Saudi Journal of Biological Sciences 28 (2021) 4717–4722

Contents lists available at ScienceDirect

Saudi Journal of Biological Sciences

journal homepage: www.sciencedirect.com

Original article

Association between the *lipoprotein lipase* rs1534649 gene polymorphism in intron one with Body Mass Index and High Density Lipoprotein-Cholesterol

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ARTICLE INFO

Article history:
Received 8 March 2021
Revised 24 April 2021
Accepted 26 April 2021
Available online 4 May 2021

Keywords:
Obesity
LPL
Kuwait
Polymorphism HDL
BMI

ABSTRACT

Lipoprotein lipase (LPL) is an enzyme involved in lipid metabolism and distribution of fatty acids hence its role in the initiation and development of dyslipidemia and adiposity. Single nucleotide polymorphisms (SNPs) across the *LPL* gene have been associated with dyslipidemia, however, the association with obesity has been limited towards specific populations. This study examined the association between *LPL* gene polymorphisms with plasma lipid levels and body mass index (BMI) in the Kuwaiti population. We examined a total of 486 adults (303 and 183 females and males respectively) with plasma lipid levels and BMI. DNA samples were genotyped for two *LPL* gene polymorphisms (rs1534649 and rs28645722) using TaqMan allelic discrimination. The relationship between the genotypes with both plasma lipid levels and BMI were assessed using linear regression using "SNPassoc" package from R statistical software. Using an additive genetic model, linear regression analysis showed the T-allele of rs1534649 to be associated with increased BMI in a dose-dependent trend $\beta = 2.13$ (95% CI 1.33–2.94); $p = 1.7 \times 10^{-7}$. In addition, a borderline significance was observed between the T-allele and low levels of high density lipoprotein-cholesterol $\beta = -0.04$ (95% CI −0.08, −0.006); $p = 0.02$. There were no associations between rs28645722 and plasma lipid levels ($p > 0.05$). However, a trend was observed between the A-allele and increased BMI $\beta = 1.75$ (95% CI 0.14–3.35); $p = 0.03$. Our study shows intron one polymorphism rs1534649 to increase the risk of obesity and dyslipidemia. Our findings warrant further investigation of the mechanism of LPL on the development of obesity along with the role of intron one and its impact on *LPL* gene activity.

1. Introduction

Obesity is a disease in which excessive accumulation of body fat results in health impairment. According to the world health organization the rate of obesity has tripled since 1975 with 50% of adults above 18 years of age are estimated to be overweight/obese (WHO, 2018). Moreover, Kuwait similar to its neighboring countries is an Arab state that is considered one of the most obese populations worldwide with an estimated prevalence of obesity reaching 50% (Ng et al. 2014). Although obesogenic environments contribute towards the increase in prevalence, however, heritability estimates suggest that 40–70% is due to genetic factors (Hill et al. 2003; Farooqi and O'Rahilly 2000). In addition, genome-

Abbreviations: LPL, Lipoprotein lipase; SNPs, Single nucleotide polymorphisms; BMI, body mass index; TG, triglycerides; VLDL-C, very low-density lipoproteins cholesterol; TC, total cholesterol, HDL-C, high-density lipoprotein cholesterol, LDL-C, low-density lipoprotein cholesterol; SD, standard deviation; HWE, Hardy and Weinberg Equilibrium; β, Beta-coefficient; CI, confidence intervals; glm, general linear model.

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Peer review under responsibility of King Saud University.

Production and hosting by Elsevier

https://doi.org/10.1016/j.sjbs.2021.04.085

wide association studies (GWAS) have identified genetic variants that increase ones' susceptibility to obesity (Locke et al. 2015). Such studies contribute towards understanding the mechanisms involved in the initiation and development of obesity.

Lipoprotein lipase (LPL) is the rate-limiting enzyme responsible for the hydrolysis of triglycerides (TG) in chylomicrons and very low-density lipoproteins cholesterol (VLDL-C) (Eckel 1989). General LPL-knockout (*lpl*-/-) experiments on mice revealed an increase in plasma TG and VLDL cholesterol levels (Weinstock et al. 1995). Whereas, mice with heterozygous (*lpl*+/-) deficiency have reduced expression of LPL and have shown an increase in fat mass/lean mass ratio developed over time (Chen et al. 2008). Although LPL is expressed in multiple tissues, including brain and central nervous system, heart, liver, adipose and skeletal muscle (Kirchgessner et al. 1989), its role in obesity is suggested as being tissue-specific (Garcia-Arcos et al. 2013; Wang and Eckel 2009)

The *LPL* gene encodes 448 amino acids and consists of 10 exons found on chromosome 8p22 (Wion et al. 1987). Single nucleotide polymorphisms (SNPs) in the *LPL* gene have been found to be associated with multiple phenotypes including coronary artery disease (Shahid et al. 2017; Bogari et al. 2020), dyslipidemia (Al-Bustan et al. 2018), Alzheimer's disease (Xu et al. 2013) and obesity (Radha et al. 2007; Prakash et al. 2018). A number of these studied polymorphisms have been located across the *LPL* gene around *cis*-acting regulatory elements in the promoter and 5 -untranslated regions which span the sequences of exon one and intron one which are known to impact *LPL* gene expression levels (Tanuma et al. 1995). In addition, recent studies sequencing the *LPL* gene have reported intron one to comprise the majority of uncommon variants (Pirim et al. 2014; Al-Bustan et al. 2018) suggesting the need to for further investigate the region. Moreover, a study by Hua et al., found intron one of the mouse *lpl* gene to comprise a response sequence element that is suggested to have a regulatory role and that the mouse *lpl* gene is mapped to a region on chromosome 8 that is homologous to that of humans (Hua et al. 1991). Due to the limited studies investigating variants in intron one of the *LPL* gene we therefore were interested in investigating common variants (minor allele frequency > 5%) within intron one in relation to plasma lipid traits and body mass index (BMI). Two SNPs in intron one were selected (rs1534649 and rs28645722). The SNP rs1534649 was previously reported to be associated with lipid traits (Liao et al. 2008) whereas rs28645722 (Tag SNP) was found to be in linkage disequilibrium (r^2 > 0.8) with 127 SNPs in the *LPL* gene (release 93 of 1000 genome project) (Zerbino et al. 2018).

