

Original Research

Genetic risk score in patients with the *APOE2/E2* genotype as a predictor of familial dysbetalipoproteinemia



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KEYWORDS

Familial
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Polymorphism;
Genetic risk score

Abstract:

Background: Familial dysbetalipoproteinemia (FD) is an autosomal recessive (rarely dominant) inherited disorder that is almost exclusively associated with the apolipoprotein E gene (*APOE*) variability. Nonetheless, only a small proportion of *APOE2/E2* subjects develop the phenotype for mixed dyslipidemia; the context of other trigger metabolic or genetic factors remains unknown.

Methods: One hundred and one patients with FD and eighty controls (all *APOE2/E2* homozygotes; rs429358) were screened for 18 single-nucleotide polymorphisms (SNPs) within the genes involved in triglyceride metabolism.

Results: Two SNPs were significantly associated with the FD phenotype (rs439401 within *APOE*; $P < 0.0005$ and rs964184 within *ZPR1/APOA5/A4/C3/AI* gene cluster; $P < 0.0001$). Unweighted genetic risk scores - from these two SNPs (GRS2), and, also, additional 13 SNPs with P-value below 0.9 (GRS15) - were created as an additional tool to improve the risk estimation of FD development in subjects with the *APOE2/E2* genotype. Both GRS2 and GRS15 were significantly ($P < 0.0001$) increased in

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patients and both GRSs discriminated almost identically between the groups ($P = 0.86$). Subjects with an unweighted GRS2 of three or more had an almost four-fold higher risk of FD development than other individuals (odds ratio (OR) 3.58, 95% confidence interval (CI): 1.78–7.18, $P < 0.0005$).

Conclusions: We identified several SNPs that are individual additive factors influencing FD development. The use of unweighted GRS2 is a simple and clinically relevant tool that further improves the prediction of FD in *APOE2/E2* homozygotes with corresponding biochemical characteristics.

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Introduction

Cardiovascular diseases (CVDs), mainly atherosclerotic diseases (ASCVDs), remain the leading cause of premature morbidity and mortality worldwide. In the Czech Republic, they account for 45 % of total standardized mortality.¹ Among other factors, such as smoking or obesity, different types of dyslipidemias (DLPs) play a pivotal role in ASCVD manifestation. The vast majority of DLPs have a significant genetic background, with many polymorphisms individually playing a relatively small but highly important role in concert.² In addition to polygenic DLPs, there are monogenically inherited disorders of blood lipid metabolism, among which familial hypercholesterolemia (FH) is the most common.³

Familial dysbetalipoproteinemia (FD), previously known according to the Fredrickson *et al.*⁴ classification as hyperlipoproteinemia type III (HLP III), seems to be the second most common, and FD subjects possess a risk of ASCVD that is comparable to that in subjects with heterozygous FH.⁵

Phenotypically, FD manifests as a mixed DLP with the presence of cholesterol-rich VLDL particles, with a VLDL-cholesterol/triglycerides (TG) ratio > 0.3 .⁵ In clinical practice, the first clue to think about possible FD should be when a patient presents with mixed DLP and has a total cholesterol (TC)/TG ratio ≤ 2 , under conditions of TC > 5 mmol/l, TG > 3 mmol/l.⁵ When FD is suspected, overall accepted diagnostic criteria can be used to help select suitable candidates for further (especially genetic) testing. Widely used criteria include the apolipoprotein B (apoB)/TC ratio < 0.15 g/mmol (sensitivity 89 %, specificity 97 %), the so-called apoB algorithm, defining FD as apoB < 1.2 g/l, TG > 2.3 mmol/l, TG/apoB < 10 and TC/apoB > 6.2 (sensitivity 93 %, specificity 99 %) or^{6,7} the non-HDL-cholesterol (non-HDL-C)/apoB ratio with comparable sensitivity and specificity and the cut-off value 3.69 mmol/g.⁸

FD is characterized by the accumulation of triglyceride-rich apoB-containing particles (mostly remnant particles) and, according to some sources, is associated with an up to 10-fold increase in the risk of developing premature CV events.^{5,9} In the study by Paquette *et al.*¹⁰ it was demonstrated that the risks of ASCVD and peripheral vascular disease (PVD) in FD are more than 3-fold and 13-fold higher, respectively, than in normolipidemic controls. Furthermore, the risk of PVD is approximately 4-fold higher in FD than in FH. Another potential clinical impact of FD or concomitant

hypertriglyceridemia (HTG) is the risk of developing acute pancreatitis, a potentially life-threatening condition.^{5,9}

