Atherosclerosis 229 (2013) 511–516

Contents lists available at SciVerse ScienceDirect

Atherosclerosis

journal homepage: www.elsevier.com/locate/atherosclerosis

Lipoprotein lipase gene polymorphism rs1059611 functionally influences serum lipid concentrations

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ARTICLE INFO

Article history:
Received 24 September 2012
Received in revised form
7 April 2013
Accepted 6 May 2013
Available online 18 May 2013

Keywords:
Lipid
Lipoprotein lipase
Expression
Transcription factor

ABSTRACT

Objective: Dozens of single nucleotide polymorphisms (SNPs) in the lipoprotein lipase (*LPL*) gene have been reported to be associated with lipid concentrations. The aim of this study was to validate the association between rs1059611 in the *LPL* gene and serum lipid concentrations in the Chinese Han population and explore the biological relevance.
Methods: A total of 5664 participants were recruited and genotyped for the SNP. Gene expression levels of *LPL* in blood cells were evaluated by real-time PCR and western blotting analysis. The functional potential of the SNP was examined by luciferase reporter assay and electrophoretic mobility-shift assay (EMSA).
Results: We observed significant associations between rs1059611 and increased HDL-C ($P = 5.65 \times 10^{-5}$) and decreased TG concentrations ($P = 2.68 \times 10^{-7}$). We also found that participants with the C allele had higher mRNA expression level ($P = 0.0334$) and protein expression level ($P = 0.0641$) of *LPL*. The luciferase activity of the rs1059611 T construct was 0.69-fold of the rs1059611 C construct ($P = 0.0009$). The EMSA showed that the binding of the transcription factor(s) differed for the alleles of the SNP.
Conclusion: The results of our study demonstrated that rs1059611 was associated with HDL-C and TG concentrations in Chinese Han population and might have a functional effect on the transcription of *LPL* by differential binding of transcription factors.

1. Introduction

Lipoprotein lipase (LPL) is a key enzyme in the metabolism of lipoproteins. It hydrolyzes plasma lipoprotein triglycerides (TG) into free fatty acids and glycerol, and converts very-low-density lipoprotein to low-density lipoprotein (LDL) [1]. The *LPL* gene is located at 8p22 and comprises 10 exons spanning about 30 kb [2,3]. Lipid concentrations are affected by both lifestyle factors, such as diet, obesity, and physical activity, and genetic factors [4]. Several functional polymorphisms in *LPL* have been studied with regard to their associations with lipid profile and risk of coronary heart disease in candidate gene association studies [5,6]. Variants in the 3′ region of the gene may play an important part in affecting gene transcription and expression [7]. It was reported that a haplotype based on 19 single nucleotide polymorphisms (SNPs), of which 15 SNPs were located in the 3′-untranslation region (3′-UTR) of the *LPL*

Abbreviations: 3′-UTR, 3′-untranslation region; BMI, body mass index; DBP, diastolic blood pressure; EMSA, electrophoretic mobility-shift assay; GLU, glucose; GWAS, Genome-Wide Association Study; HDL-C, high-density lipoprotein cholesterol; HWE, Hardy–Weinberg equilibrium; LD, linkage disequilibrium; LDL-C, low-density lipoprotein cholesterol; MAF, minor allele frequency; SBP, systolic blood pressure; SNP, single nucleotide polymorphism; TG, triglycerides.

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http://dx.doi.org/10.1016/j.atherosclerosis.2013.05.005

gene, was associated with increased LPL activity and multiple phenotypes related to the metabolic syndrome [8]. SNP rs1059611 located in the 3′-UTR of the *LPL* gene was in strong linkage disequilibrium (LD) with many lipid-associated SNPs in or near *LPL* identified by genome-wide association study (GWAS), such as rs328, and was reported to be associated with lipid levels in GWAS replication studies [9–11].

A functional SNP in non-coding regions would be most likely affecting gene regulation by altering the binding of some transcription factors. Computer-based analysis (i.e. TFSearch and TESS) showed that rs1059611 could alter the binding of a transcription factor YY1 (JASPAR ID: MA0095.1) at this site. YY1 is a ubiquitously distributed transcription factor belonging to the GLI-Kruppel class of zinc finger proteins. The protein is involved in repressing and activating a diverse number of promoters [12]. The algorithm miRanda (http://www.microrna.org/microrna/home.do) also suggested that rs1059611 resided in a putative microRNA (miR-136) binding site. Besides, this polymorphism (among others) is able to modify gene expression but also affects activity through the LD with other SNPs in *LPL* gene. Whether rs1059611 is essentially a surrogate for a functional SNP that is so well characterized or a functional SNP needs to be tested.

The association between rs1059611 and lipid concentrations was less reported in the Chinese Han population. We conducted a study to validate the associations between rs1059611 and serum lipid concentrations and the biological relevance. We now report on the lipid associations and the findings of *in vivo* and *in vitro* experiments for rs1059611.

