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Evaluation of polygenic cause in Korean patients with familial hypercholesterolemia – A study supported by Korean Society of Lipidology and Atherosclerosis

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ABSTRACT

Background/Objective: Familial hypercholesterolemia (FH) is an autosomal dominant disorder caused by mutations in *LDLR*, *APOB*, or *PCSK9*. Polygenicity is a plausible cause in mutation-negative FH patients based on LDL cholesterol (LDL-C)-associated single nucleotide polymorphisms (SNPs) identified by the Global Lipids Genetics Consortium (GLGC). However, there are limited data regarding the polygenic cause of FH in Asians.

Methods: We gathered data from 66 mutation-negative and 31 mutation-positive Korean FH patients, as well as from 2274 controls who participated in the Korean Health Examinee (HEXA) shared control study. We genotyped the patients for six GLGC SNPs and four East Asian LDL-C-associated SNPs and compared SNP scores among patient groups and controls.

Results: Weighted mean 6- and 4-SNP scores (0.67 [SD = 0.07] and 0.46 [0.11], respectively) were both significantly associated with LDL-C levels in controls ($p = 2.1 \times 10^{-4}$, $R^2 = 0.01$ and $p = 5.0 \times 10^{-12}$, $R^2 = 0.02$, respectively). Mutation-negative FH patients had higher 6-SNP (0.72 [0.07]) and 4-SNP (0.49 [0.08]) scores than controls ($p = 1.8 \times 10^{-8}$ and $p = 3.6 \times 10^{-3}$, respectively). We also observed higher scores in mutation-positive FH patients compared with controls, but the difference did not reach statistical significance.

Conclusion: The present study demonstrates the utility of SNP score analysis for identifying polygenic FH in Korean patients by showing that small-effect common SNPs may cumulatively elevate LDL-C levels.

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1. Introduction

Monogenic mutations in *LDLR, APOB,* or *PCSK9* have been identified as definitive causes of familial hypercholesterolemia (FH) [1,2]. However, in FH patients diagnosed by clinical criteria, at least 20% do not have mutations in the above-mentioned genes [3]. Researchers have been actively searching for a mutation in an additional gene that could explain the genetic cause of mutation-negative FH cases [4–8]. Nevertheless, novel FH-causing genes still account for only fractional portions of genetically undefined FH patients, possibly due to allelic rarity or heterogeneity [4,9]. Recently, studies of European patients demonstrated that a subset of mutation-negative FH patients may have a polygenic cause [10,11]. Small-effect LDL cholesterol (LDL-C)-elevating alleles may have a cumulative effect severe enough to generate signs that meet the diagnostic criteria for FH, based on LDL-C associated single nucleotide polymorphisms (SNPs) identified by the Global Lipids Genetics Consortium (GLGC). While differentiating between monogenic and polygenic causes of FH would not significantly affect a patient's course of treatment, finding the specific genetic cause of FH may determine how to select candidates for cascade screening. In monogenic FH, approximately 50% of the patients' relatives are expected to develop FH. However, when a polygenic cause is assumed, much fewer relatives are expected to be affected. Therefore, identifying the genetic cause of FH is important for developing an effective cascade screening program.

Although determining a polygenic cause of FH by LDL-C SNP score analysis has been performed in several cohorts, these studies have been confined to Caucasian subjects. Because the effects of SNPs may vary among different ethnicities, the utility of SNP score analysis for identifying polygenic FH in Asian patients remains unclear. In this study, we applied LDL-C SNP score analysis to Korean FH cases and assessed the hypothesis that common LDL-C-elevating alleles have a cumulative effect, ultimately causing FH in these patients. Additionally, we performed SNP score analysis with SNPs identified from a genome-wide association study (GWAS) of East Asians.

