Genetic association of *LPL* rs326 with BMI among the Kuwaiti population

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Lipoprotein lipase is a key enzyme in lipid metabolism with reported variants associated with obesity, hypertension, type 2 diabetes, and coronary heart disease. This study was performed to investigate the association between common lipoprotein lipase single nucleotide polymorphisms and metabolic disorders in a sample of Kuwaiti cohort (n = 494). Five lipoprotein lipase variants (rs1801177, rs295, rs326, ss2137497749, and ss2137497750) across the lipoprotein lipase gene were genotyped by real-time PCR employing the TaqMan allele discrimination assay. Genotype, allelic frequencies, and Hardy-Weinberg Equilibrium were determined for each variant in the cohort followed by multivariate and logistic regression analysis. A novel finding was observed for the G allele of single nucleotide polymorphism rs326 which was associated with increased BMI after adjusting for age and sex (β = 1.04; 95% confidence interval = 0.15–1.94; *P* = 0.02). Moreover, a significant difference in the distribution of the minor C allele of rs295 among coronary heart disease subjects compared with noncoronary heart disease, however, this significance was diminished after controlling for age, sex, and BMI. This study demonstrated that lipoprotein lipase rs326 may be indicative for the increased risk of obesity and possibly rs295 for coronary heart disease. The findings are also in agreement with other reports suggesting that intronic variants are important genetic markers in association studies. The findings warrant further studies in a large cohort to confirm and validate the results presented. *Cardiovasc Endocrinol Metab* 10: 215–221 Copyright © 2021 The Author(s). Published by Wolters Kluwer Health, Inc.

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Introduction

The *LPL* gene, encoding the lipoprotein lipase enzyme, is approximately 30 kilobases pairs (kb) in length and is localized on chromosome 8p22. It consists of 10 exons and 9 introns where exons 1 to 9 have an average size of 105–276 bp in contrast to the length of exon 10 which is 1948 bp encoding the entire 3′-untranslated region [1]. Exon 1 encodes the 5′-untranslated region, the signal peptide plus the first two amino acids of the mature protein while exons 2–9 encode the remaining 446 amino acids [2]. *LPL* belongs to the lipase gene family, which includes pancreatic lipase, hepatic lipase, and endothelial lipase [3]. The lipoprotein lipase (LPL) enzyme is expressed in the adipose tissue, cardiac, skeletal muscles and attached to endothelium of the blood capillaries, however, less amount of LPL is synthesized in the brain, adrenal, kidney, and macrophages [4]. LPL is an enzyme, that is, activated by Apo C-II and Apo A-V while it is inhibited by Apo C-III and Apo A-II. LPL catalytic activity is essential in the breakdown of TGs carried in CMs and VLDL to form free fatty acid [3]. Many common single nucleotide polymorphisms (SNPs) and other functional variants at the *LPL* gene locus have been identified [5,6]. Mutations in the *LPL* gene may result in disrupting the normal hydrolysis of TG in the body that may lead to increased levels of lipids. Previous study mentioned that a decrease in LPL activity leads to elevated TG in the plasma and decreases the HDL-C which results in atherosclerosis and coronary heart disease (CHD) [7].

It has been reported that CHD is the major cause of morbidity and mortality [8]. Both genetic and environmental factors can contribute to the development of CHD in various degrees [9]. Numerous variants at many gene loci are considered as genetic factors contributing to the increased risk of CHD [10–12]. Major CHD risk factors are due to abnormal lipid levels, and about 50% of the abnormalities of lipid levels are related to genetic factors [13]. CHD can be described as a failure of blood supply to reach the heart muscles. Risk factors for CHD have been well documented with strong implications on the role of high blood pressure, high blood cholesterol,

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smoking, diabetes, obesity, and lack of exercise [14]. Therefore, CHD can be considered a multifactorial disease, and some of the risk factors can be controlled. Obesity, a risk factor of CHD, is associated with increased morbidity and mortality [15]. In 2014, the prevalence of obesity in the adult Kuwaiti population was measured as 40.3% (men, 36.5% and women, 44.0%) and overweight was measured as 37% [16]. It has also been reported that the adult Kuwaiti population prevalence of metabolic syndrome was 36.2% [17]. A previous study has reported that malfunction disorders of the *LPL* gene can lead to obesity [18,19].

