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PLASMA APOLIPOPROTEIN H (β_2-GLYCOPROTEIN I) PHENOTYPE FREQUENCIES IN A JAPANESE POPULATION

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Summary We determined plasma apolipoprotein H (β_2-glycoprotein I) levels in 300 healthy adult individuals and evaluated the frequencies of the Bg^N and Bg^D alleles in a Japanese population. These results were then compared with the previous reports. The plasma apo H levels in the subjects showed bimodal distribution: 274 subjects were in the range 15.6–33.2 mg/dl and were considered to be homozygous for Bg^N (phenotype NN), and 26 subjects were found in the range 9.6–14.8 mg/dl and were presumably heterozygous for Bg^N and Bg^D (phenotype ND). In this study, no sample below 5 mg/dl (phenotype DD) was found. Mean plasma apo H levels in NN and ND groups were 22.1 ± 1.6 mg/dl and 12.5 ± 1.6 mg/dl, respectively. The gene frequencies of Bg^N and Bg^D in a Japanese population were 0.957 and 0.043, respectively. These results were similar to gene frequencies of Bg^N and Bg^D in Caucasoids.

Key Words apolipoprotein H, β_2-glycoprotein I, population study, gene frequencies

INTRODUCTION

β_2-Glycoprotein I was first demonstrated as a component of human serum by Schultze *et al.* (1961) and has been studied in detail. As to the function, Polz *et al.* (1979) showed that in man about 30% of β_2-glycoprotein I was associated with the serum lipoproteins. Nakaya *et al.* (1980) demonstrated β_2-glycoprotein I activation of lipoprotein lipase and designated this glycoprotein as apolipoprotein H (apo H). Lozier *et al.* (1984) reported that apo H consisted of 326 amino acids and had five attached glucosamine-containing oligosaccharides. Hoeg *et al.* (1985) investigated the plasma lipid and lipoprotein levels in patients with apo H deficiency

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and demonstrated that plasma apolipoprotein C-II levels were elevated in these patients, approximately three times as much as those in healthy individuals in spite of normotriglyceridemia. However, the precise metabolic function of apo H is yet unknown.

As for serum concentration of this glycoprotein, Cleve (1968) showed that serum apo H level is controlled by a pair of autosomal co-dominant alleles, for which the notations Bg^N and Bg^D were given: Individuals homozygous for Bg^N (phenotype NN) were found to have apo H levels between 16 and 35 mg/dl, those heterozygous for Bg^N and Bg^D (phenotype ND) had levels between 6 and 14 mg/dl, and those homozygous for Bg^D (phenotype DD) had levels below 5 mg/dl. The frequencies of phenotypes and the Bg^N and Bg^D alleles were evaluated in various populations (Cleve, 1968; Koppe *et al.*, 1970; Atkin and Rundle, 1974; Rahimi *et al.*, 1977; Walter *et al.*, 1979; Papiha, 1982). In these studies, Koppe *et al.* (1970) and Walter *et al.* (1979) demonstrated the possibility of racial differences in the distribution of the *Bg* alleles: All samples of Caucasoids had approximately similar phenotype and gene frequencies, but the samples of Mongoloids and Negroids had lower Bg^N and higher Bg^D frequencies than those of Caucasoids. Since then, as far as we know, no population study of Mongoloids, including a Japanese population, has been carried out.

We determined plasma apo H levels and evaluated the frequencies of the Bg^N and Bg^D alleles in a Japanese population. These results were then compared with the previous reports.

MATERIALS AND METHODS

Overnight fasting plasma was collected from 300 unrelated healthy adult individuals (151 males, 149 females; age 46.2 ± 9.0 y.o.) during a medical examination of workers, living in Kochi Prefecture, southwestern Japan. They were fully active and had normal medical examination results, which involved routine biochemical examinations of urine and blood, electrocardiogram and chest roentogenogram.

