ELSEVIER

Archives of Medical Research 41 (2010) 19–25

Archives of Medical Research

ORIGINAL ARTICLE

# Associations for Lipoprotein Lipase and Peroxisome Proliferator-activated Receptor-γ Gene and Coronary Artery Disease in an Indian Population

Manickaraj AshokKumar,[a] Navaneethan Gnana Veera Subhashini,[a] Sekar Kanthimathi,[a] Ramineni SaiBabu,[a] Arabandi Ramesh,[c] Kotturathu Mammen Cherian,[a] and Cyril Emmanuel[b]

[a]*International Centre for Cardiothoracic and Vascular Diseases, Frontier Lifeline, Chennai, India,* [b]*Global Hospitals and Health City, Chennai, India,* [c]*Department of Genetics, Dr. A. L. Mudaliar Postgraduate Institute of Basic Medical Sciences, University of Madras, Taramani Campus, India*

Received for publication July 29, 2009; accepted October 22, 2009 (ARCMED-D-09-00365).

*Background and Aims*. Peroxisome proliferator activated receptor-γ (PPARγ) and lipoprotein lipase (LPL) genes are important in pathways of triglyceride metabolism, insulin resistance and adipogenesis. We hypothesized that polymorphisms of PPARγ Pro12Ala, LPL HindIII and LPL Ser447X influence severity of coronary artery disease (CAD) in an Indian population.

*Methods*. PPARγ Pro12Ala, LPL HindIII and LPL Ser447X polymorphisms were genotyped in 414 patients with CAD and matched with 424 controls. The study subjects were inducted after standard diagnostic procedures and analyzed statistically for the association of polymorphisms with clinical characteristics.

*Results*. We found that PPARγ alleles were not associated with CAD among Indians although proline carriers had significantly higher levels of HDL-cholesterol ($p = 0.03$) among CAD patients. The LPL HindIII also had no significant correlations for CAD or for any clinical characteristics. The Ser447X polymorphism ($p = 0.015$) influenced lower triglyceride levels among CAD patients with significant associations (OR = 0.66, 95% CI 0.483–0.915, $p = 0.012$). This protective effect of the 447X allele was more pronounced among the CAD patients without the risk factor of diabetes (OR = 0.60, 95% CI 0.403–0.907, $p = 0.014$) along with less progression of a severe atherosclerotic disease.

*Conclusions*. PPARγ and LPL have intractable roles in pathways that lead to CAD, but their gene polymorphisms associate differently. Our results imply a significant correlation of Ser447X polymorphism and its protective effect on Indians against severity of CAD modified by the risk of diabetes, than LPL HindIII and PPARγ Pro12Ala. © 2010 IMSS. Published by Elsevier Inc.

*Key Words:* Coronary artery disease, Polymorphisms, Indians, Lipoprotein lipase, Peroxisome proliferator-activated receptor-γ, Type 2 diabetes.

## Introduction

Coronary artery disease (CAD), the leading cause of death worldwide (1), has an increasing incidence among Indian populations with a frequency of 3–10% among adults >20 years of age (2). Type 2 diabetes mellitus (T2DM), dyslipidemia, hypertension, family history, and increased body mass index are significant risk factors for CAD (3), which increases the rate of morbidity and mortality. T2DM is associated with dyslipidemia, hypertension and obesity, and augments onset of atherosclerosis, resulting in CAD and heart failure in the long term (4). Lipoprotein lipase (LPL) and peroxisome proliferator-activated receptors (PPARs) are the two important proteins playing a key role in triglyceride metabolism, insulin resistance and adipogenesis. Variations in those two genes are associated with both CAD and T2DM in various populations (5–12).

The LPL gene is located on chromosome 8p22 and codes for a 475-amino acid protein, which includes

Address reprint requests to: Dr. Cyril Emmanuel, Ph.D., Director, Research and Academics, Global Hospitals and Health City, Perumbakkam, Chennai-600100, India; Phone: +914422777000, +919444206238; Fax: +914422777100; E-mail: emmanuel69@rediffmail.com

0188-4409/10 $–see front matter. Copyright © 2010 IMSS. Published by Elsevier Inc.
doi: 10.1016/j.arcmed.2010.01.005

a 27-amino acid signal peptide (6). LPL is the key enzyme in the hydrolysis of triglycerides on chylomicron and very-low-density lipoprotein (VLDL). The released fatty acids are metabolized principally by heart, skeletal muscle and in adipose tissues, which are the principal sites of LPL synthesis (7). PPARγ, located on chromosome 3p25, is a transcription factor activated by fatty acid or fatty acid derivatives. It forms heterodimers with retinoic acid receptor to regulate expression of target genes that are active in lipid metabolism. PPARγ has two isoforms (γ1 and γ2), which differ at their N terminus (13,14). The LPL promoter is known to carry a PPAR response element within 230 bp upstream of the LPL gene (15,16).

