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APOLIPOPROTEIN AI-CIII GENE POLYMORPHISMS IN JAPANESE MYOCARDIAL INFARCTION SURVIVORS

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Summary To search for a linkage marker for the putative deleterious atherogenic gene in the apo AI-CIII-AIV gene complex in Japanese, apo CIII *Sst*-I genotypes and apo AI *Msp*-I genotypes were investigated in 69 Japanese myocardial infarction survivors, using genomic hybridization analysis, and compared with the genotypes in 82 healthy subjects. Unlike the association of the *S2* and *M2* alleles with myocardial infarction found in Caucasians, there were no differences in both the frequencies of the *S2* and *M2* alleles between Japanese myocardial infarction survivors and healthy subjects. The individual with the haplotype *S1-M2*, however, was significantly increased in myocardial infarction survivors compared with the one in healthy subjects (24% *versus* 11%; $\chi^2=4.90$, d.f.$=1$, $p<0.05$). The data suggest that the haplotype *S1-M2* may be a linkage marker for the putative atherogenic gene or may show regional differences in the frequency in Japanese.

INTRODUCTION

Apolipoprotein AI and CIII (apo AI and CIII) play an important role in the metabolism of plasma lipoproteins and lipids (Herbert *et al.*, 1983). The human apo AI and apo CIII genes are tightly linked and form a gene complex together

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with the apo AIV gene on the long arm of the chromosome 11 (Karathanasis, 1985). A mutation of the apo AI gene is associated with low plasma HDL and premature atherosclerosis (Karathanasis *et al.*, 1983a, 1983b). In addition, restriction fragment length polymorphisms related to the apo AI and apo CIII genes have been reported to be associated with coronary atherosclerosis and familial hypoalphalipoproteinemia in Caucasians (Ferns *et al.*, 1985; Rees *et al.*, 1985; Sidoli *et al.*, 1985; Ferns and Galton, 1986; Ordovas *et al.*, 1986; Buraczynska *et al.*, 1986; Deeb *et al.*, 1986; Acton *et al.*, 1986). An apo AI-CIII gene polymorphism associated with coronary atherosclerosis may be a linkage marker for an atherogenic gene in the apo AI-CIII-AIV gene complex. Such a linkage marker is likely to vary among the races.

In the previous study, we revealed that the haplotypes identified by the apo CIII *Sst*-I and apo AI *Msp*-I polymorphisms are useful genetic markers for Japanese (Onuki *et al.*, 1986). In the present study, the haplotypes identified by the apo CIII *Sst*-I and apo AI *Msp*-I polymorphisms were investigated in Japanese myocardial infarction survivors, as an attempt to find a linkage marker for the putative atherogenic gene in the apo AI-CIII-AIV gene complex in Japanese.

MATERIALS AND METHODS

Blood was collected from 69 Japanese myocardial infarction survivors without diabetes mellitus (59 males, 10 females) attending the cardiac clinics at Tsuchiura-kyodo Hospital and Tsukuba Medical Center, Ibaraki-ken. Diagnosis was confirmed by clinical history, typical electrocardiographic changes and enzyme examination. The mean ($\pm$SD) age of the myocardial infarction survivors was 59 ± 10.

DNA was isolated from whole blood cells essentially according to the method of Kunkel *et al.* (1977). The apo CIII *Sst*-I genotypes and apo AI *Msp*-I genotypes were analyzed by Southern blotting using ^{32}P-labeled human apo AI clone composing 2.2 kb *Pst*-I fragment of an apo AI genomic clone as described previously (Southern, 1975; Kessling *et al.*, 1985; Onuki *et al.*, 1986).

Association analysis between myocardial infarction and the haplotype identified by the apo AI-CIII *Sst*-I and *Msp*-I polymorphisms was performed in 2×2 tables by chi-square statistics. In the association analysis, the data on 82 unrelated healthy members of the university staffs and students (age range 20–62, mean age 30 ± 12) were used as the control; the data was described in detail previously (Onuki *et al.*, 1986).

RESULTS

Figure 1 shows simplified restriction site maps for the *Sst*-I and *Msp*-I polymorphisms in the apo AI-CIII genes. Table 1 presents the distribution and allele frequencies of the apo CIII *Sst*-I genotypes and apo AI *Msp*-I genotypes in 69 myocardial infarction survivors and 82 unrelated healthy subjects. Both the frequencies of the *S2* and *M2* alleles were high in myocardial infarction survivors and healthy

