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DNA Polymorphism in 5′-Flanking Region of Human Apolipoprotein A1 and Glutathione S-Transferase Mu1 Gene in Koreans

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The distributions of G to A substitution (G^{-75}→A) mutation in the human apolipoprotein A1 (*APOA1*) gene promoter region and glutathione S-transferase Mu1 (*GSTM1*) gene deletion were examined in subjects with Korean population. The G^{-75}→A mutation of *APOA1* was genotyped by the polymerase chain reaction (PCR) and subsequent digestion of the PCR product using either *Msp*I or *Mva*I (n=206). The observed numbers of GG, GA and AA genotypes were 132, 63 and 11, respectively. The allele frequencies of G and A were 0.794 and 0.206, respectively. The *GSTM1* gene deletion was simply examined by the PCR amplification (n=106). The observed numbers of null type (*GSTM1*0/GSTM1*0*) and positive type were 55 and 51, respectively. The allele frequency of *GSTM1*0* was 0.720.

The apolipoprotein A1 (APOA1) is a plasma protein involved in the transport of cholesterol and other lipids in the plasma. Plasma APOA1 is a cofactor for lecithin-cholesterol acyltransferase which catalyzes the esterification of plasma cholesterol (Soutar et al., 1975; Havel and Kane, 1989). The genes coding for APOA1 and two other apolipoproteins, APOC3 and APOA4, are closely linked and tandemly organized within a 15-kb DNA segment in the long arm of human chromosome 11q23-24 (Shoulders et al., 1983; Karathanasis, 1985).

The mutation of a guanine to adenine substitution (G^{-75}→A), serving a different *Msp*I and *Mva*I restriction site, within the *APOA1* promoter region has been identified (Shoulders et al., 1983; Needham et al., 1994). The mutation is located 75 bp upstream of the transcription initiation site between the putative CAT and TATA-like sequences.

The human glutathione S-transferases (GSTs) are a supergene family that is involved in the metabolism and detoxification of cytotoxic and carcinogenic compounds (Boyer and Kenney, 1985). Eight genetic loci encoding human GST isozymes have been characterized. At least two of eight loci are genetically polymorphic. *GST Mu1 (GSTM1)*, one of them, is of particular interest because it has a null allele (*GSTM1*0*), in addition to two more expressing alleles (*GSTM1*A* and *GSTM1*B*: Mannervik et al., 1992). The null allele *GSTM1*0* is due to a gene deletion, and persons having it in a homozygous state have no gene product (Seidegard et al., 1988). A simple polymerase chain reaction (PCR)-based assay to determine the presence or absence of *GSTM1* gene was developed by Comstock et al. (1990).

In the present study, the G→A mutation at position -75 of the *APOA1* gene promoter and the *GSTM1* gene deletion were examined in an unrelated Korean population. The G^{-75}→A mutation of *APOA1* was genotyped by the PCR amplification and subsequent digestion of the PCR product using either *Msp*I or *Mva*I. The *GSTM1* gene deletion was simply examined by the PCR amplification.

Materials and Methods

Subjects

Bloods samples were obtained from healthy unrelated Koreans ranging in age from 20 to 30 years. Bloods (10-ml) were collected into 15 ml-tubes containing 10 mg EDTA, and then genomic DNAs were isolated from whole blood using Wizard genomic DNA purification system (Promega).

Polymerase chain reaction

The PCR amplification of *APOA1* gene promoter region was carried out in a 20-μl reaction mixture containing

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20 pmol of each primer and 0.2-0.6 μg of genomic DNA using premix reaction mixture (Bioneer). Amplification was achieved by denaturation at 94℃ for 3 min, followed by 30 cycles of denaturation 94℃ for 1 min, annealing at 58℃ for 30 sec and extension at 72℃ for 2 min using a DNA Thermal Cycler (Perkin Elmer Cetus). The two primers used were as the followings (Pagani et al., 1990; Needham et al., 1994): AI.1: 5′-CACCCG-GGAGACCTGCAAGC-3′ and AI.2: 5′-TCTAAGCAGC-CAGCTCTTGCA-3′.

