their third decade of life in men, and 10 years later in women [2]. Untreated HeFH individuals aged 20–39 years are at 100-fold increase in mortality from coronary heart disease compared to those of a general

* Corresponding author.
E-mail address: amickiewicz@gumed.edu.pl (A. Mickiewicz).

http://dx.doi.org/10.1016/j.atherosclerosis.2016.03.025
0021-9150/

population [3]. Therefore, special efforts should be implemented to identify these patients at early stages of life and to introduce effective lipid-lowering treatment and life style changes.

Usually, the FH diagnostic process employs clinical criteria and molecular analysis of *LDLR, APOB* and *PCSK9* genes, in which mutations are known to case FH. Currently, there are three clinical diagnostic systems for FH based on the findings of: 1. the Simon Broome Register Group in the United Kingdom, 2. the US MEDPED Program, and 3. the Dutch Lipid Clinic Network [3–5]. In Poland, the Polish Lipid Experts Forum proposed diagnostic criteria for FH based on the Dutch Lipid Clinic Network score and the Simon Broome Register criteria [6]. This scale includes criteria selected in the Netherlands, modified by the assigned cut points for the concentrations of LDL-cholesterol (LDL-C) taken from the Simone Broome Register criteria. Diagnostic points that are taken into consideration include: LDL cholesterol levels, personal and family history of premature cardiovascular disease, family history of hypercholesterolemia, presence of tendon xanthomas and arcus cornealis in subject or family history, and an underlying genetic defect. The cut points for LDL-C concentrations in first-degree relatives were modified from 95th percentile to 4.9 mmol/l (190 mg/dl) in adults and 4.0 mmol/l (155 mg/dl) in children <18 years of age. These criteria classify FH patients as possible FH (3–5 points), probable FH (6–8 points) and definite FH (>8 points) making the selection for further genetic testing easier (Table 1).

To date, due to a limited accessibility of the FH genetic diagnosis, the efficacy of the Polish Lipid Experts Forum criteria in Polish population has not been assessed yet. The question also arose, if these criteria are also suitable for young adult patients (aged below 40 years), in whom clinical consequences of high LDL-C (such as symptoms of coronary artery disease) are less evident than in the older FH patients. Therefore, the aim of this study was to evaluate the utility of the proposed clinical criteria for FH diagnosis, and their ability to efficiently select index patients for the genetic *analysis* of *LDLR*, *APOB* and *PCSK9* genes in Poland.

2. Materials and methods

A total of 193 unrelated adult patients were enrolled to the study. Patients were recruited over a period 2006–2011 at 1st Department of Cardiology, at the Medical University in Gdansk. To qualify for the study, patients had to have fasting pre-treatment LDL-C level above or equal to 4.9 mmol/l (190 mg/dl). The specific exclusion criteria were: secondary hypercholesterolemia and triglyceride concentration over 4.5 mmol/l (400 mg/dl) [3]. All patients underwent physical examination. Clinical diagnosis of FH was specified according to the criteria recommended by the Polish Lipid Experts Forum (Table 1) [6].

Informed consent for participation in the study was obtained from all patients. The protocol of the study has been approved by the Local Ethics Committee.

Genomic DNA was isolated from whole blood using standard methods [7] and mutational analysis of *LDLR* and *APOB* was performed in all patients as previously described [8]. A fragment of exon 26 of the *APOB* gene located between codons 3473–3606, which covers the region of the most frequent FH mutation, was screened using Sanger sequencing [9].

Statistical analysis was performed using the Statistica software (v.10.0) [10]. Descriptive statistical evaluations were expressed as mean, standard deviation, median, minimum and maximum, for numerical variables, and as numbers and percentages for categorical variables. The difference between the groups with regards to categorical variables was determined by the Pearson $\chi 2$ and Fisher exact tests. For other variables, the difference between two groups was determined by the independent sample t-test in the case of normally distributed data, whereas the Mann-Whitney U test was used for analysis of non-normally distributed data for independent groups. The *p*-value of 0.05 was considered statistically significant, with the confidence interval (CI) 95%.

The ability of the clinical criteria to discriminate between FH mutation carriers and non-carriers was assessed by the receiver operating characteristic (ROC) curve statistics. Subsequently, ROC curves were plotted for numerical variables significant in univariate analysis in order to identify optimal cut-off values. The diagnostic criteria, such as the diagnostic sensitivity, specificity, predictive and negative value and the ROC curve were calculated. Cut-off values corresponding to the minimal false positive and false negative parameters were determined [11]. The method described by DeLong et al. was used to compare areas under curve [12]. Before undertaking ROC statistics the FH clinical criteria score were adjusted for study differences.

Table 1
Diagnostic criteria for HeFH adapted by the Polish Experts Forum.

Heterozygous familial hypercholesterolemia diagnostic criteria score *(adapted from the Dutch Lipid Clinic Network–WHO and Simon Broome Register scale)*	Score
Family history	
1. First-degree relatives with premature coronary or vascular disease (<55 years, men; <60 years, women)	1
2. First-degree relatives with LDL cholesterol >4.9 mmol/l (190 mg/dl)	1
3. First-degree relatives with tendinous *xanthoma* and/or arcus cornealis	2
4. Children <18 years of age with LDL cholesterol >4.0 mmol/l (155 mg/dl)	2
Clinical history	
1. Premature coronary heart disease (men <55 years; women <60 years)	2
2. Premature cerebral or peripheral vascular disease	1
Physical examination:	
1. Tendinous *xanthoma*	6
2. Arcus cornealis	4
Laboratory analysis:	
LDL cholesterol >8.5 mmol/l (330 mg/dl)	8
LDL cholesterol 6.5–8.4 mmol/l (250–329 mg/dl)	5
LDL cholesterol 4.9–6.4 mmol/l (190–249 mg/dl)	3
LDL cholesterol 4.0–4.9 mmol/l (155–189 mg/dl)	1
Mutation of LDLR receptor gene	8
FH diagnosis	
Definite	>8
Probable	6–8
Possible	3–5
Unconfirmed	<3