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SHORT COMMUNICATION

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Association between coronary heart disease and the apolipoprotein A-I/C-III/A-IV complex in a Japanese population

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Abstract Several studies have reported that a variant allele (S_2) of the apolipoprotein (apo) A-I/C-III/A-IV complex is associated with hyperlipoproteinemia in some populations and that the frequency of this allele is two- to fivefold higher in patients with premature coronary heart disease (CHD) than in healthy controls. In the present study in a Japanese population, we were unable to confirm the association of the S_2 allele with either coronary heart disease or elevated serum apo C-III levels, as has been previously reported in Caucasians. No genotype difference was observed among the severity of coronary heart disease, as determined by the number of involved vessels (one, two and three vessel disease), compared to controls. In addition, the frequency of the S_2 allele among Japanese, in both CHD (0.328) and controls (0.369), was quite different from that in many other populations.

Introduction

Cytosine-to-guanosine substitution in the 3′ untranslated region of the apo CIII gene distinguishes two alleles (S_1 and S_2) of the apo A-I/C-III/A-IV complex (Karathanasis et al. 1985). Several studies have reported that variant allele 2 (S_2) of the apo A-I/C-III/A-IV complex is associated with primary hypertriglyceridemia (Rees et al. 1985a), severe coronary atherosclerosis (Rees et al. 1985b), and myocardial infarction (Ferns et al. 1985). The clinical significance of this allele has been demonstrated in case-control studies that have shown a two- to fivefold increase in the frequency of the S_2 allele in patients with coronary heart disease (CHD) and peripheral vascular disease, as compared with those in control groups (Rees et al. 1985b; Ferns et al. 1985; O'Connor et al. 1987; Price et al. 1989).

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In the present study, we investigated whether the S_2 allele of the apo A-I/C-III/A-IV complex is associated with CHD, the severity of coronary atherosclerosis, or elevated serum apo C-III and other apolipoprotein levels in a Japanese population.

Materials and methods

Ninety patients with coronary heart disease (70 males, 20 females, age 63 ± 12 years), based on coronary angiography, were studied. The severity of coronary atherosclerosis was based on the number of involved vessels (single, double and triple vessels), which was defined as a 75% or greater stenosis. Sixty-five controls (39 males, 26 females, age 58 ± 12 years) consisted of both angiographically proven nonstenotic coronary artery subjects (n = 42) and healthy controls with normal electrocardiograms without episodes of chest pain (n = 23). 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. After an overnight fast, blood was drawn. Serum total cholesterol (TC) and triglyceride (TG) were measured by enzymatic methods (Allain et al. 1974; Eggestein and Kreuntz 1966). High-density lipoprotein-cholesterol (HDL-C) was determined by the heparin Ca^{2+} precipitation method (Noma et al. 1978). Apo A-I, apo A-II, apo B, apo C-II, apo C-III and apo-E were measured by the turbidity immunoassay (TIA) method (Ikeda et al. 1991). All apolipoproteins were assayed within 48 h. Genomic DNA was isolated from 500 µl peripheral blood according to the method of Higuchi (Erlich 1989). Restriction fragment length polymorphism (RFLP) analysis to determine the genotype of the subjects was performed using *Sst*I restriction enzyme digestion after production of polymerase chain reaction (PCR) DNA fragments that spanned the *Sst*I site of the apo C-III gene.

Results

The genotypes of *Sst*I polymorphism in CHD and controls are shown in Table 1 and Fig. 1. The frequency of *Sst*I allele 2 was 0.369 in controls and 0.328 in CHD patients; there was no difference in the frequency of the S_2 allele between the two groups. Even if the CHD patients were divided into one, two and three vessel diseases by the number of involved vessels, no frequency difference of genotypes were observed by the χ^2-test.

Table 1 Genotypes and frequencies for *Sst*I marker of the apo A-I/C-III/A-IV complex. Numbers in parentheses indicate number of subjects with each genotype or number of alleles of each type. 1V, 2V and 3V represent the severity of coronary artery stenosis (CHD coronary heart disease)

	Frequency				
	Controls (n = 65)	CHD (n = 90)	1V	2V	3V
Sst I genotype					
S_1S_1	0.415 (27)	0.478 (43)	48.84 (21)	37.21 (16)	13.95 (6)
S_1S_2	0.431 (28)	0.389 (35)	37.14 (13)	51.43 (18)	11.43 (4)
S_2S_2	0.154 (10)	0.133 (12)	0.50 (6)	0.25 (3)	0.25 (3)
Sst I allele					
1	0.631 (82)	0.672 (121)			
2	0.369 (48)	0.328 (59)			