Several studies have reported a significant association between *LPL* gene polymorphisms and lipids profile [20–22]. The rationale behind this study is that variants at the *LPL* gene locus can affect the transport and clearance of TG and HDL and LDL levels which are known to contribute to the increased risk to develop CHD. Therefore, we aimed to investigate the association of common *LPL* polymorphisms and the risk of obesity and CHD among a cohort of the Kuwaiti population.

Subjects and methods

A total of 494 Kuwaiti nationals' DNA samples 286 CHD (58%) and 208 non-CHD (42%) that had been recruited from various government hospitals and clinics in Kuwait during the period 2007–2014. This cohort included 494 Kuwaiti nationals (Table 1), of whom were 158 females (32%) and were 336 males (68%). The mean age of all participants was 50. The subjects were divided into two binary categories based on their BMI (kg/m^2), the normal weight samples (BMI ≤ 25) which represented 24% while the overweight/obese samples (BMI ≥ 25) represented 76%. Only 349 of the included participants had their lipid profile on record provided in Supplementary Table 1, Supplemental digital content 1, http://links.lww.com/CAEN/A31. The lipid profile levels were expressed in range, median and mean ± SD for total cholesterol, TG, LDL-C, HDL-C, and VLDL. A standard questionnaire along with the most recent lipid profile had collected (routine test done at the biochemistry lab in the hospital), medical history and family history. The inclusion criteria was Kuwaiti patients confirmed with CHD by clinical diagnosis which was provided from the medical records and based on medical history of the presence of typical chest pain, echocardiogram, and previous history of myocardial infarction, coronary angioplasty, and Kuwaiti volunteers confirmed to be devoid of CHD by clinical examination. For the association study with BMI, the inclusion criteria was Kuwaiti individuals who do not present any clinical signs of other metabolic disorders nor CHD. Demographic description of the samples is provided in Supplementary Table 1, Supplemental digital content 1, http://links.lww.com/CAEN/A31. Informed consent was obtained from each participant and in accordance with the revised Helsinki guidelines of 1975. Ethical approval with the reference number (VDR/JC/256) for the collection of samples has been granted by Kuwait Ministry of Health Ethical Committee.

Table 1 Demographic data of the Kuwaiti samples analyzed in this study (n = 494)

Variables	All participant (n = 494)
Age (y) ± SD	49.83 ± 13.01
Subjects (female)	158 (32%)
Mean BMI ± SD	29.72 ± 7.21
BMI ≤ 25	118 (24%)
BMI ≥ 25	376 (76%)
Non-CHD	208 (48%)
CHD	286 (58%)
HT	140 (49%)
T2DM	124 (43%)

BMI (kg/m^2); BMI ≤ 25, normal weight samples; BMI ≥ 25, overweight/obese samples. If percentages are not indicated, the values given indicate mean ± SD. CHD, coronary heart disease; HT, hypertension; T2DM, type 2 diabetes.