PPARγ has been shown to have roles in regulation of cholesterol uptake and efflux in macrophages. Also, PPARγ promotes uptake of oxidized non-native low-density lipoprotein (LDL) and subsequent differentiation of monocytes into foam cells (17,18). The most extensively studied polymorphism of proline to alanine change in codon12 (Pro12Ala) results in a reduced transcriptional activity of PPARγ2 (5) and also is associated with decreased risk of T2DM (19). This polymorphism is shown to greatly influence LPL activity in humans rather than that of other frequent polymorphisms within the LPL gene itself (20). However, the LPL HindIII polymorphism on intron 8 and Ser447X mutation on exon 9 play an important role in the inter-individual variability of plasma lipid concentrations (11,12,21).

Numerous studies have been performed to evaluate the impact of these gene polymorphisms individually in various populations. The combined effects of variant alleles are a means to find comprehensive associations of genes with the disease. The present study aims to find the associations of the interacting PPARγ polymorphism (Pro12Ala), LPL HindIII polymorphism and Ser447X mutation among Indian CAD patients.

## Materials and Methods

### *Patient Population*

The studied subjects were patients with CAD ($n$ = 414) who underwent elective coronary angiography at the International Centre for Cardiothoracic and Vascular Diseases, Chennai, India. Among the diagnosed subjects there were individuals with T2DM ($n$ = 181) and without diabetes as a risk factor ($n$ = 233). The following data were obtained from medical records: cigarette smoking, hypertension, family history of CAD, and dyslipidemia. These data were used to create a database of the studied population. Body mass index was calculated as weight/height$^2$. An equal procedure was applied to obtain age-matched controls ($n$ = 424) from the inpatient and outpatient departments, who ruled out CAD using a standard set of procedures including angiography, treadmill test, cardiac enzymes and/or CT scan.

Blood was collected for biochemical investigations and DNA isolation appropriately while fasting but before any administration of therapeutic drugs or an interventional procedure. Informed written consent was obtained according to the institutional ethics committee from all participants after explaining the objectives and details of the study. Plasma glucose, serum cholesterol, serum triglycerides, LDL cholesterol, and HDL cholesterol and creatinine tests were performed at the clinical biochemistry department on a Randox RX Daytona auto analyzer (GMI, Inc. Ramsey, MN), using enzymatic kits (Randox Enzymatic Kits, GMI Inc). Genomic DNA was isolated with salt and heat treatment method (22). DNA was quantified with a spectrophotometer and also by running on a 0.8% agarose gel electrophoresis.

### *Genotype Determination*

The Pro12 (CCA) → Ala (GCA) single nucleotide polymorphism was amplified and identified using primers described previously (23). The forward primer 5′-TCTGGGAG ATTCTCCTATTGGC-3′ has an additional single-base mutation located 2-bp upstream of the site of substitution. The reverse primer is normal 5′-CTGGAAGACAACTACAA GAG-3′ and the amplified product size is 154 bp. The products were digested using Hha I (Fermentas, Burlington, Ontario) and the 131- and 23-bp digested products were genotyped on a 2.5% high resolution agarose (Sigma Chemicals, St. Louis, MO) electrophoresis. The rare genotypes were confirmed using 14% polyacrylamide gel electrophoresis and ethidium bromide staining.

The HindIII polymorphism of the LPL gene was amplified using primers used by Anderson et al. (10). Forward and reverse primers were LPL-H1 5′-TGAAGCTCAAATGG AAGAGT-3′ and LPL-H2 5′-TCAAAGCAAATGACT AAA-3′, respectively. The amplified products of 770 bp were then subjected to HindIII (Fermentas) digestion. The presence of the restriction site (H+) yielded 600-bp and 170-bp fragments. The LPL Ser447X mutation was identified using the primers 5′-TACACTAGCAATGTCTAGGTGA-3′ and 5′-TCAGCTTTAGCCCAGAATGC-3′ used by Kuivenhoven et al. (24). The 489-bp product was digested with Mnl I (Fermentas) and yielded 287 and 203bp products for Ser/Ser genotype, 287, 242, 203 and 45bp products for Ser/X and 242, 203 and 45bp products for X/X.

### *Statistical Analysis*

The genotypes were double confirmed by investigators blinded to the clinical condition. Allelic and genotypic frequencies among patients were tested with $\chi^2$ test and logistic regression. Hardy–Weinberg equilibrium for the distribution of genotypes was estimated by the $\chi^2$ test. One-way ANOVA models were used to assess effects of the disease, polymorphisms and the interaction between disease group and polymorphisms. Least-squares contrasts of the ANOVA models were applied for multiple comparisons of

Download English Version:

https://daneshyari.com/en/article/3447365

Download Persian Version:

https://daneshyari.com/article/3447365

Daneshyari.com