ARTICLE

Diagnostic value of post-heparin lipase testing in detecting common genetic variants in the *LPL* and *LIPC* genes

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Post-heparin lipoprotein lipase and hepatic lipase activities are used to identify primary disorders of triglyceride and HDL-cholesterol metabolism. Their ability to identify common variants in the lipoprotein lipase (*LPL*) and hepatic lipase (*LIPC*) genes is unclear. To investigate the ability of lipase testing to detect common lipase gene variants, we included 183 patients who had undergone post-heparin lipase testing and genotyped the *LPL* D9N, N291S, *PvuII*, *HindIII*, and S447X and the *LIPC*-514CT, V73M, V133V, and N193S polymorphisms. Allele frequencies were compared with 163 controls. Polymorphisms with different allele frequencies in patients and controls or influencing lipids, were analyzed further. The diagnostic value of post-heparin lipase testing was assessed using logistic regression and receiver operating characteristic curves. We found that lipase activities did not predict the *LPL* D9N and N291S polymorphisms, but predicted the *LPL* S447X and *LIPC*-514CT polymorphisms. Adjusted for covariates, the area under the receiver operating characteristic curves was 0.643, 0.478, 0.686, and 0.657 for *LPL* D9N, N291S S447X and *LIPC*-514CT, respectively. On the basis of these findings, we conclude that high-*LPL* and low-HL activities associate with the *LPL* S447X and *LIPC*-514CT polymorphisms, but low-*LPL* activity was not related to *LPL* polymorphisms. Overall, the discriminative ability of post-heparin lipase tests in identifying carriers of common variants in the *LPL* and *LIPC* genes was limited. This indicates that conclusions on the genetic causes of lipase activities outside of the normal range should be drawn with caution.

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Introduction

In clinical practice, measurements of post-heparin *LPL* and HL activities are used to identify the cause of primary hypertriglyceridemia (HTG) and disorders of the HDL-cholesterol metabolism. In dyslipidemic patients, these tests may identify abnormalities in the *LPL* or hepatic lipase (HL) gene expression and protein function.

Hypertriglyceridemia results from increased supply to and impaired removal of triglyceride-rich lipoproteins from circulation. Although HTG is a multifactorial disorder, genetic factors clearly influence the disease phenotype.¹ Notably, it has been shown that genetic factors strongly influence variation in inter-individual HDL-cholesterol levels as well.² *LPL* plays a pivotal role in the metabolism of triglyceride-rich lipoproteins,³ whereas HL has a more prominent role in the metabolism of HDL.⁴ In addition, HL is also involved in the conversion of IDL to LDL and influences the LDL size.^{5,6} In line, decreased post-heparin *LPL* activity was associated with elevated plasma triglyceride levels and lower levels of HDL-cholesterol,^{7,8} whereas decreased post-heparin HL activity was associated

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with slightly increased triglyceride and strongly increased HDL-cholesterol levels.^{8,9}

Several common sequence variations in the lipoprotein lipase (*LPL*) and hepatic lipase (*LIPC*) genes that affect post-heparin lipase activities, have been identified.^{10–13} Subnormal levels of lipase activities are often interpreted as the result of the presence of common polymorphisms. This effect has also been referred to as ‘partial lipase deficiency’.¹⁴ However, the ability of post-heparin lipase testing to identify common genetic *LPL* and *LIPC* variants in a dyslipidemic outpatient lipid clinic population has not been investigated.

In this study, we investigated the diagnostic value of post-heparin lipase testing for the detection of common genetic variants of the *LPL* and *LIPC* genes in an outpatient lipid clinic population.

Materials and methods

Patients

In our study, we included a total of 183 Caucasian patients who had undergone post-heparin lipase testing at our outpatient clinic between 1991 and 2003. In 128 patients, the primary reason for post-heparin lipase testing was HTG. The remainder of patients had other reasons for lipase testing, such as low HDL-cholesterol ($n=21$), high HDL-cholesterol ($n=7$), combined dyslipidemia ($n=7$), screening for cardiovascular disease risk factors ($n=5$), hypercholesterolemia ($n=4$), or other ($n=11$). Family members, patients under 18 years of age and carriers of the ApoE2/E2 genotype and patients with homozygous *LPL* or *LIPC* deficiencies (defined as homozygous carriers of *LPL* or *HL* mutations that results in complete loss of function (*3)) were excluded from the analyses.

