Genetic Variation in Exon 3 of Human Apo B mRNA Editing Protein (apobec-1) Gene*

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We have investigated the genetic variation in the human apo B mRNA editing protein (apobec-1) gene. Exon 3 of the apobec-1 gene was amplified by polymerase chain reaction. After detection of an additional band by single strand conformational polymorphism (SSCP) analysis, sequencing of the SSCP-shift sample revealed a single-base mutation. The mutation was a CGG transversion at codon 80 resulting in a IleRMet substitution. This substitution was confirmed by restriction fragment length polymorphism analysis since a *Pvu*II site is abolished by the substitution. Population and family studies confirmed that the inheritance of the genotypes for apobec-1 gene polymorphism is controlled by two codominant alleles (P1 and P2). A significant difference in plasma triglyceride was detected among the different apobec-1 genotypes in the CAD patients ($P<0.05$). Our study could provide the basis for elucidating the interaction between genetic variation of the apobec-1 gene and disorders related to lipid metabolism.

Keywords: apobec-1, genetic variation, lipid level, polymorphism, SSCP

INTRODUCTION

Apolipoprotein (Apo) B is a major protein component of LDL and occupies an important role in the maintenance of cholesterol homeostasis (Herbert *et al.*, 1983). It serves as the ligand for the recognition and catabolism of plasma LDL by the LDL-receptor (Brown and Goldstein, 1984). Elevated levels of serum apo B are associated with an increased risk of premature atherosclerosis (Brunzell *et al.*, 1984). Because of its large size and insolubility in water, the gene structure and amino acid sequence of apo B100 have been difficult to determine. The primary structure of the carboxyl-terminal end has been deduced from the nucleotide sequence of the cDNA by Knott *et al.* (1986). Thus, apo B48 terminates at amino acid residue 2153 and consists of the N-terminal 48% of apoB100. ApoB48 lacks the carboxyl-terminal domain of apoB100 and thus does not bind to the LDL receptor.

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ApoB mRNA editing occurs exclusively in the small intestine of most mammal species tested although some species produce apoB48 even in the liver (Greeve *et al.*, 1993). Apo B48 is the product of a novel post-transcriptional RNA editing of a CAA (glutamine) to a UAA (stop) codon in apo B mRNA (Powell *et al.*, 1987; Chen *et al.*, 1987). In rat, apo B mRNA editing is known to be mediated by a protein complex consisted of the catalytic subunit and auxiliary factors (Bostrom *et al.*, 1990; Johnson *et al.*, 1993). The cDNA clone encoding the rat apobec-1 has been isolated (Teng *et al.*, 1993). It is a 27 kD protein that has been shown to have cytidine deaminase activity. To express its enzymatic activity, apobec-1 requires one or more auxiliary proteins (Teng and Davidson, 1992; Giannoni *et al.*, 1994). In addition, the auxiliary proteins which specifically bind to apoB mRNA were also characterized (Lau *et al.*, 1991; Navaratnam *et al.*, 1993; Harris *et al.*, 1993). Therefore, auxiliary proteins may be interacted with apobec-1, with apo B mRNA or with both. The activity of apo B mRNA editing is known to be changed according to developmental (Higuchi *et al.*, 1992), hormonal (Davidson *et al.*, 1991; Seishima *et al.*, 1991), and nutritional (Baum *et al.*, 1990; Inui *et al.*, 1994) states. The gene encoding for human apobec-1 was characterized and assigned to chromosome 1 (Hadjiagapiou *et al.*, 1994; Lau *et al.*, 1994). The cDNA for mouse and rabbit apobec-1

have also been cloned (Osuga *et al.*, 1995; Yamanaka *et al.*, 1994).

To assist the better understanding of the apobec-1 gene structure, we described the identification of a new polymorphic site by a CRG nucleotide substitution in exon 3 of the apobec-1 gene. We also investigated the relationship between this polymorphism and plasma lipid or lipoprotein levels.

MATERIALS AND METHODS

Study subjects

The study subjects were recruited from Seoul province, Korea. We have selected 127 CAD patients documented by coronary angiography from Seoul National University Hospital. The control group consisted of 127 individuals within the same age range as the patients who were randomly selected via health screening. None of the controls had a history of CAD and all had normal electrocardiograms. Mean age was 56.0±8.8 and 55.5±8.2 for cases and controls, respectively; the age difference was not statistically significant.

DNA analysis

Total genomic DNA was prepared from the leucocytes of 10 ml of blood after lysis of red blood cells (Sambrook *et al.*, 1989). Exon 3 of the apobec-1 gene was amplified by polymerase chain reaction (PCR) technique using genomic DNA (0.1 μg) as template. The primers used were designed in exon 3 according to the published apobec-1 gene sequence (Hadjiagapiou *et al.*, 1994), 5'-GAG AAG AAT CGA ACC CTG GGA-3' and 5'-GTT AAC AAG GTC CCT GAG ACC-3'. Amplification reactions were performed in a 50 μl volume containing 0.2 mM each of dCTP, dATP, dGTP, dTTP, 10 pM of each oligonucleotide primer, and 1.25 units of Taq DNA polymerase (Promega), 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM $MgCl_2$ and 0.01% gelatin. Amplification was for 30 cycles of denaturation at 94°C for 1 min, 1 min of annealing at 55°C, and 1 min of extension at 72°C . PCR products were conducted to single strand conformation polymorphism (SSCP) for mutation screening.