The amplification of *GSTM1* gene was performed in the same premix reaction mixture described above, except for using three primers instead of two. Amplification was achieved by denaturation at 94℃ for 3 min, followed by 30 cycles of denaturation 94℃ for 1 min, annealing at 50℃ for 1.5 min and extension at 72℃ for 1 min. The three primers used were as the followings (Mikelsaar et al., 1994): P1: 5′-CGCCATCTTGTGCTA-CATTGCCCG-3′, P2: 5′-ATCTTCTCCTCTTCTGTCTC-3′, and P3: 5′-TTCTGGATTGTAGCAGATCA-3′.

Digestion of APOA1 gene promoter region

The PCR products of *APOA1* gene promoter region were purified by the treatments of phenol:chloroform and chloroform, and then incubated with 10 units of restriction enzyme either *Msp*I or *Mva*I (Boehringer Mannheim) at 37℃ for 3 h. Digested PCR products were genotyped by the electrophoresis using either 2% agarose gel with 1X TAE buffer (*Msp*I- digested DNA) or 10% non-denaturing polyacrylamide gel with 1X TBE buffer (*Mva*I-digested DNA).

Statistical analysis

The distributions of allele frequencies for the *APOA1* gene were examined by χ^2 test to detemine whether the observed genotypic distributions conform to their respective Hardy-Weinberg equilibrium expectations.

Results

Genotyping of G^{-75}→A mutation of APOA1 gene promoter region

The mutation of G to A at position -75 of the APOA1 gene promoter (GGCC**G**/**A**GGGC) causes an abolishment of *Msp*I restriction site, but produces a *Mva*I site. For the examination of G^{-75}→A Mutation of *APOA1* gene promoter region, 206 unrelated Koreans were studied. The PCR amplification of the *APOA1* gene promoter region produced a 258 bp product (Fig. 1A). As shown in Fig. 1(B), digestion of the product with *Msp*I restriction endonuclease yielded two bands of 175 and 79 bp in the presence of the G allele, however, the digestion showed 254 bp single band instead of two restriction fragments in the presence of A allele. The heterozygote individuals (GA genotype) showed three bands of 254, 175 and 79 bp which were sum of

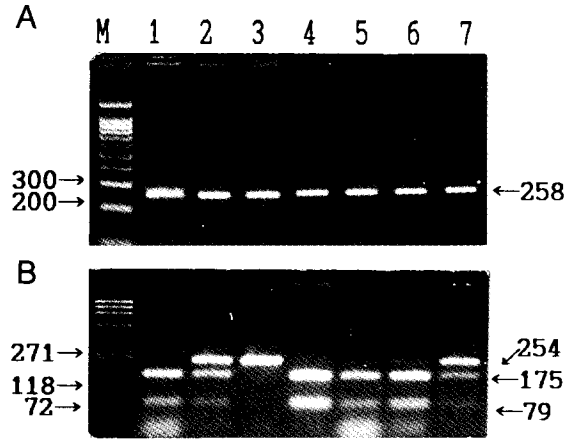

Fig. 1. PCR amplification and digestion with *Msp*I for the 5′-nontranscribed region of human *APOA1* gene. A, The amplification of the promoter region of *APOA1* gene between -258 and -1 was achieved by 30 cycles of denaturation at 94℃ for 1 min, annealing at 58℃ for 30 sec and extension at 72℃ for 2 min (M, 100 bp DNA ladder; lane 1-7, nondigested PCR product). B, The PCR products were digested with *Msp*I at 37℃ for 3 h, and then electrophoresed using 2% agarose gel (M, φx174/*Hae*III-digested marker; lanes 1, 4, 5, and 6, GG genotype; lanes 2 and 7, GA genotype; lane 3, AA genotype).

bands yielded by G and A allele. A 4 bp fragment was produced by the *Msp*I digestion, however, it was not visualized on the electrophoresed gel because of its very small size.

Confirmation of the G^{-75}→A mutation was carried out by digestion of the PCR product with *Mva*I restriction endonuclease. It yielded two invariant fragments of 110 and 42 bp, as well as either a variable fragment of 59 bp in the presence of G allele or two bands of 35 and 24 bp in the presence of the A allele. The heterozygotes showed all five bands (Fig. 2).

