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

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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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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

parametric distributions) and categorical data as the absolute number and percentage. Kolmogorov–Smirnov test was performed to assess the hypothesis of a normal distribution of variables. Differences between groups were assessed by *T*-test, non-parametric Mann–Whitney test and chi-square test, as appropriate. Genotype and allele frequencies were calculated by allele counting and departure from Hardy-Weinberg expectation was evaluated by chi-square analysis (all SNPs were in equilibrium). To test the type of association, dominant and recessive models were constructed for the rare allele of each polymorphism, and univariate odds ratio (OR) with 95% Confidence Interval (CI) were calculated. Multivariate linear and logistic regressions were performed including dominant models of SNPs (encoded as 1 = homozygous common allele and 2 = rare allele carrier), gender (1 = female and 2 = male), age in years and Body Mass Index (BMI - kg/m^2). Statistical analysis was performed with PASW version 18.0 software (SPSS Inc., Chicago, IL, USA). Haploview (www.broad.mit.edu/mpg/haploview) 3.2 software was used to calculate the differences of allele frequencies, linkage disequilibrium (a D′, a normalized measure of allelic association, equal or greater than 0.8 defines a positive association). The plot of D′ for analyzed SNPs is reported in Supplemental Fig. 1. A p value <0.05 was considered significant. In order to discriminate between FCH and healthy patients, multivariate logistic regression was also used as a classification model built on top of the most significant predictors, namely the dominant model of −1131T > C and S19W in *APOA5*, the 9996G > A in USF1, Age, Gender, Triglycerides, Total cholesterol, and BMI. Since data were not available for all patients, multivariate logistic regression was performed on 157 FCH patients and 134 controls.

As a resampling method, a 200 run of 10-fold cross-validation was performed and implemented in Matlab (www.mathworks.com) to construct a fitted model aimed at calculating the posterior probabilities of developing FCH. The ROC curve and the area under the ROC curve (AUC) were also computed with their probabilities calculated from the fitted model to assess the classifier performance and calculate accuracy, sensitivity and specificity. The ROC curve is computed on the test data (the left-out data in each fold from the cross-validation procedure) in order to show the estimated proportion of FCH class subjects correctly classified as class FCH against the estimated proportion of class healthy subjects non classified as class FCH for different thresholds of the classifier output. This method allows to find the threshold that maximizes the classification accuracy or to assess, in broad terms, how the classifier performs in the regions of high sensitivity and high specificity. The optimal threshold value was determined by the farthest point from the bisector of the ROC curve.

Table 3
Genotype and allele frequencies of SNPs in APOA5 and USF1 genes.

	FCH patients	Controls	Significance level
APOA5 −1131T > C			
Genotypes	n = 165	n = 142	
TT	111 (67.3%)	117 (82.4%)	p = 0.010
TC	49 (29.7%)	23 (16.2%)	
CC	5 (3.0%)	2 (1.4%)	
Dominant model for C allele			
TT	111 (67.3%)	117 (82.4%)	p = 0.003
TC + CC	54 (32.7%)	25 (17.6%)	
Allele frequencies			
Allele C	0.179	0.095	p = 0.003
Allele T	0.821	0.905	
APOA5 S19W			
Genotypes	n = 165	n = 142	
SS	123 (74.5%)	125 (88.0%)	p = 0.008
SW	40 (24.2%)	17 (12.0%)	
WW	2 (1.2%)	0	
Dominant model for W allele			
SS	123 (74.5%)	125 (88.0%)	p = 0.003
SW + WW	42 (25.5%)	17 (12.0%)	
Allele frequencies			
Allele S	0.867	0.940	p = 0.002
Allele W	0.133	0.060	
USF1 9996G > A			
Genotypes	n = 164	n = 135	
GG	103 (62.8%)	58 (43.0%)	p = 0.003
GA	53 (32.3%)	65 (48.1%)	
AA	8 (4.9%)	12 (8.9%)	
Dominant model for A allele			
GG	103 (62.8%)	58 (43.0%)	p = 0.001
GA + AA	61 (37.2%)	77 (57.0%)	
Recessive model for A allele			
GG + GA	156 (95.1%)	123 (91.1%)	p = 0.167
AA	8 (4.9%)	12 (8.9%)	
Allele frequencies			
Allele *G*	0.790	0.670	p = 0.001
Allele A	0.210	0.330	
USF1 11235C > T			
Genotypes	n = 164	n = 135	
CC	104 (63.4%)	58 (43.0%)	p = 0.002
CT	52 (31.7%)	65 (48.1%)	
TT	8 (4.9%)	12 (8.9%)	
Dominant model for T allele			
CC	104 (63.4%)	58 (43.0%)	p = 0.001
CT + TT	60 (36.6%)	77 (57.0%)	
Recessive model for T allele			
CC + CT	156 (95.1%)	123 (91.1%)	p = 0.167
TT	8 (4.9%)	12 (8.9%)	
Allele frequencies			
Allele C	0.793	0.670	p = 0.0007
Allele T	0.207	0.330	

3. Results

3.1. Comparison of SNP frequencies between FCH patients and controls

In order to identify differences between FCH patients and controls, the genotype and allele frequencies were calculated and compared. Dominant and recessive models for the rare allele were also constructed to verify the association type of the variants.