The aim of this study is to assess the relationship between two SNPs within intron one in relation to plasma lipid levels and BMI in a Kuwaiti population.

2. Methods

2.1. Study subjects

The study included 486 adults all of whom were of Kuwaiti nationality. For each subject information regarding history of metabolic disorders, family history, smoking, medication, and BMI, were documented with a standardized questionnaire. Blood samples were collected randomly from the study subjects attending local polyclinics, Mubarak Al-Kabeer hospital and Al-Amiri hospital during their routine blood chemistry check-up from 2012 to 2016. The inclusion criteria were: 18 years or above, lipid profile, BMI measurements and Kuwaiti nationality. Whereas exclusion criteria were: confirmed clinical diagnosis chronic heart disease, type 2 diabetes mellitus, and taking any medication that may alter plasma lipid levels.

The Ethics Committee from the Kuwait Ministry of Health approved (Reference number: VDR/JC/256) the study which adheres to the Declaration of Helsinki on ethical principles for medical research involving human subjects. The study was explained to all participants, and consents were obtained. Only subjects that gave informed consent were included in the study and were requested to provide fasting blood samples.

2.2. Anthropometric measurements

Anthropometric measurements were done for all participants by qualified investigators using standard techniques. Participant's weight (kilogram (kg)) to the nearest 0.1 kg and height (centimeters (cm)) to the nearest 0.1 cm were taken while dressed in lightweight clothing without shoes. BMI was calculated as weight (kg) divided by the square of height (m^2). Biochemical analysis and genomic DNA extraction were done on the fasting blood samples collected in Ethylenediaminetetraacetic acid (EDTA) tubes. Standard lipid profile of total cholesterol (TC), triglycerides (TG), high-density lipoprotein cholesterol (HDL-C) were analyzed on an automated analyzer (Beckman Unicel DxC 800, Beckman Corporation, Brea, CA, USA). Low-density lipoprotein cholesterol (LDL-C) and very low density lipoproteins cholesterol (VLDL-C) were calculated using the Friedewald formula (Friedewald, Levy, and Fredrickson 1972). The reference used in the current study are those set by the Kuwait Ministry of Health where: HDL-C = 0.91–2.5 mmol/L, TC = 3.0–5.17 mmol/L, TG = 0.40–1.7 mmol/L, VLDL-C = 0.1 to 1.7 mmol/L and LDL-C = 1.8–3.2 mmol/L.

2.3. Selection of SNPs and Genotyping

Samples were analyzed for two SNPs in intron one of the *LPL* gene: rs1534649 (G/T) reported to being associated with TG (Liao et al. 2008) and rs28645722 (G/A) in linkage disequilibrium (r^2 > 0.8) with 127 SNPs (Zerbino et al. 2018).

Genomic DNA was extracted from whole blood using a salt extraction method (Miller, Dykes, and Polesky 1988) and standardized to a final concentration of 10 ng/ul. DNA quality and quantity were ascertained using a nanodrop spectrophotometer. Genotyping of the two variants (rs1534649 and rs28645722) was conducted using TaqMan allelic discrimination assay (Life Technologies, CA, USA) performed by ABI 7500 fast real-time PCR system SDS software (Life Technologies, CA, USA) according to standard manufacturer protocols.

2.4. Statistical analysis

Statistical analysis was performed using the Statistical Package for the Social Sciences software (version 23; SPSS Inc., Chicago, IL, USA) and R statistics using "SNPassoc" package (Gonzalez et al. 2007). Results were expressed as mean ± standard deviation (SD), and percentages where appropriate. Deviation from Hardy and Weinberg Equilibrium (HWE) was tested in R statistics. Analysis of variance using Kruskal-Wallis ANOVA test was used to compare plasma lipid levels between the genotypic groups. Linear regression was used to assess the association between the SNPs and continuous variables represented by Beta-coefficient (β) and 95% confidence intervals (CI) after adjusting for age, gender, BMI and TG where appropriate. In addition, multivariate analysis using general linear model (glm) was further used to assess predictor factors for significant traits. Normality was assessed using Kolmogorov-Smirnov test. Where appropriate lipid parameters were log-transformed for their association with *LPL* variants to ensure an approximate normal distribution. We considered an additive genetic model to assess the dose-dependent association of the minor allele. Bonferroni correction was applied and signifi-

cance was set at $p < 0.025$. We used the Power and Sample Size Calculation Software to set the power at 80% (Dupont and Plummer 1998).

3. Results

Our cohort included 486 subjects with an average age of 29.8 7 ± 13.02 years and comprised of 62.3% women (Table 1). Genotypes were all found to be in HWE ($p > 0.05$). The frequency of the minor allele (T) of rs1534649 was 35.5%, whereas the frequency of the minor allele (A) of rs28645722 was 6.3% (Table 1).

BMI, body mass index; HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein. If percentages are not indicated, the values given indicate means ± standard deviations.

3.1. Relationship between LPL polymorphisms and plasma lipid levels

The relationship between *LPL* polymorphisms and plasma lipid levels was assessed (Table 2). After adjusting for age, gender and BMI we observed a dose-dependent trend between rs1534649 T-allele and low levels of HDL-C $\beta = -0.04$ (95% CI −0.08, −0.006); $p = 0.023$ using an additive genetic model (Table 2). No other associations were observed between rs1534649 and plasma lipid levels ($p > 0.05$). Whereas for rs28645722 no significant association with plasma lipid levels was observed ($p > 0.05$) (Table 2). We further conducted a multivariate analysis using glm to assess predictor factors of HDL-C levels (Table 3). TG, gender and *LPL* rs1534649 were predictors of HDL-C levels ($p < 0.05$) (Table 3).

3.2. Relationship between LPL gene polymorphisms and BMI

Using linear regression, we assessed the relationship of the *LPL* polymorphisms (rs1534649 and rs28645722) with BMI using an additive genetic model (Table 4). The T-allele of rs1534649 was found to be associated with increased BMI in a dose-dependent trend $\beta = 2.13$ (95% CI 1.33–2.94); $p = 1.7 \times 10^{-7}$ after adjusting for age, gender and TG (Table 4). Whereas, rs28645722 showed a dose–response trend with the A-allele increasing BMI, however this was not significant after adjusting for multiple testing (Table4). We further conducted a multivariate analysis for rs1534649 using glm to assess predictor factors of BMI (Table 5). The *LPL* rs1534649 was found among the predictor variables along with age and TG for BMI ($p < 0.0001$) (Table 5). Exploratory analysis was conducted, samples were divided into two groups based on BMI; normal (BMI < 25 kg/m^2) and overweight/obese (BMI ≥ 25 kg/m^2). The distribution of the T-allele for rs1534649 was found to be more prevalent in the overweight/obese group compared to the normal group ($p < 0.001$) (Supplementary Table S1).