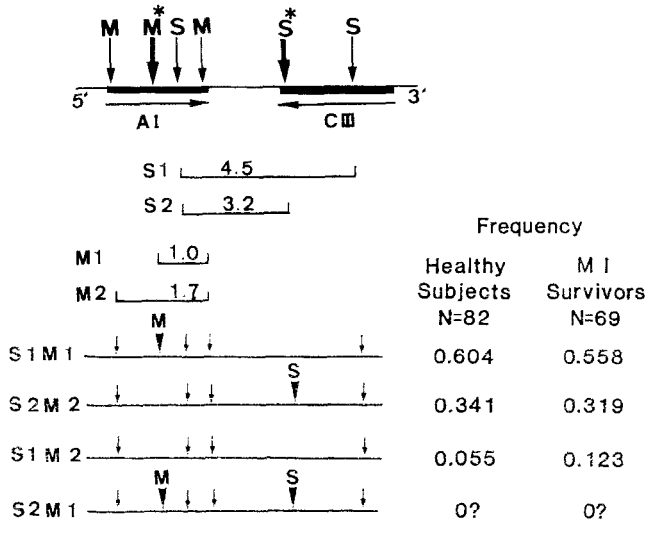

Fig. 1. Simplified restriction site maps for the *Sst*-I and *Msp*-I polymorphisms in the apo AI-CIII genes. S* and M* represent the polymorphic sites for the enzyme *Sst*-I and *Msp*-I, respectively. Two alleles for each polymorphic site and the four possible haplotypes are shown. Arrowheads on the haplotype indicate the presence of the site for the enzyme *Sst*-I (S) or *Msp*-I (M). Frequencies of each haplotype in myocardial infarction (MI) survivors and healthy subjects are also described.

Table 1. Distribution and allele frequencies of the apo CIII *Sst*-I genotypes and apo AI *Msp*-I genotypes in myocardial infarction (MI) survivors and healthy subjects.

	N	Genotypes (%)			Allele	
Apo CIII *Sst*-I		*S1S1*	*S1S2*	*S2S2*	*S1*	*S2*
MI survivors	69	20 (40.6)	38 (55.1)	3 (4.3)	0.68	0.32
Healthy subjects	82	37 (45.1)	34 (41.5)	11 (13.4)	0.66	0.34
Apo AI *Msp*-I		*M1M1*	*M1M2*	*M2M2*	*M1*	*M2*
MI survivors	69	20 (30.0)	37 (53.6)	12 (17.4)	0.56	0.44
Healthy subjects	82	32 (39.0)	35 (42.7)	15 (18.3)	0.60	0.40

subjects, and no significant differences in the frequencies of the alleles were observed between the two groups.

In the previous study we clearly demonstrated that the alleles identified by the apo CIII *Sst*-I and apo AI *Msp*-I polymorphisms are in linkage disequilibrium (Onuki *et al.*, 1986). As observed in the previous study, none of 69 subjects examined in this study had the genotypes, *S1S2M1M1*, *S2S2M1M1*, and *S2S2M1M2*. Since the haplotype *S2-M1* is considered to be absent, or, if present, very rare and

Table 2. Distribution of combined haplotype in myocardial infarction (MI) survivors and healthy subjects.

Combined haplotype	MI survivors		Healthy subjects	
	N	(%)	N	(%)
S1M1 / S1M1	20		32	
S1M1 / S2M2	29	52 (75.4)[a]	30	73 (89.0)
S2M2 / S2M2	3		11	
S1M1 / S1M2	8		5	
S2M2 / S1M2	9	17 (24.6)[a]	4	9 (11.0)
Total	69	(100)	82	(100)

[a] $\chi^2=4.90$, d.f.$=1$, $p<0.05$, when compared with healthy subjects.

negligible in Japanese, the combination of the haplotypes identified by these polymorphisms is easily determined for each individual when the genotypes of the polymorphisms are known. Table 2 shows the distribution of the combined haplotypes in myocardial infarction survivors and healthy subjects. The frequency of the individuals with the haplotype *S1-M2* (that is genotypes *S1S1M1M2*, *S1S2M2M2*) is significantly increased in myocardial infarction survivors compared with healthy subjects (24.6% *versus* 11.0%, $p<0.05$). The frequency of each haplotype in myocardial infarction survivors and healthy subjects is shown in Fig. 1; the frequencies of the haplotype *S1-M2* are 0.123 in myocardial infarction survivors and 0.055 in healthy subjects, and the difference is also significant ($\chi^2=4.30$, d.f.$=1$, $p<0.05$).

DISCUSSION

The *S2* and *M2* alleles, especially the *S2* allele, have been reported to be relatively uncommon and associated with coronary atherosclerosis in some local Caucasians (Ferns *et al.*, 1985; Rees *et al.*, 1985; Ferns and Galton, 1986; Deeb *et al.*, 1986; Acton *et al.*, 1986). In the case of Japanese, however, our data indicate that both the *S2* and *M2* alleles are common and not associated with coronary atherosclerosis. The apo CIII *Sst*-I polymorphism arises from a C-G transversion in the 3′-non-coding region of the apo CIII gene (Karathanasis *et al.*, 1983a). The apo AI *Msp*-I polymorphism arises from the presence or absence of a *Msp*-I site in the third intron of the apo AI gene (Seilhamer *et al.*, 1984). Therefore it is considered that the *S2* and *M2* alleles may represent a linkage marker for an atherogenic gene at least in some local Caucasians but not in Japanese.

The haplotypes identified by the apo CIII *Sst*-I and apo AI *Msp*-I polymorphisms are useful genetic markers for the analysis of apo AI-CIII-AIV gene complex in Japanese (Onuki *et al.*, 1986). The results of the present study suggest that the haplotype *S1-M2* may be a linkage marker for the putative atherogenic gene in

Japanese. Our data seem to be consistent with the finding reported by Rees *et al.* (1986) that the haplotype *S1-M2* is associated with hypertriglyceridemia in Japanese (Rees *et al.*, 1986). However, since the difference in the frequency of the haplotype *S1-M2* between myocardial infarction survivors and healthy subjects may reflect regional differences, it is required to perform further case-control studies to confirm the results of the present study.

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