The −1131T > C (rs662799) and S19W (rs3135506) variants in the Apolipoprotein A-V gene (APOA5), 9996G > A (rs2073658) and 11235C > T (rs3737787) in the Upstream Stimulatory Factor 1 gene (USF1) showed differences in frequencies (Table 3) while data about frequencies of not statistically different SNPs are reported in Supplemental Table 1.

The rare allele of both SNPs in APOA5 is more frequent in patients than in controls and is associated with the disease in a dominant manner (i.e. if it is present in single or double copy number) with an OR and 95% CI equal to 2.28 (1.33–3.91) for C allele carriers of −1131T > C (rs662799) and to 2.51 (1.36–4.65) for W allele carriers of S19W (rs3135506). Conversely, the rare allele of both SNPs in USF1 is more frequent in healthy controls than in patients, suggesting a protective role for these alleles in a dominant manner. The presence of allele A for 9996G > A (rs2073658) and T for 11235C > T (rs3737787) showed an OR and 95% CI of 0.45 (0.28–0.72) and 0.44 (0.28–0.70) respectively. Since the two variants in USF1 are almost completely in linkage (D′ = 1), multivariate regression analysis included only the rs2073658 SNP in order to avoid the exclusion of both variants from the model.

3.2. Association of SNPs with lipid parameters

In order to evaluate the association of SNPs to lipid values, several linear regressions were performed including the dominant model for the rare allele of SNPs −1131T > C (rs662799) and S19W (rs3135506) in APOA5 and the 9996G > A (rs2073658) SNP in USF1,

adjusted for age, sex and BMI. The presence of the rare allele of both APOA5 variants is associated with increased levels of total cholesterol, triglycerides, non-HDL cholesterol, whereas only for S19W (rs3135506) the rare allele is associated with increased LDL cholesterol, LDL score and decreased mean LDL diameter and HDL levels (Table 4) suggesting a worse lipid profile of rare allele carriers compared to non-carriers. On the other hand, the carriers of the rare allele of 9996G > A (rs2073658) in USF1 showed decreased levels of total cholesterol, triglycerides, non-HDL cholesterol, LDL cholesterol, ApoB, ApoB/total cholesterol and LDL score and increased levels of mean LDL diameter compared to homozygotes for the common allele (Table 4), highlighting the protective role of this variant.

3.3. Association of SNPs with FCH – risk evaluation

To test the association of the studied variants with FCH independently of the other parameters commonly associated with the disease, we performed a multivariate logistic regression including the variants −1131T > C (rs662799) and S19W (rs3135506) in *APOA5* and the 9996G > A (rs2073658) in USF1, age, sex, BMI, total cholesterol levels and triglycerides evaluated in the absence of lipid-lowering drugs. SNP S19W (rs3135506) in APOA5 remains significantly associated with the presence of FCH independently of the other factors with an OR of 11.03 (1.52–80.06) (Table 5). The pseudo R-squared of this model is 0.83, indicating an improvement of the full model compared to the intercept model and confirming the significance of the selected parameters. To evaluate the ability of FCH prediction based on the logistic regression model, we constructed a fitted model, using the parameters above described, and we performed a 10-fold cross-validation which was repeated 200 times. The logistic model performance can be assessed by the ROC curve calculated for patients and controls on the basis of the fitted model (Fig. 1). The AUC is 0.987 and the optimal threshold of probability to be classified as a FCH patient is 0.943 with a total accuracy of 93.91%, a sensitivity of 94.22% and a specificity of 93.54%.

Table 5
Beta-coefficients and significances obtained at multivariate logistic regression analysis.