Collection of clinical data was performed retrospectively by reviewing the patients’ medical records. HTG was defined as a serum triglyceride level above 2.3 mmol/l (204.7 mg per 100 ml).

Control allele frequencies of the tested polymorphisms were investigated in a population of 163 blood bank donors from Rotterdam Blood Bank, under the assumption that blood bank donors are generally healthy. No other characteristics of these donors were known.

All patients gave their written informed consent for participation in the study. Our local Medical Ethics Committee approved the protocol.

Post-heparin lipoprotein lipase and hepatic lipase activity

Lipase activities were determined in fasting plasma as described earlier.¹⁵ In brief, 20 min after intravenous injection of 50 IU per kg body weight heparin, blood was obtained from the other arm in heparinized tubes and was transported on wet ice to the laboratory. By centrifugation for 10 min, 3000 r.p.m. at 4°C, plasma was immediately

separated. Plasma was stored at –80°C until further analyses. All activity assays used gum acacia-stabilized [³H]triolelylglycerol as a substrate. *LPL* activity was determined after inhibition of *HL* by co-incubation with a goat antibody to human *HL*. Fatty acids were extracted as calcium salts and counted in a liquid scintillation counter. *HL* activity was measured in the presence of 1 M NaCl, which totally inhibits *LPL* activity. Activities are expressed as U/ml. One U is defined as the release of 1 nmol FFA/ml/h. In every analysis, three to four samples with known *LPL* and *HL* activity were analyzed. These controls are stable throughout a long period. New controls were standardized on the old control values so that standardization remains stable. The inter-assay variability is 1.8 and 3.1% and the intra-assay variability 5.7 and 4.2% for post-heparin *LPL* and *HL* activity measurements, respectively.

Laboratory analyses

Total cholesterol and triglycerides were measured by enzymatic colorimetric methods (CHOD-PAP and GPO-PAP, Boehringer Mannheim, Mannheim, Germany) on a Hitachi 911 analyzer (Boehringer Mannheim). HDL-cholesterol was measured using a direct enzymatic HDL-cholesterol method, using the PEG-modified enzymes method (Boehringer Mannheim) on a Hitachi 911 analyzer. Laboratory values were collected from the day of lipase testing.

DNA analysis

Genomic DNA was isolated from blood leucocytes with a salting-out procedure. The *LPL* D9N (*rs1801177*), N291S (*rs268*), PvuII (*rs285*), HindIII (*rs320*), and S447X(*rs328*) polymorphisms and *LIPC* V73M (*rs6078*), V133V (*rs690*) N193S (*rs6083*) polymorphisms were genotyped with Taqman allelic discrimination. The DNA extracted was diluted to a final concentration of 5–10 ng/μl. In the assay 1 μl DNA solution, 2.5 μl Taqman universal PCR Mix and 0.25 μl SNP genotyping assay were used. PCR was performed in 40 cycles under standard conditions provided by ABI. Reactions were performed on the Taqman Prism 7900HT platform.

The *LIPC*-514CT polymorphism was genotyped by determination of the –250GA polymorphism, because these polymorphisms are in complete linkage disequilibrium.¹⁶ Primers for PCR amplification were 5′-GAT ACT TTG TTA GGG AAG ACT GCC-3′ and 5′-GGA TCA CCT CTC AAT GGG TC-3′. PCR was performed in a 25 μl reaction mixture with initial denaturation for 5 min at 95°C. This was followed by 31 cycles of amplification for 30 s at 95°C annealing at 55°C for 30 s, extension at 72°C for 60 s, and extension at 72°C with Goldstar Taq polymerase (Eurogentec). After digestion of 15 μl of PCR mix with *DraI* (2 h at 37°C) PCR products were separated by electrophoresis on a 2% agarose gel.

Statistical analyses

Continuous variables are expressed as means $\pm$ SD. Comparisons between groups were performed with independent samples *t*-tests and χ^2 -tests for normally distributed continuous and categorical variables, respectively. ANOVA was used for comparison of continuous variables between more than two groups. Logarithmic transformation was performed on variables that were not normally distributed (ie triglycerides were approximately normally distributed after transformation (*2)). These variables are expressed as medians and their minimum and maximum values. Triglyceride levels were adjusted for the use of fibrates and statins.

Differences in lipid parameters between genotypes were analyzed in the total population, as well as in HTG patients only. Allele frequencies were estimated by gene counting. Deviations from Hardy–Weinberg equilibrium were tested by means of χ^2 testing. For all analyses, statistical significance was considered at *P*-values <0.05.

