Atherosclerosis 277 (2018) 256–261

Contents lists available at ScienceDirect

Atherosclerosis

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

Unusual genetic variants associated with hypercholesterolemia in Argentina

Pablo Corral [a], Andrew S. Geller [b], Eliana Y. Polisecki [b], Graciela I. Lopez [c], Virginia G. Bañares [d], Leonardo Cacciagiu [c], Gabriela Berg [c], Robert A. Hegele [e], Ernst J. Schaefer [b], Laura E. Schreier [c, *]

[a] Departamento de Investigación, Facultad de Medicina, Universidad FASTA, Buenos Aires, Argentina
[b] Boston Heart Diagnostics, Framingham, MA, USA
[c] Universidad de Buenos Aires, Facultad de Farmacia y Bioquímica, Departamento de Bioquímica Clínica, Laboratorio de Lípidos y Aterosclerosis, INFIBIOC-UBA, Buenos Aires, Argentina
[d] Departamento de Genética Experimental, Centro Nacional de Genética Médica "Dr Eduardo Castilla", Administración Nacional de Laboratorios e Institutos de Salud "Dr Carlos Malbrán", Buenos Aires, Argentina
[e] Robarts Research Institute, University Western Ontario, London, Ontario, Canada

ARTICLE INFO

Article history:
Received 28 March 2018
Received in revised form
17 May 2018
Accepted 7 June 2018

Keywords:
Familial hypercholestesterolemia
Low density lipoprotein cholesterol
Gene analysis
FH prevalence
Argentina

ABSTRACT

Background and aims: Marked hypercholesterolemia, defined as low density lipoprotein cholesterol (LDL-C) levels ≥ 190 mg/dL, may be due to *LDLR, APOB,* and *PCSK9* variants. In a recent analysis, only 1.7% of cases had such variants. Our goal was to identify other potential genetic causes of hypercholesterolemia.
Methods: In a total of 51,253 subjects with lipid testing, 3.8% had elevated total cholesterol >300 mg/dL and/or LDL-C≥190 mg/dL. Of these, 246 were further studied, and 69 without kidney, liver, or thyroid disease and who met Dutch Lipid Clinic Network criteria of ≥6 points had DNA sequencing done at the *LDLR, APOB, PCSK9, APOE, LDLRAP1, STAP1, ABCG5, ABCG8, CYP27A1, LIPA, LIPC, LIPG, LPL,* and *SCARB1* gene loci and also had 10 SNP analysis for a weighted high LDL-C genetic risk score.
Results: In the 69 subjects with genetic analyses, the following variants were observed in 37 subjects (53.6%): *LDLR* (n = 20, 2 novel), *ABCG5/8* (n = 7, 2 novel), *APOB* (n = 3, 1 novel), *CYP27A1* (n = 3, 1 novel), *LIPA* (n = 2, 1 novel), *APOE* (n = 2), *LIPC* (n = 1, novel), *LIPG* (n = 1, novel), and *SCARB1* (n = 1); 14 subjects (20.3%) had a high polygenic score, with 4 (5.8%) having no variants.
Conclusions: Our data indicate that in addition to variants in *LDLR, APOB, PCSK9, APOE, LDLRAP1,* and *STAP1,* variants in *ABCG5/8, CYP27A1, LIPA, LIPC,* and *LIPG* may be associated with hypercholesterolemia and such information should be used to optimize therapy.

1. Introduction

Familial hypercholesterolemia (FH) is one of the most common monogenic metabolic diseases and is characterized by lifelong elevations in plasma low-density lipoprotein cholesterol (LDL-C) levels and premature coronary heart disease (CHD). The inheritance is autosomal dominant, mainly caused by variations in the genes of LDL Receptor *(LDLR),* Apolipoprotein B *(APOB)* or Proprotein Convertase Subtilisin Kexin type 9 (*PCSK9*) [1]. Heterozygous variants of *LDLR* are present in about 90% of FH cases, while *APOB* and *PCSK9* were found in 5% and 1%, respectively [2].

