J Cardiol 1996; 28: 207–212

Analysis of a New Polymorphism in the Human Apolipoprotein A-I Gene : Association With Serum Lipoprotein Levels and Coronary Heart Disease

Huai BAI, MD
Keijiro SAKU, MD
Rui LIU, MS
Shiro JIMI, PhD*
Kikuo ARAKAWA, MD

Abstract

Systematic sequencing of the coding and exon flanking regions of the apolipoprotein (apo) A-I gene has identified a new polymorphic Msp I site (C C/T-GG). This polymorphism is situated between the transcriptional starting site and the signal peptide start coding site (intron 1), so may influence the efficiency of surrounding splicing, thereby interfering with the expression of the apo A-I gene product, or serve as a linkage marker with a hitherto unidentified mutation defect responsible for hyperlipidemia and/or premature coronary heart disease. However, there was no significant difference in the allele frequencies between control and coronary heart disease subjects in a Japanese population. The −78 G→A promoter polymorphism of apo A-I, previously reported in Western populations, has also been analyzed. The results show that neither mutation is likely to be the etiology for predisposition to a change of high-density lipoprotein cholesterol and/or variation in lipid and lipoprotein levels, or for the occurrence of coronary heart disease in Japanese populations.

Key Words
Apolipoproteins, Gene expression (A-I gene), Genetic techniques (polymorphism), Lipoproteins, Coronary heart disease

INTRODUCTION

Genetic variation of apo A-I/C-III/A-IV is associated with hyperlipidemia and coronary heart disease. Candidate genes that may contribute to inter-individual variation in plasma high-density lipoprotein-cholesterol (HDL-C) levels have been examined by comparing the HDL-C levels of unrelated individuals with different alleles of the gene under consideration[1]. In most cases, alleles have been defined by restriction fragment length polymorphisms. Several studies have indicated that a G to A substitution at position −78 in the gene encoding apo A-I confers increased plasma HDL-C[2–4].

Our sequencing study of the apo A-I gene of coronary heart disease patients has identified a new polymorphic Msp I site (C C/T-GG) at position 67 bp 3' from transcriptional starting sequence (67 Msp I).

This study investigated this polymorphism and assessed its importance in association with serum HDL-C levels, as well as other lipid and lipoprotein levels, in both controls and coronary heart disease patients. In addition, we identified the haplotypes of apo A-I gene −78 G→A promoter polymorphism and 67 Msp I polymorphism in Japanese subjects.

福岡大学医学部 第二内科，*第二病理：〒814–80 福岡市城南区七隈 7–45–1
The Second Department of Internal Medicine, *The Second Department of Pathology, Fukuoka University School of Medicine, Fukuoka
Address for reprints: SAKU K, MD, The Second Department of Internal Medicine, Fukuoka University School of Medicine, Nanakuma 7–45–1, Jonan-ku, Fukuoka 814–80
Manuscript received April 22, 1996; accepted July 15, 1996

Selected abbreviations and acronyms

apo = apolipoprotein
DNA = deoxyribonucleic acid
HDL-C = high-density lipoprotein-cholesterol
PCR = polymerase chain reaction

MATERIALS AND METHODS

Two hundred and five patients with coronary heart disease (154 males, 51 females, mean age 64±12 years), based on coronary angiography, were studied. The severity of coronary atherosclerosis was based on the number of involved vessels (single-, two- or three-) with stenosis of 75% or greater. Two hundred and eleven controls (91 males, 120 females, mean age 59±12 years) consisted of both angiographically proven non-stenotic coronary artery subjects (n=141) and healthy controls with normal electrocardiograms and without episodes of chest pain (n=70). Informed consent was obtained from each patient before entering the study, and the project was assessed and approved by the Ethics Committee of Fukuoka University.