Only polymorphisms that differed significantly in allele frequencies between patients and blood bank donors, or that significantly affected post-heparin activities and/or lipid parameters were included in subsequent analyses.

Linear regression models were constructed to estimate the influence of age, sex, environmental factors (BMI, alcohol consumption, diabetes mellitus, insulin use, smoking, use of statins, fibrates, or β -blockers) and genetic factors on LPL and HL activities. The percentage explained variance (R^2) was calculated in models with and without LPL and LIPC polymorphisms.

To calculate the risk of the presence or absence of an *LPL* or *LIPC* polymorphism associated with a certain range of post-heparin plasma lipase activity, we created a binary logistic regression model. We divided the post-heparin plasma lipase activities into tertiles. As men and women have different ranges of post-heparin lipase activities, the tertiles were calculated separately for men and women. The presence or absence of a polymorphism was the dependent variable. The tertiles were entered as categorical variables, with the lowest tertile as the reference category. In addition, we calculated the overall *P*-value (for trend) by entering the tertiles as continuous variables. Age, BMI, and alcohol use (units per day) for *LPL* polymorphisms and age, BMI and fibrate treatment for the *LIPC* polymorphism, were entered as independent variables. This resulted in odds ratios for having a polymorphism in sex-specific tertiles of post-heparin lipase activity, corrected for covariates.

For our primary analysis, receiver operator characteristic (ROC) curves were constructed by entering post-heparin LPL and HL activities as test variables and the presence or absence of a polymorphism as state variable. We did not compare diagnostic tests and therefore restricted our analyses of the ROC curve its area under the curve (AUC) (1*). An AUC of 0.5 means total absence of discriminative

ability, whereas 1.0 indicates perfect discrimination. For post-heparin LPL activities, ROC curves were made for the D9N, N291S, and S447X polymorphisms. In addition, a ROC curve was constructed for these three polymorphisms together. As 9N and 291S variant alleles are thought to reduce plasma lipase activity, whereas the 447X allele is known for its gain of function effect, we reverse-coded the S447X genotypes for analyses in the combined ROC curve. If a person carried a 9N, 291S, or 447S allele, this person was scored 1. Absence of all of these alleles was scored 0. In the *LIPC* gene, only the –514CT promoter polymorphism was used for ROC curve construction. AUCs were calculated for LPL and HL activities without adjustment for covariates. Subsequently, factors that significantly influenced variation in post-heparin lipase activities in the linear regression models (age, sex, alcohol intake, and the use of fibrates) were included in the model to calculate standardized residuals. Standardized residuals from a linear regression model, with covariates that significantly affect post-heparin lipase activities, reflect the remaining deviation of each person from the regression line. These residuals can therefore be considered to reflect the remaining variation in LPL and HL activities after correction for covariates. To calculate AUCs, we constructed ROC curves adjusted for these covariates by inserting the standardized residuals of each linear regression model as test variable and the presence or absence of a polymorphism as a state variable.¹⁷

SPSS 12.01 software was used for all statistical analyses.

Results

General characteristics

General characteristics of the patients are shown in Table 1. Of all the patients, 31.1% had a personal history of cardiovascular disease, 47.5% had a family history of cardiovascular disease, and 48.6% were known to have a family history of dyslipidemia. Ten patients (5.5%) had a history of pancreatitis. Diabetes was present in 10.4% of patients. On the day of post-heparin lipase testing, 26.2% of patients were using statins, fibrates, or both.

Allele frequencies

All allele frequencies were in Hardy–Weinberg equilibrium. The *LPL* 291S allele was significantly more frequent in patients than in blood bank donors (*P*=0.007, Table 2). In the patient group, we observed significantly less carriers of the *LPL* 447X allele (*P*=0.003). There were no significant differences in allele frequencies of the other polymorphisms between patients and blood bank donors.

Laboratory values

Table 3 shows plasma lipids and LPL and HL activities according to genotype in the total patient population.