Total genomic DNA samples were extracted using the salt extraction method by Miller *et al.* [23]. The final volume of extracted DNA was aliquoted in 1 ml and has been stored at –20° in the established DNA bank. Quality assurance has been done for the genotypes by random selection of different samples in which Sanger sequencing was done to confirm the obtained genotypes. Subsequently, these samples were used as positive controls in further real-time PCR runs. For each DNA sample quality assurance, both NanoDrop Spectrophotometers quantitative analysis and gel electrophoresis qualitative analysis were done to ensure the suitability of the DNA for genotyping using real-time PCR. Allelic discrimination was performed and analyzed using 3130xl Genetic Analyzer, ViiA7 fast real-time PCR system QuantStudio software. The protocol of the real-time PCR [24] was followed according to the manufacturer's instructions (PE Applied Biosystems, Foster City, California). A total of 5 variants including intronic and exonic SNPs spanning across the *LPL* region were selected based on having a minor allele frequency (MAF) of > 0.05, previously reported for association with CHD, and representing different regions across the gene locus. In addition, the two novel variants reported by Al-Bustan *et al.*, 2018, were included in Table 2. The position of the targeted variants at the *LPL* gene including the introns and exons are demonstrated (see Fig. 1). The primers/probes are provided in Supplementary Table 2, Supplemental digital content 1, http://links.lww.com/CAEN/A31 used for genotyping each variant were either commercially available (common reported variants) or custom designed (novel variants). The primer sequences of the five variants at the *LPL* gene locus are provided in Supplementary Table 2, Supplemental digital content 1, http://links.lww.com/CAEN/A31.

Statistical analysis

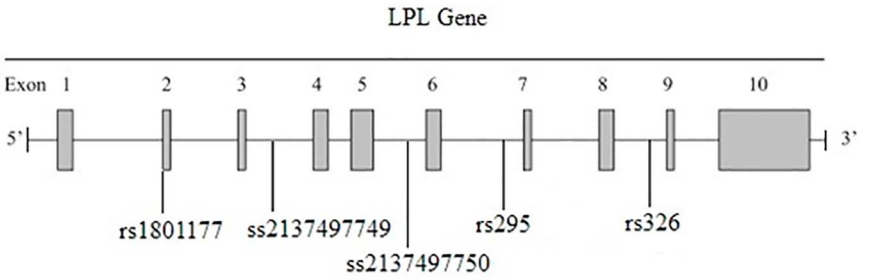
Allele and genotype frequencies were determined by the simple gene-counting method for all the five variants at the *LPL* gene locus in the 494 samples. The Hardy-Weinberg

Table 2 The *LPL* variants summary

Variants	Alleles	Gene location	Gene position	Global MAF	Kuwait MAF
rs1801177	G>A	Exon 2	14127	A:0.0176	A:0.018
rs295	A>C	Intron 6	24657	C:0.2746	C:0.222
rs326	A>G	Intron 8	27858	G:0.3494	G:0.354
ss2137497749	C>A	Intron 3	18704	NA	A:0.009
ss2137497750	C>T	Intron 5	21426	NA	T:0.017

MAF, minor allele frequency; NA, not available.

Fig. 1



The position of the targeted variants at the *LPL* gene including the introns and exons.

Equilibrium was estimated with the chi-squared goodness of fit test by comparing the observed genotype frequencies to the expected in the total studied population for all the *LPL* gene variants genotyped with a set statistical significance level of $P < 0.05$ using GENEPOP software (Version 4.0.10). Variants with MAF above 5% were selected for further statistical analysis to investigate the genetic association with obesity and CHD. All the statistical analysis was performed using IBM SPSS software (version 24; SPSS Inc, Chicago, Illinois). The means of the genotype distribution for the *LPL* variants were compared with continuous BMI (kg/m^2) by using one-way analysis of variance, the results were expressed as mean ± SD. Linear regression was used to assess the association between the *LPL* variants and BMI as continues variable represented by the β-coefficient and 95% confidence interval (CI) after adjusting for both age and sex. The association of the *LPL* variants and CHD was assessed by binary logistic regression represented as odds ratio and 95% CI after adjusting for age, sex, and BMI. Statistical significance was considered at a $P < 0.05$ for all the following tests performed.