Blood samples were taken after an overnight fast. Serum total cholesterol and triglyceride were measured by enzymatic methods[5,6]. High-density lipoprotein-cholesterol was determined by the heparin Ca^{2+} precipitation method[7]. Apo A-I, apo A-II, apo B, apo C-II, apo C-III and apo E were measured by the turbidity immunoassay method[8]. All apolipoproteins were assayed within 48 hours.

Genomic deoxyribonucleic acid (DNA) was isolated from 500 μl peripheral blood according to the method of Erlich[9]. 5'-AGGGACAGAGCTGATCCTTGAACTCTTAAG-3' (327-356) and 5'-TTAGGGGACACCTACCCGTCAGGAAGAGCA-3' (760-731)[10] were used as the sense and antisense primers, respectively. Direct sequencing was performed in an ABI DNA sequencer (Model 373A) with a dye-terminator kit according to the protocol of the kit manufacturer. Restriction fragment length polymorphism analysis to determine the genotype of the subjects was performed using Msp I restriction enzyme digestion after production polymerase chain reaction (PCR) DNA fragments which spanned the 67 Msp I site of the apo A-I gene. Msp I restriction enzyme digestion can also be used to detect G→A substitution of apo A-I gene −78 promoter polymorphism in PCR products amplified with the same primers. Statistical analysis used SAS statistical software (SAS Institute Inc., Cary, NC).

RESULTS

The genotypes of 67 Msp I polymorphism in controls and coronary heart disease patients are shown in **Table 1** and **Fig. 1**. The frequency of 67 Msp I allele 2 (M2 allele) was 0.043 in controls and 0.039 in coronary heart disease patients, with no significant difference between the two groups. The coronary heart disease patients were further divided into single-, two-, and three-vessel disease groups, but no difference in the frequency of the genotypes was observed by χ^2-test. The genotypes of −78 G→A promoter polymorphism are also shown in **Table 1** and **Fig. 2**. No significant differences in these parameters were observed within or between the two groups. Baseline parameters of coronary heart disease patients, such as age, body mass index, hypertension, smoking and diabetes mellitus, with both polymorphisms were not different between the three genotypes (**Table 2**).

Controls with the 1/2 genotype of 67 Msp I site had a higher serum HDL-C level than controls with the 1/1 genotype (59±22 vs 51±15 mg/dl), but this

Table 1 Genotypes and frequencies for the 67 Msp I marker and the −78 G→A promoter marker of the apo A-I gene

67 Msp I genotype	Frequency					−78 genotype	Frequency				
	Controls	Coronary heart disease					Controls	Coronary heart disease			
	(n=211)	(n=205)	1V	2V	3V		(n=211)	(n=205)	1V	2V	3V
1/1	0.915 (193)	0.927 (190)	47.37 (90)	36.32 (69)	16.31 (31)	G/G	0.744 (157)	0.683 (140)	40.71 (57)	39.29 (55)	20.00 (28)
1/2	0.085 (18)	0.068 (14)	50.00 (7)	50.00 (7)	0	G/A	0.242 (51)	0.268 (55)	61.82 (34)	36.36 (20)	1.82 (1)
2/2	0.000	0.005 (1)	0	0	100 (1)	A/A	0.014 (3)	0.049 (10)	50.00 (5)	20.00 (2)	30.00 (3)
67 Msp I allele						−78 allele					
1	0.957 (404)	0.961 (394)				G	0.865 (365)	0.817 (335)			
2	0.043 (18)	0.039 (16)				A	0.135 (57)	0.183 (75)			

Numbers in parentheses indicate number of subjects with each genotype or number of alleles of each type.
1V = single-vessel disease; 2V = two-vessel disease; 3V = three-vessel disease.