Table 1 General characteristics of 183 patients of the lipid clinic

Variables	Lipid clinic patients (n = 183)
Age (years)	46.1 ± 10.7
Sex (M/F) (%)	72/28
BMI (kg/m ²)	27.4 ± 5.2
Presence of diabetes mellitus (%)	10.4
Personal history of CVD (%)	31.1
Family history of CVD (%)	47.5
Family history of dyslipidemia (%)	48.6
Use of statin or fibrate therapy (%)	26.2
Statin use (%)	16.2
Fibrate use (%)	6.1
Statin and fibrate use (%)	3.9
Use of β-blocking agents (%)	26.8
Smoking (%)	35.5
Total cholesterol (mmol/l)	6.98 ± 3.1
Triglycerides (mmol/l)	7.87 (0.44–63.0)
HDL-cholesterol (mmol/l)	0.98 ± 0.6
LDL-cholesterol (mmol/l)	3.32 ± 1.6
Post-heparin lipoprotein lipase activity (U/ml)	119 ± 68
Post-heparin hepatic lipase activity (U/ml)	428 ± 177

Data are expressed as means ± SD. Triglycerides are expressed as median, minimum–maximum between brackets.

Table 2 Minor allele frequencies compared between lipid clinic patients and controls (*4)

Gene	Polymorphism	% Patients (n carriers)	% Controls (n carriers)	P-value
LPL	D9N	2.7 (10)	1.8 (7)	0.41
	N291S	7.6 (28)	3.0 (10)	0.007
	PvuII	44.8 (129)	50.3 (128)	0.15
	HindIII	19.3 (61)	24.4 (76)	0.1
	S447X	4.6 (17)	10.4 (36)	0.003
LIPC	–514CT	21.7 (72)	22.4 (64)	0.84
	V73M	2.2 (8)	4.6 (14)	0.08
	V133V	39.4 (118)	43.1 (119)	0.33
	N193S	36.7 (114)	35.5 (101)	0.76

Carriers of the *LPL* 9N allele had significantly higher plasma triglyceride levels than non-carriers. Plasma HDL-cholesterol levels were not different between the genotype groups. The mean LPL activity was lower in 9N carriers, but this did not reach statistical significance. The 291S allele did not show a significant association with plasma HDL-cholesterol, triglycerides, or plasma post-heparin LPL activity in the total population. The carriers of the 447X allele had significantly lower plasma triglyceride levels and higher plasma HDL-cholesterol levels, than non-carriers. In addition, 447X carriers had significantly higher plasma LPL activities. In the *LIPC* gene, only the –514T allele showed a significant association with plasma HL activity.

The highest plasma HL activities were found in carriers of the –514T allele. There were no differences in plasma lipids between carriers and non-carriers of this allele. None of the other genotyped polymorphisms in the *LPL* and *LIPC* genes showed a significant association with lipid levels and lipase activities (Supplementary information 1).

We also performed analyses restricted to the 128 patients with HTG (Supplementary information 2). The association with triglyceride levels originally observed in carriers of the LPL 9N allele in the whole population was borderline significant ($P=0.07$). The 291S allele was associated with significantly lower plasma HDL levels (0.85 vs 0.67 mmol/l; $P=0.01$) in patients with HTG.

Seven out of the 17 LPL 447X carriers had HTG. Despite normal to high-plasma LPL activities, these carriers had severe dyslipidemia with mean triglyceride levels of 9.5 ± 3 mmol/l. Although none of the differences between HTG and normotriglyceridemic (NTG) carriers of the 447X allele were statistically significant, the HTG patients tended to be more overweight (26.3 vs 24.9 kg/m²), more often to be smokers (4/7 HTG patients vs 1/10 NTG patients), more often suffering from type 2 diabetes (2/7 HTG patients vs 0/10 NTG patients). (Supplementary information 3).

Linear regression models

Linear regression models showed that post-heparin LPL activity was significantly influenced by age and alcohol consumption. The percentage of explained variance was 17.1% in the model without genetic factors, which increased to 24% after adding the D9N, N291S, and S447X polymorphisms. Post-heparin HL activity was significantly influenced by sex and fibrate treatment. The explained variance was 25.5% before, and 30.8% after adding the –514CT polymorphism to the model.

Logistic regression models

Table 4 shows the odds ratios for the presence of a polymorphism associated with the different LPL and HL activity tertiles. The D9N and N291S polymorphisms could not be predicted based on LPL tertiles. After adjustment for covariates, the upper tertile showed a significant odds ratio compared with the lowest tertile for the 447X allele (adjusted P -value for trend: 0.03, without adjustment P -value for trend: 0.1). The HL activity tertiles significantly predicted the presence of the *LIPC*-514T allele (P -value for trend; 0.002).

Except for the S447X polymorphism, all results were similar with and without adjustment for covariates.

ROC curves

Figure 1 shows uncorrected and corrected ROC curves. In the corrected ROC curves age, sex, BMI, and alcohol consumption were included for LPL activity, and age, sex, BMI, and fibrate treatment were included for HL activity.