Results

Population genotypes for the *LPL* variants

The genotypes for the five variants in the cohort were determined based on the generated amplification plots for each variant (see Supplementary Figures S1–S5, Supplemental digital content 2, http://links.lww.com/CAEN/A32). The genotype frequency distributions for the five *LPL* variants are shown in Table 3. The homozygous genotypes for the minor alleles of the "rare" variants were confirmed by Sanger sequencing for quality assurance. This cohort included 158 females (32%) and 336 males (68%) with a mean age of 50.

Table 3 Genotypic and allelic frequencies of the identified genetic variants of the *LPL* gene in Kuwait samples (n = 494)

Genetic variants	Genotypes and alleles	*f* (n)	HWE (*P* value)
rs1801177	GG	0.966 (477)	0.697
	GA	0.034 (17)	
	AA	0	
	G	0.983 (971)	
	A	0.017 (17)	
rs295	AA	0.626 (309)	0.081
	AC	0.314 (155)	
	CC	0.061 (30)	
	A	0.782 (773)	
	C	0.218 (215)	
rs326	AA	0.423 (209)	0.180
	AG	0.433 (214)	
	GG	0.144 (71)	
	A	0.640 (632)	
	G	0.360 (356)	
ss2137497749	CC	0.984 (486)	0.856
	CA	0.016 (8)	
	AA	0	
	C	0.992 (980)	
	A	0.008 (8)	
ss2137497750	CC	0.968 (478)	0.714
	CT	0.032 (16)	
	TT	0	
	C	0.984 (972)	
	T	0.016 (16)	

The significance of the HWE results is indicated by *P* value.
HWE, Hardy-Weinberg equilibrium.

The investigated *LPL* SNPs (rs1801177, rs295, rs326, ss2137497749, and ss2137497750) were all found to be in Hardy-Weinberg equilibrium ($P > 0.05$) in the cohort (n = 494). Two variants (rs295 A>C and rs326 A>G) had MAF ≥ 0.05 and two (rs1801177 G>A and ss2137497750 C>T)

had MAF ≤ 0.05 while one variant (ss2137497749 C>A) had MAF ≤ 0.01 (Table 3). The "common" and the novel *LPL* variants reported by [25] were subjected to further statistical analysis to assess their effect on obesity and CHD.

Association of *LPL* variants and coronary heart disease

The association of the selected *LPL* variants (MAF > 0.01) with the risk to develop CHD were analyzed using the Pearson χ^2 test by comparing the genotype distribution (Table 4). A χ^2 test showed a statistical significance in the distribution of the *LPL* rs295 variant between both CHD and non-CHD categories (P = 0.001; Table 4). However, the distribution of CC genotype of the rs295 was found to be higher (10.1%) in the non-CHD group compared with CHD group (3.1%). The AA genotype distribution of the rs295 variant was found to be significantly (P = 0.001) higher in the non-CHD group (64.4%) compared with CHD group (61.2%). The differences in the genotype distribution of the other three variants (rs1801177, rs326, and ss2137497750) were found insignificant (P > 0.05). In the studied cohort, the genotype distribution for homozygous wild-type of the three insignificant variants was found to be similar between CHD and non-CHD samples while no homozygous minor genotypes were observed for rs1801177 (Table 4).

Logistic regression analysis was done to estimate the effect on the dependent variable CHD with the independent risk factors or the predictor variables (SNPs, age, sex, and BMI) which provided in Supplementary Table 3, Supplemental digital content 1, http://links.lww.com/CAEN/A31. The significant association of rs295 with CHD was diminished (P > 0.05) after adjusting for age, sex, and BMI (Supplementary Table 3, Supplemental digital content 1, http://links.lww.com/CAEN/A31). Moreover, logistic regression for the other SNPs did not reveal any significant association with CHD (Supplementary Table 3, Supplemental digital content 1, http://links.lww.com/CAEN/A31). The analysis, however, revealed age and BMI to be significant (P < 0.05) predictor variables for the risk of CHD. BMI was found to be a highly significant predictor variable associated with CHD (odds ratio = 0.96; 95% CI = 0.94–0.99; P = 0.01) (Supplementary Table 3, Supplemental digital content 1, http://links.lww.com/CAEN/A31). The mean BMI in the females (31.44 ± 8.11) was higher than the mean of the males (28.92 ± 6.60). In contrast, the regression analysis showed age to be a significant (P < 0.001) predictor variable to with the risk of CHD higher in males than in females (Supplementary Table 3, Supplemental digital content 1, http://links.lww.com/CAEN/A31). The mean age of CHD females (48.58 years ± 11.2) was lower than the mean of males (50.42 years ± 13.01).