FD is primarily determined by a polymorphism (rs429358) within apolipoprotein E (*APOE*; OMIM acc. ID 617,347) gene. The most frequent alleles of the *APOE* gene are *E2*, *E3* and *E4*, with the *E3* allele being the most common in the general population (77–82 %, sometimes incorrectly¹¹ referred to as wild-type), followed by the *E4* allele (11–15 %) and the *E2* allele (7–8 %).^{9,12,13} In the majority of cases, FD is associated with the homozygous *APOE2/E2* genotype, which is why it was long thought to be only an autosomal recessively inherited disease (see Supplementary Table S1 for other rare variants). Less than one-fifth of patients with *APOE2/E2* manifest FD in the context of other environmental, metabolic, or yet undescribed genetic factors (see Supplementary Table S2 for more details).^{5,14–16}

Considering the literature reporting the prevalence of *APOE2/E2* (up to 1 % in Caucasians), up to 10,000 patients with FD can be expected in the Czech Republic only.¹³ However, their detection rate is dramatically lower.⁹

Genetic predispositions to FD behind the *APOE* genotype remain almost completely unknown. Only a few papers have focused on other genetic variants that could participate in FD development. Potentially, only *APOA5* variability seems to play an important role.^{14–17}

Genetic risk scores (GRS) have recently often been mentioned as a powerful tool for discriminating between patients and controls, but this concept has not yet been applied to FD.

Generally, the accumulation of risk alleles of several to thousands common DNA variants (mostly SNPs), each of which has a relatively small effect, occurs in the background of almost all phenotypes/diseases. As number of SNPs involved in disease development could reach hundreds, creation of GRS could express more complex risk estimation (based on the sum of risks caused by single SNPs) and seems to be a promising tool to implement complex results from genetic screening into the clinical practice.¹⁸

To date, dozens of SNPs associated with increased plasma TG levels in the general population have been detected using different approaches (candidate gene studies, comparative sequencing, genome-wide association studies (GWAS)).^{19–21} Based on these results and several confirmatory studies, we selected a set of 18 common SNPs whose accumulation of their risk alleles may lead to the clinical manifestation of FD.^{22,23}

The aim of our study was to analyze the potential effect of preselected SNPs on the development of FD with an effort to create a specific unweighted GRS as an additional genetic determinant of FD development in subjects with *APOE2/E2* genotype.

Materials and methods

Study subjects

The study was designed as a case–control study.

The cases are represented by 101 subjects with the *APOE2/E2* genotype and typical FD phenotype, i.e., mixed DLP (laboratory criteria of FD: TC > 5 mmol/l, TG > 3 mmol/l, TC/TG ratio ≤ 2, non-HDL-C/apoB > 3.69 mmol/g), 11 patients also had FD verified by lipoprotein ultracentrifugation. Data were collected in patients both before and during treatment at 2 centers - at the 3rd Department of Internal Medicine - Department of Endocrinology and Metabolism, 1st Faculty of Medicine, Charles University in Prague and at the Department of Clinical Biochemistry, St. Anne University Hospital in Brno.

The control group consisted of 80 probands with the *APOE2/E2* genotype without documented DLP (i.e. TC/TG > 2). Controls were selected from the large population/biobank of the post-MONICA and HAPPIEE studies with known *APOE* genotypes. All clinical data from the controls were obtained as self-reported.^{24–27}

All subjects were unrelated adult Caucasians. The study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Board (or Ethics Committee) of General University Hospital in Prague, First Faculty of Medicine Charles University (protocol code 89/19, date of approval 21 October 2019). Informed consent with genetic testing for research medical purposes was obtained from all subjects involved in the study.

DNA analyses

DNA was isolated from whole blood (collected in EDTA) using the conventional desalting method.²⁸

The *APOE2/E2* genotype (rs429358) was confirmed as described by Hixson and Vernier.²⁹ Individual genetic variants were determined by PCR-RFLP or using TaqMan assays as described in detail by Hubacek et al.²³

Statistical analyses

Hardy-Weinberger equilibrium for individual SNPs was not analyzed for either probands or controls, as patients with the *APOE2/E2* genotype represent a selected proportion of the population and deviation from this equilibrium is likely.

