Atherosclerosis 243 (2015) 53–58

Contents lists available at ScienceDirect

Atherosclerosis

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

Clinical features of familial hypercholesterolemia in Korea: Predictors of pathogenic mutations and coronary artery disease – A study supported by the Korean Society of Lipidology and Atherosclerosis

Dong Geum Shin [a, 1], Soo Min Han [b, 1], Doo Il Kim [c], Moo-Yong Rhee [d], Byoung-Kwon Lee [e], Young Keun Ahn [f], Byung Ryul Cho [g], Jeong-Taek Woo [h], Seung-Ho Hur [i], Jin-Ok Jeong [j], Yangsoo Jang [a, k], Ji Hyun Lee [l, **], Sang-Hak Lee [a, k, *]

[a] Division of Cardiology, Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
[b] Department of Pharmacology, Pharmacogenomic Research Center for Membrane Transporters, Brain Korea 21 PLUS Project for Medical Sciences, Severance Biomedical Science Institute, Yonsei University College of Medicine, Seoul, Republic of Korea
[c] Cardiology Division, Department of Internal Medicine, Haeundae Paik Hospital, Inje University College of Medicine, Busan, Republic of Korea
[d] Cardiovascular Center, Dongguk University Ilsan Hospital, Goyang, Republic of Korea
[e] Division of Cardiology, Department of Internal Medicine, Gangnam Severance Hospital, Yonsei University College of Medicine, Seoul, Republic of Korea
[f] Heart Center of Chonnam, National University Hospital, Gwangju, Republic of Korea
[g] Cardiology Division, Department of Internal Medicine, Kangwon National University Hospital, Kangwon National University College of Medicine, Chuncheon, Republic of Korea
[h] Endocrinology Division, Department of Internal Medicine, Kyunghee University School of Medicine, Seoul, Republic of Korea
[i] Cardiology Division, Department of Internal Medicine, Keimyung University Dongsan Medical Center, Daegu, Republic of Korea
[j] Cardiology Division, Department of Internal Medicine, School of Medicine, Chungnam National University, Chungnam National University Hospital, Daejeon, Republic of Korea
[k] Cardiovascular Research Institute, Yonsei University Health System, Seoul, Republic of Korea
[l] Department of Oral Biology, College of Dentistry, Yonsei University, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 27 April 2015
Received in revised form
2 August 2015
Accepted 24 August 2015
Available online xxx

Keywords:
Hyperlipoproteinemia type II
Diagnosis
Mutation
Sensitivity and specificity
Coronary artery disease

ABSTRACT

Background: Proper screening and diagnosis of familial hypercholesterolemia (FH) is of critical importance for cardiovascular prevention. However, the clinical diagnosis of FH remains difficult partly because its phenotype can vary between different ethnicities. The aim of this study was to determine the clinical features and the best diagnostic approach in Korean FH patients. The predictors of putative pathogenic mutations and coronary artery disease (CAD) were also identified.

Methods and Results: Ninety-seven patients with low-density lipoprotein-cholesterol >190 mg/dL and xanthoma or FH-compatible family history were included. Putative pathogenic mutations in *LDLR*, *APOB*, or *PCSK9* genes were identified in 32% of the enrolled patients. The subjects were classified according to four sets of clinical criteria (Simon Broome, Dutch, MEDPED, Japanese). The mutation rates in definite type FH of Simon Broome or Dutch criteria were 35%–37% and lower in our patients than in those of other countries. The mutation detection rate by MEDPED criteria was 67%–75% and higher than those based on other criteria. The best low-density lipoprotein-cholesterol (LDL-C) threshold for predicting mutations was 225 mg/dL. LDL-C was found to be the only independent predictor of mutation carriers, while hypertension and low high-density lipoprotein-cholesterol were predictive of CAD.

Conclusions: The conventional clinical criteria showed limited mutation detection power and low specificities in Korean FH patients, in whom the best LDL-C threshold for putative mutation was 225 mg/dL. Traditional cardiovascular risk factors were also significantly associated with CAD risk in this population.

* Corresponding author. Division of Cardiology, Department of Internal Medicine, Severance Hospital, Yonsei University College of Medicine, 134 Shinchon-dong, Seodaemun-gu, Seoul, 120-752, Republic of Korea.
** Corresponding author.
E-mail addresses: jihyni@yuhs.ac (J.H. Lee), shl1106@yuhs.ac (S.-H. Lee).
[1] These first two authors contributed equally to this work.

http://dx.doi.org/10.1016/j.atherosclerosis.2015.08.033
0021-9150/

1. Introduction

Familial hypercholesterolemia (FH) is a well-known, autosomal dominantly-inherited disease. As preventive interventions for cardiovascular diseases can provide outcome benefits, proper screening of index cases and affected family members is invaluable for all countries. With the aid of recent technical progress in genetic testing, several studies have reported improved efficiency in the diagnosis of FH patients [1]. However, in the medical practice, clinical diagnosis is still responsible for most cases of FH. There is difficulty in the diagnostic approach due to differences in prevalence among countries and ethnicities, complexities in the genetic variants, and polygenicity [2,3]. In this regard, the rate of diagnosis and treatment of FH is far from satisfactory [4]. Although several sets of clinical criteria with acceptable logic have been developed by expert committees, all criteria have imitations and are not universally used [5,6].