2. Materials and methods

2.1. Study populations

A group of 5664 participants were randomly selected from the International Collaborative Study of Cardiovascular Disease in Asia (InterASIA) [13] and judged to be free of coronary, cerebrovascular, renal diseases and other major chronic diseases by medical history. Standard questionnaire were used by trained interviewers to obtain information on demographic characteristics including age, sex, ethnicity, details of medical history, smoking and alcohol consumption. Blood pressure, weight and height were recorded. This study was approved by the local bioethics committee, and all subjects gave written informed consent.

2.2. Biochemical measurements

Overnight fasting blood samples were drawn by venipuncture to measure serum biochemical measurements including total cholesterol, High-density lipoprotein cholesterol (HDL-C), TG and glucose (GLU). Blood specimens were processed in the central clinical laboratory at the Department of Population Genetics at Fuwai Hospital of the Chinese Academy of Medical Sciences in Beijing. This laboratory participates in the Lipid Standardization Program of the US Centers for Disease Control and Prevention. Total cholesterol, HDL-C, TG and GLU were analyzed enzymatically on a Hitachi 7060 Clinical Analyzer (Hitachi High-Technologies Corp). The LDL-C concentrations were calculated by use of the Friedewald equation.

2.3. DNA extraction and genotyping

Genomic DNA was isolated from white blood cells according to a standard procedure using a DNA extraction kit (Tiangen Biotech, Beijing, China). SNP was genotyped using genomic DNA with the fluorogenic 5′-nuclease TaqMan allelic discrimination assay system (Applied Biosystems, Foster City, CA). The assays were performed under standard conditions on a 7900 HT Fast Real-Time PCR instrument. Probe and primer sequences can be found in Supplementary Table 1. Successful genotyping rate was over 98%. For quality control, 2% of the samples were randomly selected and genotyped in duplicate. A comparison of the duplicate results showed that the discrepancy was no more than 1%.

2.4. RNA extraction and real-time PCR

To analyze the mRNA level of the *LPL*, we separated peripheral blood mononuclear cells (PBMC) from fresh blood samples of 309 healthy individuals. Total RNA was extracted with TRIzol (Invitrogen, Carlsbad, CA). After quantification, 1 μg of total RNA was reverse transcribed using Moloney Murine Leukemia Virus Reverse Transcriptase (Tiangen Biotech, Beijing, China) with oligo dT according to the manufacturer's instructions.

Real-time PCR was performed using the Applied Biosystems 7900 HT Fast Real-Time PCR System (Applied Biosystems, USA). Expression of all assays was measured in triplicates and average values of the triplicates were used for the analysis. Gene expression was quantified using the comparative Ct method, which standardized the Ct values to an internal housekeeping gene (*ACTB*) and calculated the relative expression values (ΔCt method).

2.5. Western blotting analysis

Proteins were extracted from PBMC in trizol lysis and soluted in 1% SDS. Proteins were separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE), and then transferred by electroblotting to a nitrocellulose membrane. The membrane was blocked in 5% milk and then incubated with anti-LPL (sc32885, Santa Cruz, USA) and anti-ACTB (sc47778, Santa Cruz, USA) in 5% milk overnight at 4 °C, then washed and incubated with horseradish peroxidase-labeled second antibodies for 1 h at room temperature. Bands were visualized by the use of a super-western sensitivity chemiluminescence detection system (Pierce). Autoradiographs were quantitated by densitometry (Science Imaging System, Bio-Rad). *ACTB* was the internal control for protein normalization.

2.6. Construction of the luciferase reporter gene

The genomic sequences of *LPL* containing the site of rs1059611 (374-bp) were amplified by PCR from one individual homozygous for the major allele and one individual homozygous for the minor allele. PCR primers were presented in Supplementary Table 2. The PCR products were then purified and digested with the two designated restriction endonucleases (BamHI and SalI), and further sub-cloned into the firefly luciferase expressing pGL3-promoter vector (Promega) to create plasmid: pGL3-promoter-CC and pGL3-promoter-TT. Although there are SNPs in LD with rs1059611 in the region, the constructs have been sequenced to verify that the only ambiguity was the polymorphic site.

2.7. Cell cultures, transfections and luciferase assays

NIH3T3-L1 cells (mouse preadipocyte cells) were obtained from Dr. Peng's laboratory (Peking Union Medical College). Cells were grown in Dulbecco's modified Eagle's medium (DMEM; Invitrogen) supplemented with 10% fetal bovine serum (Hyclone, Logan, UT) at 37 °C in a humidified incubator containing 5% CO_2. Cells were plated at a density of 4×10^4 cells per well in 24-well plates for transfection. Transfections were performed using Attractene transfection reagent (Qiagen, Valencia, CA) according to the