Despite great progress on FH through the last years, the disease is still underestimated, underdiagnosed, and thus undertreated worldwide, being crucial its early detection [2]. There is a lack of data on the prevalence of FH in several countries, including ours, because there are no national policies with regard to registers or screening strategies [3].

Diagnosis of FH is based on the evaluation in clinical practice and/or genotyping. Clinical assessment takes into account LDL-C levels, the presence of premature CHD in patients or in first-degree relatives, as well as clinical examination for tendon

* Corresponding author. Laboratorio de Lípidos y Aterosclerosis, Facultad de Farmacia y Bioquímica, Universidad de Buenos Aires, Junín 956, C1113AAD, Buenos Aires, Argentina.
E-mail address: lschreier@ffyb.uba.ar (L.E. Schreier).

https://doi.org/10.1016/j.atherosclerosis.2018.06.009
0021-9150/

xanthomas and corneal arcus at a young age [4]. The Dutch Lipid Clinic Network (DLCN) Criteria are widely used and recommended for FH diagnosis [5].

The genetic analysis of the phenotypic FH patients has become widely applied [6]. However, variant detection rates vary considerably when only classic genes such as *LDLR, APOB* or *PCSK9* are assessed. Between 20 and 40% of individuals with phenotypic heterozygous FH have no variants [7,8].

An extended panel of genes related to increased LDL-C such as *APOE, ABCG5, ABCG8, LIPA, CYP27A1* or *STAP1*, in addition to other genes related to lipid metabolism, for example *LIPG and LIPC*, could explain some of the hypercholesterolemia cases, beyond the traditional genes. In addition, other patients could carry a high burden of multiple small-effect common variants (SNP) that raise LDL-C similar to FH levels, but are the result of polygenic causes [8,9].

The aim of this study was to assess the epidemiology and genetics of hypercholesterolemia in a defined district of Argentina in order to detect potential FH index cases and to ascertain genetic causes using an extended gene panel plus a polygenic score.

2. Patients and methods

Patients were selected from a database of 51,253 subjects over the age of 18 years provided by the Secretary of Health from the district of General Pueyrredón, Buenos Aires, Argentina. Subjects were seen at the Centro de Especialidades Médicas Ambulatorias from July 2013 to February 2016, with data on serum total cholesterol and/or LDL-C values and contact information being provided. Participants were eligible to participate in this study if they had a total serum cholesterol level >300 mg/dL and/or a LDL-C >190 mg/dL. A total of 1967 patients (3.84%) met these inclusion criteria, and up to the moment 246 subjects agreed to participate in further studies. Informed consent was obtained from all patients using a protocol and consent form approved by the Ethics Committee of the University of Buenos Aires (Res CD 4705/14).

In these 246 subjects, an extensive history was obtained with a special focus on cardiovascular disease (CVD) (angina pectoris, myocardial infarction, angioplasty, coronary artery bypass grafting, stroke, and/or peripheral vascular or carotid disease), hospitalizations, hypertension, diabetes mellitus, and current lipid-lowering treatment. A careful physical examination was carried out with special care being taken to detect the presence of tendon xanthomas and/or corneal arcus. Medical records of the index cases, and when available, of their first degree relatives (parents, offspring and siblings) were obtained and examined. Premature CVD was defined as the presence angina, myocardial infarction, coronary angioplasty or coronary artery bypass, or other vascular disease occurring prior to age 55 years in male index cases and family members, and prior to age 65 years in female index cases and family members. The score according to DLCN criteria was calculated in each patient considering the highest LDL-C value noted [5]. A total of 98 subjects (40%) were receiving lipid-lowering treatment, and when LDL-C data off medication were not available, a LDL-C correction was applied based on the dose and potency of the statin being used [10]. In addition 21 subjects were excluded: 8 (3.3%) had a TSH value > 10 mUI/L, 4 (1.6%) had a serum creatinine >1.7 mg/dL, 2 (0.8%) had an alkaline phosphatase >200 U/L, and 1 (0.4%) had serum triglyceride levels >1000 mg/dL. Three (1.2%) women were excluded because of pregnancy, and 3 subjects (1.2%) declined to have their blood drawn. Therefore, a total of 225 subjects with potential FH were included in the research analysis. All these subjects had their blood drawn in the non-fasting state, and samples were sent to the Lipid and Atherosclerosis Laboratory at the University of Buenos Aires for biochemical analysis.