Chi-square and individual ORs (odds ratio, 95 % confidence interval [CI]) were determined using a four-field contingency table and STATISTICA software (Statsoft, Prague,

Czech Republic). When the number of individuals with minor genotypes was below five, these were pooled with heterozygotes and analyzed together.

Regardless of nominal significance, only SNPs with P-value over 0.9 (see section Results for details) were excluded from creation of the unweighted GRS. Two scores have been created - GRS2 includes only two most powerful SNPs (within *APOE* and *ZPR1/APOA5/A4/C3/A1* gene cluster); GRS15 includes 15 SNPs set with above mentioned restriction. For the analysis, the "protective" genotype was replaced with a value of 0, carriers of one risk allele were designated 1, and finally, the risk genotype was designated 2. In the case, that minor homozygotes were not present (in at least one of the groups), carriers of the minor alleles have been pooled together in both examined groups and only risk values "0" and "2" have been implemented. A final unweighted GRS value is represented as a simple sum of these values. A P-value ≤ 0.05 was considered statistically significant.

Results

Population characteristics

A detailed description of the study subjects is summarized in Table 1. In accordance with the criteria for the diagnosis of FD, the ratios of the mean pre-treatment TC and TG levels was ≤ 2, non-HDL-C/apoB > 3.69 mmol/g. About one-fifth of the probands had known diabetes, and both groups had mean body mass index (BMI) at borderline for obesity definition. One-third of the patients were smokers and there was higher prevalence of manifest ASCVD (25.7% vs. 11.2 %, $P < 0.02$).

Single nucleotide polymorphisms

Detailed genotype frequencies of the 18 SNPs analyzed are summarized within Table 2. Only variants within *APOE* and *ZPR1/APOA5/A4/C3/A1* gene cluster reached significantly different genotype frequencies between the patients and controls (Table 3). The strongest determinant of the development of FD beyond the *APOE2/E2* genotype was the *APOE* rs439401 polymorphism: OR (95 % CI) for the association between homozygotes and minority heterozygotes was 9.51 (CI: 2.08 - 43.51, $P < 0.0005$). The second polymorphism significantly associated with FD was within the *ZPR1/APOA5/A4/C3/A1* gene cluster (rs964184, OR 3.62 (CI: 1.85 - 7.10, $P < 0.0001$ for +G vs. CC comparison)).

Additional variants potentially associated with FD (despite the relatively high ORs, but genotype differences were only borderline significantly different between the groups) were variants within the *GCKR*, *FRMD5*, *GALNT2* and *LPL* genes (for more details see Tables 2 and 3).

Table 1 Basic characteristics of patients with familial dysbetalipoproteinemia (FD) and controls.

	Patients	Controls	P
Number (% females)	101 (38.6 %)	80 (48.7 %)	
Age (years)	61.2 ± 15.8	52.6 ± 7.4	0.01
Active smoking; N (%)	31 (30.7 %)	23 (28.8 %)	n.s.
Arterial hypertension; N (%)	45 (44.6 %)	38 (47.5 %)	n.s.
Type 2 diabetes; N (%)	21 (20.8 %)	9 (11.2 %)	n.s.
ASCVD in anamnesis; N (%)	26 (25.7 %)	9 (11.2 %)	0.02
BMI (kg/m ²)	29.0 ± 3.9	28.3 ± 4.6	n.s.
Total cholesterol (mmol/L)	8.9 ± 2.8	4.8 ± 1.1	0.001
Triglycerides (mmol/L)	6.7 ± 6.0	1.9 ± 0.9	0.001
HDL-cholesterol (mmol/L)	1.4 ± 0.5	1.4 ± 0.4	0.61
Apolipoprotein B (g/L)	1.1 ± 0.5	N/A	N/A
non-HDL-cholesterol (mmol/L)	7.1 ± 3.3	3.1 ± 1.5	0.001

ASCVD – atherosclerotic cardiovascular disease in anamnesis (history of coronary artery disease, stroke, peripheral vascular disease included), BMI – body mass index; HDL, high-density lipoprotein; N/A, not applicable; and n.s., not significant.