Table 1
Characteristics of the studied cohort.

Characteristics	Subjects (n = 486)
Age, years	29.87 ± 13.02
Females (%)	303 (62.3%)
Males (%)	183 (38.7%)
BMI (kg/m^2)	27.17 ± 6.62
Weight (kg)	74.94 ± 20.08
Height (cm)	165.73 ± 8.49
TC (mmol/L)	4.67 ± 0.93
HDL-C (mmol/L)	1.18 ± 0.32
LDL-C (mmol/L)	3.08 ± 0.79
TG (mmol/L)	0.93 ± 0.72
VLDL (mmol/L)	0.39 ± 0.32
rs1534649	
GG	207 (42.5%)
GT	213 (44%)
TT	66 (13.5%)
rs28645722	
GG	429 (88.2%)
GA	53 (11%)
AA	4 (0.8%)

4. Discussion

In this study, we observed an association between rs1534649 polymorphism with both obesity and dyslipidemia. The T-allele was strongly found to be associated with increased BMI in a dose-dependent trend. In addition, we observed a borderline association between the T-allele and low levels of plasma HDL-C. After multiple testing we did not observe any association between the variant rs28645722 and any of the phenotypic traits analyzed.

Various *LPL* gene polymorphisms have been found to be associated with lipid traits including those found in GWAS (Shahid et al. 2017; Ayyappa et al. 2017; Al-Bustan et al. 2018; Middelberg et al. 2011). However, association with obesity has only been reported in two studies (Radha et al. 2007; Prakash et al. 2018). These studies found the *LPL* promoter SNP rs1800590 to be associated with obesity in an Asian population, suggesting that the relation between *LPL* polymorphisms and obesity may be population specific. Our intron one SNP rs1534649 has been investigated in three independent studies to date (Smith et al. 2010; Voruganti et al. 2010; Liao et al. 2008). Only one study assessed the relationship with BMI and failed to report any association (Voruganti et al. 2010). For the tag SNP rs28645722, prior to multiple testing the variant was found to be associated with increased BMI however this was not considered significant after adjusting for $p < 0.025$ in our study. This finding should not be overlooked, especially that the variant is in LD ($r^2 > 0.8$) with 127 SNPs. Our association with obesity is consistent with the role of LPL in the initiation and development of obesity which has been reviewed intensively (Wang and Eckel 2009). Briefly, LPL impact on obesity is tissue-specific, with studies showing both deletion and overexpression of LPL in adipose and muscle tissues impacts body weight and composition (Wang and Eckel 2009; Garcia-Arcos et al. 2013). Fatty acids (FA), products of the TG-hydrolysis by LPL are taken up by adipose and muscle tissues where they are stored and oxidized respectively (Hollenberg 1966; Wang and Eckel 2009), hence the role of LPL in distribution of fatty acids in the body. A study by Garcia-Acros et al., demonstrated tissue-specific knockout experiments where mice with LPL adipose tissue deletion showed an increase in white adipose tissue (Garcia-Arcos et al. 2013). However, mice with deleted LPL in adipose tissue showed reduction in fat accumulation only when LPL activity was high in skeletal muscles (Garcia-Arcos et al. 2013). Moreover, mice with overexpression of LPL in skeletal muscle are protected from weight gain and have an increased metabolic rate (Wang and Eckel 2009). Such findings support that *LPL* is an obesity gene.

The relationship between rs1534649 and plasma lipid levels has been investigated in three independent populations (Voruganti et al. 2010; Smith et al. 2010; Liao et al. 2008). The association of this variant with TG was observed in Caribbean Hispanics (Liao et al. 2008) and Alaskan Eskimos populations (Voruganti et al. 2010), whereas, no association was observed in a UK population (Smith et al. 2010) which is consistent with our study. In addition, all three studies reported no association with HDL-C. Findings from a GWAS has reported associations between variants in the *LPL* gene with HDL-C and TG (Middelberg et al. 2011). However, the GWAS did not report any associations with our studied variant. This can be explained by the differences in allelic frequencies found between different populations which are reported by the 1000

Table 2
Relationship between *LPL* polymorphisms and plasma lipid levels using linear regression.

rs1534649	**GG (n = 207)**	**GT (n = 213)**	**TT (n = 66)**	**β (95% CI)**	***P***
TC (mmol/L)	4.73 ± 0.95	4.6 ± 0.95	4.76 ± 0.76	−0.07(−0.19, 0.04)	0.33
HDL-C (mmol/L)	1.21 ± 0.34	1.17 ± 0.31	1.12 ± 0.29	−0.04(−0.08, −0.006)	**0.02**
LDL-C (mmol/L)	3.1 ± 0.77	3.02 ± 0.82	3.2 ± 0.74	−0.02(−0.12, 0.08)	0.71
TG (mmol/L)	0.95 ± 0.75	0.91 ± 0.73	0.97 ± 0.57	−0.01(−0.08, 0.04)	0.57
VLDL (mmol/L)	0.4 ± 0.33	0.38 ± 0.33	0.4 ± 0.24	−0.01(−0.05, 0.01)	0.32
rs28645722	**GG (n = 420)**	**GA (n = 61)**	**AA (n = 5)**	**β (95% CI)**	***P***
TC (mmol/L)	4.67 ± 0.95	4.71 ± 0.8	4.46 ± 0.46	−0.07 (−0.3, 0.15)	0.51
HDL-C (mmol/L)	1.18 ± 0.32	1.17 ± 0.35	1.16 ± 0.48	0.009(−0.06, 0.08)	0.81
LDL-C (mmol/L)	3.08 ± 0.79	3.1 ± 0.77	2.93 ± 0.55	−0.06(−0.26, 0.12)	0.5
TG (mmol/L)	0.93 ± 0.74	0.99 ± 0.59	0.91 ± 0.4	−0.001(−0.12, 0.12)	0.98
VLDL (mmol/L)	0.39 ± 0.32	0.42 ± 0.26	0.38 ± 0.15	−0.01(−0.08, 0.05)	0.7

HDL-C, high-density lipoprotein cholesterol; LDL-C, low-density lipoprotein cholesterol; TC, total cholesterol; TG, triglyceride; VLDL, very low-density lipoprotein. The values given indicate means ± standard deviations.