Independent variables	Significance	Or	95% CI
APOA5 −1131T > C	$p = 0.018$	11.03	1.52–80.06
APOA5 S19W	ns	2.38	0.55–10.32
USF1 11235C > T	ns	0.43	0.12–1.58
Age (years)	ns	1.02	0.96–1.09
Male gender	$p = 0.002$	11.54	2.39–55.7
Total cholesterol (mmol/L)	$p < 0.0001$	70.05	15.18–323.35
Triglycerides (mmol/L)	$p < 0.0001$	23.01	6.46–81.91
BMI (kg/m^2)	ns	0.97	0.82–1.16

4. Discussion

Twenty SNPs in genes involved in lipid metabolism have been studied in relation to the presence of FCH. We show that the frequencies of variants −1131T > C (rs662799) and S19W (rs3135506) in APOA5, and of variants 9996G > A (rs2073658) and 11235C > T (rs3737787) in USF1 differ between patients and control subjects. Patients bearing the rare allele of both SNPs in APOA5 and the homozygotes for the common allele of both SNPs in USF1 had a higher risk of FCH compared to carriers of the other genotypes. These data have been obtained in a well selected population in which the presence of gene defects causing other dyslipidemias with a similar phenotype was excluded as recommended in the diagnostic procedures [4]. The presence of LDLR mutations or of the E2/E2 genotype was an exclusion criterion, whereas this exclusion criterion was not applied in most of the previous studies. Since each SNP accounts for a tiny part of the dyslipidemic phenotype, the presence of a more relevant variant could mask the role of the SNPs studied herein.

After the first evidence of linkage between USF1 variants and FCH [13], various studies have confirmed the protective role of the rare allele of USF1 SNPs in different populations of FCH patients [14–16]. Here we report the first evidence of an association of USF1 variants in an Italian population affected by FCH. Linear regression analysis showed that the presence of the SNPs in APOA5 and *USF1* is directly associated to the lipid levels, which indicates that these various play a role in phenotype development, and partially explain its lipid variability. In particular, carriers of the rare allele of both SNPs in *APOA5* and homozygotes of the common allele of 9996G > A (rs2073658) in *USF1* had a worse lipid profile compared to carriers of other genotypes. The association of both 9996G > A (rs2073658) and 11235C > T (rs3737787) in *USF1* with triglyceride levels was previously reported in FCH patients [14,17,18] as well as in patients with cardiovascular disease [19], carotid artery intima-media thickness [20], type 2 diabetes [21] or obesity [22].

It was recently demonstrated that the rare allele of USF1 9996G > A (rs2073658) induces FOXOA expression less efficiently than the common allele, thereby leading to a minor expression of

Table 4
Beta-coefficients and significances obtained at multivariate linear regression analysis performed using different lipid parameters as dependent variable.

Independent variables	Total cholesterol		Triglycerides		Non-HDL cholesterol		HDL cholesterol		LDL cholesterol	
	β	Significance	β	Significance	β	Significance	β	Significance	β	Significance
APOA5 -1131T > C	0.388	$p = 0.050$	0.468	$p = 0.028$	0.425	$p = 0.035$	−0.035	ns	0.211	ns
APOA5 S19W	0.663	$p = 0.003$	0.746	$p = 0.002$	0.780	$p = 0.001$	−0.114	$p = 0.008$	0.439	$p = 0.025$
USF1 9996G > A	−0.485	$p = 0.005$	−0.470	$p = 0.012$	−0.536	$p = 0.002$	0.053	ns	−0.328	$p = 0.032$
Age	0.031	$p < 0.0001$	0.013	ns	0.032	$p = 0.0001$	0.000	ns	0.025	$p = 0.0005$
Male gender	0.291	ns	0.817	$p < 0.0001$	0.582	$p = 0.002$	−0.287	$p < 0.0001$	0.236	ns
BMI	0.048	$p = 0.040$	0.085	$p = 0.001$	0.072	$p = 0.003$	−0.023	$p < 0.0001$	0.036	ns

Independent variables	LDL score		Mean LDL diameter		ApoB		ApoB/total cholesterol	
	β	Significance	β	Significance	β	Significance	β	Significance
APOA5 -1131T > C	2.155	ns	−1.027	ns	0.048	ns	−0.002	ns
APOA5 S19W	7.653	$p = 0.0003$	−3.396	$p = 0.000$	0.061	ns	−0.007	ns
USF1 9996G > A	−4.848	$p = 0.003$	2.033	$p = 0.003$	−0.147	$p = 0.0002$	−0.008	$p = 0.028$
Age	0.281	$p = 0.0003$	−0.115	$p = 0.001$	0.008	$p < 0.0001$	0.000	$p = 0.034$
Male gender	7.152	$p < 0.0001$	−3.754	$p < 0.0001$	0.083	$p = 0.045$	0.007	$p = 0.071$
BMI	0.401	ns	−0.202	$p = 0.032$	0.011	$p = 0.040$	0.000	ns

ns = not significant.