Association between *LPL* variants and BMI

The means of genotype distribution were compared for the selected *LPL* variants with BMI using analysis of variance. In the total number samples (n = 494) analyzed, SNPs rs1801177, ss2137497749, and ss2137497750 showed no significant association (P > 0.05) with BMI (Table 5). However, two of the variants (rs295 and rs326) showed a nonsignificant trend with BMI. The CC genotype for rs295 showed a possible association (P = 0.21) for a higher BMI value of (BMI: 31.98 ± 9.96) when compared to the AC genotype (BMI: 29.69 ± 8.15) and the AA genotype (BMI: 29.52 ± 6.33). Similarly, the GG genotype for rs326 showed a possible association (P = 0.08) for a higher BMI (BMI: 31.40 ± 10.1) when compared to the AG genotype (BMI: 29.71 ± 6.95) and the AA genotype (BMI: 29.17 ± 6.20) (Table 5).

Table 4 Genotype distribution of the *LPL* variants in the coronary heart disease patients using crosstab

	CHD		
SNP	Yes, n (%)	No, n (%)	*P* value
rs1801177			0.35
GG	278 (97.2%)	199 (95.7%)	
GA	8 (2.8%)	9 (4.3%)	
AA	0	0	
rs295			0.001
AA	175 (61.2%)	134 (64.4%)	
AC	102 (35.7%)	53 (25.5%)	
CC	9 (3.1%)	21 (10.1%)	
rs326			0.05
AA	112 (39.2%)	97 (46.6%)	
AG	137 (47.9%)	77 (37%)	
GG	37 (12.9%)	34 (16.3%)	
ss2137497750			0.09
CC	280 (97.9%)	198 (95.2%)	
CT	6 (2.1%)	10 (4.8%)	
TT	0	0	

CHD, coronary heart disease; SNP, single nucleotide polymorphism.

Assessing the potential effect of rs295 and rs326 on BMI using linear regression

Further analysis using linear regression was used to evaluate the relationship between the selected *LPL* SNPs and BMI after adjusting for age and sex. The variant rs326 was found to be associated with BMI (Table 6). The G allele of the variant rs326 was found to be associated with increased BMI (Table 5) after adjusting for age and sex with a β-coefficient of 1.04 (95% CI = 0.15–1.94; P = 0.02) (Table 6). No further association was observed between the remaining SNPs (rs1801177, rs295, ss2137497749, and ss2137497750) and BMI after adjusting for age and sex (P > 0.05). The regression analysis revealed that sex was the only predictor variable to be associated with BMI (P < 0.001) in the studied cohort (n = 494). The mean of BMI in females (31.44 ± 8.11) was significantly higher than that for males (28.92 ± 6.60).

Discussion

LPL is a key enzyme involved in lipid metabolism that has been found associated with obesity, dyslipidemias, hypertension, type 2 diabetes, and CHD [26]. In this present study, the association of *LPL* variants was analyzed in relation to CHD and BMI in a subset of Kuwaiti subjects.

Common variants were selected from previous studies based on their association with cardiometabolic traits [20,27,28] for replication in a cohort of the Kuwaiti Arab population in addition to the novel variants at the *LPL* gene loci discovered by Al-Bustan *et al.* [25].

