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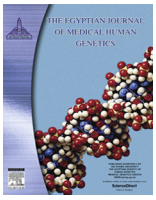

Original article

An association between apo-A4 gene polymorphism (Thr347Ser and Gln360His) and coronary artery disease in northern India

Pramod Kumar [a], Arbind Kumar Choudhary [b,*], Nibhriti Das [a]

[a] Department of Biochemistry, All India Institute of Medical Sciences, New Delhi, India
[b] Department. of Physiology, L.N. Medical College and J.K. Hospital, Bhopal, India

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ABSTRACT

Background: Coronary artery disease (CAD) is emerging as a major health problem in India. It is predicted that by 2020, India will be at the verge of CAD epidemic. A low level of high-density lipoproteins (HDL) is prevalent in Asian Indians and is the major lipid risk factor for CAD. HDL contains Apo A, E, C and antioxidant enzymes. The genetic variants of these proteins appear to influence the occurrence and frequency of CAD. In this context APO A4 have drawn much attention. The polymorphisms at Thr347Ser and Gln360His of the apo A4 gene are under investigation in different parts of the world in relation to dyslipidemias, diabetes and CAD. Since data are conflicting and no conclusive data is available from India.
Objective: We aimed at studying the relationship between apoA4 gene polymorphisms (Thr347Ser and Gln360His) and coronary artery disease in northern Indian participants.
Method: We recruited 200 control (Group-I) and 200 patients (Group-II) and used PCR-RFLP to study the gene polymorphisms. Enzymatic Kits were used to estimate the lipids and lipoproteins.
Result: We observed not any significant association for ApoA4 Thr347Ser polymorphism as well as lipid profile [total cholesterol (TC), triglyceride (TG), HDL, low-density lipoproteins (LDL) and HDL/LDL] levels among AA, AT and TT Individuals in controls and patients. However, after adjusting for age and sex, among control AA genotype had significantly lower levels of oxidised LDL (OXLDL) as compared to AT genotype and in patients, levels of OXLDL in AT genotype was lower than with AA genotype and for ApoA4 Gln360His polymorphism, after adjusting for age and sex, and no significant difference was observed in TC, TG, HDL, LDL and HDL/LDL, OXLDL and LDL / OXLDL levels among 1–1, and 1–2. Individuals in controls and patients.
Conclusion: To accomplish, this preliminary study brought the information on the ApoA4 polymorphism in the Asian Indians residing in Delhi and adjacent areas. The minor alleles of the Ser347 and His360 showed significant association with lipid risk factors like high levels of OXLDL, TC, and low HDL levels. However neither of these polymorphisms showed an association with CAD.

1. Introduction

The impairment of cardiac function due to inadequate blood flow to the heart, leads to Coronary artery disease (CAD) [1]. Hypercholesterolemia as well as high level of LDL cholesterol considered as major risk factors for development and progression of atherosclerosis [2]. low-density lipoproteins (LDL) driven atherosclerotic plaque formation result in tissue ischemia and end organ damage in coronary artery disease [3]. Among Indians, CAD has been found to be severe, diffuse and associated with serious complications and increased mortality at younger age [4]. Among Asian Indians, Low levels of HDL and hypertriglyceridemia have emerged as major risk factors for CAD [5]. Genetic factors such as Cluster of apolipoprotein gene has been identified as a potential genetic contributing factor for inter-individual differences in the levels of high-density lipoproteins (HDL) cholesterol and triglyceride (TG) and predisposition to cardiovascular diseases [6].

ApoA4 has been known to play a major role in lipid metabolism at several steps of reverse cholesterol transport [7] and acting as potent inhibitor of lipid peroxidation and thus provides protection against the risk of atherosclerosis [8]. ApoA4 gene is polymorphic at codons 127, 130, 347 and 360 [9] and most commonly studied

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* Corresponding author at: Dept. of Physiology, L.N. Medical College and J.K. Hospital, Bhopal 462007, India.
E-mail address: arbindchoudhary111@gmail.com (A.K. Choudhary).

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polymorphisms in relation to cardiovascular disorders and dislipidemias are apoA4-347 and apoA4-360 [10]. The association of apoA4 Thr347Ser with coronary artery disease and other cardiovascular disease had been studied in different population [6,11].

ApoA4 is a polymorphic gene and this polymorphism arises due to a substitution of base adenine (A) to thymine (T) in the apoA4 gene. Due to this base change the amino acid changes from threonine to serine. Three genotypes are observed. These are AA, AT, TT. Where AA represents threonine and TT represents serine.

A Polymorphism of Thr347Ser (of apoA4 gene) lowered antioxidant status and associated with cardiovascular disease (CVD) [12]. Ser347 allele associated with lower plasma Apo B, Apo A4 level and LDL-C level, and increased level of LDL [12–15]. A Polymorphism Gln360His (of apoA4 gene) has been associated with triglycerides (TG), HDL-C, LDL-C, glucose and ApoA4 level [11,12,16]. However, polymorphisms apoA4-347 and apoA4-360 have been studied mostly in Western countries and information as well as contradictory in different population studies. Up till now, study is scanty from north Indian population.