Table 3
Multivariate analysis using linear regression model to assess the predictor factors associated with HDL-C.

Variable	**β (95% CI)**	***P***
Gender	0.77 (0.73–0.82)	**<0.0001**
Age (+1 year)	1.001 (0.99–1.003)	0.18
TG (mmol/L)	0.94 (0.9–0.98)	**0.01**
LPL rs1534649	0.9 (0.82–0.98)	**0.02**
BMI (kg/m^2)	0.99 (0.9–0.98)	0.16

TG, triglyceride; BMI, body mass index; LPL, lipoprotein lipase

Table 4
Relationship between *LPL* polymorphisms and BMI using linear regression.

rs1534649	**BMI** (kg/m^2)	**β *(95% CI)***	***P***
GG (2 0 7)	26.06 ± 5.5		
GT (2 1 3)	27.03 ± 6.4	2.13 (1.33–2.94)	**1.7×10^{-7}**
TT (66)	31.17 ± 8.5		
rs28645722	**BMI** (kg/m^2)	**β (95% CI)**	***P***
GG (4 2 0)	26.99 ± 6.5		
GA (61)	27.86 ± 6.5	1.75 (0.14–3.35)	0.03
AA (5)	38.17 ± 9.5		

Table 5
Multivariate analysis using linear regression model to assess the predictor factors associated with BMI.

Variable	**β (95% CI)**	***P***
Gender	−0.035 (−1.18, −1.11)	0.95
Age (+1 year)	0.09 (0.05–0.13)	**<0.0001**
TG (mmol/L)	2.01 (1.2–2.82)	**<0.0001**
LPL rs1534649	2.13 (1.33–2.94)	**<0.0001**

TG, triglyceride; LPL, lipoprotein lipase

genome project for the variant rs1534649 suggesting that our findings are population specific. Moreover, a positive correlation between HDL-C and LPL activity has been previously reported with mutations in the *LPL* gene resulting in LPL-deficiency showing decreased HDL-C concentrations (Bijvoet et al. 1996). The hydrolysis of TG via the LPL enzyme produces remnants required for the maturation of HDL particles such as free cholesterol, phospholipids and apolipoproteins and therefore low activity of LPL may result in less mature HDL (Brunzell 1995).

The association of the T-allele of rs1534649 with increased BMI and low levels of HDL-C in our study may suggest the SNP to be involved in lowering *LPL* gene activity through gene regulation. However, functional studies are required to assess the role of this variant in relation to the *LPL* gene activity. The polymorphism rs1534649 is located in intron one of the *LPL* gene. Intron one lies between two important regions, exon one, encodes for the signal peptide and exon two, includes the protein domain that binds the lipoprotein substrate which are all pivotal for transportation (Deeb and Peng 1989). A study by Pirim et al., reported that intron one comprises the majority of variants in the *LPL* gene (Pirim et al. 2014). Moreover, a recent study by Al-Bustan et al., reported a similar finding with intron one of the *LPL* gene to also comprise the majority of variants, along with a novel polymorphism in an intronic region to be associated with HDL-C (Al-Bustan et al. 2018). In addition, intron one of the mouse *lpl* gene comprises a response sequence element that is suggested to have a regulatory role (Hua et al. 1991) and that the mouse *lpl* gene is mapped to a region on chromosome 8 that is found to be homologous to that of humans (Liu et al. 2008). Moreover, well studied intron one polymorphisms associated with obesity such as those of the *fat-mass obesity (FTO)* gene have been suggested to act as long range targets of other genes related to lipid metabolism (Smemo et al. 2014) which may suggest our selected variant may have a similar mechanism. Therefore, it is possible that the variant rs1534649 is surrounded by regulatory sequence elements however such claim needs to be further validated. In addition, data from the 1000genome project indicate our variant to be in linkage disequilibrium (LD) ($r^2 > 0.8$) with a regulatory SNP (rs10091649) upstream the *LPL* promoter region. The SNP (rs10091649) is located approximately 42 kb upstream the promoter region and is surrounded by transcription factor binding sites suggesting a regulatory role of our studied variant which may be acting as a proxy SNP (Zerbino et al. 2018).

A limitation of this study is that we lacked any LPL activity measures that could be assessed with our selected SNPs. Although we found a borderline association with HDL-C, power calculation showed that to detect a power of 80% we require a larger sample size. On the other hand, the strength of this study is the association with BMI, power calculation showed that the sample size was sufficient to detect a power over 80% for the relationship between rs1534649 and BMI. Moreover, we took multiple-testing into account to avoid any type one errors in our study.

In conclusion, this is the first study to observe an association between rs1534649 with both BMI and HDL-C and therefore replication studies are necessary to validate our findings. Moreover, no previous studies have reported an association between *LPL* polymorphisms and obesity in an Arab state. Such findings have major metabolic consequences and is of great interest for understanding the mechanisms involved in the development of obesity.

Declarations

Ethics approval and consent to participate

The Ethics Committee from the Kuwait Ministry of Health approved (Reference number: VDR/JC/256) the study which adheres to the Declaration of Helsinki on ethical principles for medical research involving human subjects. The study was explained to all participants, and consents were obtained. Only subjects that gave informed consent were included in the study and were requested to provide fasting blood samples.

Consent for publication

All authors have reviewed and consented to publication of the paper

Availability of data

The datasets supporting the conclusions of this article are included within the article and can be provided for meta-analysis.

Funding

This project was partially funded by Kuwait University (KURS SL04/11).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The authors would like to extend their appreciation and gratitude to Kuwait University Research Sector (KURS SL04/11) and to the technical staff of Mubarak Al-Kabeer and Al-Amiri hospital for providing the samples and lipid profiles. We would also like to thank the Biotechnology center for use of facilities (GS01/02).

