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ASSOCIATION OF THE APOLIPOPROTEIN E4 ALLELE WITH HYPERCHOLESTEROLEMIA IN APPARENTLY HEALTHY MALE ADULTS IN TOKYO

Shigeru TSUCHIYA,[1] Yasuko YAMANOUCHI,[2] Ryunosuke MIYAZAKI,[3] Hisako YANAGI,[2] Kimiko YAMAKAWA,[2] Kenji YUZAWA,[2] Minoru OHNUKI,[1] and Hideo HAMAGUCHI[2]

[1]*Institute of Community Medicine and* [2]*Institute of Basic Medical Sciences, University of Tsukuba, Tsukuba 305, Japan*
[3]*Kudanzaka-Hospital, Chiyoda-ku, Tokyo 102, Japan*

Summary This study was performed to investigate whether the apolipoprotein (apo) E4 allele is associated with hypercholesterolemia in Japanese male adults in large cities. The apo E phenotypes and serum lipid levels were analyzed on 142 apparently healthy male civil servants working in Tokyo. The prevalence of hypercholesterolemia (serum total cholesterol level > 250 mg/dl) were 4.7% in 106 apo E4-absent subjects and 19.4% in 36 apo E4-present subjects ($p=0.012$). The prevalence of hypercholesterolemia were 5.3% in 94 subjects with the apo E3/3 phenotype and 20.0% in 30 subjects with the apo E3/4 phenotype ($p=0.023$). In addition, mean serum total cholesterol levels were significantly higher in the apo E4-present subjects than in the apo E4-absent subjects (213 ± 29.1 *versus* 199 ± 36.0, $p<0.05$). The data suggest that the apo E4 allele is associated with hypercholesterolemia in Japanese male adults working in large cities.

INTRODUCTION

Human apolipoprotein (apo) E is a single chain of 299 amino acids and a major protein constituent of plasma very low density lipoprotein (VLDL) (Shelburne and Quarfordt, 1974; Rall *et al.*, 1982). Apo E is also of a normal protein constituent of chyromicron remnants, intermediate density lipoproteins, and a subfraction of the high density lipoproteins (Weisgraber and Mahley, 1978; Mahley and Innerarity, 1983). Apo E serves as a mediator of the cellular uptake of specific plasma lipoproteins through an interaction with apo E receptor and apo B, E receptor (Mahley and Innerarity, 1983). In addition, apo E is involved in the conversion of VLDL to the low density lipoprotein (LDL) (Ehnholm *et al.*, 1984). In the apo E gene,

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there are three common alleles, designated ε2, ε3 and ε4, which code for apo E2, apo E3, and E4, respectively (Zannis *et al.*, 1982). The apo E3 allele is the most common and the apo E4 allele is the second commonest in Japanese (Asakawa *et al.*, 1985; Tsuchiya *et al.*, 1985; Eto *et al.*, 1986). The apo E4 allele is present in about 20% of populations. Besides the evidence for the association of the apo E2 allele with type III hyperlipoproteinemia (Havel, 1982), there is growing evidence that the apo E4 allele is involved in predisposing one to hyperlipidemia (Assmann *et al.*, 1984; Utermann *et al.*, 1984; Leren *et al.*, 1985; Sing and Davignon, 1985; Tsuchiya *et al.*, 1986; Boerwinkle *et al.*, 1987; Ordovas *et al.*, 1987; Pagnan *et al.*, 1987). In addition, an association of the apo E4 allele with hypercholesterolemia has been demonstrated in Caucasian populations (Utermann *et al.*, 1984; Leren *et al.*, 1985). The purpose of this study is to investigate whether the apo E4 allele is associated with hypercholesterolemia in Japanese male adults working in large cities.

MATERIALS AND METHODS

The subjects were 142 apparently healthy Japanese male civil servants working in Tokyo. They were selected consecutively from those who visited the health care center of Kudanzaka Hospital, Tokyo, for their annual health examinations. The subjects were not excluded by any other criterion except those with diabetes mellitus, abnormal thyroid function or abnormal kidney function. They ranged in age from 36 to 64 years with an average age of 46.7 years. No individuals indicated use of antihyperlipidemic drugs.

Blood samples were obtained after an overnight fast. The phenotype of apo E was determined by two-dimensional gel electrophoresis as described previously (Tsuchiya *et al.*, 1985). Serum total cholesterol and triglyceride were measured enzymatically (Allain *et al.*, 1974; Sampson *et al.*, 1975) on an automated analyzer (Hitachi Model 712, Hitachi Ltd., Tokyo). HDL cholesterol was determined after isolating the HDL fraction by the dextran sulphate-magnesium chloride precipitation (Kostner, 1976). Statistical differences in the prevalence of hypercholesterolemia were assessed by the Fisher's exact test. Statistical differences in mean serum total cholesterol levels were assessed by the unpaired Student *t*-test (Armitage, 1977).