Hence, the present study was designed to elucidate the relationship of the Thr347Ser and Gln360His polymorphisms with coronary artery disease. Objectives were (I) To study the frequency distribution of apoA4 Thr347Ser, Gln360His genotype and alleles in healthy individuals (control) and patients with CAD; (II) To determine the levels of cholesterol, TG, HDL, LDL and oxidised LDL in study subjects; (III) To evaluate the correlations of apoA4 Thr347Ser and Gln360His polymorphisms with lipid profiles; and (IV) To evaluate the correlations of apoA4 Thr347Ser and Gln360His polymorphisms with CAD.

2. Subjects and methods

2.1. Biological reagents

ApoA4 Thr347Ser, Gln360His sense and antisense primers, Hinf1, Fnu4HI restriction enzymes, deoxynucleotides, Taq DNA polymerase with buffers and DNA markers were purchased from Promega Corporation, USA. Proteinase K was obtained from Banglore Genei.

2.2. Ethnic statement

The study was approved by ethical committee and the research advisory committee of All India Institute of Medical Science and Hospital. The present study was conducted in the Department of Biochemistry, All India Institute of Medical Science, Delhi, India. The work was carried out in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki). Detailed written consent of all the participants was taken and the purpose of study was explained to the participants and assurance was given to the participants that test is not hurtful.

2.3. Participants

2.3.1. Inclusion and exclusion criteria

Participants meeting the following criteria were included: All patients were assessed angiographically, patients with $\geq$ 70% diameter stenosis in $\geq$ 1 coronary artery involvement, and Age > 40 years, and patients with other complication such as diabetes and hypertension were included and, patients with < 70% Stenosis, Cardiomyopathy and Drugs abusers were excluded. For Healthy individuals, subjects free from clinical diagnosis for any major chronic or acute illness were included and individuals age < 40 years and pregnant women were excluded. Finally, all selected participants were divided randomly into the following groups, Group I-(n = 200)-normal healthy individual and the study group were patients suffering with CAD in Group II (n = 200).

2.3.2. Sample collection, processing and storage

Under aseptic conditions, venous blood (5 ml) was collected in EDTA and serum tube, from each participant after overnight fasting. Plasma was separated by centrifugation at 4 °C, and store at −70 °C until further analysis. The packed cells were processed for DNA isolation. Clotted blood was allowed to centrifuge at 2000 rpm for 10 min and separated serum was stored at 4 °C until further analysis.

2.4. Protocol

2.4.1. DNA analysis by PCR-RFLP for elucidating Apo A4 T347S and G360H polymorphisms

Gnomic DNA samples were isolated from the leukocyte of blood samples. RBC was lysed using RBC lysis buffers (Tris, Mgcl2, Nacl, pH 7.6). The DNA from leukocytes was extracted by proteinase K digestion and ethanol extraction. The genomic DNA was amplified by polymerase chain reaction (PCR) using primers of ApoA4 gene. Primer pairs were used: [sense- 5′- CGGGT GGAGCCCTACGGGGA-3′] and [antisense- 5′- TGGGGCCAGTGCACCAGGGG-3]′ (Boerwinkle et al. [17], Zaiou et al. [18]). DNA was initially denatured for 4 min at 94 °C, followed by 35 cycles at 94 °C for 30 s and 65 °C for 75 s and then finally at 65 °C for 10 min. Amplified products were run on a check gel with 0.8% agarose. The amplified product was 300 bp. For Thr347Ser polymorphism the amplified product was digested with HinfI which gave rise to 183 bp and 117 bp band in homozygous allele (+/+) where as in homozygous rare allele (−/−) site for HinfI get lost so it gives only 300 bp band. In heterozygous stateb (+/−) it gives all the three bands of 300 bp, 183 bp and 117 bp. For Gln360His polymorphism amplified product was digested with Fnu4HI it gave rise to 180 bp, 65 bp, 43 bp and 12 bp band in homozygous allele (+/+). Whereas in homozygous rare allele site for one site of Fnu4HI get lost which give rise to 192 bp, 65 bp and 43 bp. In heterozygous state it gives all the five band of size 192 bp, 180 bp, 65 bp, 43 bp and 12 bp (+/−). Digested PCR fragment was visualised using silver staining.

2.4.2. Analysis of lipid profile

Triglycerides and Cholesterol estimation was done by enzymatic kit method (Centronic GmbH); HDL cholesterol estimation was done by Wilson and Spiger method, LDL cholesterol was calculated by = Total cholesterol – TG/5-HDL cholesterol, and oxidised LDL (OXLDL) was estimated by ELISA kit method (Mercodia).

2.5. Statistical methods

Statistical methods were done according to Statistical Methods for Medical Research using SPSS-9 software programme. Frequency of genotypes (AA, AT, TT) and alleles (A, T) of Thr347Ser and (1–1, 1–2, 2–2) and alleles (1, 2) of Gln360His of apoA4 gene were assessed using Fisher's exact test and chi-square. The statistical analysis was performed by student *t* test or Mann-Whitney test as relevant. $P \leq 0.05$ was considered statistically significant.

3. Result

3.1. The identification of apoA4 Thr347Ser alleles and genotypes

The Fig. 1 represents the PCR product of apoA4 gene of 300 bp. After digestion with Hinf1 the resulting fragments [AA(lane2) = 183 bp; 117 bp, AT(lane3) = 300 bp; 183 bp; 117 bp and TT