

## APOLIPOPROTEIN E PHENOTYPES AND SERUM LIPID LEVELS IN APPARENTLY HEALTHY MALE ADULTS IN TOKYO

Shigeru TSUCHIYA,<sup>1</sup> Yasuko YAMANOUCHI,<sup>2</sup> Ryunosuke MIYAZAKI,<sup>3</sup>  
Mieko ONUKI,<sup>2</sup> Kimiko YAMAKAWA,<sup>2</sup> Ikuko KONDO,<sup>2</sup>  
Minoru OHNUKI,<sup>1</sup> and Hideo HAMAGUCHI<sup>2</sup>

<sup>1</sup>*Department of Nursing and Rehabilitation Medicine, Institute of  
Community Medicine, and*

<sup>2</sup>*Department of Human Genetics, Institute of Basic Medical Sciences,  
University of Tsukuba, Sakura-mura, Ibaraki 305, Japan*

<sup>3</sup>*Kudanzaka-Hospital, Kudanzaka-minami, Chiyoda-ku,  
Tokyo 102, Japan*

*Summary* This study was performed to investigate whether the  $\epsilon 4$  allele of the apolipoprotein (apo) E predisposes Japanese male adults in large cities to hyperlipidemia. The apo E phenotypes and serum total lipid levels were determined using blood samples obtained after an overnight fast from 85 apparently healthy Japanese male civil servants in Tokyo. The frequency of hyperlipidemia was 50.0% in 20 subjects with the apo E4-positive phenotype and 25.8% in 62 subjects with the apo E3/3 phenotype. The difference is statistically significant ( $p < 0.05$ ). The mean ( $\pm$ SD) serum triglyceride levels, which were calculated on the subjects with serum triglyceride lower than 250 mg/dl, was  $132.8 \pm 5.1$  mg/dl in 18 subjects with the apo E4-positive phenotype and  $108.4 \pm 43.0$  mg/dl in 58 subjects with the apo E3/3 phenotype. The difference is also statistically significant ( $p < 0.05$ ). The data suggest that the  $\epsilon 4$  allele might predispose Japanese adults in large cities to hyperlipidemia.

### INTRODUCTION

Apolipoprotein (apo) E is a normal protein constituent of human plasma very low density lipoproteins (VLDL), intermediate density lipoproteins (IDL), chylomicrons, chylomicron remnants, and a subfraction of the high density lipoproteins (HDL) (Shelburne and Quarfordt, 1974; Weisgraber and Mahley, 1978; Mahley and Innerarity, 1983). Apo E plays a central role in the metabolism of cholesterol and triglyceride: It serves as a mediator of the cellular uptake of specific plasma lipoproteins through an interaction with apo B,E receptor and apo E receptor, and

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is also involved in the conversion of VLDL to the low density lipoproteins (LDL) (Mahley and Innerarity, 1983; Ehnholm *et al.*, 1984). In man, the locus for apo E is polymorphic (Utermann *et al.*, 1977; Zannis *et al.*, 1981, 1982). Three common alleles, designated  $\epsilon 2$ ,  $\epsilon 3$ , and  $\epsilon 4$ , code for three common apo E isoforms, apo E2, apo E3 and apo E4, respectively, and determine six apo E phenotypes (Zannis *et al.*, 1982). The three common alleles have been demonstrated to exist in various populations (Utermann *et al.*, 1982; Wardell *et al.*, 1982; Ghiselli *et al.*, 1982a; Cumming and Robertson, 1984; Sing and Davignon, 1985; Asakawa *et al.*, 1985; Tsuchiya *et al.*, 1985).

In Japanese, the  $\epsilon 3$  allele is the most common and the  $\epsilon 4$  allele is the second commonest, as in other populations studied thus far (Asakawa *et al.*, 1985; Tsuchiya *et al.*, 1985). In Caucasian populations, there is growing evidence that the  $\epsilon 4$  allele might be involved in predisposing one to hyperlipidemia (Ghiselli *et al.*, 1982a, 1982b; Utermann *et al.*, 1984; Sing and Davignon, 1985). The purpose of this study is to investigate whether the  $\epsilon 4$  allele predisposes Japanese male adults in large cities to hyperlipidemia.

