

FREQUENCIES OF APOLIPOPROTEINS E5 AND E7 IN APPARENTLY HEALTHY JAPANESE

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Summary Human apolipoprotein (apo) E plays an important role in the metabolism of cholesterol and other lipids. Apo E5 and apo E7 are genetic variants of apo E and have been detected in about 5% of Japanese patients with hyperlipidemia and ischemic heart disease. The existence of apo E5 and apo E7, however, had not been reported in apparently healthy individuals except for a few family members of the patients with apo E5 or apo E7. It has been suggested that apo E5 and apo E7 are closely related to the development of atherosclerosis. The purpose of this study is to investigate the frequency of apo E5 and apo E7 in apparently healthy Japanese and to analyze serum lipid levels of the individuals with apo E5 or apo E7.

The apo E phenotypes of 197 apparently healthy Japanese adults were determined by two-dimensional gel electrophoresis. The gene frequencies of apo E were: $\epsilon 3$, 0.843; $\epsilon 4$, 0.112; $\epsilon 2$, 0.038; $\epsilon 5$, 0.006; $\epsilon 7$, 0.0035. Three out of 187 subjects (1.5%) were found to have apo E5 or apo E7 in heterozygous state. Two of them were heterozygous with apo E3 (apo E3/5 and apo E3/7) and the both had normal serum lipid levels, though they were more than 50 years old. The other individual was heterozygous with apo E2 (apo E2/5) and had mild hypertriglyceridemia. As to myocardial infarction, angina pectoris and cerebral infarction, no clinically abnormal findings were detected in all the three individuals with apo E5 or apo E7.

The data suggest that the frequency of the individuals with apo E5 or apo E7 is of the order of 1% and much higher than that of the homozygotes with apo E2/2 in Japanese. The data also indicate that further genetic, epidemiologic and clinical studies are required to determine whether $\epsilon 5$ and $\epsilon 7$ act as a dominant major gene, as a recessive major gene, or as

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one of polymeric genes, in predisposing one to hyperlipidemia and atherosclerosis.

INTRODUCTION

Apolipoprotein (Apo) E is a major protein constituent of human plasma very low density lipoproteins (VLDL) (Shelburne and Quarfordt, 1974). It is also a normal constituent of chylomicrons and a subfraction of the high density lipoproteins (HDL) (Weisgraber and Mahley, 1978; Mahley and Innerarity, 1983). Human apo E is a single chain polypeptide (Mr 34,000) of 299 amino acids and its complete amino acid sequences are known (Rall *et al.*, 1982b). The locus for human apo E is closely linked to the loci for apo CII and the Lutheran blood group on chromosome 19 (Myklebost *et al.*, 1984; Gedde-Dahl *et al.*, 1984). Recently, the complete nucleotide sequence of the human apo E gene has been determined (Paik *et al.*, 1985). The major function of apo E is its mediation of the cellular uptake of specific plasma lipoproteins through an interaction with apo B,E (LDL) receptor on extrahepatic and hepatic cell surface and with distinct hepatic apo E receptor (Mahley and Innerarity, 1983). In addition, apo E is involved in the normal conversion of VLDL to the low density lipoproteins (LDL) (Ehnholm *et al.*, 1984). Thus, apo E plays an important role in the metabolism of cholesterol and other lipids.

In man, the locus for apo E is polymorphic (Utermann *et al.*, 1977; Zannis *et al.*, 1981; Zannis *et al.*, 1982). Three common alleles, designated ϵ_2 , ϵ_3 and ϵ_4 , have been demonstrated to exist in various populations (Utermann *et al.*, 1982; Wardell *et al.*, 1982; Ghiselli *et al.*, 1982; Cumming and Robertson, 1984; Asakawa *et al.*, 1985; Sing and Davignon, 1985). The three common alleles, ϵ_2 , ϵ_3 and ϵ_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.*, 1981; Zannis *et al.*, 1982; Utermann *et al.*, 1982). Furthermore, rare mutant genes have been described (Chiselli *et al.*, 1982; Weisgraber *et al.*, 1984; Gregg *et al.*, 1983; Yamamura *et al.*, 1984a; Yamamura *et al.*, 1984b). Besides an association of apo E2 with type III hyperlipidemia (Havel, 1982), most of apo E isoforms encoded by these rare mutant genes have been reported to be associated with hyperlipidemia or atherosclerotic diseases or both. They include apo E Bethesda (apo E1) detected in a Caucasian family and apo E5 and apo E *suita* (apo E7) found in Japanese families (Gregg *et al.*, 1983; Yamamura *et al.*, 1984a; Yamamura *et al.*, 1984b). Apo E5 and apo E7 have been thus far detected only in Japanese patients with myocardial infarction and hyperlipidemia and in their family members (Yamamura *et al.*, 1984b).