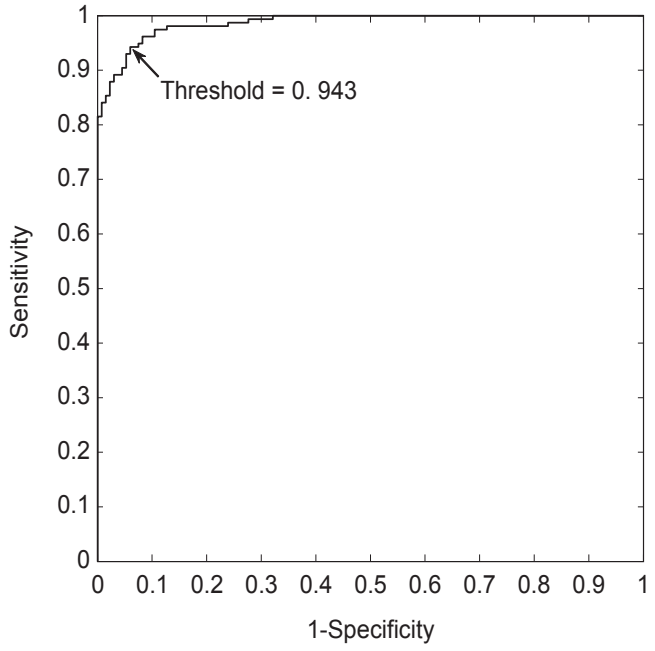

Fig. 1. ROC curve of probabilities of having FCH calculated by a 10-fold cross-validation repeated 200 times The curve describes the variation of sensitivity and specificity obtained with each value of probability of having FCH. The arrow indicates the optimal threshold (i.e. the point farthest from the bisector of the ROC curve).

microsomal triglyceride transfer protein that mediates the incorporation of triglycerides into VLDL [22] and their secretion. However, other studies report conflicting results regarding the role of the rare allele of USF1 9996G > A (rs2073658). In fact, the minor allele of this SNP was often considered the risk allele [19,23–25], while other did not find an association between the minor allele and the disease [26].

Different inclusion criteria for FCH cases or the lack of appropriate control groups (i.e., absence of family history of dislipidemia, medical history based on questionnaires, etc.), together with the specific ethnic characteristics of the populations examined, may explain these discordant findings. The absence of an association of the other studied SNPs with the disease could be explained in the same way. In light of the above considerations, further studies are needed to provide unbiased information for SNP selection.

Several studies of the association of the −1131T > C (rs662799) and S19W (rs3135506) SNPs in the APOA5 gene with FCH found that FCH patients more frequently carried variants rs662799 [27], rs3135506 [26,28] or both [29,30] compared to controls. The association of the rare allele of both SNPs with increased triglyceride levels was also demonstrated in other studies [26,28,30–32]. Zheng at al [33]. observed that most of the studies evaluating the association of APOA5 SNPs with metabolic syndrome or its components lacked the correction for triglyceride levels or that the association disappeared after this correction.

In our study, logistic regression analysis showed that also after correction for BMI, age, sex, cholesterol and triglyceride levels, the S19W polymorphism in APOA5 remained significantly associated with the presence of the disease. This result highlights that this SNP plays an important role in the identification of subjects at risk of developing FCH. The diagnosis of FCH is still difficult due to the need for repeated measurements of cholesterol and triglyceride levels that have a high biological variability. In addition, lipid levels are increased in different dyslipidemias, including non-familial or secondary dyslipidemias. As the genotype is a permanent factor that is not influenced by such biological variability as biochemical factors, the typing of an SNP may help to select patients at a high risk of FCH.

The association of −1131T > C (rs662799) and S19W (rs3135506) in APOA5, and of 9996G > A (rs2073658) in USF1 with anthropometric factors and cholesterol and triglyceride levels is demonstrated by the high value of the pseudo R-squared obtained at the logistic regression and confirmed by the cross-validation analysis. Using the probabilities calculated with the fitted model obtained through a cross-validation analysis, we constructed a ROC curve with an AUC of 0.987, indicating a good ability to discriminate patients from controls. Using the results of a multivariate logistic regression, Veerkamp et al. [34] constructed a Nomogram based on biochemical measurements to calculate the probability of FCH. Our model includes the genetic variants together with biochemical and anthropometric factors to calculate FCH risk and the resulting threshold of 0.943 shows an accuracy of 93.91% with a sensitivity of 94.22% and a specificity of 93.54%.

5. Study limitations

A limitation of this study is the small sample size, which may diminish the relevance of our conclusions. Although the frequencies of the APOA5 19W and −1131C alleles are rather low, our results were obtained in a well selected population. In fact, the presence of gene defects causing other dyslipidemias with a similar phenotype was excluded. Another limitation of the study is the selection of SNP, since we typed the SNPs most frequently associated to dyslipidemia. In particular, we did not examine the involvement of other SNPs such as rs2516839 in USF1 which is associated to high triglyceride levels and atherosclerosis [32]. More extensive investigations are required to verify and extend our data.

6. Conclusions

Our results show that USF1 and APOA5 polymorphisms are associated with FCH and that the S19W SNP (rs3135506) in the APOA5 gene is associated to the disease independently of total cholesterol, triglycerides and BMI.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.mcp.2014.10.002.

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