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2021.04.085.

References

Al-Bustan, S.A., Al-Serri, A., Annice, B.G., Alnaqeeb, M.A., Al-Kandari, W.Y., Dashti, M., 2018. A novel LPL intronic variant: g.18704C>A identified by re-sequencing Kuwaiti Arab samples is associated with high-density lipoprotein, very low-density lipoprotein and triglyceride lipid levels. PLoS ONE 13, e0192617.

Ayyappa, K.A., Shatwan, I., Bodhini, D., Bramwell, L.R., Ramya, K., Sudha, V., Anjana, R.M., Lovegrove, J.A., Mohan, V., Radha, V., Vimaleswaran, K.S., 2017. High fat diet modifies the association of lipoprotein lipase gene polymorphism with high density lipoprotein cholesterol in an Asian Indian population. Nutr Metab (Lond) 14, 8.

Bijvoet, S., Gagne, S.E., Moorjani, S., Gagne, C., Henderson, H.E., Fruchart, J.C., Dallongeville, J., Alaupovic, P., Prins, M., Kastelein, J.J., Hayden, M.R., 1996. Alterations in plasma lipoproteins and apolipoproteins before the age of 40 in heterozygotes for lipoprotein lipase deficiency. J Lipid Res 37, 640–650.

Bogari, N.M., Aljohani, A., Dannoun, A., Elkhateeb, O., Porqueddu, M., Amin, A.A., Bogari, D.N., Taher, M.M., Buba, F., Allam, R.M., Bogari, M.N., Alamanni, F., 2020. Association between HindIII (rs320) variant in the lipoprotein lipase gene and the presence of coronary artery disease and stroke among the Saudi population. Saudi J Biol Sci 27, 2018–2024.

Brunzell, J. D. 1995. Familial lipoprotein lipase deficiency and other causes of the chylomicronemia syndrome.

Chen, Y., Zhu, J., Lum, P.Y., Yang, X., Pinto, S., MacNeil, D.J., Zhang, C., Lamb, J., Edwards, S., Sieberts, S.K., Leonardson, A., Castellini, L.W., Wang, S., Champy, M. F., Zhang, B., Emilsson, V., Doss, S., Ghazalpour, A., Horvath, S., Drake, T.A., Lusis, A.J., Schadt, E.E., 2008. Variations in DNA elucidate molecular networks that cause disease. Nature 452, 429–435.

Deeb, S.S., Peng, R.L., 1989. Structure of the human lipoprotein lipase gene. Biochemistry 28, 4131–4135.

Dupont, W.D., Plummer Jr., W.D., 1998. Power and sample size calculations for studies involving linear regression. Control Clin Trials 19, 589–601.

Eckel, R.H., 1989. Lipoprotein lipase A multifunctional enzyme relevant to common metabolic diseases. *N Engl J Med* 320, 1060–1068.

Farooqi, I.S., O'Rahilly, S., 2000. Recent advances in the genetics of severe childhood obesity. Arch Dis Child 83, 31–34.

Friedewald, W.T., Levy, R.I., Fredrickson, D.S., 1972. Estimation of the concentration of low-density lipoprotein cholesterol in plasma, without use of the preparative ultracentrifuge. Clin Chem 18, 499–502.

Garcia-Arcos, I., Hiyama, Y., Drosatos, K., Bharadwaj, K.G., Hu, Y., Son, N.H., O'Byrne, S.M., Chang, C.L., Deckelbaum, R.J., Takahashi, M., Westerterp, M., Obunike, J.C., Jiang, H., Yagyu, H., Blaner, W.S., Goldberg, I.J., 2013. Adipose-specific lipoprotein lipase deficiency more profoundly affects brown than white fat biology. J Biol Chem 288, 14046–14058.

Gonzalez, J.R., Armengol, L., Sole, X., Guino, E., Mercader, J.M., Estivill, X., Moreno, V., 2007. SNPassoc: an R package to perform whole genome association studies. Bioinformatics 23, 644–645.

Hill, J.O., Wyatt, H.R., Reed, G.W., Peters, J.C., 2003. Obesity and the environment: where do we go from here?. Science 299, 853–855.

Hollenberg, C.H., 1966. The origin and glyceride distribution of fatty acids in rat adipose tissue. J Clin Invest 45, 205–216.

Hua, X.X., Enerback, S., Hudson, J., Youkhana, K., Gimble, J.M., 1991. Cloning and characterization of the promoter of the murine lipoprotein lipase-encoding gene: structural and functional analysis. Gene 107, 247–258.

Kirchgessner, T.G., LeBoeuf, R.C., Langner, C.A., Zollman, S., Chang, C.H., Taylor, B.A., Schotz, M.C., Gordon, J.I., Lusis, A.J., 1989. Genetic and developmental regulation of the lipoprotein lipase gene: loci both distal and proximal to the lipoprotein lipase structural gene control enzyme expression. J Biol Chem 264, 1473–1482.

Liao, Y.C., Lin, H.F., Rundek, T., Cheng, R., Hsi, E., Sacco, R.L., Juo, S.H., 2008. Multiple genetic determinants of plasma lipid levels in Caribbean Hispanics. Clin Biochem 41, 306–312.

Liu, D., Deschamps, A., Korach, K.S., Murphy, E., 2008. Estrogen-enhanced gene expression of lipoprotein lipase in heart is antagonized by progesterone. Endocrinology 149, 711–716.

