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Association of Apolipoprotein B Gene Signal Peptide Insertion-Deletion Polymorphism and the Associated Lipid levels with Coronary Artery Disease

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Abstract :

Background and objectives - Apolipoprotein B (apo B) is the principal structural protein of low density lipoprotein (LDL), involved in the lipoprotein metabolism and in maintaining the cholesterol homeostasis. It functions as a ligand for the LDL-receptor, thus mediating the cellular uptake of cholesterol. A polymorphism located in the coding part of the signal peptide of APOB gene has been associated with altered lipid levels, mainly increased plasma total cholesterol, LDL and apo B concentrations. We carried out this study to determine the association of APOB signal peptide Ins- Del polymorphism and its associated lipid levels with coronary atherosclerosis. Methods - Genotype analysis was done on 100 patients with angiographically proven coronary atherosclerosis and 100 control subjects by polymerase chain reaction. Serum lipids and apolipoprotein B were measured. Results - Patients had significantly higher frequency of Del (ID and DD) genotype than control subjects (0.51 versus 0.27, p - 0.001) with odds ratio of 2.8 (1.6 to 5.1, P - 0.001) between Del (ID and DD) genotype and II genotype for developing coronary atherosclerosis. Significantly higher levels of apo B (148.2 mg per dL versus 106.4, p - 0.000) was observed in coronary atherosclerosis patients as compared to control subjects. Conclusions - The APOB signal peptide Ins-Del polymorphism and its associated high level of apo B were significantly associated with coronary artery disease. The high level of apo B may be an independent risk factor for coronary atherosclerosis.

Keyword :apolipoprotein B, APOB gene, signal peptide, insertion-deletion, coronary artery disease, polymorphism.

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Introduction

Coronary artery disease (CAD) has become a major public health problem in many developing countries (1, 2). CAD is a multifactorial disease caused by genetic and environmental factors (3). Lipoproteins play a central role in the development of atherosclerotic cardiovascular disease in humans. The high level of low density lipoprotein (LDL) is an important risk factor for CAD (4). Apolipoprotein B (apo B) is the principal structural apoprotein of LDL, involved in the lipoprotein metabolism and in maintaining the cholesterol homeostasis.

ApoB-100 is one of the largest monomeric proteins, having 4536 amino acid residues, with a molecular weight of 550 kDa. It is synthesised in liver and functions as a ligand for the LDL-eceptor, thus mediating the cellular uptake of cholesterol (5). High level of apo B is a better indicatorfor CAD risk than LDL (6). Due to its central role in lipid transport and metabolism, examining the genetic variations of the APOB gene could help to explain inter -individual difference in lipid levels and susceptibility to CAD. The gene for human apo B contains 43 kb genomic DNA and is composed of 29 exons and 28 introns (7). It has been localized on chromosome 2p24 (8). The longest coding sequence within the gene is exon 26 and more than one-half of the apoB-100 protein molecule is coded by this exon (9). Two forms of the apo B protein, apoB-48 and apoB-100, are coded by a single APOB gene, but in the intestine, the mRNA undergoes editing, so as to produce the apoB-48 protein (10, 11, 12). ApoB-48 is so named because it is only 48% of the size of apo B -100. Numerous polymorphisms of the apolipoprotein B gene have been described (13, 14, 15). The APOB signal peptide Ins/Del polymorphism consists of an insertion or deletion of three codons involving three amino acids, leucine-alanine-leucine (16). The APOB signal peptide is coded by exon 1 and contains either 24 or 27 amino acids. Its function is mainly concerned with secretion of the mature protein across the cell membrane, and it is cleaved off shortly after the insertion of the protein at a membrane site (17). The presence or absence of three amino acids in the hydrophobic region of the signal peptide could affect the degree of hydrophobicity and efficiency of apo B processing. This polymorphism was first typed directly using polymerase chain reaction (PCR) by Boerwinkle and Chan (18). The common alleles are the insertion (Ins) allele (93 bp), which contains a 27-amino acid signal peptide, and the deletion (Del) allele (84 bp), with a 24-amino acid signal peptide resulting from the deletion of three amino acids (19). In epidemiological studies, the Del allele has been associated with altered lipid levels, mainly increased plasma total cholesterol, LDL and apo B concentrations (20-27). A meta-analysis has shown that subjects with the Del/Del APOB genotype had higher cholesterol levels and an increased risk of coronary heart disease (6). The aim of the present study was to determine the association of apolipoprotein B gene signal peptide insertion/ deletion polymorphism and the concerned lipid levels with coronary artery disease.

