Molecular and Cellular Probes 29 (2015) 19–24

Contents lists available at ScienceDirect

Molecular and Cellular Probes

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

Association of USF1 and APOA5 polymorphisms with familial combined hyperlipidemia in an Italian population

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ARTICLE INFO

Article history:
Received 26 May 2014
Accepted 1 October 2014
Available online 13 October 2014

Keywords:
Familial combined hyperlipidemia
Single nucleotide polymorphism (SNP)
Apolipoprotein A-V (APOA5)
Upstream stimulatory factor 1 (USF1)

ABSTRACT

Background: Familial combined hyperlipidemia (FCH) is a polygenic and multifactorial disease characterized by a variable phenotype showing increased levels of triglycerides and/or cholesterol.
The aim of this study was to identify single nucleotides (SNPs) in lipid-related genes associated with FCH.
Methods and results: Twenty SNPs in lipid-related genes were studied in 142 control subjects and 165 FCH patients after excluding patients with mutations in the LDLR gene and patients with the E2/E2 genotype of APOE. In particular, we studied the 9996G > A (rs2073658) and 11235C > T (rs3737787) variants in the Upstream Stimulatory Factor 1 gene (USF1), and the −1131T > C (rs662799) and S19W (rs3135506) variants in the Apolipoprotein A-V gene (APOA5). We found that the frequencies of these variants differed between patients and controls and that are associated with different lipid profiles. At multivariate logistic regression SNP S19W in APOA5 remained significantly associated with FCH independently of age, sex, BMI, cholesterol and triglycerides.
Conclusions: Our results show that the USF1 and APOA5 polymorphisms are associated with FCH and that the S19W SNP in the APOA5 gene is associated to the disease independently of total cholesterol, triglycerides and BMI. However, more extensive studies including other SNPs such as rs2516839 in USF1, are required.

1. Introduction

Familial combined hyperlipidemia (FCH) is the most frequent familial dyslipidemia with a prevalence of 1% in the general population and 38% in young patients with myocardial infarct [1,2]. Increased levels of total cholesterol, triglycerides or both and a variable lipid profile over time and in the different members of the affected families are the main characteristics of FCH [3]. These characteristics make FCH diagnosis particularly difficult due to its overlapping with other metabolic diseases and to the variability of its phenotype [4]. The presence of small dense LDLs (sdLDL) and increased levels of Apolipoprotein B (ApoB) [5] have been considered hallmarks of FCH, although it has been recently demonstrated that they are also common to other hyperlipidemias [5]. The genetic background of FCH is heterogeneous as it includes many genes having a different impact on the disease development [1]. Owing to a partially overlapping phenotype, mutations in the LDL receptor and Apo E genes usually causing other familial dyslipidemias, such as familial hypercholesterolemia and dysbetalipoproteinemia, have been equally identified in patients with a clinical diagnosis of FCH [6,7]. In most of the previous association studies performed in FCH populations including patients with different types of dyslipidemias, the impact of rare variants has lead to potentially

Abbreviationlist: APOA5, Apolipoprotein A-V; ApoB, Apolipoprotein B; APOC3, Apolipoprotein C-III; APOE, Apolipoprotein E; CETP, cholesteryl ester transfer protein, plasma; FCH, familial combined hyperlipidemia; HMGCR, 3-hydroxy-3-methylglutaryl-CoA reductase; KIF6, Kinesin family member 6; LDLR, LDL receptor; LPL, lipoprotein lipase; PCSK9, proprotein convertase subtilisin/kexin type 9; PPARG, peroxisome proliferator-activated receptor gamma; sdLDL, small dense LDL; SNP, single nucleotide polymorphisms; USF1, upstream stimulatory factor 1.

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http://dx.doi.org/10.1016/j.mcp.2014.10.002
0890-8508/

underestimated results. Since hyperlipidemia is related to a growing number of cardiovascular diseases, some Single nucleotide polymorphisms (SNPs) in lipid-related genes have been studied for their association with cardiovascular markers [8].

The aim of this paper is to perform an association study of 20 SNPs in lipid-related genes in a well selected population of FCH patients. The study was performed after excluding patients with mutations in the LDLR gene or with the E2/E2 genotype of APOE since these variants could have an impact on the dyslipidemic phenotype.

2. Materials and methods

2.1. Studied population

One hundred sixty-five unrelated patients with FCH, among those consecutively admitted to the outpatient Lipid Clinic of the University of Naples, were enrolled in the study, after excluding patients with secondary causes of dyslipidemia, patients with mutations in the LDLR gene or with the E2/E2 genotype for APOE as well as patients taking any drug known to affect lipid metabolism. Genetic screening of LDLR was performed as previously described [9], whereas the analysis of the APOE polymorphisms was performed by Real-Time PCR on Light Cycler (Roche, Mannheim, Germany), using specific hybridization probes and subsequent melting curve analysis.

Familial combined hyperlipidemia was diagnosed according to the criteria suggested by Gaddi et al. [4]: serum triglyceride levels higher than 200 mg/dL (2.3 mmol/L) and/or LDL cholesterol higher than 160 mg/dL (4.1 mmol/L) and/or ApoB higher than 130 mg/dL in the proband, plus phenotype variability in at least one first-degree relative. Exclusion criteria are related to a possible secondary dyslipidemia, i.e. thyroid dysfunction, renal or hepatic diseases. Metabolic syndrome was diagnosed as previously described [10]. The features of FCH patients and the 142 healthy subjects from the same ethno-geographic origin used as the reference population, are reported in Table 1. The study was performed according to the current version of the Helsinki Declaration. Informed consent was obtained for each patient or control.