RESULTS

Two-dimensional electrophoresis patterns of three common apo E phenotypes are shown in Fig. 1. Table 1 presents the apo E phenotype frequencies, average serum lipid levels, and prevalence of hypercholesterolemia (serum total cholesterol > 250 mg/dl) in the samples of 142 unrelated males. Besides three common genetic types of apo E, that is, apo E2, apo E3 and apo E4, apo E5 was detected in one subject and apo E7 in another one subject. The observed phenotype frequencies did not differ from those expected based on Hardy-Weinberg equilibrium. Hypercholes-

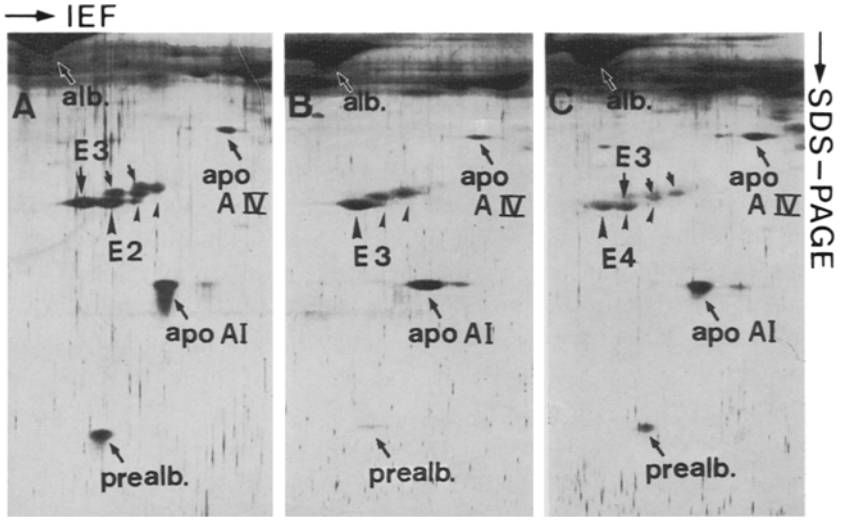

Fig. 1. Three common phenotypes of apo E in the two-dimensional electrophoresis pattern. A: Apo E2/3, B: Apo E3/3, C: Apo E3/4. E2, E3, and E4 indicate apo E2, apo E3 and apo E4, respectively. Albumin (alb), prealbumin (prealb), apolipoprotein AI (apo AI), and apolipoprotein AIV (apo AIV) are also shown. Isoelectric forcusing (IEF) was from left to right, and molecular weight separation by SDS-polyacrylamide gel electrophoresis (SDS-PAGE) was from top to bottom.

Table 1. Apo E phenotype distribution, serum lipid levels, and prevalence of hypercholesterolemia.

Apo E phenotype	n (%)	Age (mean±SD)	Total Ch (mg/dl)	Total TG (mg/dl)	HCH	
					n	(%)
E3/3	94 (66.2)	47 ± 6.8	198 ± 29.6	127 ± 76.5	5	(5.3)
E3/4	30 (21.1)	46 ± 7.6	211 ± 38.4	152 ± 98.7	6	(20.0)[a]
E2/3	10 (7.0)	43 ± 4.6	202 ± 24.2	127 ± 55.7	0	
E2/4	3 (2.1)	45 ± 4.7	233 ± 16.3	133 ± 63.2	1	(33.3)
E4/4	3 (2.1)	47 ± 5.4	209 ± 7.7	135 ± 47.9	0	
E2/5	1 (0.7)	37	178	188	0	
E3/7	1 (0.7)	54	235	144	0	

Values are mean±SD. Ch, cholesterol; TG, triglyceride; HCH, hypercholesterolemia (total serum cholesterol >250 mg/dl). [a] Significant difference ($p=0.023$) as compared with E3/3.

terolemia was detected in five of the 94 subjects with the apo E3/3 phenotype (5.3%) and six of the 30 subjects with the apo E3/4 phenotype (20.0%). The difference was statistically significant ($p=0.023$). The mean (±SD) serum total cholesterol levels were 198±29.6 mg/dl in the 94 subjects with the apo E3/3 phenotype and

211±38.4 mg/dl in the 30 subjects with the apo E3/4 phenotype. Although the difference was not quite significant at the 5% level ($t=1.92$, $0.05<p<0.10$), subjects with the apo E 3/4 phenotype tended to have a higher serum cholesterol level than subjects with the apo E3/3 phenotype.