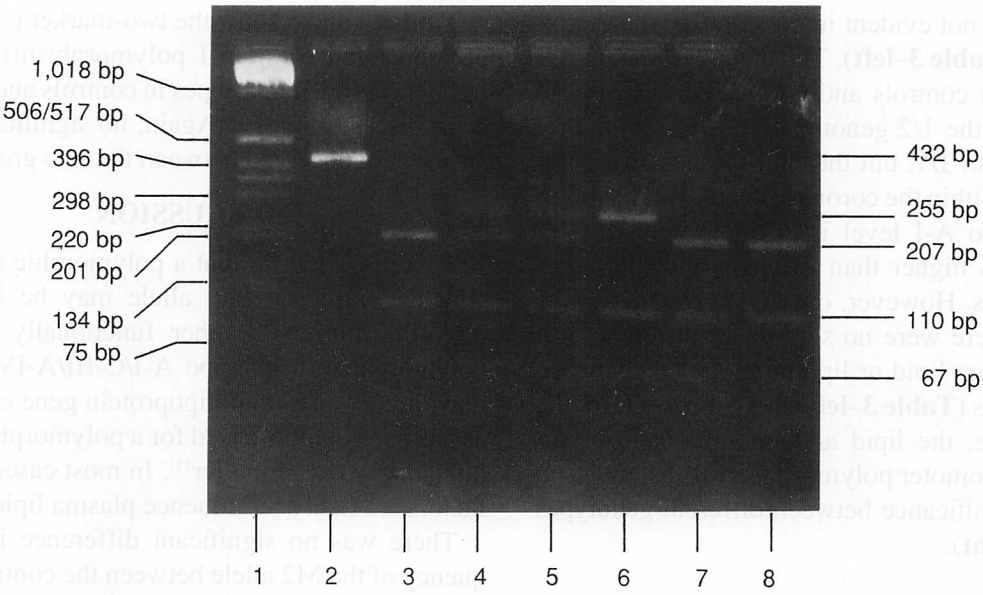

Fig. 1 C to T replacement in intron 1 of the apo A-I gene

This mutation was analyzed by Msp I restriction fragment length polymorphism, and was identified by the loss of a 432 bp DNA fragment and the formation of a characteristic pattern of 207 bp and/or 255 bp fragments. Lanes 3, 7 and 8 show the wild type (207 bp fragment); lanes 4 and 5, heterozygote mutation (207 bp and 255 bp); and lane 6, homozygote mutation (255 bp).
Lane 1 : molecular-weight marker. Lane 2 : uncut control. The other fragments at 110 bp and 67 bp have no significance in genotype determination.