Locke, A. E., B. Kahali, S. I. Berndt, A. E. Justice, T. H. Pers, F. R. Day, C. Powell, S. Vedantam, M. L. Buchkovich, J. Yang, D. C. Croteau-Chonka, T. Esko, T. Fall, T. Ferreira, S. Gustafsson, Z. Kutalik, J. Luan, R. Magi, J. C. Randall, T. W. Winkler, A. R. Wood, T. Workalemahu, J. D. Faul, J. A. Smith, J. H. Zhao, W. Zhao, J. Chen, R. Fehrmann, A. K. Hedman, J. Karjalainen, E. M. Schmidt, D. Absher, N. Amin, D. Anderson, M. Beekman, J. L. Bolton, J. L. Bragg-Gresham, S. Buyske, A. Demirkan, G. Deng, G. B. Ehret, B. Feenstra, M. F. Feitosa, K. Fischer, A. Goel, J. Gong, A. U. Jackson, S. Kanoni, M. E. Kleber, K. Kristiansson, U. Lim, V. Lotay, M. Mangino, I. M. Leach, C. Medina-Gomez, S. E. Medland, M. A. Nalls, C. D. Palmer, D. Pasko, S. Pechlivanis, M. J. Peters, I. Prokopenko, D. Shungin, A. Stancakova, R. J. Strawbridge, Y. J. Sung, T. Tanaka, A. Teumer, S. Trompet, S. W. van der Laan, J. van Setten, J. V. Van Vliet-Ostaptchouk, Z. Wang, L. Yengo, W. Zhang, A. Isaacs, E. Albrecht, J. Arnlov, G. M. Arscott, A. P. Attwood, S. Bandinelli, A. Barrett, I. N. Bas, C. Bellis, A. J. Bennett, C. Berne, R. Blagieva, M. Bluher, S. Bohringer, L. L. Bonnycastle, Y. Bottcher, H. A. Boyd, M. Bruinenberg, I. H. Caspersen, Y. I. Chen, R. Clarke, E. W. Daw, A. J. M. de Craen, G. Delgado, M. Dimitriou, A. S. F. Doney, N. Eklund, K. Estrada, E. Eury, L. Folkersen, R. M. Fraser, M. E. Garcia, F. Geller, V. Giedraitis, B. Gigante, A. S. Go, A. Golay, A. H. Goodall, S. D. Gordon, M. Gorski, H. J. Grabe, H. Grallert, T. B. Grammer, J. Grassler, H. Gronberg, C. J. Groves, G. Gusto, J. Haessler, P. Hall, T. Haller, G. Hallmans, C. A. Hartman, M. Hassinen, C. Hayward, N. L. Heard-Costa, Q. Helmer, C. Hengstenberg, O. Holmen, J. J. Hottenga, A. L. James, J. M. Jeff, A. Johansson, J. Jolley, T. Juliusdottir, L. Kinnunen, W. Koenig, M. Koskenvuo, W. Kratzer, J. Laitinen, C. Lamina, K. Leander, N. R. Lee, P. Lichtner, L. Lind, J. Lindstrom, K. S. Lo, S. Lobbens, R. Lorbeer, Y. Lu, F. Mach, P. K. E. Magnusson, A. Mahajan, W. L. McArdle, S. McLachlan, C. Menni, S. Merger, E. Mihailov, L. Milani, A. Moayyeri, K. L. Monda, M. A. Morken, A. Mulas, G. Muller, M. Muller-Nurasyid, A. W. Musk, R. Nagaraja, M. M. Nothen, I. M. Nolte, S. Pilz, N. W. Rayner, F. Renstrom, R. Rettig, J. S. Ried, S. Ripke, N. R. Robertson, L. M. Rose, S. Sanna, H. Scharnagl, S. Scholtens, F. R. Schumacher, W. R. Scott, T. Seufferlein, J. Shi, A. V. Smith, J. Smolonska, A. V. Stanton, V. Steinthorsdottir, K. Stirrups, H. M. Stringham, J. Sundstrom, M. A. Swertz, A. J. Swift, A. C. Syvanen, S. T. Tan, B. O. Tayo, B. Thorand, G. Thorleifsson, J. P. Tyrer, H. W. Uh, L. Vandenput, F. C. Verhulst, S. H. Vermeulen, N. Verweij, J. M. Vonk, L. L. Waite, H. R. Warren, D. Waterworth, M. N. Weedon, L. R. Wilkens, C. Willenborg, T. Wilsgaard, M. K. Wojczynski, A. Wong, A. F. Wright, Q. Zhang, Study LifeLines Cohort, E. P. Brennan, M. Choi, Z. Dastani, A. W. Drong, P. Eriksson, A. Franco-Cereceda, J. R. Gadin, A. G. Gharavi, M. E. Goddard, R. E. Handsaker, J. Huang, F. Karpe, S. Kathiresan, S. Keildson, K. Kiryluk, M. Kubo, J. Y. Lee, L. Liang, R. P. Lifton, B. Ma, S. A. McCarroll, A. J. McKnight, J. L. Min, M. F. Moffatt, G. W. Montgomery, J. M. Murabito, G. Nicholson, D. R. Nyholt, Y. Okada, J. R. B. Perry, R. Dorajoo, E. Reinmaa, R. M. Salem, N. Sandholm, R. A. Scott, L. Stolk, A. Takahashi, T. Tanaka, F. M. van 't Hooft, A. A. E. Vinkhuyzen, H. J. Westra, W. Zheng, K. T. Zondervan, A. DIPOGen Consortium, Agen-Bmi Working Group, C. ARDIOGRAMplusC4D Consortium, C. KDGen Consortium, Glgc, Icbp, Magic Investigators, Ther Consortium Mu, M. IGen Consortium, Page