Table 3 Lipid profile and lipase activities compared between genotypes in all lipid clinic patients

Gene	Polymorphism	Genotype (n)	Total cholesterol	Triglycerides ^a	HDL-cholesterol	Lipoprotein lipase activity	Hepatic lipase activity
LPL	D9N	GG (173)	6.95 ± 3.2	8.0 ± 9.5	0.99 ± 0.63	120 ± 69	
		GA (10)	7.6 ± 1.8	14.5 ± 7.1	0.77 ± 0.28	100 ± 46	
	N291S	AA (155)	6.96 ± 3.1	8.0 ± 9.1	1.02 ± 0.62	117 ± 63	
		AG (28)	7.2 ± 3.5	10.1 ± 11.7	0.79 ± 0.42	129 ± 91	
	S447X	CC (166)	7.0 ± 3.2	8.8 ± 9.7	0.95 ± 0.54	115 ± 63	
		CG (17)	6.5 ± 2.8	4.6 ± 6.4	1.3 ± 0.93	156 ± 100	
LIPC	-514CT		<i>P</i> =0.53	<i>P</i> =0.004	<i>P</i> =0.31	<i>P</i> =0.36	
			<i>P</i> =0.73	<i>P</i> =0.19	<i>P</i> =0.08	<i>P</i> =0.39	
			<i>P</i> =0.49	<i>P</i> =0.004	<i>P</i> =0.02	<i>P</i> =0.02	
			<i>P</i> =0.06	<i>P</i> =0.19	<i>P</i> =1.0		
							461 ± 168
							384 ± 186
							328 ± 151
							<i>P</i> =0.005

^aCorrected for statin and fibrate therapy. *P*-value calculated after logarithmic transformation.**Table 4** Odds ratios for the presence of an LPL or LIPC polymorphism according to lipase activity tertiles

Gene	Polymorphism	Tertile		Odds ratio	95% CI	<i>P</i> -values for trend
		Men	Women			
LPL	Combined: D9N, N291S.	0–86	0–84	1.0	Reference	
		87–134	85–118	0.69	0.26–1.81	
		134–Highest	119–Highest	0.76	0.30–1.94	0.54
LPL	S447X	0–86	0–84	1.0	Reference	
		87–134	85–118	4.5	0.84–24.7	
		134–Highest	119–Highest	6.4	1.19–34.9	0.03
LIPC	-514CT	0–395	0–253	1.0	Reference	
		396–544	254–359	0.60	0.29–1.25	
		545–Highest	360–Highest	0.27	0.12–0.61	0.002

LPL corrected for age, BMI and alcohol consumption; LIPC corrected for age, BMI and fibrate treatment.

Figure 1 ROC curves for the LPL D9N, S447X, and LIPC-514CT polymorphisms adjusted and unadjusted for covariates.

The ROC curve for the D9N polymorphism had an unadjusted AUC of 0.58 and adjusted AUC of 0.64. For the N291S polymorphism the unadjusted AUC was 0.46

and adjusted AUC was 0.48. (Figures not shown) The uncorrected ROC curve for the LPL S447X polymorphism had an AUC of 0.62, which increased to 0.69 after

adjustment for covariates. Combination of these three LPL polymorphisms resulted in adjusted and unadjusted curves identical to the curves for the S447X polymorphism alone.

The ROC curves constructed for the LIPC-514CT polymorphism showed an unadjusted AUC of 0.65 that increased to 0.66 after adjustment for covariates.

Discussion

In this study, we found that post-heparin LPL and HL activity measurements associate with the presence of the LPL S447X and LIPC-514CT polymorphisms. LPL activity lowering polymorphisms (D9N, N291S) did not show significant associations with LPL activity tertiles. Nonetheless, ROC curve analyses showed low AUCs, for all polymorphisms, indicating that the test has limited diagnostic value for the identification of carriers and non-carriers in clinical practice.