#### MATERIALS AND METHODS

Blood samples were obtained after an overnight fast from 85 apparently healthy Japanese male civil servants working in a government agency in Tokyo. They visited the health care center of Kudanzaka Hospital, Tokyo, for their annual health examinations. Their mean ( $\pm$ SD) age was  $47.1 \pm 7.1$  (range 35-64). The phenotypes 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). Statistical analysis of the frequency of hyperlipidemia was done by  $\chi^2$ -analysis. Statistical analysis of the serum lipid concentration was done by a one-way analysis of *t* test.

#### RESULTS

In the 85 males examined, 62 males were the apo E3/3 phenotype, 18 males were the apo E3/4 phenotype, two males were the apo E4/4 phenotype, two males were the apo E2/3 phenotype, and one male was the apo E2/5 phenotype. All the subjects had normal thyroid, liver, and kidney function. Four apo E3/3 and one apo E3/4 phenotype subjects were found to have diabetes mellitus. Since serum total cholesterol and triglyceride concentrations were not substantially influenced by age in this study, serum lipid levels were not adjusted for age. Table 1 presents frequencies of hyperlipidemia (serum total cholesterol > 250 mg/dl and/or serum total triglyceride > 160 mg/dl) in 62 subjects with the apo E3/3 phenotype and in 20 subjects with the apo E4-positive phenotype (apo E3/4 and apo E4/4). The

Table 1. Frequencies of hyperlipidemia in subjects with the apo E3/3 phenotype and apo E4-positive phenotype.

	Apo E3/3 (n=62)	Apo E4-positive (E3/4 & E4/4) (n=20)
Hypercholesterolemia <sup>a</sup> without hypertriglyceridemia	2 (3.2%)	2 (10.0%)
Hypercholesterolemia and hypertriglyceridemia	2 (3.2%)	2 (10.0%)
Hypertriglyceridemia <sup>b</sup> without hypercholesterolemia	12 (19.4%)	6 (30.0%)
Total	16 (25.8%)	10 (50.0%)*

<sup>a</sup> Hypercholesterolemia: serum total cholesterol > 250 mg/dl. <sup>b</sup> Hypertriglyceridemia: serum total triglyceride > 160 mg/dl. \*  $\chi^2=4.09$ ,  $p<0.05$ .

Table 2. Mean serum total cholesterol and triglyceride levels in subjects with the apo E3/3 phenotype and apo E4-positive phenotype.

	Apo E3/3	Apo E4-positive (E3/4 & E4/4)
Serum cholesterol	201.0 ± 30.1 (n=62)	209.7 ± 37.2 (n=20)
Serum triglyceride	108.3 ± 43.1 (n=58)	132.8 ± 51.1* (n=18)

The values are mean concentrations ± SD (mg/dl). Mean triglyceride concentrations were calculated on the subjects with serum triglyceride lower than 250 mg/dl. \*  $t=1.836$ ,  $p<0.05$ .

mean (±SD) age was 47.1 ± 7.5 (range 36–63) in the apo E3/3 phenotype group and 48.4 ± 8.7 (range 35–64) in the apo E4-positive phenotype group. In the 62 subjects with the apo E3/3 phenotype, 16 subjects (25.8%) had hypercholesterolemia or hypertriglyceridemia or both. In the case of 20 subjects with the apo E4-positive phenotype, however, 10 subjects (50.0%) had hyperlipidemia. The difference is statistically significant ( $\chi^2=4.09$ ;  $p<0.05$ ).