The major complication induced by FH is atherosclerotic cardiovascular disease; therefore, early and effective detection of coronary artery disease (CAD) is critical for this population. Several prior studies have analyzed predictors of CAD, revealing that conventional risk factors were generally important in FH. However, the factors varied to some extent, depending on the study population [7–9].

In Korea, several studies conducted among small numbers of FH patients have reported certain novel *LDLR* mutations [10–12]. However, a comprehensive investigation has not been undertaken to identify clinical features, including CAD and the best clinical diagnostic approach in Korean FH patients. We recruited and analyzed the clinical features of 97 unrelated Korean FH patients. The values of four sets of clinical diagnostic criteria were evaluated, and predictors of pathogenic mutations and CAD were identified.

2. Methods

2.1. Study population

The Korean Society of Lipidology and Atherosclerosis supported this study and nine university hospitals in Korea participated. The study protocol was approved by the institutional review board at each center, and all subjects provided written informed consent. From January 2009 to July 2014, 97 consecutive unrelated men and women older than 19 years who had 1) low-density lipoprotein cholesterol (LDL-C) >190 mg/dL without lipid lowering agents and tendon xanthoma or 2) the same LDL-C levels and a family history of CAD or hypercholesterolemia were included in this study. Investigators checked patients' hands, elbows, pretibial areas and Achilles tendons during physical examination. Only tendon xanthoma was included in the classification. Secondary causes of lipid disorder were excluded through clinical and biochemical filtering.

2.2. Clinical and laboratory data collection

At the time of enrollment, each study subject underwent history taking, physical examination, and laboratory assessment. Clinical variables included age, sex, history of diabetes mellitus, hypertension, CAD, smoking, family history of LDL-C >190 mg/dL and premature coronary artery disease (<60 years in first-degree relatives), body mass index, presence of tendon xanthoma, and lipid profile. A history of CAD was defined as angiographically documented coronary artery stenosis >50% at more than one coronary artery or positive results of exercise or pharmacologic stress tests. Patients without a history of CAD were screened with stress tests, and those who revealed negative results were regarded as CAD-negative. Patients fasted for at least 12 h prior to blood sampling and the samples were analyzed within 4 h by laboratories certified by the Korean Society of Laboratory Medicine.

2.3. Diagnostic classification by clinical criteria

Patients were classified based on four different sets of clinical diagnostic criteria for FH: 1) The Simon Broome Register Group criteria [13], 2) The Dutch Lipid Clinic Network criteria [13], 3) The Make Early Diagnosis to Prevent Early Deaths (MEDPED) criteria [5], and 4) Japanese criteria [14]. The Simon Broome criteria are based on lipid values, the presence of tendon xanthomas, and a family history of hypercholesterolemia or CAD. Using these criteria, patients were classified as having definite or possible FH. The Dutch criteria have a scoring system based on parameters similar to the Simon Broome criteria. With the Dutch criteria, patients were categorized as having definite, probable, or possible FH. The MEDPED criteria are composed of age and specific levels of total cholesterol and LDL-C. The Japanese criteria consist of items similar to the Simon Broome criteria except for a slightly lower LDL-C cutoff value.

2.4. Pathogenic mutation analysis

Genomic DNA was extracted using a commercially available isolation kit (QuickGene SP kit DNA Whole blood, Fujifilm, Tokyo, Japan). We obtained DNA sequences of 3 FH genes (*LDLR*, *APOB*, and *PCSK9*) by whole exome sequencing (WES) for 68 subjects, targeted exome sequencing (customized hybridization capture) of 3 FH genes for 26 subjects and Sanger sequencing for 3 subjects. For WES, the Agilent SureSelect Enrichment System (SureSelect All Exon 50 Mb or SureSelect All Exon V4+UTRs kit) was used according to the manufacturer's instructions. Sequencing reads obtained from Illumina HiSeq 2000/2500 platforms were further analyzed using Novoalign (v2.07.18), Genome Analysis Toolkit (v2.3.6), Picard (v1.6.7) and CoNIFER (COpy Number Inference From Exome Reads). For targeted sequencing, DNA fragments were enriched by solution-based hybridization capture and sequenced with an Illumina Hiseq2500 platform (Illumina, San Diego, CA, USA), using the 2 × 150 bp paired-end read module. The target region included all coding exons and flanking intron regions of the *LDLR*, *APOB* and *PCSK9* genes. Capture probes were generated by Celemics, Inc. (Seoul, Korea). The hybridization capture procedure was also performed at Celemics, Inc. according to the manufacturer's standard protocol. After non-synonymous variants were detected by the Unified GATK Genotyper (v2.3.6), mutations were classified as “known pathogenic” according to public databases (LOVD-LDLR, LSBD-UMD-LDLR, LOVD2-LDLR, and HGMD) of the three FH genes. For mutations without previous reports, we confirmed the pathogenicity using one of the following methods: 1) inevitably deleterious effects of amino acid changes such as frame shift insertions/deletions and copy number deletions or 2) co-segregation of the same variants in family members with FH. Finally, each variant was classified according to The American College of Medical Genetics and Genomics guidelines [15]. All variants listed as either known or novel pathogenic mutations were validated by Sanger sequencing.

2.5. Statistical analysis

The number of enrolled population was 97; thus it was not sufficiently large to represent the total FH community in Korea. However, to reduce potential bias, this study was performed in nine university hospitals throughout the country. Continuous variables