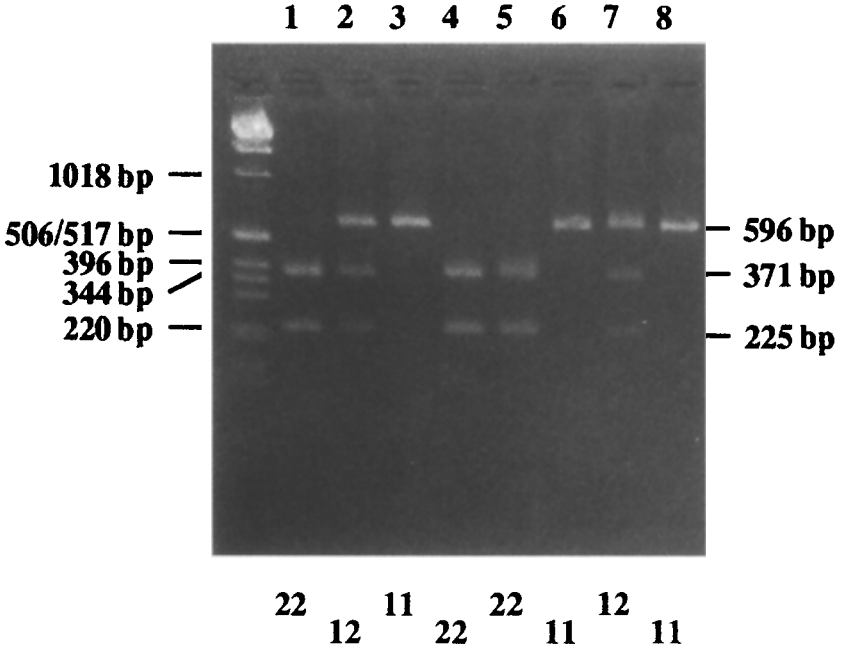

Fig. 1 C-to-G replacement of the 3′ end of apo C-III gene. This mutation was analyzed by *Sst*I RFLP, and was identified by the loss of a 596-bp DNA fragment and the formation of 371-bp and 225-bp fragments. Numbers below the lanes denote genotypes: *11* S_1S_1; *12* S_1S_2, *22* S_2S_2

Table 2 Serum lipoprotein and apolipoprotein levels for *Sst*I marker of the apo A-I/C-III/A-IV complex (mean±SD in mg/dl)

	Controls (n = 65)			CHD (n = 90)		
	S_1S_1	S_1S_2	S_2S_2	S_1S_1	S_1S_2	S_2S_2
TC	190 ± 45	210 ± 37	198 ± 35	184 ± 41	196 ± 40	172 ± 22
TG	112 ± 55	118 ± 54	147 ± 176	114 ± 59	139 ± 62	103 ± 22
HDL-C	47 ± 14	50 ± 18	42 ± 14	37 ± 11	36 ± 8	40 ± 14
Apo AI	123 ± 33	127 ± 31	113 ± 25	100 ± 20	99 ± 15	95 ± 16
Apo AII	32 ± 9.9	30 ± 5.8	30 ± 6.7	27 ± 5.7	26 ± 4.3	22 ± 4.2[a]
Apo B	120 ± 125	108 ± 28	103 ± 27	105 ± 31	114 ± 34	90 ± 10.9[b]
Apo CII	3.5 ± 1.8	3.7 ± 1.7	4.3 ± 2.9	4.1 ± 5.2	3.7 ± 1.9	2.5 ± 0.9[c]
Apo CIII	10.8 ± 7.5	10.7 ± 4.0	12.3 ± 7.2	8.4 ± 3.7	9.4 ± 3.9	7.8 ± 1 .5

[a] S_2S_2 of apo AII vs S_1S_1 P = 0.006 (t-test) vs S_1S_2 P = 0.021
[b] S_2S_2 of apo B vs S_1S_2 P = 0.02
[c] S_2S_2 of apo CII vs S_1S_2 P = 0.041

The S_1S_2 genotype tended to have a higher serum TC level than the other genotypes in both controls and CHD groups, but this difference was not significant (Table 2). Mean serum apo C-III levels in S_2S_2 were higher than those in S_1S_1 or S_1S_2 in the control group, but this difference was not statistically significant, and the tendency was not observed in the CHD group. The mean serum apo A-I levels in S_2S_2 tended to be lower both in CHD and controls, but again, these differences were not significant. In the CHD group, serum apo A-II, apo B and apo C-II values were significantly lower ($P < 0.05$) in S_2S_2 than in the other genotypes, while such tendencies were not observed in the controls.

Discussion

There is some evidence that a polymorphic nucleotide identifying a rare allele is in linkage disequilibrium with a mutation defect that directly predisposes the carrier to develop elevated plasma lipid levels, and is thus the primary defect responsible for the association of this rare allele with hyperlipidemia and premature CHD. In most situations, multiple factors are likely to influence plasma lipid levels.

The frequency of the S_2 allele in the Japanese population is quite different from that in many other populations (Paul et al. 1987). In this study, the frequency of allele 2 was 0.369 and 0.328 in the control and CHD groups, respectively. These findings are in line with the Japanese data by Rees et al. 1986, allele 2 frequency has been previously reported to be 0.01 in Caucasians, 0.27 in Negroes and 0.19 in Indian Asians (Paul et al. 1987). In the present study in a Japanese population, we were unable to confirm the association of the S_2 allele with either CHD or an elevation of serum apo C-III levels, as has been previously reported in Caucasians (Rees et al. 1985b; Shoulders et al. 1991). No genotype difference was observed among the severity of coronary heart disease, as determined by the number of involved vessels (one, two and three vessel disease), compared to controls. Furthermore, the observed variations in serum apo A-II, apo B and apo C-II levels in S_2S_2 in the CHD group may not be directly associated with S_2 of the apo A-I/C-III/A-IV complex, since no such tendencies were observed in controls. These are issues that remain to be resolved. As a distinct ethnic group, Japanese show a lower prevalence of CHD, compared to western populations. This suggests the possibility that CHD is associated with a specific genetic background. Confirmation of disease associations will require more information regarding polymorphism of the apo A-I/C-III/A-IV gene locus, to establish an association between an independent predictor and hyperlipidemia or CHD.

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