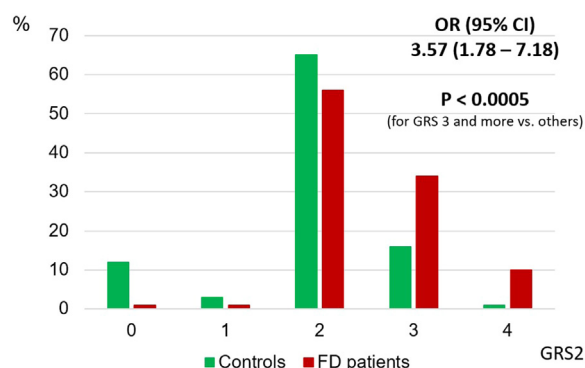
The values are n (%) or mean ± SD.

Unweighted GRS

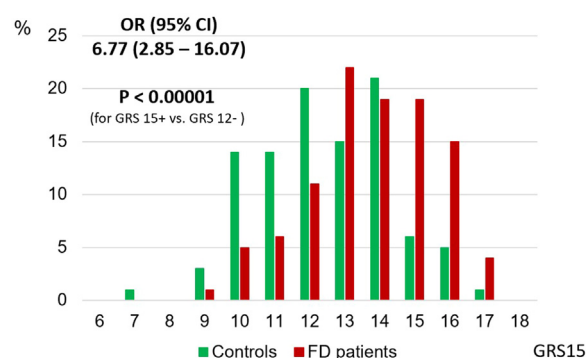
Two unweighted GRSs were created (based on the results presented in Table 2) as an additional tool to improve the assessment of the risk of FD development in subjects with the *APOE2/E2* genotype. The simplest one includes just the two most powerful SNPs (GRS2, within genes for *APOE*, rs439401 and *ZPR1/APOA5/A4/C3/A1* gene cluster, rs964184); the second exclude the three SNPs (within *LRP1*, *MAP3K1* and *CAPN3* genes) where no differences between patients and controls have been observed yielding 15 SNPs gene score (GRS15).

The mean GRSs were significantly different in both cases (both $P < 0.0001$). Mean GRS2 was 2.50 ± 0.73 in patients vs. 1.86 ± 0.91 in controls; values for GRS15 were than 6.94 ± 1.22 vs. 5.96 ± 1.29 . The detailed distribution of unweighted GRS values is summarized in Figs. 1 and 2. Importantly, adjustment for sex, type 2 diabetes (T2D) or BMI did not change the results significantly.

Finally, and rather surprisingly, both GRSs discriminated almost identically between both groups. AUC (area under curve) for GRS2 was (mean ± SD) 0.676 ± 0.03 and 0.684 ± 0.04 for GRS15 ($P = 0.86$).

**Fig. 1** Distribution of unweighted GRS2 in FD patients and controls.

CI, confidence interval; FD, familial dysbetalipoproteinemia; GRS, genetic risk score; OR, odds ratio.

**Fig. 2** Distribution of unweighted GRS15 in FD patients and controls.

CI, confidence interval; FD, familial dysbetalipoproteinemia; GRS, genetic risk score; OR, odds ratio.

Discussion

Our study examined the role of common gene polymorphisms influencing TG rich lipoprotein metabolism and led to construction of a GRS for the determination of susceptibility to FD (HLP III). It is well known that the *APOE2/E2* genotype is considered necessary for development of this pathology, but, as only a minor part of *APOE2/E2* subjects is affected, simultaneous presence of other susceptibility factors, either genetic or environmental, is thought to be essential.

Out of the 18 analyzed SNPs within genes with a role in TG plasma levels regulation, only two were highly associated with the FD phenotype. It is not a surprise that these two significant SNPs are located at *APOE* and *ZPR1/APOA5/A4/C3/A1* gene cluster loci. These genes are very plausible candidates for future detailed genetic testing (in-depth sequencing).

In addition to *APOE*, the *ZPR1/APOA5/A4/C3/A1* gene cluster, as the most powerful determinant of plasma TG and a proven genetic risk factor for ASCVD manifestation, is the second most confirmed candidate.³⁰⁻³³ Indeed, similar to our study, Evans et al.¹⁴ associated a common (rs662799

Table 2 Genotype frequencies of individual SNPs within the FD patients and controls.