Materials and methods

Study population

The study sample comprised 100 unrelated coronary artery disease patients (90 male, 10 female) of mean age 50.79±9.3 years. Inclusion criteria was more than 50% stenosis of at least one of the major coronary arteries. Patients with recent episode of myocardial infarction (less than 3 months) were excluded. 100 control subjects were recruited from out-patient department during their visit for non- cardiac cases. Age, Sex and other confounding

factors like diabetes, hypertension, smoking, alcoholism were matched. For all diabetic controls, tread mill test was done. Only those with negative tread mill test result were included in the study.

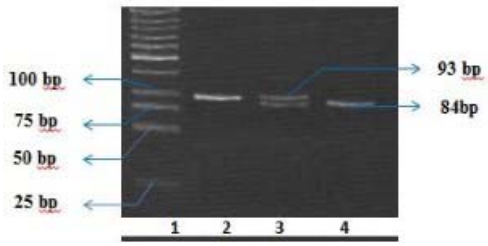
Methods

Recumbent blood pressure and 12 lead electrocardiogram (ECG) were recorded on each subject after a thirty minute rest on the couch. Height and weight were recorded and blood samples were collected by venipuncture after fortnight fasting in two test tubes. One was collected into plain nonadditive tube and the other anticoagulated with ethylene diamine tetra-acetic acid (EDTA). Plain tube was centrifuged at 2000 rpm for 20 minutes and serum was used for lipid profile estimation. EDTA-containing tube was centrifuged at 2000 rpm for 20 minutes to get the buffy coat for DNA extraction.

Lipid analysis

Lipid profile was measured by enzymatic methods with an auto analyser (XL 300) and manufacturers Agent kits. Serum total cholesterol was estimated by Esterase Oxidase method and triglycerides by colorimetric enzymatic method. High Density Lipoprotein cholesterol (HDL-C) and Low density lipoprotein cholesterol (LDL-C) were estimated by a modified polyvinyl sulfonic acid (PVS) and polyethylene-glycol methyl ether (PEGME) coupled classic precipitation method. Apolipoprotein B was measured by turbidimetric immunoassay.

APOB signal peptide Ins/Del polymorphism analysis



DNA was extracted from buffy coat by modified high salt method (28). The Ins/Del site in exon 1 of the APOB gene was analysed by amplifying a fragment of 93/84 bp using two primers designed by Boerwinkle et al. (18) : 5'-CAGCTGGCGATGGACCCGCCGA-3' (forward primer) and 5'-ACCGGCCCTGGCGCCCGCCAGCA-3' (reverse primer).

Figure 1 shows APOB signal peptide Ins/Del polymorphism genotyping. Lane 1: Ladder, Lane

2: II genotype, Lane 3: ID genotype, Lane 4: DD genotype

Genomic DNA (200 ng) was amplified in 25 L reaction mixture containing 10 pmol of each primer and red dye master mix (Bangalore Genei) containing 100 mol/L of each dNTP, 2.5 L of 10X reaction buffer and 0.6 unit of Taq DNA polymerase. After the DNA was denatured for 5 minutes at 95°C, the reaction mixture was subjected to 30 cycles of denaturation for 1 minute at 94°C, 1.5 minutes of annealing at 64°C and 1 minute of extension at 72° C. Final extension was carried over at 72° C for 10 minutes. The amplified products were separated on the 12% native Poly Acrylamide Gel Electrophoresis and visualized under ultraviolet light after ethidium bromide staining (figure 1). The PCR product is the 84 bp fragment in the presence of a deletion (D) allele, and a 93 bp fragment in the presence of the Insertion (I) allele. Thus, each DNA sample revealed one of the three possible patterns after electrophoresis: a 93bp band (II genotype), 84 bp band (DD genotype), or both 93 and 84 bp bands (ID genotype).