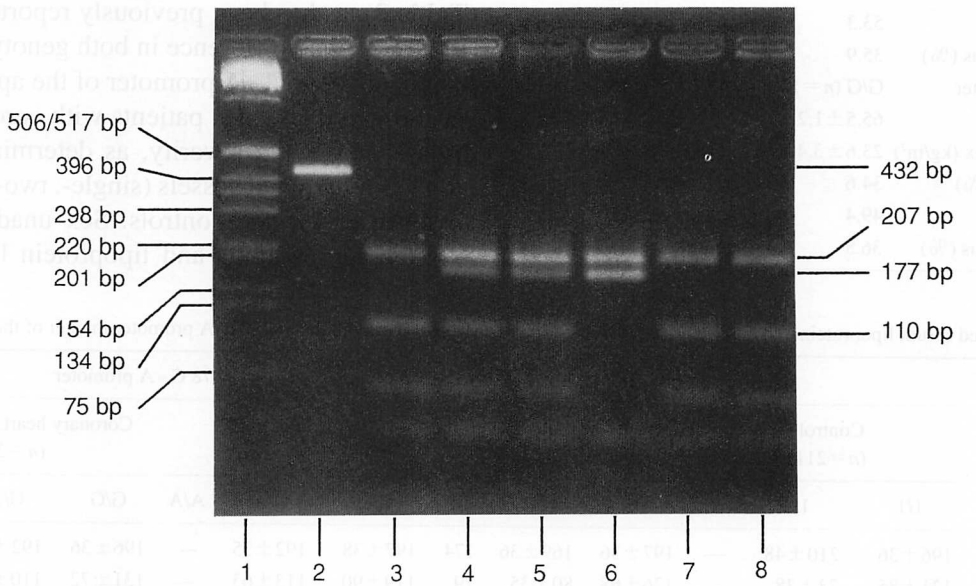

Fig. 2 G to A replacement in −78 G→A promoter of the apo A-I gene

This mutation was analyzed by Msp I restriction fragment length polymorphism, and was identified by the loss of a 432 bp DNA fragment and the formation of a characteristic combination pattern of 207 bp, 177 bp and 110 bp fragments. Lanes 3, 7 and 8 show the wild type (207 bp and 110 bp fragments); lanes 4 and 5, heterozygote mutation (207 bp, 177 bp and 110 bp); and lane 6, homozygote mutation (207 bp and 177 bp).
Lane 1 : molecular-weight marker. Lane 2 : uncut control. The other fragment at 67 bp has no significance in genotype determination.

tendency was not evident in the coronary heart disease group (**Table 3–left**). The mean serum apo A-I levels in both controls and coronary heart disease patients with the 1/2 genotype tended to be higher than those with 1/1, but these differences were not significant. Within the coronary heart disease group, the serum apo A-I level in patients with the 2/2 genotype was higher than in those with the other two genotypes. However, only one 2/2 sample was available. There were no significant differences in any of the other lipid or lipoprotein levels between the two groups (**Table 3–left**). Like the result for the 67 Msp I site, the lipid and lipoprotein levels of −78 G→A promoter polymorphism also showed no statistical significance between different genotypes (**Table 3–right**).

Table 2 Baseline characteristics of coronary heart disease patients according to apolipoprotein A-I polymorphism

Variables	Genotypes		
67 Msp I	1/1 (n=190)	1/2 (n=14)	2/2 (n=1)
Age (yr)	62.8±10.1	67.0±6.7	70.0
Body mass index (kg/m^2)	23.5±3.3	23.6±2.3	26.2
Hypertension (%)	37.0	50.0	100.0
Smoking (%)	53.3	50.0	0
Diabetes mellitus (%)	35.9	0	0
−78 G→A promoter	G/G (n=140)	G/A (n=55)	A/A (n=10)
Age (yr)	65.5±1.2	63.2±2.1	59.8±4.3
Body mass index (kg/m^2)	23.6±3.4	23.3±3.1	23.4±2.7
Hypertension (%)	34.6	37.9	75.0
Smoking (%)	49.4	65.5	25.0
Diabetes mellitus (%)	36.8	27.6	50.0

Tables 4 and **5** show the two-marker (−78 G→A promoter and 67 Msp I polymorphism) genotype frequencies and haplotypes in controls and coronary heart disease subjects. Again, no significant differences were observed between the two groups.

DISCUSSION

There is evidence that a polymorphic nucleotide which identifies a rare allele may be in linkage disequilibrium with other functionally important polymorphism in the apo A-I/C-III/A-IV locus, or may directly affect apolipoprotein gene expression, as has been demonstrated for a polymorphism in the human apo A-I promoter[11]. In most cases, multiple factors are likely to influence plasma lipid levels.

There was no significant difference in the frequency of the M2 allele between the control (0.043) and coronary heart disease (0.039) groups. In the present study, we were unable to find a significant association between the M2 allele and either coronary heart disease or a change in serum HDL-C or apo A-I levels in a Japanese population. We also did not confirm that the A allele of −78 G→A promoter polymorphism affects HDL-C elevation (**Table 3**), as has been previously reported in other studies[2–4,12]. No difference in both genotypes for 67 Msp I and −78 G→A promoter of the apo A-I gene was observed between patients with coronary heart disease of varying severity, as determined by the number of involved vessels (single-, two- and three-vessel disease), and controls. Sex unadjusted and adjusted serum lipids and lipoprotein levels were