The observed numbers of GG, GA and AA genotypes were 132, 63 and 11, respectively (Table 1), thus the

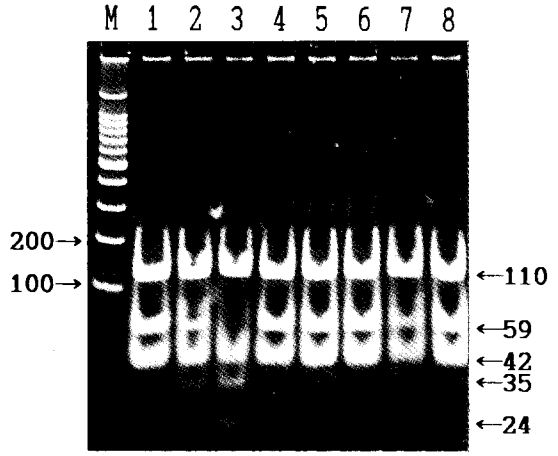

Fig. 2. Electrophoretic patterns of alleles for the G^{-75}→A mutation of *APOA1* gene following digestion with *Mva*I. The PCR products were digested with *Mva*I at 37℃ for 3 h, electrophoresed using 10% non-denatured polyacrylamide gel with 1X TBE buffer, and then visualized by SYBR Green I staining (M, 100 bp DNA ladder; lanes 1, 4, 5, 6, and 8, GG genotype; lanes 2 and 7, GA genotype; lane 3, AA genotype).

Table 1. Distribution of genotypes and allele frequencies for the *APOAI* G^{-75}→A mutation

Subject	Sample size	Genotype			Allele frequency		Reference
		GG	GA	AA	G	A	
Korean	206	132 (129.9)[1]	63 (67.4)	11 (8.7)	0.794	0.206	Present study
Japanese	21	17	3	1	0.881	0.119	Needham et al. (1994)
Caucasian (UK)	56	41	13	2	0.848	0.152	Needham et al. (1994)
Caucasian (American)	409	261	130	18	0.797	0.203	Barre et al. (1994)

[1]The expected genotype numbers are presented in parentheses.

allele frequencies of G and A were 0.794 and 0.206, respectively. The heterozygosity for these alleles was 0.306. The observed number of each genotype well agreed with the Hardy-Weinberg equilibrium with no significant asymmetry in the genotype distribution (χ^2 =0.929, df=2, 0.50<P<0.80).

Detection of GSTM1 gene deletion

In order to examine the *GSTM1* gene deletion, 106 unrelated Koreans were studied. A set of three primers was used to amplify *GSTM1* gene. Primer P1 annealed to the 5′ region of exon 4 of *GSTM1* as well as 5′ region of exon 4 of *GSTM4*. Primer P1 with primer P3 annealing to the 3′ region of exon 5 of *GSTM1* produced a 231 bp product in *GSTM1*-positive individuals. Primer P1 with primer P2 hybridizing to the 3′ region of exon 5 of *GSTM4* produced a 158 bp product in all persons, which serves an internal control (Fig. 3).

As shown in Table 2, the observed numbers of *GSTM1* null and positive phenotypes were 55 (frequency 0.519) and 51 (frequency 0.481), respectively. The allele frequencies of *GSTM1*0* and the sum of *GSTM*1* and *GSTM*2* were 0.720 and 0.280, respectively.

Discussion

The allele frequencies of *APOA1* gene were compared with the results obtained by Barre et al. (1994) and Needham et al. (1994). As shown in Table 1, the

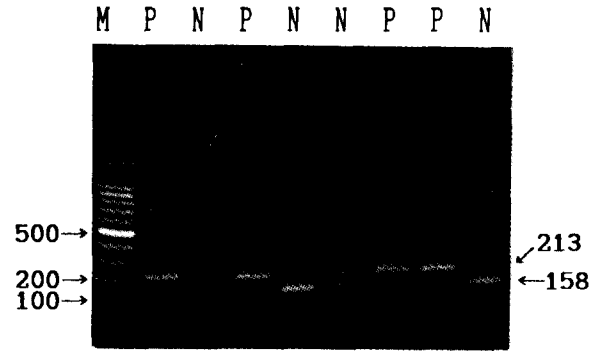

Fig. 3. Detection of the *GSTM1* gene deletion with PCR amplification. The amplification of *GSTM1* gene was achieved by 30 cycles of denaturation at 94℃ for 1 min, annealing at 50℃ for 1.5 min and extension at 72℃ for 1 min. The PCR products were electrophoresed on 2% agarose gel (M, 100 bp DNA ladder; P, positive phenotype; N, null phenotype).

mutated allele (A type) frequency of Korean was highest (0.208) among the populations studied so far (Caucasian UK, 0.152; Japanese, 0.119). However, test for χ^2 analysis revealed no significant allele distributional asymmetry between populations.