Statistical Analysis:

Direct gene counting method was used to determine the frequency of genotypes and alleles. Age, BMI, serum lipid levels were compared between control

subjects and patients by Student's t-test. Genotype frequency distribution between cases and controls were compared using chi-square test; $p < 0.05$ was considered significant. Serum apo B and LDL-C levels were compared between genotypes by using Student's t-test. The association between the genotypes and CAD risk was analysed by calculating the odds ratio (OR) and 95% confidence interval (95% CI) using the chi-square test. Logistic regression analysis was performed to evaluate the interaction between APOB signal peptide Ins/Del genotypes and other variables in relation to the prevalence of coronary artery disease. Independent variables included in the analysis were age (quantitative), sex (male/female), smoking (yes/no), Alcoholism (Yes/No), Hypertension (Yes/No), Diabetes (Yes/No), Serum levels of total cholesterol, triglycerides (Quantitative). The analysis was executed by SAS Statistical program Version 6.10 for Macintosh.

Results

Table 1 shows Age, Sex, BMI, Total cholesterol, Triglycerides, HDL-C, LDL-C, apo B levels and conventional risk factor distribution among patients and control subjects. Since all the confounding factors were matched there were no significant differences between the two groups. There was a significant difference in the Total cholesterol, Triglycerides, HDL-C, LDL-C, apo B levels among patients and control subjects.

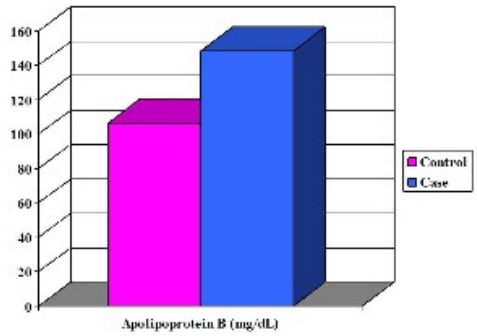


Figure 2 shows the comparison of serum apolipoprtein B levels among controls and cases. There is a significantly high level of apo B among cases (148.2±43.8 mg/dL) when compared to controls (106.4±41.5 mg/dL). P value was 0.000.

Table 2 shows genotype distribution and allele frequencies of human APOB gene signal peptide Ins/Del polymorphism in patients with CAD and control subjects. The allele frequencies were Ins/Ins (II) = 122, Ins/Del (ID) = 68 and Del/Del (DD) = 10. This was found to be in Hardy Weinberg equilibrium. DD genotype was more frequent among cases (7%) when compared to controls (3%). In contrast II was more common among controls (73%) when compared to cases (49%). There was a significant difference in the distribution of ID genotype also between cases (44%) and controls (24%). P value is 0.002. In short D+ (ID+DD) genotype is more common among cases (51%) when compared to controls (27%). P value is 0.001.

Table 2: Distribution of APOB signal peptide Ins/Del genotypes and allele frequencies among CAD patients and controls Figure 3 shows the relationship between serum levels of apo B, LDL-C and genotypes

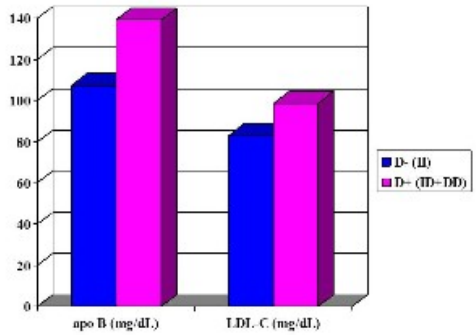


Table 1: Characteristics of patients with CAD and of control subjects

Variables	Case (n=100)	Control (n=100)	P value
Age (years)	50.79 ± 9.3	50.76 ± 9.1	0.99 –NS
Sex male (%)	90	88	0.82 –NS
Female (%)	10	12	
DM (%)	51	48	0.77 –NS
HT (%)	47	50	0.77 –NS
DM+HT (%)	16	14	0.84 – NS
SMK (%)	54	47	0.39 –NS
ALC (%)	53	48	0.48 –NS
BMI (kg/m^2)	25.3 ± 3.2	24.9 ± 3.1	0.37 –NS
Total cholesterol (mg/dL)	179.1 ± 24.6	157.8 ± 23.1	0.000 –S
Triglycerides (mg/dL)	160.1 ± 41.7	127 ± 29.6	0.000 –S
High Density lipoprotein (mg/dL)	38.7 ± 9.1	48.3 ± 9.7	0.000 –S
Low Density lipoprotein (mg/dL)	106.9 ± 24.9	83.3 ± 24	0.000 –S
Apolipoprotein B (mg/dL)	148.2 ± 43.8	106.4 ± 41.5	0.000 –S

CAD patients and controls

Genotype	Control (n = 100)	Case (n = 100)	P value
II	73	49	Chi sq = 12.2 p= 0.002 – S
ID	24	44	
DD	3	7	

