The major lipids of human body are phospholipids, cholesterol, triglycerides and cholesteryl esters. These insoluble lipids are transported through blood as lipoprotein complexes of lipids and one or more specific proteins, called apolipoproteins. By actively exchanging certain lipids and apolipoproteins with each other, the lipoproteins are synthesized and degraded at a constant rate. Among lipoproteins, low density lipoprotein (LDL) is 75% lipid (cholesterol and cholesteryl esters) and 25% protein. LDL is the metabolic product of very low density lipoprotein (VLDL). The high level of LDL is a risk factor for cardiovascular disease.

ApoB is the major protein component of LDL and plays an important role in the maintenance of cholesterol homeostasis (1). It serves as the ligand for the recognition and catabolism of plasma LDL by the LDL-receptor (2). Elevated levels of serum apoB are associated with an increased risk of cardiovascular disease. ApoB circulates in two distinct forms (apoB100 and apoB48) encoded by a single gene localized in chromosome 2 pter-24 (5). ApoB100, the larger form, is synthesized in the liver as a translational product of the entire apoB mRNA. The smaller form, apoB48, is produced from the small intestine by a novel posttranscriptional RNA editing of a CAA (glutamine) to a UAA (stop) codon in apoB mRNA (6, 7). Thus, apoB48 terminates at amino acid residue 2153 and consists of the N-terminal 48% of apoB100. ApoB48 lacks the C-terminal domain of apoB100. As a result, it does not bind to the LDL-receptor. ApoB mRNA editing occurs exclusively in the small intestine of most mammalian species tested, although some species produce apoB48 even in the liver (8).

Until now, over ten common polymorphisms within or flanking apoB gene have been detected. Several polymorphic loci have been recognized in association with total cholesterol, LDL cholesterol, and apoB levels. In this study, six polymorphic sites of the apoB gene were analyzed in 235 patients with coronary artery disease (CAD) and 216 normal control subjects. There were no significant differences in the allele frequencies of apoB polymorphisms between the control and patient groups. However, haplotype frequencies were significantly different between the CAD patients and control (p<0.05). In addition, the allelic distributions of both EcoRI and MspI polymorphisms in Koreans were similar to those in Chinese but significantly different from those in Caucasians. ApoB polymorphisms showed no association with plasma lipid levels. In conclusion, haplotype analysis of the apoB gene using multiple diallelic markers might be a useful marker for Korean CAD patients.

**Key Words:** Apolipoproteins B; Variation (Genetic); Coronary Disease

**INTRODUCTION**

The major lipids of human body are phospholipids, cholesterol, triglycerides and cholesteryl esters. These insoluble lipids are transported through blood as lipoprotein complexes of lipids and one or more specific proteins, called apolipoproteins. By actively exchanging certain lipids and apolipoproteins with each other, the lipoproteins are synthesized and degraded at a constant rate. Among lipoproteins, low density lipoprotein (LDL) is 75% lipid (cholesterol and cholesteryl esters) and 25% protein. LDL is the metabolic product of very low density lipoprotein (VLDL). The high level of LDL is a risk factor for cardiovascular disease.

ApoB is the major protein component of LDL and plays an important role in the maintenance of cholesterol homeostasis (1). It serves as the ligand for the recognition and catabolism of plasma LDL by the LDL-receptor (2). Elevated levels of serum apoB are associated with an increased risk of premature atherosclerosis (3). Because of its large size and insolubility in water, the gene structure and amino acid sequence of apoB have been difficult to determine. The primary structure of the carboxyl-terminal end had been deduced from the nucleotide sequence of the cDNA by Knott et al. (4). The apoB gene is clearly distinct from other genes of the soluble apolipoproteins (apoAI, AII, AIV, CI, CII, CIII, and E). The distribution of the introns in the apoB gene is asymmetric. No sequence homology has been identified between apoB and other apolipoproteins.

ApoB circulates in two distinct forms (apoB100 and apoB48) encoded by a single gene localized in chromosome 2 pter-24 (5). ApoB100, the larger form, is synthesized in the liver as a translational product of the entire apoB mRNA. The smaller form, apoB48, is produced from the small intestine by a novel posttranscriptional RNA editing of a CAA (glutamine) to a UAA (stop) codon in apoB mRNA (6, 7). Thus, apoB48 terminates at amino acid residue 2153 and consists of the N-terminal 48% of apoB100. ApoB48 lacks the C-terminal domain of apoB100. As a result, it does not bind to the LDL-receptor. ApoB mRNA editing occurs exclusively in the small intestine of most mammalian species tested, although some species produce apoB48 even in the liver (8).