2. Methods

2.1. Subjects

We collected data from 97 unrelated Korean patients from nine locations in Korea who had 1) low-density lipoprotein cholesterol (LDL-C) >4.9 mmol/L without lipid lowering agents and tendon xanthoma or 2) the same LDL-C levels and family history of CAD or hypercholesterolemia (Table 1). In addition, data from 2274 control participants were gathered from the Korean Health Examinee (HEXA) shared controls study (Table 2) [12]. All FH patients were tested for mutations in *LDLR, APOB,* or *PCSK9* by whole exome sequencing (WES) [13] or by custom-designed hybridization capture of 3 FH genes. The WES was carried out using the SureSelect All Exon 50 Mb or All Exon V4+UTRs kit (Agilent Technologies, Santa Clara, CA). Three genes were covered at a mean read-depth of 56.7x for exons and nearby regions. Those regions were covered over 99.9% (median), except the first and last exons of *LDLR* and *APOB* and exons 1, 6, and 10–12 of *PCSK9*. The custom hybridization capture of the 3 FH genes covered almost 100% of targets with a mean read-depth of 1,420x, including exon–intron junctions of *LDLR* (−90 bp/+50 bp), *APOB* (3 bp/+6 bp), and *PCSK9* (3 bp/+6 bp). No patients were expected to have familial combined hyperlipidemia or familial dysbetalipoproteinemia. Among the 97 enrolled FH patients, 66 patients did not have an FH-causing mutation, and 31 patients carried a mutation in one of the three FH-causing genes. All clinical investigations were conducted according to the Declaration of Helsinki. This study was approved by the institutional review board of Severance Hospital at Yonsei University College of Medicine in Korea (IRB No.: 4-2008-0267).

2.2. Genotyping

Patients were genotyped for 12 SNPs used in prior studies assessing polygenic causes of FH in Caucasian patients (Supplementary Table S1) [10]. Among the 12 SNPs, six refined SNPs were included that performed equally to the 12-SNP score as a diagnostic tool for FH patients in a follow-up study by the same group [11]. Additionally, we genotyped four SNPs associated with LDL-C concentration in East Asians (Supplementary Table S2) [12]. The SNPs from each patient were genotyped using the SNaPshot or Taqman SNP genotyping assay (Applied Biosystems/Life Technologies, USA). Controls were genotyped using an Affymetrix SNP array (version 6.0), and SNPs not available from the array were imputed with IMPUTE2 by using the 1000 Genome (February 2012 release) as reference haplotypes [14].

2.3. SNP score calculation

Weighted mean SNP scores of patients and controls were calculated based on LDL-C elevating alleles and their beta-coefficients reported by the GLGC (Supplementary Table S1) and the East Asian GWAS (Supplementary Table S2) as described by Talmud et al. [10,12,15]. Because rs3757354 and rs8017377 have opposite directional effects between Europeans and East Asians [15], opposite alleles of these SNPs were selected for SNP score calculation in our study.

2.4. Statistical analysis

Linear regression was used to assess the association between different sets of SNP scores and LDL-C levels in the control group. We tested the hypothesis that weighted mean LDL-C scores would be higher in mutation-negative FH patients when compared to healthy controls. Welch's two-sample t-test was used to compare SNP scores among mutation-negative and mutation-positive patient groups and control subjects. R 3.1.2 (http://www.r-project.org) was used for statistical analysis.

3. Results

3.1. Clinical characteristics and pathogenic mutations of FH patients

The characteristics of enrolled FH patients and HEXA controls are shown in Tables 1 and 2, respectively. The mean age of FH patients was 54 years, and 38 (39%) were male, while the mean age of HEXA controls was 51 years, and 1022 (45%) were males. Twenty-seven (28%) FH patients had a history of coronary artery disease (CAD), and 50 patients (54%) revealed a family history of premature CAD. Tendon xanthomas were observed in 19 (20%) patients. The mean LDL-C level in FH patients was 5.9 mmol/L. Among the 97 enrolled FH patients, 66 patients did not have a known FH-causing mutation, and 31 patients carried a mutation in one of the three known FH-causing genes. Specifically, 27 (87.1%) mutation-positive patients had one of 21 mutations in *LDLR*, and of these, 17 were mutations previously reported and 4 were novel. In terms of functional consequences of mutations, nine of *LDLR* mutations are class I (null) mutations, disrupting the synthesis of LDLR. Those include 2 copy number deletions, 2 small in-dels, 2 nonsenses, 2 splicing variants and a promoter variant known to reduce