The main purpose of this study is to investigate the frequency of apo E5 and apo E7 in apparently healthy Japanese. Our data suggest that the frequency of individuals with apo E5 or apo E7 is of the order of 1% and much higher than that of homozygotes with apo E2/2 in Japanese.

MATERIALS AND METHODS

Subjects and samples. We studied 197 apparently healthy Japanese: 85 males and 8 females aged 35–64 years who visited the health care center of Kudanzaka hospital in Tokyo; 53 males aged 40–57 years who visited the health care center of Tsuchiura-kyodo hospital in Tsuchiura city; 51 staffs (30 males and 21 females), aged 22–43 years, of University of Tsukuba. Blood samples were taken after an overnight fast.

Laboratory procedures. Ten μl of VLDL-rich fraction was separated from 175 μl of serum by ultracentrifugation for 3 hr at $160,000 \times g$ using Beckmann Airfuge Ultracentrifuge (Beckman Instruments, Inc., Irvine, California). To determine the phenotype of apo E, 10 μl of VLDL-rich fraction was subjected to two-dimensional (two-D) gel electrophoresis. Two-D gel electrophoresis was performed essentially according to the method of O'Farrell (1977) as previously described (Hamaguchi *et al.*, 1982) with minor modifications: Ampholine mixtures of 1.4% pH range 5–8 and 0.6% pH range 3–10 were used in the first dimensional disc gel. After two-D gel electrophoresis, polypeptides were visualized on slab gels by the silver stain technique of Merril *et al.* (1981). The apo E spots on slab gels were identified by the method of immuno-blotting (Towbin *et al.*, 1979), using goat anti-human apo E (Daiichi Pure Chemicals Ltd., Tokyo) and fluorescein-conjugated rabbit anti-goat IgG (Cappel Laboratories Inc., Malvern, Pennsylvania). Serum total cholesterol and triglyceride were measured enzymatically (Allain *et al.*, 1974; Sampson *et al.*, 1975): The samples from Kudanzaka hospital were analyzed on an automated analyzer (Hitachi Model 712, Hitachi Ltd., Japan) and the other samples manually using commercial kits (Cholesterol E-Test and Triglyceride CII-Test, Wako Pure Ltd., Tokyo).

RESULTS

Figure 1 shows the two-D gel electrophoresis pattern of the three most common apo E phenotypes, apo E3/3, apo E3/4 and apo E2/3. In this survey, apo E5 was found in heterozygous state with apo E2 or apo E3 in two males. Figure 2 shows the two-D gel electrophoresis pattern of apo E 2/5 phenotype, together with the data on the identification of the apo E spot on the gel by immuno-blotting. In addition to apo E5, apo E7 was also found in heterozygous state with apo E3 in one male. The two-D gel electrophoresis pattern of apo E3/7 phenotype is shown in Fig. 3. The apo E7 spot on the slab gel was also identified as apo E by immuno-blotting (data not shown). No apo E1 was found.

Table 1 presents the distribution of apo E phenotypes in 197 subjects. The gene frequencies of apo E are: ϵ_3 , 0.843; ϵ_4 , 0.112; ϵ_2 , 0.038; ϵ_5 , 0.005; ϵ_7 , 0.0035. It can be seen that the observed numbers are close to those calculated on the assump-