Association between *LPL* variants and BMI

A positive association was observed between *LPL* rs326 gene polymorphism and BMI. The G allele of the variant rs326 was associated with increased BMI after adjusting for age and sex. Our finding is consistent with other studies demonstrating an association between *LPL* variants and obesity [25,29]. Although no studies have previously reported an association between rs326 and obesity, other studies have reported an association between *LPL* variants and obesity including a promoter variant –93 T<G (rs1800590) in the *LPL* gene with obesity [25] two promoter region variants of the *LPL* gene (–T93G and –G53C) with obesity in a cohort of 1350 [30] and a variant of rs320 with higher BMI in obese subjects [29].

The significant association of rs326 further supports a potential role for intronic variants at the LPL gene locus to contribute to the development of obesity. Functional analysis of *LPL* showed that it is expressed in multiple tissues based on knockout experiments is specific targeted tissues. LPL is an important multifunctional enzyme secreted from various tissues such as the skeletal muscles, adipose tissues, and cardiac muscles [4]. Furthermore, regulations of the *LPL* gene occur at different levels including transcriptional, posttranscriptional, and posttranslational levels and are tissue-specific. Hence, variation in the *LPL* gene sequence may influence, expression and regulation subsequently leading to comprised LPL function and increased risk to develop a metabolic disorder 18,31. Moreover, *LPL* knockout mice in skeletal muscles reduced TG in muscles and resulted in insulin resistance and obesity [19]. Such findings may suggest some explanation on how rs326 may lower *LPL* gene activity leading to increased TG levels that are subsequently stored in the adipose tissue [32]. In addition, Cruz *et al.* reported that abnormal *LPL* gene developed dyslipidemia that affected the regulation of the pancreatic β-cell function, β-cell apoptosis, and the insulin signal cascades, which induced insulin resistance and later cause type 2 diabetes [33]. In contrast, mice with overexpression of *LPL* in skeletal muscles have been demonstrated to have a protective role against overweight or obesity [34]. The studied population is known for their high-fat rich diets [35] so it may be possible that the association of rs326 with obesity is an outcome from such gene-environment interaction. There has been growing evidence showing the interaction between *LPL* variants and environmental factors (diet and physical activity) [21,36–38]. This is also supported by the findings from a study of Gao *et al.* who showed an interaction between *LPL* rs283 and exercise. They found that individuals with GG genotypes had higher physical activity than those compared with GA genotype ($P < 0.01$) [37].

Such gene-environmental interactions may suggest that our current findings in relation to obesity is limited to populations with similar environments supported by other studies [30,39] that reported similar findings of associated *LPL* variants with obesity. Asian and Middle Eastern diets include high amounts of carbohydrates and fat [40,41] and most people have a sedentary life-style. Thus, those factors could be interacting with *LPL* variants increasing the risk to develop obesity. In the same region different genetic variants (FTO gene) found to be associated with risk of overweight/obesity among the young UAE Arab population [42].

Table 5 Association of the *LPL* single nucleotide polymorphisms with BMI

SNP	BMI (mean ± SD)	*P* value
rs1801177		0.36
GG	29.67 ± 7.1	
GA	31.29 ± 10.12	
AA	0	
rs295		0.21
AA	29.52 ± 6.33	
AC	29.69 ± 8.15	
CC	31.98 ± 9.96	
rs326		0.08
AA	29.17 ± 6.20	
AG	29.71 ± 6.95	
GG	31.40 ± 10.1	
ss2137497749		0.72
CC	29.74 ± 7.18	
CA	28.83 ± 8.94	
AA	0	
ss2137497750		0.48
CC	29.68 ± 7.20	
CT	30.99 ± 7.52	
TT	0	

SNP, single nucleotide polymorphism.