The mean (±SD) serum total cholesterol levels were 201.0 ± 30.1 mg/dl (range 139–285) in the 62 apo E3/3 phenotype subjects and 209.7 ± 37.1 mg/dl (range 138–271) in the 20 apo E4-positive subjects (Table 2). The difference is not significant, probably because of a small sample size. The mean (±SD) serum total triglyceride levels were 125.6 ± 79.5 (range 46–422) in the apo E3/3 phenotype group and 154.8 ± 83.1 (range 66–354) in the apo E4-positive phenotype group. Since a few subjects with serum triglyceride greater than 250 mg/dl raised the mean triglyceride levels by about 20 mg/dl in both groups and since the values for triglyceride of these subjects were distributed apart from the values for triglyceride of the other subjects, we excluded these subjects from the analysis of mean triglyceride levels. The mean (±SD) triglyceride levels, which were calculated on the subjects with serum triglyc-

eride lower than 250 mg/dl, were  $108.4 \pm 43.0$  in the apo E3/3 phenotype group and  $132.8 \pm 51.1$  in the apo E4-positive phenotype group (Table 2). The difference is statistically significant ( $t=1.836$ ;  $p<0.05$ ).

All the four diabetic males with the apo E3/3 phenotype were normolipidemic; the one diabetic male with the apo E3/4 phenotype was 42 years old and hypertriglyceridemic (serum total triglyceride 354 mg/dl). Two subjects with the apo E2/3 phenotype were normolipidemic. The one subject with the apo E2/5 phenotype had a mild degree of hypertriglyceridemia, as described elsewhere (Tsuchiya *et al.*, 1985).

#### DISCUSSION

Among the three common apo E alleles, only the  $\epsilon 3$  allele has yet to be associated with any of the lipid disorders. It is well established that the  $\epsilon 2$  allele, especially the homozygote for  $\epsilon 2$ , is associated with type III hyperlipidemia (Breslow *et al.*, 1982; Ghiselli *et al.*, 1982a; Havel, 1982). As to the  $\epsilon 4$  allele, Ghiselli *et al.* (1982a, 1982b) reported an association with type V hyperlipoproteinemia and Utermann *et al.* (1984) observed an association with hypercholesterolemia and mixed hyperlipidemia. Furthermore, it is suggested that the  $\epsilon 4$  allele raises plasma total cholesterol in normolipidemic individuals (Sing and Davignon, 1985). Since the  $\epsilon 4$  allele is considered to act as a polymeric gene in predisposing one to hyperlipidemia, if the  $\epsilon 4$  allele is associated with hyperlipidemia, and since a diet of a population has a considerable effect on serum lipid levels of the population, the results obtained in Caucasian populations may not be applied to Japanese. Therefore, we have examined serum lipid levels of apparently healthy Japanese civil servants working in Tokyo, in connection with the  $\epsilon 4$  allele. In the present study, significantly higher frequency of hyperlipidemia and mean serum triglyceride level were observed in the subjects with the apo E4-positive phenotype, as compared to the subject with the apo E3/3 phenotype. The results suggest that the  $\epsilon 4$  allele might predispose Japanese adults in large cities to hyperlipidemia, too.

Our results are consistent with the concept that the  $\epsilon 4$  allele predisposes one to the development of hyperlipidemia. At present, however, it is difficult to explain the mechanism by which the  $\epsilon 4$  allele predisposes one to hyperlipidemia. Apo E4 differs from apo E3 by an amino acid substitution at residue 112 on the 299 amino acid chain of the apo E molecule: Apo E3 has cysteine at site 112, while apo E4 has arginine (Rall *et al.*, 1982a, 1982b; Weisgraber *et al.*, 1982). However, no difference has been observed in the binding activity to apo B,E receptor between apo E3 and apo E4 (Weisgraber *et al.*, 1982). As to the binding activity to apo E receptor and the function for the conversion of VLDL to LDL, it is not determined whether there are some differences between apo E3 and apo E4. In addition, the type of hyperlipidemia associated with the  $\epsilon 4$  allele is not established yet. Further biochemical, genetic, clinical, and epidemiological investigations are necessary to establish and characterize the association of the  $\epsilon 4$  allele and hyperlipoproteinemia.

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