Gene	SNP alleles	Group	MM		Mm		mm		P
			N	%	N	%	N	%	
Included in the GRS2									
<i>APOE</i>	rs439401	Controls	67	83.8	13	16.5	0	0.0	0.00007
<i>ZPR1/APOA5/A4/C3/A1 gene cluster</i>	M – C; m – T	FD patients	99	98.0	2	2.0	0	0.0	
	rs964184	Controls	64	80.0	15	18.8	1	1.3	0.0001
	M – C; m – G	FD patients	53	52.5	36	35.6	12	1.9	
Extended GRS15									
<i>GCKR</i>	rs1260326	Controls	27	33.8	32	40.0	21	26.3	0.03
	M – C; m – T	FD patients	22	21.8	60	59.4	19	18.8	
<i>FRMD5</i>	rs2929282	Controls	74	92.5	6	7.5	0	0.0	0.04
	M – A; m – T	FD patients	83	82.2	18	17.8	0	0.0	
<i>GALNT2</i>	rs1321257	Controls	41	51.3	27	33.8	12	15.0	0.06
	M – A; m – G	FD patients	37	36.6	52	51.5	12	11.9	
<i>LPL</i>	rs12678919	Controls	65	81.3	14	17.5	1	1.3	0.09
	M – A; m – G	FD patients	91	90.1	10	9.9	0	0.0	
<i>TYW1B</i>	rs13238203	Controls	74	92.5	6	7.5	0	0.0	0.24
	M – C; m – T	FD patients	88	87.1	7	6.9	6	5.9	
<i>HLA</i>	rs2247056	Controls	43	53.8	30	37.5	7	8.8	0.29
	M – T; m – C	FD patients	65	64.4	27	26.7	9	8.9	
<i>CTF1</i>	rs11649653	Controls	29	36.3	42	52.5	9	11.3	0.35
	M – C; m – G	FD patients	29	23.7	54	53.5	18	17.8	
<i>TRIB1</i>	rs2954029	Controls	25	31.3	40	50.0	15	18.8	0.38
	M – A; m – T	FD patients	34	33.7	41	40.6	26	25.7	
<i>CILP2</i>	rs10401969	Controls	71	88.8	9	11.3	0	0.0	0.48
	M – T; m – C	FD patients	86	85.1	13	12.9	2	2.0	
<i>CYP26A1</i>	rs2068888	Controls	21	26.3	40	50.0	19	23.8	0.53
	M – G; m – A	FD patients	25	24.8	58	57.4	18	17.8	
<i>CETP</i>	rs7205804	Controls	27	33.8	32	40.0	21	26.3	0.61
	M – G; m – A	FD patients	40	39.6	40	39.6	21	20.8	
<i>LIPC</i>	rs261342	Controls	45	56.3	23	28.8	12	15.0	0.69
	M – G; m – C	FD patients	53	52.5	35	34.7	13	12.9	
<i>NAT2</i>	rs1495743	Controls	47	58.8	29	36.3	4	5.0	0.84
	M – C; m – G	FD patients	55	54.5	40	39.6	6	5.9	
SNPs excluded from calculations									
<i>LRP1</i>	rs11613352	Controls	52	65.0	25	31.3	3	3.8	0.93
	M – C; m – T	FD patients	65	64.4	31	30.7	5	5.0	
<i>MAP3K1</i>	rs9686661	Controls	52	65.0	24	30.0	4	5.0	0.96
	M – C; m – T	FD patients	66	65.3	32	31.7	3	3.0	
<i>CAPN3</i>	rs2412710	Controls	77	96.3	3	3.7	0	0.0	1.00
	M – G; m – A	FD patients	97	96.0	4	4.0	0	0.0	

FD, familial dysbetalipoproteinemia and SNP, single nucleotide polymorphism; M – major allele; m – minor allele (based on the frequency in controls); P values are calculated for MM vs. Mm vs. mm comparison, if there are at least 5 minor homozygotes. If there were fewer than 5 minor homozygotes, the MM vs. (Mm+mm) model was used.

and rs3135506) *APOA5* polymorphism with FD. It is likely that analysis of the degree of linkage disequilibrium and/or *APOE-APOA5* interactions will further improve the FD diagnostics.