Table 6 Linear regression model on the association of the *LPL* variants with BMI and cofactors

Variables	β (95% CI)	*P* value
rs1801177	1.77 (–1.68 to 5.17)	0.32
Age	0.03 (–0.01 to 0.09)	0.13
Sex	–2.60 (–3.95 to –1.25)	< 0.001
rs295	0.84 (–0.19 to 1.88)	0.11
Age	0.03 (–0.01 to 0.08)	0.12
Sex	–2.65 (–4.00 to –1.30)	< 0.001
rs326	1.04 (0.15 to 1.94)	0.02
Age	0.03 (–0.01 to 0.08)	0.15
Sex	–2.67 (–4.02 to –1.33)	< 0.001
ss2137497749	–1.32 (–6.32 to 3.66)	0.60
Age	0.03 (–0.01 to 0.08)	0.12
Sex	–2.60 (–3.95 to –1.25)	< 0.001
ss2137497750	1.62 (–1.93 to 5.18)	0.37
Age	0.03 (–0.01 to 0.08)	0.11
Sex	–2.60 (–3.95 to –1.25)	< 0.001

CI, confidence interval.

Association between *LPL* variants and coronary heart disease

Of the three *LPL* variants assessed for their association with CHD, a potentially positive association for the C allele distribution of rs295 was observed between CHD and non-CHD patients. However, the significance was diminished after controlling for confounders. Other studies have reported an association of rs295 with plasma lipid profile [20] and with metabolic syndrome [43], but similar to this study non have reported an association with CHD. However, it may be worthwhile to reinvestigate the association of rs295 in a bigger cohort to confirm if the initial observed association was by chance or related to interaction with other risk factors.

We did not find any associations with the novel variants ss2137497749 and ss2137497750 previously reported to have an association with lipid levels in a Kuwaiti cohort [25] with CHD. This could be due to the low MAF ≤ 0.05 observed in the cohort and thus larger sample sizes for association studies of these variants are needed.

The inconsistency between association studies reported above could be due to differences in frequencies of the studied variants between populations [44]. We, therefore, compared our variant frequencies with those reported in other populations. The observed frequencies for rs295 A/C were found to be closest to the American and East Asian MAF and much lower than that reported in Africans. Interestingly, the MAF for rs326 A/G was closest to Europeans and the global MAF and much lower than that of the African MAF. These findings suggest that the distribution of the allele frequency could be ethnic specific and any genetic association on such variants should be adjusted for ethnicity in logistic regression analysis. Furthermore, more data analysis is needed to investigate if the frequency of these are under any form of selection pressure.

Conclusion

This study reported an association between rs326 of the *LPL* gene with BMI yet failed to confirm the association of other selected *LPL* variants with the increased risk to develop CHD. Nonetheless, the findings from this study provide further support to the role of LPL in adiposity. The association between an *LPL* variants and obesity is consistent with other populations with similar environments suggesting that some variants are ethnic and population specific, especially since there are no previous reports on the association of rs326 with BMI. Limitations of this study include the sample size (under powered less than 80%), lack of premedication lipid profile measurements along with measurements of LPL activity to assess the role of our selected variants on *LPL*. Our findings bring us a step closer to understanding the mechanisms involved in the development of obesity. It is recommended that a functional analysis of rs326 in animal models or expression profiling in different tissues be performed to assess the role of rs326 in developing obesity.

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S.H.M. performed most of the experiments and data analysis as well as assisted in drafting the manuscript. A.E.A.-S. assisted with the study design data analysis, interpretation of the results and in revising and editing the manuscript. S.A.A.-B. conceptualized the project idea, planned the study design and prepared the project proposal, supervised the molecular genetic studies and phenotypic documentation, supervised the data analysis and interpretation, and assisted in drafting the manuscript. All the authors have read and approved the final manuscript.

Data availability: Raw genotypic data is available upon request for conducting meta-analysis. Any additional data also be made available upon request.

List of abbreviations: A list of abbreviations used in this manuscript are provided in a Supplementary file, Supplemental digital content 3, http://links.lww.com/CAEN/A33.

Conflicts of interest

There are no conflicts of interest.

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