A number of studies have shown that the *GSTM1* null allele is very common and the frequency of null phenotype is considerably variable between the studied populations, ranging from 30 to 60% (Board, 1981; Laisney et al., 1984; Seidegard and Pero, 1985; Afan asyeva and Spitsyn, 1990; Zhong et al., 1991; Brockmöller et al., 1992; Harada et al., 1992; Mikelsaar et

Table 2. Distribution of *GSTM1* null phenotype in different populations

Population	Sample size	Phenotype		Reference
		Null	Positive	
Korean[1]	106	55(51.9)	51(48.1)	Present study
Indians	43	28(61.5)	15(34.9)	Board (1981)
Chinese	96	40(41.7)	56(58.3)	Board (1981)
Japanese	84	44(52.4)	40(47.6)	Harada et al. (1992)
Caucasians (Australia)	40	14(35.0)	26(65.0)	Board (1981)
Caucasians (England)	225	131(58.0)	94(42.0)	Zhong et al. (1991)
Caucasians (France)	56	32(57.0)	24(43.0)	Laisney et al. (1984)
Caucasians (Russia)	100	51(51.0)	49(49.0)	Afanasyeva and Spitsyn (1990)
Caucasians (Estonia)	151	75(49.7)	76(50.3)	Mikelsaar et al. (1994)
Caucasians (Sweden)	248	114(46.0)	134(54.0)	Seidegard and Pero (1985)
Caucasians (Germany)	145	65(44.8)	80(55.2)	Brockmöller et al. (1992)

[1]Allele frequency of *GSTM1*0* was 0.720 in Korean population.

al., 1994). Table 2 shows the percentage of individuals lacking the *GSTM1* isozyme in several populations. The present study showed that 51.9% of the Korean population have the homozygous deletion of *GSTM1* gene, which is a similar value to that of the Japanese (52.4%: Harada et al., 1992), but, higher than that of Chinese (41.7%: Board, 1981).

Epidemiological studies suggested that low levels of plasma APOA1 or HDL cholesterol increase the risk of developing atherosclerotic heart disease (Schaefer, 1984). Jeenah et al. (1990) suggested that the substitution of G to A is associated with elevated serum APOA1 and HDL cholesterol concentrations. The $G^{-75} \rightarrow A$ mutation has been shown to decrease *APOA1* expression *in vivo* and *in vitro* (Smith et al., 1992; Tuteja et al., 1992). Down regulation of the mutation may lead to reduced HDL turnover and LDL clearance, so causing the accumulation of triglyceride, manifested as primary hypertriglyceridaemia. (Needham et al., 1994).

It has been suggested that those people with the GSTM1 null phenotype have an increased risk for several kinds of cancers. It has been shown that the null phenotype is more common in smokers with lung cancer (Kihara et al., 1994). It is also suggested that the null phenotype is associated with increased risk of stomach cancer (Harada et al., 1992) and alcoholic hepatitis (Afanasyeva and Spitsyn, 1990).

Since this study was performed in subjects with healthy individuals, no information concerning APOA1 $G^{-75} \rightarrow A$ mutation- and GSTM1 null phenotype-related genetic disorders were obtained. However, further studies should be carried out to reveal any correlation between increased risk for some types of disorders and genotypes.

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References

Afanasyeva JS and Spitsyn VA (1990) Glutathione S-transferase hereditary polymorphism in normal and alcoholic hepatitis liver. *Genetika* 26: 1309-1315.

Barre DE, Guetrra R, Verstraete R, Wang Z, Grundy SM, and Cohen JC (1994) Genetic analysis of a polymorphism in the human apolipoprotein A-1 gene promoter: effect on plasma HDL-cholesterol levels. *J Lipid Res* 35: 1292-1296.

Board PG (1981) Biochemical genetics of glutathione S-transferase in man. *Am J Hum Genet* 33: 36-43.

Boyer TD and Kenney WC (1985) Preparation, characterization and properties of glutathione S-transferases. In: Zakim D and Vessey D (eds), Biochemical Pharmacology and Toxicology, John Wiley & Sons, New York, pp 297-364.

Brockmöller J, Gross D, Kerb R, Drakoulis N, and Roots I (1992) Correlation between *trans*-stilbene oxide-glutathion conjugation activity and the deletion mutation in the glutathione S-transferase class *Mu* gene deletion by polymerase chain reaction. *Biochem Pharmacol* 43: 647-650.