Total cholesterol, triglycerides, HDL-cholesterol, direct LDL-C, creatinine, and alkaline phosphatase were measured in serum samples using standardized enzymatic methods with assay kits (Roche Diagnostics, Mannheim/Germany) using a Cobas C-501 autoanalyzer. Mean coefficients of variation (CV) values for these parameters were< 2.3% for intra-assay CVs and <3.0% for inter-assay CVs. Serum apolipoproteinB (apoB) levels were determined using Roche immunoturbidimetric assays on the same automated analyzer, with intra-assay and interassay CVs of <2.5%. TSH was measured by chemiluminescence (DPC, Immulite, Los Angeles, CA, USA) with intra- and inter-assay CVs of <3.5%.

Genomic DNA was extracted from whole blood by the Salting Out protocol [11]and library preparation was performed using Nextera Rapid Capture Custom Enrichment kit (Illumina, San Diego, CA). Next generation DNA sequencing was carried out on all subjects that had a DLCN score of ≥6. DNA sequencing was carried at the following gene loci (exons and intron padding): *LDLR, APOB, PCSK9, LDLRAP1, STAP1, ABCG5, ABCG8, APOE, LIPA, CYP27A1, LIPC, LIPG, LPL*, and *DHCR24* as previously described [12]. In addition, 10 SNPs (rs6544713 in *ABCG8*, rs515135 in *APOB*, rs12740374 in *CELSR2*, rs3846663 in *HMGCR*, rs2650000 in *HNF1*, rs6511720 in *LDLR*, rs6102059 in *MAFB*, rs10401969 in *NCAN*, rs11206510 in *PCSK9*, and rs1501908 in *TIMD4)* were included for the calculation of a Genetic Risk Score developed to assess polygenic contribution to increased LDL-C as previously described [6].

Sequencing was performed on an Illumina MiSeq Dx instrument using 2 × 150 paired end reads. The median read depth was 600X, with 100%sensitivity and specificity for SNV's and 100% sensitivity and 91% specificity for Indels. FASTQ files were processed using a custom workflow in CLC Biomedical Genomics Workbench (v3.2, Qiagen). All mapping, variant calling and filtering, and quality control parameters were performed using validated protocols. VCF files containing all identified variants in targeted regions were annotated using Ingenuity Variant Analysis (Qiagen) with a customized filtering cascade. The variants were classified according to the American College of Medical Genetics and Genomics (ACMG) [13,14]. In addition, assessment of CNV's and large structural variants was performed using built in tools from Biomedical Genomics Workbench and VarSeq CNV Analysis from Golden Helix.

With regard to novel variants, further analysis was sometimes required to determine whether these variants were pathogenic. Software used for these assessments included: 1) Genomic Evolutionary Rate Profiling (GERP) scores to evaluate conservation, 2-DANN pathogenicity scores (which use computational deep learning methodologies to classify variants), and 3- dbNSFP which aggregates several informatics prediction algorithms and uses a proportion to indicate the number of scores predicting the mutation to be deleterious versus the number predicting low functional impact. The variant classifications were based on ACMG guidelines, and were made using aggregated data from public databases and computational tools in VarSome (Saphetor) and Ingenuity Variant Analysis (Qiagen). Calculation of the Polygenic Risk Score (GRS) at particular loci associated with LDL-C levels applied for each SNV using previous methods. The cutoff score for the 90th percentile was 1.96, with maximum GRS of 2.42 as described [15].

All data are expressed as median (range) according to their distribution and percentage or prevalence. The statistical analysis was performed using SPSS 19.0. Frequency differences between ranges were assessed by Chi2 test. A $p < 0.05$ was considered statistically significance.

3. Results

In the population studied (n 51,253 subjects), 1967 or 3.8% had a total cholesterol >300 mg/dL and/or an LDL-C ≥190 mg/dL.