Consortium, Consortium ReproGen, Genie Consortium, Consortium International Endogene, A. C. Heath, D. Arveiler, S. J. L. Bakker, J. Beilby, R. N. Bergman, J. Blangero, P. Bovet, H. Campbell, M. J. Caulfield, G. Cesana, A. Chakravarti, D. I. Chasman, P. S. Chines, F. S. Collins, D. C. Crawford, L. A. Cupples, D. Cusi, J. Danesh, U. de Faire, H. M. den Ruijter, A. F. Dominiczak, R. Erbel, J. Erdmann, J. G. Eriksson, M. Farrall, S. B. Felix, E. Ferrannini, J. Ferrieres, I. Ford, N. G. Forouhi, T. Forrester, O. H. Franco, R. T. Gansevoort, P. V. Gejman, C. Gieger, O. Gottesman, V. Gudnason, U. Gyllensten, A. S. Hall, T. B. Harris, A. T. Hattersley, A. A. Hicks, L. A. Hindorff, A. D. Hingorani, A. Hofman, G. Homuth, G. K. Hovingh, S. E. Humphries, S. C. Hunt, E. Hypponen, T. Illig, K. B. Jacobs, M. R. Jarvelin, K. H. Jockel, B. Johansen, P. Jousilahti, J. W. Jukema, A. M. Jula, J. Kaprio, J. J. P. Kastelein, S. M. Keinanen-Kiukaanniemi, L. A. Kiemeney, P. Knekt, J. S. Kooner, C. Kooperberg, P. Kovacs, A. T. Kraja, M. Kumari, J. Kuusisto, T. A. Lakka, C. Langenberg, L. L. Marchand, T. Lehtimaki, V. Lyssenko, S. Mannisto, A. Marette, T. C. Matise, C. A. McKenzie, B. McKnight, F. L. Moll, A. D. Morris, A. P. Morris, J. C. Murray, M. Nelis, C. Ohlsson, A. J. Oldehinkel, K. K. Ong, P. A. F. Madden, G. Pasterkamp, J. F. Peden, A. Peters, D. S. Postma, P. P. Pramstaller, J. F. Price, L. Qi, O. T. Raitakari, T. Rankinen, D. C. Rao, T. K. Rice, P. M. Ridker, J. D. Rioux, M. D. Ritchie, I. Rudan, V. Salomaa, N. J. Samani, J. Saramies, M. A. Sarzynski, H. Schunkert, P. E. H. Schwarz, P. Sever, A. R. Shuldiner, J. Sinisalo, R. P. Stolk, K. Strauch, A. Tonjes, D. A. Tregouet, A. Tremblay, E. Tremoli, J. Virtamo, M. C. Vohl, U. Volker, G. Waeber, G. Willemsen, J. C. Witteman, M. C. Zillikens, L. S. Adair, P. Amouyel, F. W. Asselbergs, T. L. Assimes, M. Bochud, B. O. Boehm, E. Boerwinkle, S. R. Bornstein, E. P. Bottinger, C. Bouchard, S. Cauchi, J. C. Chambers, S. J. Chanock, R. S. Cooper, P. I. W. de Bakker, G. Dedoussis, L. Ferrucci, P. W. Franks, P. Froguel, L. C. Groop, C. A. Haiman, A. Hamsten, J. Hui, D. J. Hunter, K. Hveem, R. C. Kaplan, M. Kivimaki, D. Kuh, M. Laakso, Y. Liu, N. G. Martin, W. Marz, M. Melbye, A. Metspalu, S. Moebus, P. B. Munroe, I. Njolstad, B. A. Oostra, C. N. A. Palmer, N. L. Pedersen, M. Perola, L. Perusse, U. Peters, C. Power, T. Quertermous, R. Rauramaa, F. Rivadeneira, T. E. Saaristo, D. Saleheen, N. Sattar, E. E. Schadt, D. Schlessinger, P. E. Slagboom, H. Snieder, T. D. Spector, U. Thorsteinsdottir, M. Stumvoll, J. Tuomilehto, A. G. Uitterlinden, M. Uusitupa, P. van der Harst, M. Walker, H. Wallaschofski, N. J. Wareham, H. Watkins, D. R. Weir, H. E. Wichmann, J. F. Wilson, P. Zanen, I. B. Borecki, P. Deloukas, C. S. Fox, I. M. Heid, J. R. O'Connell, D. P. Strachan, K. Stefansson, C. M. van Duijn, G. R. Abecasis, L. Franke, T. M. Frayling, M. I. McCarthy, P. M. Visscher, A. Scherag, C. J. Willer, M. Boehnke, K. L. Mohlke, C. M. Lindgren, J. S. Beckmann, I. Barroso, K. E. North, E. Ingelsson, J. N. Hirschhorn, R. J. F. Loos, and E. K. Speliotes. 2015. 'Genetic studies of body mass index yield new insights for obesity biology', Nature, 518: 197-206.

Middelberg, R.P., Ferreira, M.A., Henders, A.K., Heath, A.C., Madden, P.A., Montgomery, G.W., Martin, N.G., Whitfield, J.B., 2011. Genetic variants in LPL, OASL and TOMM40/APOE-C1-C2-C4 genes are associated with multiple cardiovascular-related traits. BMC Med Genet 12, 123.

Miller, S.A., Dykes, D.D., Polesky, H.F., 1988. A simple salting out procedure for extracting DNA from human nucleated cells. Nucleic Acids Res 16, 1215.

Ng, M., Fleming, T., Robinson, M., Thomson, B., Graetz, N., Margono, C., Mullany, E.C., Biryukov, S., Abbafati, C., Abera, S.F., Abraham, J.P., Abu-Rmeileh, N.M., Achoki, T., AlBuhairan, F.S., Alemu, Z.A., Alfonso, R., Ali, M.K., Ali, R., Guzman, N.A., Ammar, W., Anwari, P., Banerjee, A., Barquera, S., Basu, S., Bennett, D.A., Bhutta, Z., Blore, J., Cabral, N., Nonato, I.C., Chang, J.C., Chowdhury, R., Courville, K.J., Criqui, M.H., Cundiff, D.K., Dabhadkar, K.C., Dandona, L., Davis, A., Dayama, A., Dharmaratne, S.D., Ding, E.L., Durrani, A.M., Esteghamati, A., Farzadfar, F., Fay, D. F., Feigin, V.L., Flaxman, A., Forouzanfar, M.H., Goto, A., Green, M.A., Gupta, R., Hafezi-Nejad, N., Hankey, G.J., Harewood, H.C., Havmoeller, R., Hay, S., Hernandez, L., Husseini, A., Idrisov, B.T., Ikeda, N., Islami, F., Jahangir, E., Jassal, S.K., Jee, S.H., Jeffreys, M., Jonas, J.B., Kabagambe, E.K., Khalifa, S.E., Kengne, A.P., Khader, Y.S., Khang, Y.H., Kim, D., Kimokoti, R.W., Kinge, J.M., Kokubo, Y., Kosen, S., Kwan, G., Lai, T., Leinsalu, M., Li, Y., Liang, X., Liu, S., Logroscino, G., Lotufo, P. A., Lu, Y., Ma, J., Mainoo, N.K., Mensah, G.A., Merriman, T.R., Mokdad, A.H., Moschandreas, J., Naghavi, M., Naheed, A., Nand, D., Narayan, K.M., Nelson, E.L., Neuhouser, M.L., Nisar, M.I., Ohkubo, T., Oti, S.O., Pedroza, A., Prabhakaran, D., Roy, N., Sampson, U., Seo, H., Sepanlou, S.G., Shibuya, K., Shiri, R., Shiue, I., Singh, G.M., Singh, J.A., Skirbekk, V., Stapelberg, N.J., Sturua, L., Sykes, B.L., Tobias, M., Tran, B.X., Trasande, L., Toyoshima, H., van de Vijver, S., Vasankari, T.J., Veerman, J.L., Velasquez-Melendez, G., Vlassov, V.V., Vollset, S.E., Vos, T., Wang, C., Wang, X., Weiderpass, E., Werdecker, A., Wright, J.L., Yang, Y.C., Yatsuya, H., Yoon, J., Yoon, S.J., Zhao, Y., Zhou, M., Zhu, S., Lopez, A.D., Murray, C.J., Gakidou, E., 2014. Global, regional, and national prevalence of overweight and obesity in children and adults during 1980–2013: a systematic analysis for the Global Burden of Disease Study 2013. Lancet 384, 766–781.