Comstock KE, Sanderson BJS, Claflin G, and Henner WD (1990) *GST1* gene deletion determined by polymerase chain reaction. *Nucleic Acids Res* 18: 3670.

Harada S, Misawa S, Nakamura T, Tanaka N, Ueno E, and Nozoe M (1992) Detection of *GSTM1* gene deletion by the polymerase chain reaction and its possible correlation with stomach cancer in Japanese. *Hum Genet* 90: 62-64.

Havel RJ and Kane JP (1989) Structure and metabolism of plasma lipoproteins. In: Seriver CR, Beaudet AL, Sly WS, and Valle D (eds), The Metabolic Basis of Inherited Disease, McGraw-Hill Book Co, New York, pp 1129-1138.

Jeenah M, Kessling A, Miller N, and Humphries S (1990) G to A substitution in the promoter region of the apolipoprotein AI gene is associated with elevated serum apolipoprotein AI and high density apolipoprotein cholesterol concentrations. *Mol Biol Med* 7: 233-241.

Karathanasis SK (1985) Apolipoprotein multigene family: tandem organization of human apolipoprotein *AI, CIII,* and *AIV* genes. *Proc Natl Acad Sci USA* 82: 6374-6378.

Kihara M, Kihara M, and Noda K (1994) Lung cancer risk of *GSTM1* null genotype is dependent on the extent of tobacco smoke exposure. *Carcinogenesis* 15: 415-418.

Laisney V, Van Cong N, Gross MS, and Frezal J (1984) Human genes for glutathione S-transferases. *Hum Genet* 68: 221-227.

Mannervik B, Awasthi YC, Board PG, Hayes JD, Di Ilio C, Ketterer B, Lisotowsky I, Morgenstern R, Muramatsu M, Widersten M, and Wolf CR (1992) Nomenclature for human glutathione transferases. *Biochem J* 282: 305-306.

Mikelsaar A-V, Tasa G, Pärlist P, and Uusküla M (1994) Human glutathione S-transferase *GSTM1* genetic polymorphism in Estonia. *Hum Hered* 44: 248-251.

Needham EWA, Mattu RK, Rees A, Stocks J, and Galton DJ (1994) A polymorphism in the human apolipoprotein *A1* promoter region: a study in hypertriglyceridaemic patients. *Hum Hered* 44: 94-99.

Pagani F, Sidoli A, Giudici GA, Barenghi L, Vergani C, and Baralle FE (1990) Human apolipoprotein *A-I* gene promoter polymorphism: association with hyperalphalipoproteinemia. *J Lipid Res* 31: 1371-1377.

Schaefer EJ (1984) Clinical, biochemical, and genetic features in familial disorders of high density lipoprotein deficiency. *Arteriosclerosis* 4: 303-322.

Seidegard J, and Pero RW (1985) The hereditary transmission of high glutathione transferase activity towards *trans*-stilbene oxide in human mononuclear leukocytes. *Hum Genet* 69: 66-68.

Seidegard J, Vorachek WR, Pero RW, and Pearson WR (1988) Hereditary differences in the expression of the human glutathione transferase active on *trans*-stilbene oxide are due to a gene deletion. *Proc Natl Acad Sci USA* 85: 7293-7297.

Shoulders CC, Kornblihtt AR, Munro BS, and Baralle FE (1983) Gene structure of human apolipoprotein *AI. Nucleic Acids Res* 11: 2827-2837.

Smith JD, Briton EA, and Breslow JL (1992) Polymorphism in the human apolipoprotein *A-I* gene promoter region: association of the minor allele with decreased production rate *in vivo* and promoter activity *in vitro. J Clin Invest* 89: 1796-1800.

Tuteja R, Tuteja N, Melo C, Casari G, and Baralle FE (1992) Transcription efficiency of human apolipoprotein *A-I* promoter varies with naturally occurring A to G transition. *FEBS Lett* 304: 98-101.

Zhong S, Howie AF, Ketterer B, Taylor J, Hayes JD, Geoffrey JB, Wathen CG, Wolf CR, and Spurr NK (1991) Glutathione S-transferase *mu* locus: use of genotyping and phenotyping assays to assess association with lung cancer susceptibility. *Carcinogenesis* 12: 1533-1537.

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