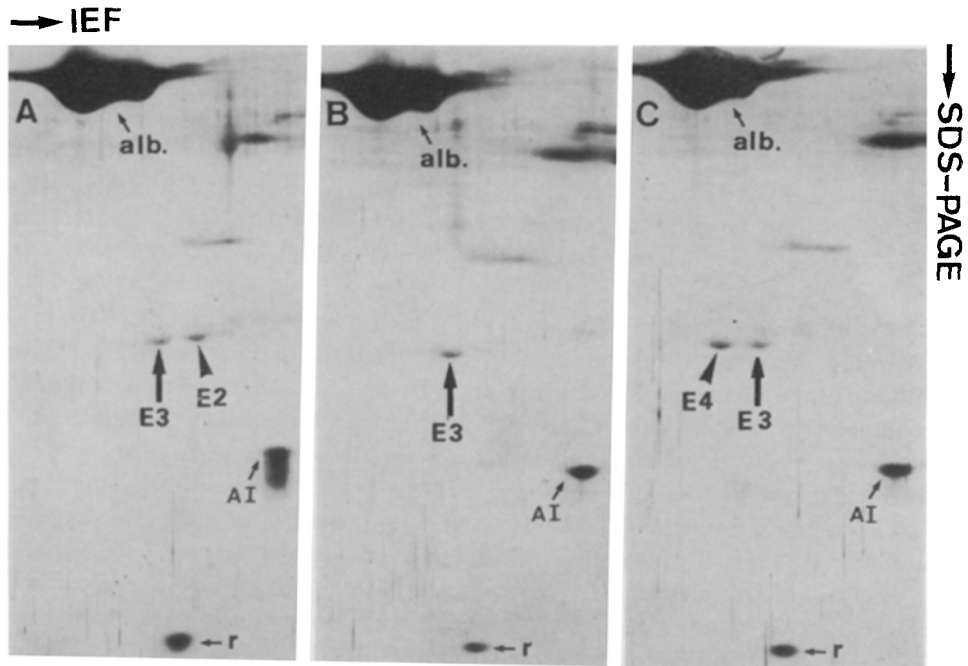


Fig. 1. Two-D gel electrophoresis patterns of the three most common apo E phenotypes. A, apo E2/3; B, apo E3/3; C, apo E3/4. Apo E2, apo E3, and apo E4 are indicated by E2, E3, and E4, respectively. Albumin and apolipoprotein A-I are shown by alb and AI, respectively. The polypeptide indicated by m is a useful marker for the determination of apo E phenotypes. Isoelectric focusing was from left to right and molecular weight separation from top to bottom.

tion that Hardy-Weinberg equilibrium is observed. The frequencies of the three common alleles, $\epsilon 2$, $\epsilon 3$ and $\epsilon 4$, are similar to those observed in Hiroshima (Asakawa *et al.*, 1985). However, it is noteworthy that three out of 187 subjects (1.5%) were found to have apo E5 or apo E7 in heterozygous state.

Table 2 shows the serum total cholesterol and triglyceride levels of the three subjects with apo E5 or apo E7. In two individuals with apo E3/5 and apo E3/7, the serum lipid levels were normal. On the other hand, one individual with apo E2/5 had mild hypertriglyceridemia.

DISCUSSION

Apo E5 and apo E7 have been detected in about 5% of Japanese patients with hyperlipidemia and ischemic heart disease, while they were not found among 100 healthy Japanese (Yamamura *et al.*, 1984a; Yamamura *et al.*, 1984b). We also found four cases with apo E5 or apo E7 (4.3%) out of 92 patients with myocardial

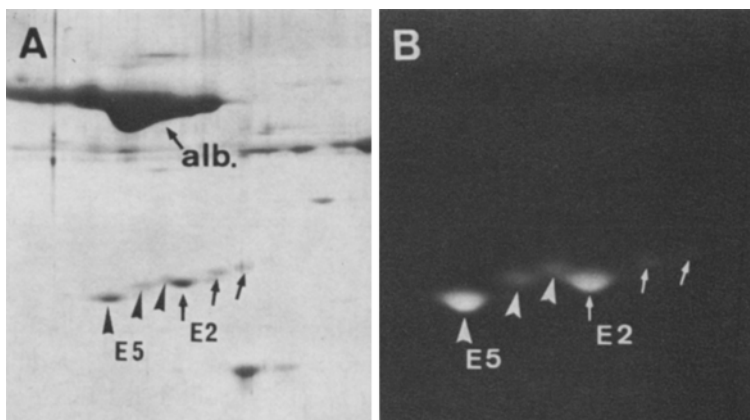


Fig. 2. Two-D gel electrophoresis pattern of apo E2/5. A, silver staining; B, immunoblotting. Apo E2 and apo E5 are indicated by E2 and E5, respectively. Albumin is shown by alb.

