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

Ahmad Al-Serri a,⇑, Suzanne A. Al-Bustan b, Salman K. Al-Sabah c, Babitha G. Annice b, Majed A. Alnaqeeb b, Olusegun A. Mojiminiyi d

a Human Genetics Unit, Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait, PO Box 24923, Safat 13110, Kuwait
b Department of Biological Sciences, Faculty of Science, Kuwait University, Safat, Kuwait
c Department of Surgery, Faculty of Medicine, Kuwait University, Kuwait, PO Box 24923, Safat 133110, Kuwait
d Department of Pathology, Faculty of Medicine, Kuwait University, Kuwait, PO Box 24923, Safat 13110, Kuwait

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.

© 2021 The Author(s). Published by Elsevier B.V. on behalf of King Saud University. This is an open access article under the CC BY-NC-ND license (http://creativecommons.org/licenses/by-nc-nd/4.0/).

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-
wide association studies (GWAS) have identified genetic variants that increase one’s 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), dystipidemia (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 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 TG (Liao et al. 2008) and very low density lipoprotein cholesterol (VLDL-C) were analyzed 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 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 (r² > 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 the 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-
3. Results

Our cohort included 486 subjects with an average age of 29.87 ± 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 β = −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 between 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 β = 2.13 (95% CI 1.33–2.94); p = 1.7 × 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/m²) and overweight/obese (BMI ≥ 25 kg/m²). 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).

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 (r² > 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

| Characteristics | Subjects (n = 486) |
|-----------------|-------------------|
| Age, years      | 29.87 ± 13.02     |
| Females (%)     | 303 (62.3%)       |
| Males (%)       | 183 (37.7%)       |
| BMI (kg/m²)     | 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%)          |

4719
Lower levels of HDL-C in our study may suggest the SNP to be associated with less mature HDL (Brunzell 1995). Apolipoprotein activity and therefore low activity of LPL may result in the maturation of HDL particles such as free cholesterol, phospholipids and apolipoproteins and therefore low activity of LPL may result in the degradation of TG via the LPL enzyme producing remnants required for the uptake by the liver. The mechanisms involved in the development of obesity is of great interest for understanding the metabolic consequences and is of great interest for understanding the metabolic consequences.

### Table 2

| rs1534649 | GG       | GT       | TT       | 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 |

**Note:** 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 (0.79–0.82) | <0.0001 |
| Age (+1 year)             | 1.00 (0.99–1.003) | 0.16 |
| TG (mmol/L)               | 0.04 (0.09–0.98) | 0.01 |
| LPL rs1534649 TT          | 0.9 (0.82–0.98) | 0.02 |
| BMI (kg/m²)               | 0.99 (0.09–0.98) | 0.16 |

### Table 4

Relationship between LPL polymorphisms and BMI using linear regression.

| rs1534649 | BMI (kg/m²) | β (95% CI) | P   |
|-----------|-------------|------------|-----|
| TT (n = 66) | 26.06 ± 5.5 | 27.03 ± 6.4 | 21.17 ± 8.5 | 1.7 ± 10^-7 |
| GG (n = 66) | 24.4 ± 6.1 | 23.7 ± 5.6 | 22.1 ± 7.8 | 1.6 ± 10^-5 |
| GA (n = 61) | 26.99 ± 6.5 | 27.18 ± 6.5 | 27.37 ± 8.5 | 1.3 ± 10^-4 |
| AA (n = 5)  | 23.87 ± 6.5 | 24.76 ± 6.5 | 25.67 ± 8.5 | 1.1 ± 10^-3 |

### 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 (1.3–2.94) | <0.0001 |

### Note:

TG, triglyceride; BMI, body mass index; LPL, lipoprotein lipase
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 medically explained to all 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.jsb.2021.04.085.

References

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

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

Bivoet, S., Gagne, S.E., Moorjani, S., Gagne, C., Henderson, H.E., Frucht, J.C., Dal longeville, J., Alauapovic, 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, O., Elkhateeb, O., Forqued, M., Amin, A.A., Bogari, D.N., Sahar, M., Bulba, F., Allam, R.M., Bogari, M.N., Almannami, F., 2020. Association between HindIII (rs3220) 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.