Table 3 Adjusted serum lipoprotein and apolipoprotein levels for the 67 Msp I marker and the −78 G→A promoter marker of the apo A-I gene

	67 Msp I						−78 G→A promoter					
	Controls (n=211)			Coronary heart disease group (n=205)			Controls (n=211)			Coronary heart disease group (n=205)		
(mg/dl)	1/1	1/2	2/2	1/1	1/2	2/2	G/G	G/A	A/A	G/G	G/A	A/A
Total cholesterol	196±36	210±48	—	197±36	169±36	174	197±38	192±35	—	196±36	192±36	203±49
Triglyceride	121±85	73±38	—	126±68	80±35	79	119±90	113±63	—	131±72	110±55	121±55
HDL-C	51±15	59±22	—	40±12	43±5	58	45±18	49±15	—	40±12	40±15	42±5
Lipoprotein (a)	20±14	21±29	—	27±19	19±20	29	20±16	16±12	—	26±20	29±18	19±6
Apo A-I	121±29	150±43	—	108±23	110±15	138	119±31	127±32	—	109±22	104±25	113±8
Apo A-II	31±7.5	34±12.8	—	28±5.5	25±5.6	29	31±7.8	31±8.5	—	28±5.5	27±5.7	30±4.4
Apo B	102±25	87±31	—	114±31	87±28	80	101±25	96±25	—	114±31	108±29	118±34
Apo C-II	3.7±2.0	3.1±0.8	—	3.6±1.7	2.6±1.8	2.2	3.7±2.0	3.4±1.8	—	3.7±1.7	3.4±1.6	3.5±1.1
Apo C-III	10.9±6.3	10.7±1.9	—	9.6±4.0	7.1±2.6	6.9	11.0±6.9	9.9±3.3	—	9.8±4.1	8.6±3.7	9.8±2.4
Apo E	5.3±1.9	5.7±1.3	—	5.0±1.6	3.8±1.0	4.4	5.4±1.8	5.1±2.1	—	5.0±1.7	4.6±1.5	4.9±2.0

Values are mean±standard deviation.

Table 4 Two-marker genotype frequencies in normal controls and coronary heart disease patients

−78 G→A promoter	67 Msp I	Controls (n=211)	Coronary heart disease group (n=205)
G/G	1/1	0.654 (138)	0.561 (115)
G/A	1/1	0.242 (51)	0.317 (65)
G/G	1/2	0.080 (17)	0.059 (12)
G/A	1/2	0.005 (1)	0.010 (2)
G/G	2/2	0	0.005 (1)
A/A	1/1	0.019 (4)	0.049 (10)

Numbers in parentheses indicate number of subjects with each genotype.
Pairwise linkage disequilibrium : −78 G→A promoter and 67 Msp I, Q=0.488 in normal group and Q=0.325 in coronary heart disease group. Q=0, no disequilibrium; Q=1, complete disequilibrium[13].

Table 5 Proportion of two-marker −78 G→A promoter/67 Msp I haplotypes in controls and coronary heart disease patients

Haplotypes		Proportion	
−78 G→A promoter	67 Msp I	Controls (n=211)	Coronary heart disease (n=205)
G	1	0.817	0.754
A	1	0.140	0.207
G	2	0.043	0.039
A	2	0	0

consistent according to the genotypes both for 67 Msp I and −78 G→A promoter polymorphisms. Combining the two markers, we determined the two-marker frequencies and haplotypes in normal and coronary heart disease groups. However, no strong informative association was observed. The polymorphic sites in the normal and coronary heart disease groups, as well as in the combination of the normal and coronary heart disease data, showed no significant deviation from the Hardy-Weinberg equilibrium. As a distinct ethnic group, Japanese show a lower prevalence of coronary heart disease than Western populations. This suggests the possibility that coronary heart disease is associated with a specific genetic background. However, confirmation of disease associations will require additional information regarding polymorphism of the apo A-I/C-III/A-IV gene locus, to establish an association between an independent predictor and hyperlipidemia and/or coronary heart disease.