SSCP and sequencing analyses for apobec-1 mutation screening

For mutation analysis of the apobec-1 gene by SSCP, PCR products were denatured and electrophoresed on nondenaturing 8% polyacrylamide gels containing 5% glycerol at 10 watts for approximately 24 h at room temperature. The banding patterns were visualized by silver staining. Samples showing SSCP-shifts were then sequenced manually.

Determination of plasma lipid levels

Levels of plasma cholesterol and triglycerides were measured by enzymatic colorimetry methods using commercial kits (Boehringer Mannheim, Germany) and a Hitachi 747 automatic chemistry analyzer. HDL cholesterol levels were determined by measuring cholesterol in the supernatant liquid after precipitation of plasma with $MgCl_2$ 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. (1972). Plasma Lp(a) levels were determined using the polyclonal competitive ELISA method (Kim *et al.*, 1992).

Data analysis

The counting method was used for the frequency estimation of the apobec-1 genotypes. The X^2 test was used for Hardy-Weinberg equilibrium, while the one-way analysis of variance (ANOVA) test was performed to compare the mean levels of lipid among apobec-1 genotypes. Statistical significance was accepted at P=0.05 level.

RESULTS AND DISCUSSION

As shown in Fig. 1A, an abnormal SSCP fragment (lane 5 of Fig. 1A, arrow) was detected. The sequence of the sample showing SSCP-shift was analysed by manual sequencing (Fig. 1B). When our data were compared to previously published human apobec-1 gene sequences (Hadjiagapiou *et al.*, 1994; Lau *et al.*, 1994), a mutation (CRG, arrow of Fig. 1B) at codon 80 was identified, resulting in an Ile for Met substitution. Furthermore, the single-base substitution at codon 80 causes the abolition of a *Pvu*II cleavage site. The *Pvu*II polymorphic patterns of human apobec-1 gene are shown in Fig. 1C. In subjects homozygous for absence of a *Pvu*II site, 364 bp fragment of the corresponding PCR amplification product can be visualized upon electrophoresis (Fig. 1C, lane 4). This genotype is referred to as P1P1, whereas the genotype of subjects homozygous for the other allele is referred to as P2P2. The fragments of the P2P2 genotype are 198 bp and 166 bp (Fig. 1C, lanes 2 and 3) respectively. Heterozygotes (genotype P1P2) appeared as a combination of the both alleles (Fig. 1C, lane 1).

We determined allele frequencies in the samples of 127 Korean patients with CAD and 127 unrelated healthy individuals. Allele frequencies of the study subjects are given in Table 1. The P2 allele appears more frequently

than the P1 allele. The observed heterozygosity was 0.46 while value of polymorphism information content was 0.35. The genotype distributions did not differ from that expected for Hardy-Weinberg proportions. On the other hand, no polymorphic pattern for *HinfI*, *StuI*, *MspI*, *AluI*, *BglII*, *TaqI* and AvaII restriction enzymes was detected for the same PCR products (each 15 individuals).

The effects of a *PvuII* polymorphism on plasma lipid and lipoprotein levels were investigated by ANOVA test. Table 2 presents the comparison of lipid levels according to PvuII genotypes. A significant difference in plasma triglyceride was detected among the different apobec-1 polymorphic genotypes in the CAD patients ($P<0.05$).

Apobec-1 has the cytidine deaminase activity although auxiliary proteins are required. The structural and functional alterations of apobec-1 might have phenotypic effects on lipoprotein metabolism. Recently, there were some evidences that apobec-1 has some effect in relation to lipoprotein metabolism. The knock-out mice models have established that apobec-1 is the key component of the apo B mRNA editing (Oka *et al.*, 1997; Chan *et al.*, 1997). A report on the human apobec-1 *PvuII* polymorphism using different primers was published (Hirano *et al.*, 1997). They demonstrated that this common polymorphism is functionally silent. Thus, the lack of association between apobec-1 genotypes and plasma lipid levels in healthy subjects of this study was compatible with the work of Hirano *et al.* In the CAD group of this study, however, plasma triglyceride levels were varied significantly among apobec-1 genotypes. It is likely that the observed association with CAD group are due to the allelic association of this polymorphism with other functional sequence changes in the apobec-1 gene. Such allelic association may be involved in controlling the expression of the apobec-1 gene, thereby resulting in variations of plasma triglyceride levels of CAD group.