Table 2 shows the prevalence of hypercholesterolemia and mean serum total cholesterol levels in the apo E4-absent and apo E4-present subjects. Hypercholesterolemia was detected in five of 106 apo E4-absent subjects (4.7%) and seven of 36 apo E4-present subjects (19.4%). The difference was significant ($p=0.012$). Furthermore, mean serum total cholesterol levels were significantly higher in the apo E4-present subjects than in the apo E4-absent subjects (213±29.1 *versus* 199±36.0, $t=2.32$, $p<0.05$). The individual data on serum lipid levels of the hypercholes-

Table 2. Prevalence of hypercholesterolemia and mean serum total cholesterol levels in the apo E4-absent males and apo E4-present males.

Apo E phenotype	n	Age (mean±SD)	Hypercholesterolemia		Total cholesterol (mean±SD) (mg/dl)
			n	(%)	
E4-absent (E3/3, E2/3, E2/5, E3/7)	106	46 ± 6.8	5	(4.7)	199 ± 36.0
E4-present (E3/4, E2/4, E4/4)	36	46 ± 7.3	7	(19.4)[a]	213 ± 29.1[b]

[a] Significant difference ($p=0.012$). [b] Significant difference ($t=2.32$, $p<0.05$).

Table 3. Individual data on serum lipid levels in hypercholesterolemic subjects.

	Apo E phenotypes	Cholesterol (mg/dl)		Total TG (mg/dl)
		Total	HDL	
HCD- 35 48	E3/3	274	40	408
94 55	3/3	276	44	130
97 44	3/3	260	45	105
313 54	3/3	254	78	35
343 44	3/3	251	52	189
HCD- 15 52	E3/4	265	45	169
25 58	3/4	264	64	82
40 35	3/4	255	42	192
167 54	3/4	291	90	53
336 42	3/4	257	66	119
340 39	3/4	274	55	157
HCD-319 48	E2/4	256	46	212

terolemic subjects are given in Table 3. In the case of hypercholesterolemic subjects with the apo E4, all individuals had moderate hypercholesterolemia with a normal triglyceride level or with mild hypertriglyceridemia.

DISCUSSION

As to the association of the apo E4 allele with hypercholesterolemia, Utermann *et al.* (1984) reported that apo E4 was significantly more frequent in patients with hypercholesterolemia than in blood donors in Federal Republic of Germany. Apo E4 was also significantly more frequent among male subjects with multifactorial hypercholesterolemia than among normocholesterolemic controls in Norway (Leren *et al.*, 1985). It is well established that both the individual's genotype and nutritional factors are involved in determining serum total cholesterol levels. Since the apo E4 allele is present in about 20% of Japanese populations and since the apo E4 allele may act as one of polymeric genes in predisposing one to multifactorial hypercholesterolemia, it is important to investigate whether the apo E4 allele is associated with hypercholesterolemia in Japanese. An association of the apo E4 allele with hypercholesterolemia in Japanese, if present, would be more easily verified in subjects working in large cities than in agricultural districts, because mean serum total cholesterol levels in middle-aged Japanese are generally higher in large cities than in agricultural districts. This study has shown that the prevalence of hypercholesterolemia was significantly higher in the apo E4-present subjects than in the apo E4-absent subjects in apparently healthy male civil servants working in Tokyo. Furthermore, hypercholesterolemia was significantly more frequent among the apo E3/4 phenotype group than among the apo E3/3 phenotype. Considering that only the apo E3 allele has yet to be associated with any of the lipid disorders, these findings suggest that the apo E4 allele is associated with hypercholesterolemia at least in Japanese males working in large cities.

In the present study, it was observed that hypercholesterolemic subjects with the apo E4 had moderate hypercholesterolemia with a normal triglyceride level or with mild hypertriglyceridemia. In addition, mean serum total cholesterol levels were significantly higher in the apo E4-present subjects than in the apo E4-absent subjects. Mean serum total cholesterol levels also tended to be higher in the apo E3/4 phenotype group than in the apo E3/3 phenotype group, though the difference was not quite significant at the 5% level probably due to small sample sizes. It has been demonstrated that mean serum total cholesterol levels have a tendency to be higher in the apo E3/4 phenotype as compared with the apo E3/3 phenotype in Caucasians (Sing and Davignon, 1985; Boerwinkle *et al.*, 1987; Pagnan *et al.*, 1987). Considering that most of multifactorial hypercholesterolemia is not severe and that many individuals with apo E4 are normocholesterolemic, these findings suggest that the apo E4 allele acts as a polymeric gene in predisposing one to hypercholesterolemia. The apo E4 molecule differs from the apo E3 molecule by an amino acid substitution

from cysteine to arginine at residue 112 (Rall *et al.*, 1982). The mechanism underlying the association of the apo E4 allele with hypercholesterolemia might be related to a phenomenon observed by Gregg *et al.* (1986) that apo E4 is catabolized more rapidly than apo E3.

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