There is no existing literature reporting on the associations of SNPs located on other loci/gene clusters and the risk of FD. Despite the relative low number of patients and controls, our study pointed at four additional genes of interest – namely *GCKR*, *FRMD5*, *GALNT2* and *LPL*. Variants within these four genes were slightly (P-values between 0.03 and 0.09) associated with FD. This is consistent with our previous results²³ – all the 4 SNPs detected herein were signifi-

cantly associated also with extremely high plasma TG levels in a non-FD population. This further underline their importance, as previous GWAS^{21,34,35} have found that these SNPs are powerful determinants of increased TG levels at the population level. The lack of information about other important polymorphisms associated with FD could also be (and not in our study only) a consequence of the relatively low number of examined subjects, as FD is a relatively rare phenotype.

The effect of individual SNPs on phenotype often differs between populations.^{22,23,36-39} To avoid this problem, we selected SNPs with already confirmed effects on TG levels in the Czech population.²³

Table 3 Effects of individual SNPs potentially associated with FD development.

Gene	SNP	Calculated for	OR	95 % CI	P
<i>APOE</i>	rs439401	CC vs. +T	9.51	2.08 – 43.51	0.0005
<i>ZPR1/APOA5/A4/C3/A1</i>	rs964184	+G vs. CC	3.62	1.85 – 7.10	0.0001
<i>gene cluster</i>					
<i>GCKR</i>	rs1260326	+T vs. CC	1.83	0.94 – 3.54	0.07
<i>FRMD5</i>	rs2929282	+T vs. AA	2.67	1.01 – 7.10	0.04
<i>GALNT2</i>	rs1321257	+G vs. AA	1.87	1.03 – 3.40	0.04
<i>LPL</i>	rs12678919	AA vs. +G	2.10	0.89 – 4.97	0.09
<i>TYW1B</i>	rs13238203	+T vs. CC	1.82	0.66 – 5.03	0.24
<i>HLA</i>	rs2247056	TT vs. +C	1.55	0.85 – 2.83	0.15
<i>CTF1</i>	rs11649653	+G vs. CC	1.41	0.75 – 2.64	0.28
<i>TRIB1</i>	rs2954029	TT vs. +A	1.50	0.73 – 3.08	0.26
<i>CILP2</i>	rs10401969	+C vs. TT	1.38	0.57 – 3.33	0.48
<i>CYP26A1</i>	rs2068888	+A vs. GG	1.08	0.55 – 2.12	0.82
<i>CETP</i>	rs7205804	GG vs. +A	1.29	0.70 – 2.37	0.42
<i>LIPC</i>	rs261342	+C vs. GG	1.16	0.65 – 2.10	0.61
<i>NAT2</i>	rs1495743	+G vs. CC	1.19	0.66 – 2.16	0.56
<i>LRP1</i>	rs11613352	+T vs. CC	1.03	0.56 – 1.90	0.93
<i>MAP3K1</i>	rs9686661	CC vs. +T	1.02	0.55 – 1.88	0.96
<i>CAPN3</i>	rs2412710	+A vs. GG	1.06	0.23 – 4.87	0.94

Abbreviations: CI, confidence interval; FD, familial dysbetalipoproteinemia; OR, odds ratio; SNP, single nucleotide polymorphism.

The use of individual SNPs as disease predictors is often criticized because of the relatively low risk associated with individual alleles, and, thus, an OR greater than 2 is rarely achieved.^{33,40} In fact, however, “traditional” CV risk factors, such as high cholesterol or arterial hypertension, are not better predictors.^{41,42} In contrast, as demonstrated in our study, a much larger OR can be achieved if patients and subjects are selected according to very strict inclusion criteria.

Even the most powerful individual gene polymorphism would not be able to predict the risk of any polygenic disease with the required accuracy. Therefore, in the past decade, much effort has been put into the analysis of GRSs,^{18,43} which shall predict the complex genetic risk more precisely.

Briefly, GRSs are based on a sum of disease-associated alleles presented in each individual. GRS could be calculated simply on the presence of each (unweighted GRS) risky allele; in the case that sum is based not only on risk status but also on the effect size (usually based on hazard ratio/odds ratio or on β -coefficient), weighted GRS is more suitable.