Allele frequencies of the apobec-1 polymorphism might be varied among racial or ethnic groups. The frequency of the P2 allele in Korean and Caucasian populations is higher than that of the P1 allele. In contrast, African-American population showed higher P1 allele frequency. The P2 allele frequency of Korean (0.64) population had intermediate value among Caucasian (0.75), Chinese (0.50), and African-American (0.47) populations. As possible explanation for the differences of the apobec-1 polymorphism among populations, it may be due to

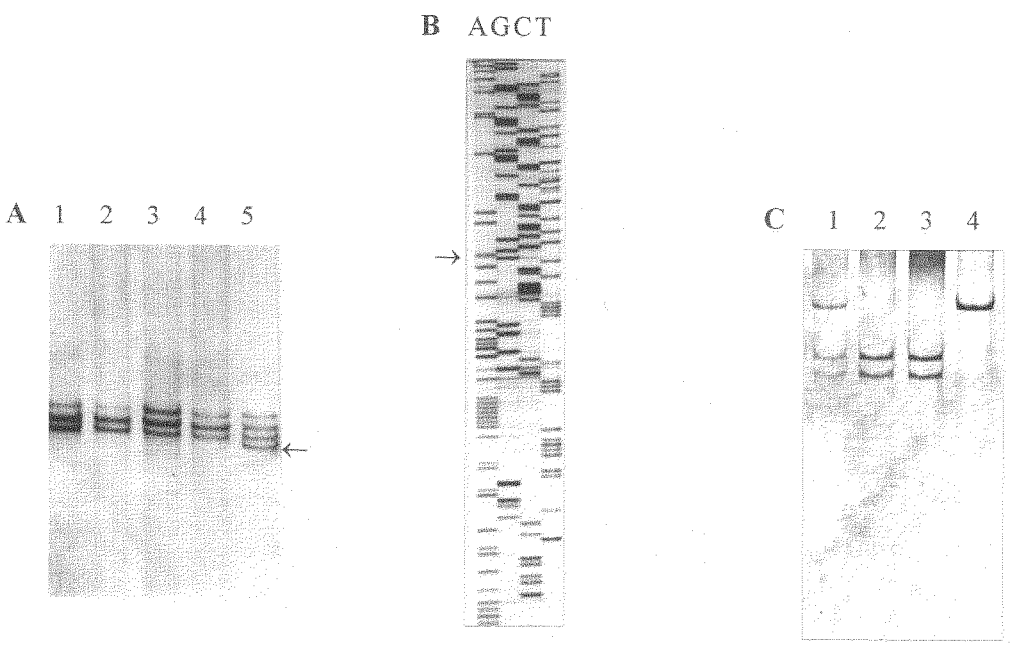

Fig. 1. (A) Identification of an abnormal band by the PCR-SSCP analysis. Band showing the SSCP-shift are shown by arrow (lane 5). (B) Identification of a mutation site in the apobec-1 gene using manual sequencing. A mutation site was identified to be a C to G transversion (arrow) at codon 80, resulting in substitution of Met (ATG) for Ile80 (ATC). (C) Electrophoretic separation patterns of a *PvuII* digests after amplification of genomic DNA; P1P1 homozygote (lane 4), P1P2 heterozygote (lane 1), and P2P2 homozygote (lanes 2 and 3).

Table 1. Genotype and allele frequencies of the *Pvu*II polymorphic site in the apobec-1 gene

Genotypes	Controls	CAD
P1P1	15	17
P1P2	61	70
P2P2	51	40
Allele frequencies		
P1	0.36	0.41
P2	0.64	0.59

Table 2. Comparison of lipid and lipoprotein levels (mg/dl) according to *Pvu*II genotypes of the apobec-1 gene

Variables	Gynotypes		
	P1P1	P1P2	P2P2
	Controls/CAD		
Cholesterol	192.1±12.9	189.6±11.9	192.5±11.9
	171.4±32.7	172.1±28.2	183.4±41.8
Triglyceride	144.4±48.3	144.5±53.0	142.9±51.7
	155.5±37.7	129.2±61.9	142.5±84.7
HDL-Cholesterol	37.4±7.8	38.2±11.7	36.2±8.2
	37.5±9.1	34.9±9.8	36.2±8.2
LDL-cholesterol	125.7±16.4	122.4±16.7	128.9±16.4
	106.5±33.7	110.8±24.9	116.1±26.8
Lp(a)	15.9±13.6	14.9±11.9	14.4±13.0
	16.9±12.6	15.6±12.1	15.1±12.9

Abbreviation: HDL, high density lipoprotein; LDL, low density lipoprotein; Lp, lipoprotein. Values are mean ±SD (mg/dL).

Significant difference in triglyceride level among three genotypes in the CAD group ($P<0.05$).

differences in the genetic background as well as environmental factors. That is, it might be due to genetic drift by a founder effect or a selective mechanism.

In conclusion, we have identified a *Pvu*II polymorphism in exon 3 of the apobec-1 gene. The *Pvu*II polymorphism of the apobec-1 gene showed lack of association with plasma lipid levels in Koreans. Further studies in other racial or ethnic groups will be of great interest.

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