Post-heparin plasma LPL and HL activity measurements are performed when a genetic cause of HTG or low HDL-cholesterol is suspected, to anticipate the response to treatment. Familial LPL and LIPC deficiency are rare disorders that are caused by homozygosity or compound heterozygosity for mutations that result in complete loss of function.^{18,19} We have excluded these patients from the present analyses. More subtle changes in post-heparin plasma lipase activities are expected in carriers of common, less detrimental, genetic variation that leads to less severe dyslipidemic phenotypes. In clinical practice, subnormal levels of post-heparin lipase activities are often referred to as partial lipase deficiencies.¹⁴

The patients in our study were recruited from our tertiary outpatient clinic and selected towards severe dyslipidemia. The nature of the dyslipidemic phenotypes in our population was heterogeneous, which introduced large variance of post-heparin lipase values, as is usually is observed when using these tests in clinical practice. By genotyping the nine common genetic variants in the LPL and LIPC lipase genes, we tested the majority of known functional variants in these genes. To our knowledge, this is the first study to investigate the discriminative ability of post-heparin lipase testing in the detection of genetic variants in the LPL and LIPC genes in clinical practice.

Our study population was unfortunately too small to study gene–environment interactions, which have been described for a number of the selected polymorphisms.^{20–23} For instance, the effects of the D9N and N291S polymorphisms become more apparent when the lipolytic system is challenged by environmental factors, such as high BMI and insulin resistance. However, the small effect sizes of the interactions described so far may not substantially influence diagnostic accuracy. Nonetheless, diagnostic value might improve when taking these interactions into account. Another possible limitation of our study is the number of polymorphisms studied. We have genotyped

nine polymorphisms, four in the LIPC gene and five in the LPL gene. Especially the LPL gene is highly polymorphic.²⁴ Haplotype analyses would require a large number of tag SNP's and a larger study population. Large populations with post-heparin lipase measurements are scarce. We cannot exclude that other, to date unknown, variants in these genes exert significant effects on LPL and HL function. We analyzed the currently known potentially functional genetic variants.

As expected, in our study the 9N and 291S alleles were more frequent, whereas the 447X allele was significantly less frequent in dyslipidemic patients than in control subjects. Remarkably, the 447X allele was present in seven patients with severe HTG. This clearly indicates that other factors, can overrule its beneficial effects. However, overall, our findings are largely in agreement with meta-analyses on the effects of these polymorphisms on plasma lipids and post-heparin lipase activities.^{11–13,23}

Logistic regression models of lipase activity tertiles showed significant odds for predicting the presence of the LPL S447X and LIPC-514CT polymorphisms, whereas the tertiles did not significantly associate with LPL activity lowering polymorphisms. Despite these findings, the discriminative ability of the LPL and HL activity measurements, assessed by ROC curve analyses, was low for all polymorphisms. In a study on the effects of the N291S polymorphism in normolipidemic subjects, Mero *et al*²⁵ drew a similar conclusion, being unable to distinguish carrier status by post-heparin LPL activity measurements, because of the wide range of activities in both carriers and non-carriers of this polymorphism.

There are a number of possible explanations for our findings. First, post-heparin plasma lipase testing may not accurately reflect *in vivo* LPL and HL function. Post-heparin lipase activity measurement is based on the concept that heparin released the active lipase proteins from their proteoglycan-bound sites at the endothelium, reflecting the total putative *in vivo* catalytic activity at a certain time point. However, it does not reflect the actual availability required under general and pathophysiological circumstances.²⁶ Furthermore, non-catalytic ligand and bridging functions of LPL and HL are not reflected by post-heparin lipase activity measurements.^{27,28} If polymorphisms affect non-catalytic functions of the enzymes, we will not observe this by measuring post-heparin lipase activities. Second, lipase activities can be affected by other factors, such as age, sex, BMI, smoking, alcohol consumption, insulin resistance, use of medication,^{8,29–32} and proteins such as ApoCII, ApoCIII, AngPTL3, and LMF1.^{33–36} We adjusted for covariates that had a significant effect on post-heparin lipase activities. BMI was included as a covariate, because it is frequently described as a factor influencing post-heparin plasma lipase activities. Correction for covariates resulted in an increase in AUCs, thereby increasing the diagnostic accuracy of the test. AUCs, nonetheless,

remained below 0.7. Third, there might be other, to date unknown, gene variants affecting LPL and HL activity. Adding these molecular variants to the analyses and performing haplotype analyses might further improve the diagnostic value. However, whether adding additional covariates and genetic variants to the models will substantially improve the diagnostic value is difficult to predict.

In conclusion, we have shown that, although certain polymorphisms associate with post-heparin lipase activities, the tests are unable to accurately identify carriers of well-known common variants in the *LPL* and *LIPC* genes in a dyslipidemic population. This indicates that conclusions on the genetic causes of lipase activities outside of the normal range should be drawn with caution.

Disclosure

The authors have nothing to disclose.

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