2.2. SNP genotyping

The selected SNPs were the most frequently associated with lipid metabolism alterations, such as hypertriglyceridemia, FCH and metabolic syndrome.

SNPs listed in Table 2 were assayed, in duplicate, by the real time TaqMan® method; primers and probes were chosen among predeveloped assays or were taken from custom assays service of Applied Biosystems. Real time PCR was performed as previously described [11] on an ABI Prism 7900-HT instrument with the Sequence Detection System 2.3 (Applied Biosystems, Foster City, CA, USA).

2.3. Biochemical analysis

Biochemical markers were measured on serum after an overnight fast. Total cholesterol, HDL cholesterol, triglyceride and glucose levels were evaluated by standard methods using an automated analyzer (Modular P3, Roche, Mannheim, Germany). LDL cholesterol concentrations were calculated according to the Friedewald method or measured using a homogeneous enzymatic colorimetric assay if triglycerides levels were higher than 400 mg/dL. ApoB and ultra sensitive C-reactive protein were measured on serum (Dade-Behring, Marburg, Germany). LDL particles separation was performed by Lipoprint System (Quantimetrix Inc., Redondo Beach, CA, USA). The diameter of LDL particles at the cut-off point separating subfractions 1–2 from subfractions 3–7 (sdLDL) was 251 Å [12]. The proportion of sdLDL particles to the whole LDL area was calculated in our sample (LDL score).

Table 1
Demographic, biochemical and clinical features of control subjects and FCH patients.

Parameter	Controls $n = 142$	FCH patients $n = 165$	Significance
Age (years)	43.9 ± 9.6	47.5 ± 12.2	$p = 0.005$
Gender ($n°$ males and %)	65 (46%)	121 (73%)	$p < 0.0001$
BMI (kg/m^2)	25.5 ± 4.1	27.2 ± 3.4	$p < 0.0001$
Triglycerides (mmol/L)[a]	0.88 (0.63–1.24)	2.28 (1.71–3.38)	$p < 0.0001$
Total cholesterol (mmol/L)	4.85 ± 0.72	6.97 ± 1.39	$p < 0.0001$
LDL cholesterol (mmol/L)	3.08 ± 0.66	4.66 ± 1.35	$p < 0.0001$
HDL cholesterol (mmol/L)	1.30 ± 0.33	1.03 ± 0.30	$p < 0.0001$
Non-HDL cholesterol (mmol/L)	3.54 ± 0.75	5.94 ± 1.35	$p < 0.0001$
ApoB (g/L)	0.88 ± 0.19	1.36 ± 0.30	$p < 0.0001$
ApoB/total cholesterol (g/mmol)[a]	0.18 (0.17–0.20)	0.20 (0.18–0.21)	$p < 0.0001$
LDL score (% sdLDL/LDL)[a]	1.34 (0–3.95) $n = 128$	20.5 (11.5–30.8) $n = 80$	$p < 0.0001$
LDL diameter (Å)[a]	272 (270–273) $n = 128$	263 (259–266) $n = 80$	$p < 0.0001$
Glucose (mmol/L)[a]	5.19 (4.83–5.49)	5.27 (4.94–5.88)	$p = 0.001$
C-reactive protein (mg/L)[a]	1.0 (0.6–2.1)	1.9 (1.0–3.5)	$p < 0.0001$
Diabetes n (%)	1 (0.7%)	14 (8.5%)	$p = 0.002$
Obesity n (%)	22 (15.5%)	23 (13.9%)	n.s.
Hypertension n (%)	1 (0.7%)	41 (24.8%)	$p < 0.0001$
Metabolic syndrome n (%)	8 (6.7%)	111 (67.3%)	$p < 0.0001$

Continuous variables with a parametric distribution are reported as mean ± standard deviation.
n.s. Difference not statistically significant.
[a] Data are reported as median and interquartile range (non-parametric distribution).

2.4. Statistical analysis

Continuous variables were expressed as a mean ± SD (parametric distributions) or median value and interquartile range (non-

Table 2
List of analyzed SNPs.

Gene symbol	Full gene name	Variant	Position	SNP ID
APOA5	Apolipoprotein A-V	−1131T > C	5′ Gene/promoter	rs662799
		S19W	Coding	rs3135506
APOC3	Apolipoprotein C-III	−482T > C	5′ gene/promoter	rs2854117
		2342 *G* > C	3′ UTR	rs5128
		2373 *G* > T	3′ UTR	rs4225
LPL	Lipoprotein lipase	−280T > G	5′ UTR	rs1800590
		D9N	Coding	rs1801177
		N291S	Coding	rs268
		S474X	Coding	rs328
HMGCR	3-hydroxy-3-methylglutaryl-CoA reductase	10173A > T	Intronic	rs12654264
		12654A > G	Intronic	rs3846662
PCSK9	Proprotein convertase subtilisin/kexin type 9	25467958T > C	5′ Gene/promoter	rs11206510
		R46L	Coding	rs11591147
CETP	Cholesteryl ester transfer protein, plasma	−656C > A	5′ Gene/promoter	rs1800775
		V405I	Coding	rs5882
		R451Q	Coding	rs1800777
USF1	Upstream stimulatory factor 1	9996G > A	intronic	rs2073658
		11235C > T	3′ UTR	rs3737787
PPARG	Peroxisome proliferator-activated receptor gamma	P12A	Coding	rs1801282
KIF6	Kinesin family member 6	W719R	Coding	rs20455