Genotype	Control	Case	P value
	(n = 100)	(n = 100)	
D- (II)	73	49	Chi sq = 12.11 P = 0.001 – S
D+ (ID+DD)	27	51	

Table 3: Comparison of apo B and LDL-C levels between APOB signal peptide Ins/Del genotypes

Variable	D- genotype (n = 122)	D+ genotype (n = 78)	P value
Apo B (mg/dL)	107.4 ± 41.6	139.2 ± 43.1	0.000 –S
LDL-C (mg/dL)	82.9 ± 24.2	98.6 ± 24.4	0.000 –S

Table 4: Univariate analysis

Genotype	Controls (n = 100)	Cases (n = 100)	Odds ratio (95% CI)
D- (II)	73	49	2.8 (1.6 – 5.1) p = 0.001
D+ (ID+DD)	27	51	

On multivariate analysis, low HDL-C (p = 0.01), high LDL-C (p = 0.000) and high apo B (p = 0.000) were independent correlates of the presence of coronary artery disease, whereas the APOB signal peptide Ins/Del polymorphism (p = 0.094) was not an independent predictor of CAD.

Discussion:

Both genetic and environmental factors play an important role in the pathogenesis of CAD in humans. These factors may vary depending on race and ethnic group (29, 30). The susceptibility to CAD is a complex trait (28, 31). Worldwide studies have been done correlating the APOB signal peptide Ins/Del polymorphism with risk for CAD (6, 32). In view of this, the present study was performed to determine the association of APOB signal peptide Ins/Del polymorphism and its associated serum lipid levels with coronary atherosclerosis. The three human APOB signal peptide Ins/Del genotypes and the phenotypes were determined in 100 patients with CAD confirmed by angiography and 100 control subjects. The insignificant p value with respect to all the confounding variables like age, sex, BMI, history of diabetes, hypertension, smoking, alcoholism showed that the cases and control groups had been perfectly matched. The significantly low HDL level (38.7±9.1), high LDL (106.9±24.9) in cases reemphasises the fact that HDL is protective and LDL causes atherogenesis.When genotype analysis was performed, distribution of D+ genotype was significantly higher among cases (51%) when compared to controls (27%). P value was 0.001 showing that it is significant. This proved that D+ genotype of APOB signal peptide Ins/Del polymorphism is significantly associated with coronary atherosclerosis. D+ here includes both ID and DD, when analysed separately also, we found that both ID and DD were found more among cases (44% and 7% respectively). The inference of this is that presence of even a single Del allele is significantly associated with atherosclerosis. The evidence available showed that there is a significantly high level of apo B among cases (148.2±43.8) when compared to controls (106.4±41.5). p value was 0.000. This showed that high level of apo B is an independent risk factor for atherosclerosis. When the apo B levels were compared between D+ and D- genotypes there was a significantly high level of apo B among D+ genotypic individuals (139.2±43.1) when compared to D- genotypic individuals (107.4±41.6). P value was 0.000, suggesting the fact that D+ genotype is associated with high apo B level and this high level makes a person more susceptible to atherosclerosis. The mechanism underlying the effect of the Del allele on serum lipid levels or CAD risk is not completely understood. This variant may affect the rate of translocation of newly synthesized apo B from cytoplasm into the endoplasmic reticulum and reduces the rate of biosynthesis and secretion of apo B from hepatocytes in the form of LDL or lipoprotein (a). Given the role of apo B as a ligand for removal of LDL from the circulation via the LDL receptor, the presence of the Del allele might cause hyperlipidemia.

When the LDL-C levels were compared between D+ and D- genotypes, D+ genotype was associated with high LDL -C levels (98.6±24.4) when compared to D- genotype (82.9±24.2), p value was 0.000. Many large-scale studies have reported an association between apo B levels, LDL levels and the APOB signal peptide Ins/Del polymorphism (20-23, 33, 34). On univariate analysis, the odds ratio for D+ genotype was 2.8 (95% CI

1.6 to 5.1; p = 0.001). This shows that D+ genotype favours atherosclerosis. Multivariate analysis showed that this association was not independent of other factors related to atherosclerosis risk. In conclusion, the results of the present study indicate significant association of APOB gene signal peptide Ins/Del polymorphism and the associated high apo B level with coronary artery disease. The high level of apo B may be an independent risk factor for coronary atherosclerosis.

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