Until now, over ten common polymorphisms within or flanking apoB gene have been detected. Several polymorphic loci have been recognized in association with total cholesterol, LDL cholesterol, and apoB levels. In this study, six polymorphisms (HinII, PstII, AluI, MspI, EcoRI, and 3' hypervariable region) of the apoB gene were analyzed in Korean patients with coronary artery disease (CAD), and their association with plasma lipid traits was investigated. Secondly, we compared our data to those in other racial or ethnic populations studied previously.
MATERIALS AND METHODS

Study subjects

The study subjects were recruited from the Seoul city in Korea. We selected 235 CAD patients (164 males and 71 females) from Seoul National University Hospital, Korea, documented by coronary angiography because of recent myocardial infarction or angina. None of the selected CAD patients was on a lipid lowering therapy at the time of blood sampling. In myocardial infarction patients, blood samples were obtained two months after the occurrence of the myocardial infarction. Patients with hypertension, diabetes, and endocrine or metabolic disorders were excluded from this group. The control group consisted of 216 individuals (162 males and 54 females), within the same age range as the patients, who were randomly selected by health screening at the same hospital to screen out those who had a history of chest pain, diabetes, hypertension, and general illness. Mean ages of CAD patients and controls were 53.2 ± 9.3 and 51.1 ± 9.1, respectively: the age difference was not statistically significant. Clinical details for these groups are summarized in Table 1. Blood samples were collected from all subjects after a fast of 12-16 hr to exclude the fluctuations of lipid levels. Plasma samples were stored at -70 °C until the time of lipid assay.

DNA analysis

Total genomic DNA was prepared from leukocytes of 10 mL blood after lysis of red blood cells (9). Polymorphic regions of the apoB gene were amplified by polymerase chain reaction (PCR) from genomic DNA (Fig. 1). Primer sequences and procedures for PCR amplification have previously been described (10). Five polymorphic sites are caused by single base substitutions in various exons and introns in the apoB gene. The amplified PCR products were digested at specific restriction sites of endonucleases; namely, HincII, PvuII, AluI, MspI, and EcoRI. Genotype analyses were done by electrophoresis on an agarose gel, except for use of 8% polyacrylamide gel in case of 5′ hypervariable region (HVR) polymorphism of the apoB gene. Alleles of each polymorphic site were classified as (+) or (−) according to the presence or absence at the cutting site of each restriction enzyme, respectively. The 5′ HVR polymorphism is produced by differences of 15-bp repeat at 491 bp 3′ of the translational termination site. Alleles of the 3′ HVR polymorphism were determined according to the nomenclature of Ludwig et al. (11) based on their 15 base repeats. Haplotype frequencies of the apoB gene in Koreans were also determined according to method of Thompson et al. (12).

Determination of lipid levels

Levels of plasma cholesterol and triglyceride were measured by enzymatic colorimetry methods using commercial kits (Boeringer Mannheim, FRG) on a Hitachi 747 automatic chemistry analyzer. HDL-cholesterol was determined by measuring cholesterol in the supernatant liquid after precipitation of the plasma with MgCl2 and dextran-sulfate, using a Gilford Impact 400E automatic analyzer with reagents and calibrators from Boeringer Mannheim, Germany. LDL-cholesterol levels were calculated by using the formula of Friedwald et al. (13).

Data analysis

Heterozygosity (H) and polymorphism information content (PIC) values of the apoB gene were calculated according to the method of Botstein et al. (14). Five diallelic markers within the apoB gene were used for haplotyping. The degree of nonrandom association was determined by calculation of the delta value (Δ) between the two polymorphic sites at the apoB gene (15). The χ² test was used to apply for Hardy-Weinberg equilibrium and to compare allele frequencies between the CAD and control groups, while the one-way analysis of variance (ANOVA) test was performed to compare the mean levels of lipid parameters among different genotypes. All statistical analyses were performed using the Statistical Analysis System software (SAS Institute, Inc). Statistical significance was accepted at the p=0.05 level.

RESULTS

Allele frequencies of the six polymorphic sites of the apoB gene in CAD patients and controls are shown in Table 2. Any
of the polymorphisms in this study did not show significant differences in allele frequencies between patients and controls. The values of heterozygosity (H) and polymorphism information content (PIC) values based on the allele frequencies of each polymorphism were also estimated. The H and PIC values for the 3′ HVR polymorphism were relatively high in the control and CAD groups. Genotype distributions did not differ from those expected for Hardy-Weinberg proportions at all polymorphic sites. Unequivocal assignments of haplotypes can be made for all but the doubly heterozygous individuals. Two polymorphisms (insertion/deletion and XbaI) previously reported by authors (16) were added to the haplotype analysis. From 7 diallelic markers within the structure