Pirim, D., Wang, X., Radwan, Z.H., Niemsiri, V., Hokanson, J.E., Hamman, R.F., Barmada, M.M., Demirci, F.Y., Kamboh, M.I., 2014. Lipoprotein lipase gene sequencing and plasma lipid profile. J Lipid Res 55, 85–93.

Prakash, J., Mittal, B., Srivastava, A., Awasthi, S., Srivastava, N., 2018. The Association of a Rare Variant of -93, -53 Promoter Gene Polymorphisms of Lipoprotein Lipase gene with Obesity and Insulin Resistance. Oman Med J 33, 401–408.

Radha, V., Vimaleswaran, K.S., Ayyappa, K.A., Mohan, V., 2007. Association of lipoprotein lipase gene polymorphisms with obesity and type 2 diabetes in an Asian Indian population. Int J Obes (Lond) 31, 913–918.

Shahid, S.U., Shabana, N.A., Cooper, J.A., Rehman, A., Humphries, S.E., 2017. Common variants in the genes of triglyceride and HDL-C metabolism lack association with coronary artery disease in the Pakistani subjects. Lipids Health Dis 16, 24.

Smemo, S., Tena, J.J., Kim, K.H., Gamazon, E.R., Sakabe, N.J., Gomez-Marin, C., Aneas, I., Credidio, F.L., Sobreira, D.R., Wasserman, N.F., Lee, J.H., Puviindran, V., Tam, D., Shen, M., Son, J.E., Vakili, N.A., Sung, H.K., Naranjo, S., Acemel, R.D., Manzanares, M., Nagy, A., Cox, N.J., Hui, C.C., Gomez-Skarmeta, J.L., Nobrega, M.A., 2014. Obesity-associated variants within FTO form long-range functional connections with IRX3. Nature 507, 371–375.

Smith, A.J., Palmen, J., Putt, W., Talmud, P.J., Humphries, S.E., Drenos, F., 2010. Application of statistical and functional methodologies for the investigation of genetic determinants of coronary heart disease biomarkers: lipoprotein lipase genotype and plasma triglycerides as an exemplar. Hum Mol Genet 19, 3936–3947.

Tanuma, Y., Nakabayashi, H., Esumi, M., Endo, H., 1995. A silencer element for the lipoprotein lipase gene promoter and cognate double- and single-stranded DNA-binding proteins. Mol Cell Biol 15, 517–523.

Voruganti, V.S., Cole, S.A., Ebbesson, S.O., Goring, H.H., Haack, K., Laston, S., Wenger, C.R., Tejero, M.E., Devereux, R.B., Fabsitz, R.R., MacCluer, J.W., Umans, J.G., Howard, B.V., Comuzzie, A.G., 2010. Genetic variation in APOJ, LPL, and TNFRSF10B affects plasma fatty acid distribution in Alaskan Eskimos. Am J Clin Nutr 91, 1574–1583.

Wang, H., Eckel, R.H., 2009. Lipoprotein lipase: from gene to obesity. Am J Physiol Endocrinol Metab 297, E271–E288.

Weinstock, P.H., Bisgaier, C.L., Aalto-Setala, K., Radner, H., Ramakrishnan, R., Levak-Frank, S., Essenburg, A.D., Zechner, R., Breslow, J.L., 1995. 'Severe hypertriglyceridemia, reduced high density lipoprotein, and neonatal death in lipoprotein lipase knockout mice. Mild hypertriglyceridemia with impaired very low density lipoprotein clearance in heterozygotes. *J Clin Invest* 96, 2555–2568.

Wion, K.L., Kirchgessner, T.G., Lusis, A.J., Schotz, M.C., Lawn, R.M., 1987. Human lipoprotein lipase complementary DNA sequence. Science 235, 1638–1641.

Xu, X., Wang, Y., Wang, L., Liao, Q., Chang, L., Xu, L., Huang, Y., Ye, H., Xu, L., Chen, C., Shen, X., Zhang, F., Ye, M., Wang, Q., Duan, S., 2013. Meta-analyses of 8 polymorphisms associated with the risk of the Alzheimer's disease. PLoS ONE 8, e73129.

Zerbino, D.R., Achuthan, P., Akanni, W., Amode, M.R., Barrell, D., Bhai, J., Billis, K., Cummins, C., Gall, A., Giron, C.G., Gil, L., Gordon, L., Haggerty, L., Haskell, E., Hourlier, T., Izuogu, O.G., Janacek, S.H., Juettemann, T., To, J.K., Laird, M.R., Lavidas, I., Liu, Z., Loveland, J.E., Maurel, T., McLaren, W., Moore, B., Mudge, J., Murphy, D.N., Newman, V., Nuhn, M., Ogeh, D., Ong, C.K., Parker, A., Patricio, M., Riat, H.S., Schuilenburg, H., Sheppard, D., Sparrow, H., Taylor, K., Thormann, A., Vullo, A., Walts, B., Zadissa, A., Frankish, A., Hunt, S.E., Kostadima, M., Langridge, N., Martin, F.J., Muffato, M., Perry, E., Ruffier, M., Staines, D.M., Trevanion, S.J., Aken, B.L., Cunningham, F., Yates, A., Flicek, P., 2018. Ensembl 2018. Nucleic Acids Res 46, D754–D761.

WHO, 2018. 'Obesity and overweight'. http://www.who.int/news-room/fact-sheets/detail/obesity-and-overweight.