In our study, unweighted GRSs created from two, as well as fifteen, SNPs were highly significant predictors of FD development. In fact, there was no nominal improvement in FD prediction, if additional 13 SNPs have been included in GRS2 (AUC (area under curve) for GRS2 was (mean $\pm$ SD) 0.676 ± 0.03 and 0.684 ± 0.04 for GRS15 ($P = 0.86$)). It is likely caused by the fact, that the effect of two strongest SNPs was extremely powerful in comparison with other included SNPs. In view of the above, the use of unweighted GRS2 further improves the prediction of FD in *APOE2/E2* homozygotes with corresponding biochemical characteristics.

Early recognition of subjects at high risk of FD development will be of special importance, as it is known that FD subjects are under a similar risk of ASCVD and PVD

development to patients with heterozygous FH.³ Detection of risky GRS2 in *APOE2/E2* carriers shall lead to early detection of individuals susceptible to FD development enabling initiation of preventive measures to avoid premature CV morbidity and, also, reduce the risk of HTG associated acute pancreatitis.

It might be speculated the results of the observations could be influenced by differences in age, sex, BMI, dietary or exercise habits. In our study, however, adjustment for age, gender, BMI or prevalence of T2D did not change the results significantly. In the future, to maximize the subject's profit from genetic screening, genetic testing should be performed in young individuals. With timely primary prevention, suitable lifestyle changes could be implemented at a young age. This, in turn, will lead to improved profile of modifiable risk factors, which do contribute to the development of FD (e.g. abdominal adiposity, insulin resistance).

Our results were potentially influenced by several limitations. First, we did not screen patients for the presence of monogenic mutations leading to the development of severe mixed DLP. However, as the expected mutation prevalence is approximately 1: 1 000 000, it is very unlikely that such subjects have been included. Additionally, the diagnosis of FD was made based on the simultaneous presence of mixed DLP and *APOE2/E2* genotype; only 11 patients also had FD verified by lipoprotein ultracentrifugation. To minimize the impact of false positive FD diagnoses we employed the commonly recommended diagnostic algorithm to assess the presence of FD. Another limitation of the study was that only TC, TG, apoB and HDL-cholesterol were measured, and non-HDL-C was calculated. Finally, this study did not also include a confirmatory group, as both probands and controls were a very specific subset of the general population.

Conclusions

We conclude that several gene SNPs represent additional genetic factors underlying the development of FD in *APOE2/E2* individuals. The use of unweighted GRS2 is a simple and clinically relevant tool that further improves the prediction of FD in *APOE2/E2* homozygotes with corresponding biochemical characteristics (e.g., non-HDL-C/apoB > 3.69 mmol/g). Moreover, identification of positive GRS2 in these patients may help prevent the development of FD by early initiation of intensive lifestyle changes.

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Data availability statement

Raw anonymized data are available upon reasonable request from the corresponding author.

Ethics statement

The studies involving human participants were reviewed and approved by the Ethics Committee of General University Hospital in Prague, First Faculty of Medicine Charles University (protocol code 89/19, date of approval 21 October 2019). Informed consent with genetic testing for research medical purposes was obtained from all subjects involved in the study.

Use of AI and AI-assisted technologies statement

During the preparation of this work the author(s) not used artificial intelligence.

CRedit authorship contribution statement

Martin Satny: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Writing – original draft, Writing – review & editing, Visualization, Project administration, Funding acquisition. **Veronika Todorovova:**

Software, Validation. **Tereza Altschmiedova:** Formal analysis, Investigation. **Jaroslav A. Hubacek:** Conceptualization, Methodology, Validation, Resources, Writing – original draft, Visualization. **Lucie Dlouha:** Formal analysis. **Vera Lanska:** Formal analysis. **Vladimir Soska:** Investigation, Writing – review & editing. **Ondrej Kyselak:** Investigation. **Tomas Freiburger:** Data curation, Writing – review & editing. **Martin Bobak:** Investigation, Resources. **Michal Vrablik:** Conceptualization, Formal analysis, Investigation, Writing – review & editing, Supervision.

Declaration of interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript; or in the decision to publish the results.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at [doi:10.1016/j.jacl.2023.11.010](https://doi.org/10.1016/j.jacl.2023.11.010).

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