Determination of plasma apo H was made by the single radial immunodiffusion (Takamatsu *et al.*, 1987). Briefly, the isolation of apo H from pooled human serum was performed based on the report by Polz *et al.* (1981). Subsequently, a rabbit was immunized with pure apo H and anti-human apo H-rabbit serum was gained. Warm 1% agarose gel, containing 4% of this anti-human apo H-rabbit serum, was poured on a glass plate to give a 2 mm thick layer. After gelation, circular wells with a diameter of 2.5 mm were punched out. Exactly 10 μl of plasma diluted with 0.01 M phosphate buffered saline (pH 7.4) 1:2, were applied into each of the wells by a micropipette. After the plate was incubated for 72 hr at room temperature, the diameters of visible rings of precipitate around the well were measured. In order to estimate these levels, standard curves were set up by the application of stabilized human sera, which contains a known quantity of apo H. We employed standard human sera from the Behring (Lot No. 041017), containing an apo H

level of 25 mg/dl. Undiluted serum, 1:2 and 1:4 dilutions were used to get these standard curves. We confirmed good reproducibility (coefficient variability in between and within assays were 1.2 and 0.8%, respectively) and no significant difference of apo H levels in plasma and serum with this method.

RESULTS AND DISCUSSION

The distribution of the plasma apo H levels in the 300 subjects is shown in Fig. 1. Bimodal distribution without overlapping was found: The values for 274 subjects were in the range 15.6–33.2 mg/dl and were considered to be homozygous for Bg^N; and the values for 26 subjects were found in the range 9.6–14.8 mg/dl and were presumably heterozygous for Bg^N and Bg^D. In this study, no sample below 5 mg/dl was found. This distribution is the similar to results in most of the previous reports (Cleve, 1968; Koppe *et al.*, 1970; Atkin and Rundle, 1974; Walter *et al.*, 1979; Papiha, 1982). Mean plasma apo H levels in NN and ND groups were 22.1 ± 1.6 and 12.5 ± 1.6 mg/dl, respectively.

Using the method of maximum likelihood, the gene frequencies of Bg^N and Bg^D in a Japanese population were 0.957 and 0.043 (standard deviation 0.008), respectively, and the distribution did not differ significantly from the Hardy-Weinberg law ($\chi^2=0.616$, f=1, $0.50>p>0.30$) (Table 1). The gene frequencies of Bg^N and

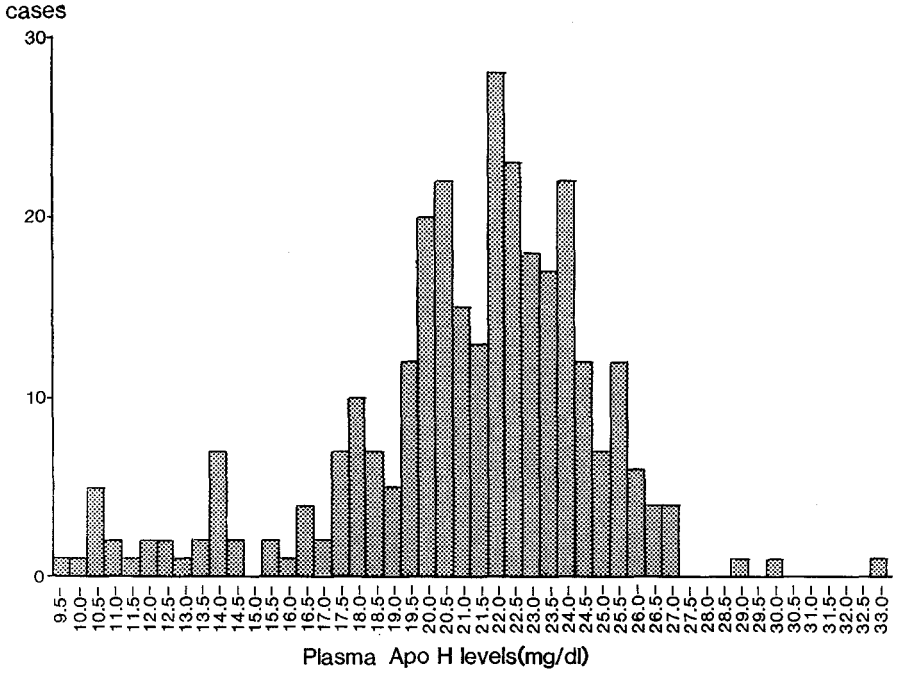

Fig. 1. Distribution of plasma apo H levels in healthy adult Japanese individuals.