| Polymorphic Site | Genotype frequency | Allele frequency | H | PIC |
|------------------|--------------------|------------------|---|-----|
|                  | Control | CAD | Control | CAD | Control/CAD | Control/CAD |
| I/D*             | + -     | 32   | 16      | - | 0.38 | 0.26 | 0.47/0.38 | 0.36/0.31 |
|                  | + +     | 85   | 0       | + | 0.62 | 0.74 |
|                  | + +     | 56   | 66      | + | 0.13 | 0.14 |
|                  | + +     | 0     | 0       | + | 0.96 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  | HinfII  | - -   | 160     | - | 0.87 | 0.86 | 0.23/0.24 | 0.20/0.21 |
|                  |         | + +   | 55      | + | 0.13 | 0.14 |
|                  | PvuII   | - -   | 208     | - | 0.98 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  |         | + +   | 8      | + | 0.02 | 0.04 |
|                  |         | + +   | 0      | + | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  | Alu*    | - -   | 148     | - | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  |         | + +   | 65     | + | 0.16 | 0.17 |
|                  |         | + +   | 0      | + | 0.95 | 0.94 | 0.10/0.11 | 0.10/0.11 |
|                  | Xbal*   | - -   | 195     | - | 0.05 | 0.06 |
|                  |         | + +   | 21     | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  |         | + +   | 0      | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  | MspI    | - -   | 0      | - | 0.02 | 0.05 | 0.04/0.10 | 0.04/0.10 |
|                  |         | + +   | 0      | + | 0.98 | 0.95 |
|                  | EcoRI   | - -   | 0      | - | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  |         | + +   | 8      | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |

Table 3. Haplotype frequencies of the apoB gene in the control and CAD group

| Haplotype | Number (%) |
|-----------|------------|
| I/D* HinfII PvuII Alu* Xbal* MspI EcoRI | Control | CAD |
| + - - - - - + + + + + + + + + + + | 3 (2.9) | 68 (58.5) |
| - - - - - + + + + + + + + + + + | 0.13 (12.3) |
| - - - - - + + + + + + + + + + + | 0.1 (2.9) |
| - - - - - - + + + + + + + + + + | 2 (1.7) |
| Total number | 116 (100.0) | 105 (100.0) |

Table 4. Standardized nonrandom association statistics (D′, Δ) between pairs of DNA polymorphisms of the apoB gene

| Polymorphic Site | Genotype frequency | Allele frequency | H | PIC |
|------------------|--------------------|------------------|---|-----|
|                  | Control | CAD | Control | CAD | Control/CAD | Control/CAD |
| I/D*             | + -     | 32   | 16      | - | 0.38 | 0.26 | 0.47/0.38 | 0.36/0.31 |
|                  | + +     | 85   | 0       | + | 0.62 | 0.74 |
|                  | + +     | 56   | 66      | + | 0.13 | 0.14 |
|                  | + +     | 0     | 0       | + | 0.96 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  | HinfII  | - -   | 160     | - | 0.87 | 0.86 | 0.23/0.24 | 0.20/0.21 |
|                  |         | + +   | 55      | + | 0.13 | 0.14 |
|                  | PvuII   | - -   | 208     | - | 0.98 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  |         | + +   | 8      | + | 0.02 | 0.04 |
|                  |         | + +   | 0      | + | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  | Alu*    | - -   | 148     | - | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  |         | + +   | 65     | + | 0.16 | 0.17 |
|                  |         | + +   | 0      | + | 0.95 | 0.94 | 0.10/0.11 | 0.10/0.11 |
|                  | Xbal*   | - -   | 195     | - | 0.05 | 0.06 |
|                  |         | + +   | 21     | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  |         | + +   | 0      | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  | MspI    | - -   | 0      | - | 0.02 | 0.05 | 0.04/0.10 | 0.04/0.10 |
|                  |         | + +   | 0      | + | 0.98 | 0.95 |
|                  | EcoRI   | - -   | 0      | - | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  |         | + +   | 8      | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |

| Polymorphic Site | Genotype frequency | Allele frequency | H | PIC |
|------------------|--------------------|------------------|---|-----|
|                  | Control | CAD | Control | CAD | Control/CAD | Control/CAD |
| I/D*             | + -     | 32   | 16      | - | 0.38 | 0.26 | 0.47/0.38 | 0.36/0.31 |
|                  | + +     | 85   | 0       | + | 0.62 | 0.74 |
|                  | + +     | 56   | 66      | + | 0.13 | 0.14 |
|                  | + +     | 0     | 0       | + | 0.96 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  | HinfII  | - -   | 160     | - | 0.87 | 0.86 | 0.23/0.24 | 0.20/0.21 |
|                  |         | + +   | 55      | + | 0.13 | 0.14 |
|                  | PvuII   | - -   | 208     | - | 0.98 | 0.96 | 0.04/0.08 | 0.04/0.08 |
|                  |         | + +   | 8      | + | 0.02 | 0.04 |
|                  |         | + +   | 0      | + | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  | Alu*    | - -   | 148     | - | 0.84 | 0.83 | 0.27/0.28 | 0.24/0.24 |
|                  |         | + +   | 65     | + | 0.16 | 0.17 |
|                  |         | + +   | 0      | + | 0.95 | 0.94 | 0.10/0.11 | 0.10/0.11 |
|                  | Xbal*   | - -   | 195     | - | 0.05 | 0.06 |
|                  |         | + +   | 21     | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |
|                  |         | + +   | 0      | + | 0.00 | 0.00 | 0.00/0.00 | 0.00/0.00 |

There was no significant linkage disequilibrium between each pair of polymorphism.
The haplotype analysis using multiple markers of the apoB gene was mainly conducted in 3,500 mutation study (17). However, the association between apoB polymorphisms and plasma lipid levels in Koreans was not associated with plasma lipid levels. Any of the polymorphic sites were not associated with plasma lipid levels.

**DISCUSSION**

CAD is a multifactorial disease that may differ in each race or ethnic population. For example, the prevalence of CAD vary widely among different population, and the frequencies of the apoB gene polymorphisms have been reported to vary among ethnic groups. Thus, we investigated polymorphisms of the apoB gene in Korean CAD patients.

Haplotype determination with multiple markers could possibly help to define more specific genotypes associated with high CAD risk than a single marker. From seven allelic polymorphic sites, we could identify the eleven different haplotypes out of a possible total of 128 (Table 4). The \(+/-/-/-/+\/+\/+\) haplotype was the most common in both groups. The second most common haplotype, \(-/-/-/-/+\/+\/+\), was significantly more frequent in the controls than in patients. However, each polymorphic pair did not show linkage disequilibrium (Table 4).

We also examined whether five diallelic and 3’ HVR polymorphisms of the apoB gene were associated with plasma lipid levels in Koreans. Any of the polymorphic sites were not associated with plasma lipid levels.

Five diallelic apoB polymorphisms of this study were not associated with plasma lipid levels (20, 30-32). However, apoB polymorphisms in Caucasian populations show more polymorphic than those in Oriental populations as we described at above. Actually associations between apoB polymorphisms and lipid levels were mainly reported in Caucasian populations. For example, Glisic et al. (33) have reported association between EcoRI and MspI polymorphisms of the apoB gene and lipid levels in Yugoslavian population. This associations were confirmed in English (34), Finns (35), Canadian (36, 37), Norwegian (38) and Danish (39) populations. Also, HVR polymorphism of this study showed the lack of association with lipid levels. Japanese population show the same trend (40). However, an association between the larger 3’ HVR alleles and CAD patients was reported in Austrian individuals (41). This population also showed associations with serum cholesterol and apoB levels. Alavantic et al. (42) have reported association between 3’ HVR polymorphism and lipid levels. Thus, polymorphisms of the apoB gene may be Caucasian-specific. That is, they may originate from Caucasian populations. Therefore, it raises the possibility that lack of association between apoB polymorphisms and lipid levels in non-Caucasian populations may, at least in Koreans, partly explain the rarity of CAD.

As a possible explanation for the differences of allele frequency and lipid association of the apoB polymorphisms among populations studied, the differences in the genetic background may be a more important factor than environmental variations, such as diet or lifestyle. That is, the results suggest that this genetic link may, at least in part, explain the differences in prevalence rate of atherosclerosis among populations. Another possibility is that they may be due to the differences in linkage disequilibrium between the two polymorphic sites of the apoB gene among populations. Genetic drift by a founder effect or selective mechanism may cause different levels of linkage disequilibrium. And they could be caused by the differences of sample numbers and the bias of sample selection for the populations studied. In other words, to compare the exact allele heterogeneity among populations, large sample sizes in order to maintain a statistical power is required, making it possible to identify more exact distributions of the polymorphisms among racial or ethnic populations. Thus, polymorphism studies should be performed ideally on samples from a population of homogenous origin. Population admixture may cause a falsely positive genetic association. In this respect, Koreans have had a very low rate of interracial marriage, maintaining a homogeneous population for a long time, suggesting that they are appropriate for polymorphism study.

In conclusion, the results of this study suggest that, at least in the Korean population, a single polymorphism of the apoB gene is unlikely to be a useful marker for CAD patients. However, haplotypes of the apoB gene are likely to be more useful markers for Korean CAD patients.
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