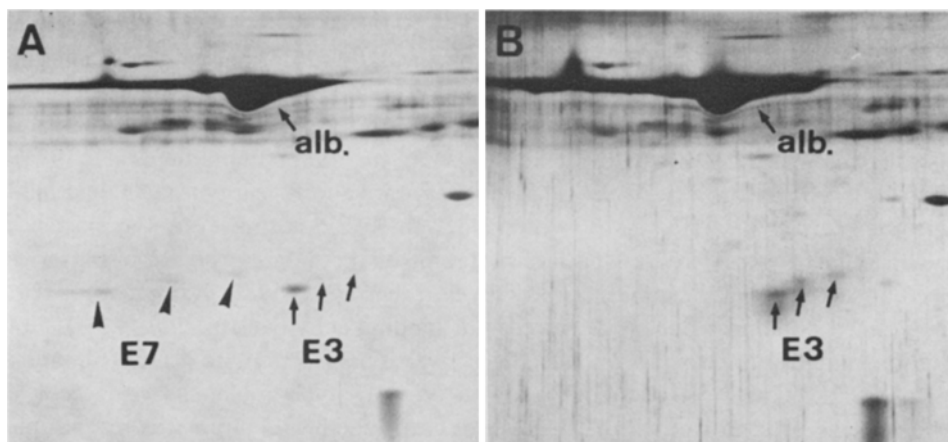


Fig. 3. Two-D gel electrophoresis pattern of apo E3/7 (A) and apo E3/3 (B). Apo E3 and apo E7 are indicated by E3 and E7, respectively. Albumin is shown by alb.

Table 1. Apo E phenotypes among 197 apparently healthy adults.

	Phenotypes							
	3/3	3/4	2/3	4/4	2/4	3/5	2/5	3/7
Observed	141	37	11	2	3	1	1	1
Expected	139.9	37.1	12.6	2.4	1.6	1.6	0.07	0.9

Expected values calculated from gene frequencies: $\epsilon_3=0.843$, $\epsilon_4=0.111$, $\epsilon_2=0.038$, $\epsilon_5=0.005$, and $\epsilon_7=0.0025$.

Table 2. Serum lipid values in subjects with apo E5 or apo E7.

Subject	Age (years)	Sex	Apo E phenotypes	Cholesterol (mg/dl)	Triglyceride (mg/dl)
HS-T11	60	M	3/5	174	116
HS-T32	51	M	3/7	231	154
HS-K64	36	M	2/5	178	188 ↑

Arrow indicates an increase in lipid value.

infarction (Hamaguchi *et al.*, in preparation). Yamamura *et al.* (1984b) observed that five of seven patients with apo E5 or apo E7 had overt atherosclerotic disease. Based on their observations, Yamamura *et al.* (1984b) suggested that apo E5 and apo E7 are closely related to the development of atherosclerosis. In the present study, apo E5 and apo E7 were detected for the first time in apparently healthy subjects, with a frequency of 1.5%. According to our data, the expected frequency of homozygotes with apo E2/2 is 0.14%. Our data suggest that the frequency of individuals with apo E5 or apo E7 is of the order of 1% and much higher than that of homozygotes with apo E2/2 in Japanese.

Two of three individuals with apo E5 or apo E7 had normal values for plasma lipid levels, though they both were more than 50 years old. They are heterozygous with apo E3. Apo E3 has normal function in both receptor binding activities and the conversion of VLDL to LDL (Weisgraber *et al.*, 1982; Ehnholm *et al.*, 1984). On the other hand, the other individual with very rare apo E genotype, $\epsilon 2/5$, had mild hypertriglyceridemia. He was 36 years old. In this case, however, the existence of $\epsilon 2$ should be considered in the interpretation of the data. Apo E2 displays reduced apo B,E receptor binding activity (Weisgraber *et al.*, 1982; Rall *et al.*, 1982a; Rall *et al.*, 1983) and reduced apo E receptor binding activity (Hui *et al.*, 1984). In addition, apo E2 appears to impede the conversion of VLDL to LDL (Ehnholm *et al.*, 1984). Usually $\epsilon 2$ is associated with type III hyperlipidemia as a recessive gene. In the present case with $\epsilon 2/5$, it is not clear whether $\epsilon 5$ alone was responsible for hypertriglyceridemia or the combination of $\epsilon 5$ with $\epsilon 2$ was important as the etiology of hyperlipidemia. As to myocardial infarction, angina pectoris and cerebral infarction, no clinically abnormal findings were detected in all the three individuals with apo E5 or apo E7. Further genetic, epidemiologic and clinical studies as well as the analysis of functions are required to determine whether $\epsilon 5$ and $\epsilon 7$ act as a dominant major gene, as a recessive major gene, or as one of polymeric genes, in predisposing one to hyperlipidemia and atherosclerosis.

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