Table 1 Gene frequencies of apo H in various populations.

Population	n	Bg^N	Bg^D	χ^2	p	Authors
Icelanders	97	0.938	0.062	0.420	0.70>p>0.50	Koppe *et al.* (1970)
Irish	107	0.948	0.052	0.309	0.70>p>0.50	Koppe *et al.* (1970)
Germans (Mainz)	210	0.953	0.047	0.542	0.50>p>0.30	Koppe *et al.* (1970
Germans (Mainz)	152	0.951	0.049	0.416	0.70>p>0.50	Walter *et al.* (1979)
Germans (Mainz)	150	0.947	0.053	0.482	0.50>p>0.30	Walter *et al.* (1979)
Germans (Marburg)	260	0.969	0.031	0.259	0.70>p>0.50	Cleve (1968)
English	381	0.941	0.059	0.089	0.90>p>0.70	Atkin and Rundle (1974)
Hungarians (Bodrogköz)	151	0.940	0.060	0.600	0.50>p>0.30	Koppe *et al.* (1970)
Greek (Creta)	157	0.924	0.076	0.009	0.95>p>0.90	Koppe *et al.* (1970)
Iranians	141	0.886	0.114	3.326	0.10>p>0.05	Koppe *et al.* (1970)
Afghanistans	816	0.869	0.131	2.389	0.20>p>0.10	Rahimi *et al.* (1977)
Indians (Kumaon)	108	0.954	0.046	0.258	0.70>p>0.50	Koppe *et al.* (1970)
Indians (Himachali)	473	0.987	0.013	0.078	0.80>p>0.70	Papiha (1982)
Pakistanis	79	0.949	0.051	0.222	0.70>p>0.50	Koppe *et al.* (1970)
Koreans	105	0.780	0.220	1.342	0.30>p>0.20	Koppe *et al.* (1970)
Philippinos	88	0.937	0.063	0.386	0.70>p>0.50	Walter *et al.* (1979)
Negroes (Mozambique)	151	0.742	0.258	9.042	0.01>p>0.001	Koppe *et al.* (1970)
Negroes (S. Africa)	250	0.950	0.050	0.693	0.50>p>0.30	Walter *et al.* (1979)
Negroes (S. Africa)	192	0.800	0.200	12.126	p<0.001	Walter *et al.* (1979)
Japanese (Kochi)	300	0.957	0.043	0.616	0.50>p>0.30	this investigation

Bg^D in various populations are demonstrated in Table 1. In these investigations, Koppe *et al.* (1970) and Walter *et al.* (1979) reported the possibility of racial differences in the distribution of the *Bg* alleles: All samples of Caucasoids had approximately similar gene frequencies but the sample of Negroids had lower Bg^N and higher Bg^D frequencies. However, the gene frequencies of Bg^N and Bg^D among Negroids in these reports were not always similar. Since the distribution of the two alleles differed significantly from the Hardy-Weinberg equilibrium in two of the three studies (Table 1), more data on Negroids are necessary to clarify the real gene distribution. As for gene frequencies in Mongoloids, Koppe *et al.* (1970)

reported that Koreans had lower Bg^N and higher Bg^D than those of Caucasoids. The sample size analyzed, however, was small. Until now, no other investigation of Mongoloids, including Japanese populations, had been carried out. We first demonstrated the gene frequencies of Bg^N and Bg^D in a Japanese population. This result was similar to the result in Caucasoids but differed from the result in Koreans. In order to clarify whether significant differences in gene frequencies of *Bg* alleles exists among Mongoloids, more population studies are required.

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