Acknowledgments

This work was supported by a grant-in-aid from the Ministry of Education, Science and Culture of Japan (No. 04671503). We wish to thank Dr. Noboru Takami for his continuing interest in our project and for his valued advice.

要　約

アポリポ蛋白 A-I の新しい遺伝子多型と血清脂質および冠動脈硬化症との関連について

白　怀　朔　啓二郎　劉　瑞　自見　至郎　荒川規矩男

アポリポ蛋白 A-I 遺伝子のコーディング部分およびエキソンフランキング部分の DNA シークエンスにより，新しい遺伝子多型 [イントロン 1，MspI(CC/T-GG)] を発見するとともに，同遺伝子多型の血清脂質レベル，冠動脈硬化症に及ぼす効果を検討した．また西欧では以前より報告されている−78 G→A プロモーター部分のアポ A-I 遺伝子変異も同時に検索した．今回の検討では，どちらのアポリポ A-I の遺伝子変異も，血清脂質やリポ蛋白レベルに何ら変化を及ぼさず，冠動脈硬化症患者においても同様に日本人では有意な関連はなかったが，新しい遺伝子多型を冠動脈疾患患者で初めて報告した．

J Cardiol 1996; 28: 207–212

References

1) Humphries SE : DNA polymorphisms of the apolipoprotein : Their use in the investigation of the genetic component of hyperlipidemia and atherosclerosis. Atherosclerosis 1988; **72** : 89–108

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

3) Pagani F, Sidoli A, Giudici GA, Barenghi L, Vergani C, Baralle FE : Human apolipoprotein A-I gene promoter polymorphism : Association with hyperalphalipoproteinemia. J Lipid Res 1990; **31** : 1371–1377
4) Paul-Hayase F, Rossenneu M, Robinson D, Van Bervliet J, Deslypere JP, Humphries SE : Polymorphisms in the apolipoprotein (apo) A-I–C-III–A-IV gene cluster in determining plasma apo A-I, apo C-III and apo A-IV concentrations. Hum Genet 1992; **88** : 439–446
5) Allain CC, Poon LS, Chan CS : Enzymatic determination of total cholesterol. Clin Chem 1974; **20** : 470–475
6) Eggestein M, Kreuntz FH : Ein neue Bestimmung der Neutralfette in Blutserum und Gewebe : I. Mitt. Prinzip, Durchführung und Besprechung der Methode. Klin Wochenschr 1966; **44** : 262
7) Noma A, Nezu-Nakayama K, Kita M, Okabe H : Simultaneous determination of serum cholesterol in high- and low-density lipoproteins with use of heparin Ca^{2+}, and anion-exchange resin. Clin Chem 1978; **24** : 1504–1508
8) Ikeda T, Shibuya U, Sugiuchi H, Araki S, Uji Y, Okabe H : Automated immunoturbidimetric analysis of six plasma apolipoproteins : Correlation with radial immunodiffusion assays. J Clin Lab Anal 1991; **5** : 90–95
9) Erlich HA : PCR technology. *in* Principles and Applications for DNA Amplification, 1st Ed. Stockton Press, New York, 1989; pp 35–36
10) Shoulders CC, Kornblihtt AR, Sean Munro B, Baralle FE : Gene structure of human apolipoprotein A-I. Nucleic Acids Res 1983; **11** : 2827–2837
11) Smith JD, Brinton EA, Breslow JL : 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 1992; **89** : 1796–1800
12) Sigurdsson G, Gudnason V, Sigurdsson G, Humphrise SE : Interaction between a polymorphism of the apo A-I promoter region and smoking determines plasma levels of HDL and apo A-I. Arterioscler Thromb Vasc Biol 1992; **12** : 1017–1022
13) Yule GU : Mathematical and physical science. Philos Trans R Soc Lond B Biol Sci 1900; Ser A **194** : 257–319