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DISTRIBUTION OF α_1-ANTITRYPSIN PHENOTYPES IN JAPANESE: DESCRIPTION OF Pi M SUBTYPES BY ISOELECTRIC FOCUSING

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Summary Serum α_1-antitrypsin phenotypes were determined by polyacrylamide gel slab isoelectric focusing in a sample from 1,271 unrelated healthy Japanese living in Tokyo. Three common Pi M subtypes (M_1M_1, M_1M_2 and M_2M_2) were found, and the allele frequencies estimated ($Pi^{M1}=0.83$, $Pi^{M2}=0.17$) were found to be similar to those observed in the European populations. A large χ^2 value, however, may indicate the presence of further microheterogeneity among Pi M subtypes. In contrast with Pi M subtypes, rare variants were found only in 0.55% of the samples examined, and no Z gene was detected. A new fast variant of α_1-antitrypsin named Pi E_{Tokyo} is reported.

INTRODUCTION

α_1-Antitrypsin which inhibits a variety of proteases is known to be a polymorphic serum protein with a high degree of genetically determined heterogeneity. A severe deficient level of α_1-antitrypsin in serum has been found to be associated with pulmonary emphysema (Laurell and Eriksson, 1963) and liver cirrhosis (Sharp *et al.*, 1969).

Fagerhol and Braend (1965) first described genetic variants of this protein using acid starch gel electrophoresis. More than 24 alleles have so far been identified. Aside from the interest in the association between α_1-antitrypsin levels and diseases, a number of studies have been carried out in various populations, since the frequencies of the various Pi alleles show geographical differences. Fagerhol (1976) tabulated the incidences of major α_1-antitrypsin variants determined by the acid starch gel electrophoresis in several populations and noted a low incidence of non-M alleles in east Asia and India.

Recently, a new method of isoelectric focusing for α_1-antitrypsin phenotype determination has been developed, which gains an advantage over the acid starch

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gel electrophoresis (Arnaud and Creyssel, 1975; van den Broek *et al.*, 1976). Small charge differences of proteins which are not separated by electrophoretic techniques can be detected by isoelectric focusing in a narrow pH gradient.

Thus, the common Pi phenotype MM can be classified into three subtypes, namely, M_1M_1, M_1M_2, M_2M_2 by this method (Frants and Eriksson, 1976). Harada *et al.* (1977) using the same method recently reported the distribution of Pi phenotypes in Japanese. However, since development of the improved conditions in the isoelectric focusing and the more cautious examination of Pi patterns have resulted in a better resolution of Pi phenotypes, we decided to reexamine the distribution of Pi phenotypes and frequencies of the common and rare Pi variants in the Japanese population using an independent sample.

MATERIALS AND METHODS

Sera from 1,271 unrelated, apparently healthy adult Japanese living in Tokyo were examined. Samples were stored at −20°C for up to 5 months until tested. Family studies were performed in two cases, Pi E_{Tokyo} M_1 and Pi M_2P. The variants were reconfirmed on the fresh samples taken several times from the same persons, and compared with the Pi control sera which were kindly provided by Dr. M. K. Fagerhol, Oslo. Some of them (Pi E_{Tokyo} M_1, Pi IM_1, Pi M_1N, Pi M_2P) were sent to the independent laboratories to have them checked according to the suggestion of Dr. M. K. Fagerhol (personal communication, 1976). The nomenclature used for Pi M subtypes was according to Frants and Eriksson (1976) and the International Pi Committee (Dr. A. M. Johnson, personal communication, 1977).

The slab gel (16 × 10 × 0.1 cm) was prepared by mixing 4 ml acrylamide solution (29.1 g/100 ml), 4 ml N,N′-methylenebisacrylamide solution (0.9 g/100 ml), 8 ml 20% sucrose solution containing 16 mg ammonium persulfate, 1.6 ml Ampholine, pH 4–6 (LKB). The gel was polymerized within 20 min at room temperature. Serum samples were applied on the cathodic side of the gel using 5 × 4 mm filter paper pieces (Whatman 3MM). Paper strips saturated with 0.5% ethylenediamine and 0.5% phosphoric acid were used as the cathode and the anode, respectively. Isoelectric focusing was carried out under the cooling system at 5°C for 6 h with increasing voltage settings from an initial 300 to 1,000 V in 2.5 hr and the final voltage was maintained for 3.5 hr. After completion of focusing, the gel was placed in 200 ml of staining solution (Coomassie Brilliant Blue 0.75 g, methanol 225 ml, water 465 ml, sulphosalicylic acid 22.5 g, trichloroacetic acid 75.0 g) for 60 min at room temperature. The stain was removed and the gel destained in several changes of destaining solution (water 1,950 ml, ethanol 750 ml, acetic acid 240 ml).

Initial localization and identification of α_1-antitrypsin bands were accomplished by antigen-antibody crossed electrophoresis after isoelectric focusing and by immunofixation print technique on cellulose acetate strips (Arnaud *et al.*, 1977). Specific antiserum against α_1-antitrypsin was obtained from Behringwerke (Marburg).

Table 1. Distribution of phenotypes of Pi M subtypes and rare variants in Japanese.

Total	M subtypes					Gene frequencies
		M_1	M_1M_2	M_2	Total	
1,271	Obs. No.	886	316	62	1,264	$Pi^{M1}=0.83$
	%	70.1	25.0	4.9	100	
	Exp. No.	862.2	363.5	38.3	1,264.0	$Pi^{M2}=0.17$
	$\chi^2=21.5$ df=1			p<0.001		
	Rare variants					
	E_{Tokyo} M_1	IM_1	M_1N	M_1S	M_2P	Total
	2	1	2	1	1	7 (0.55%)

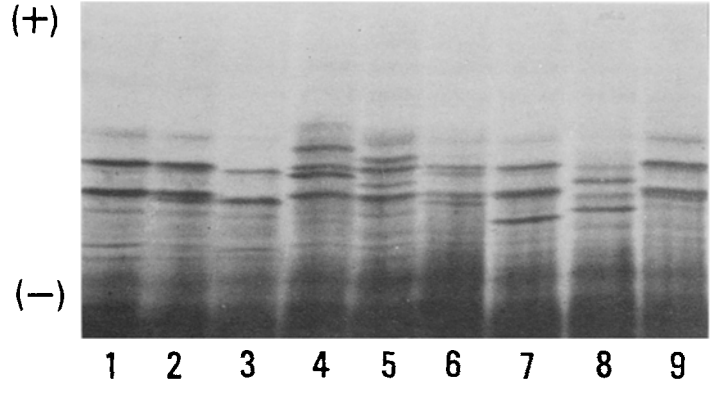

Fig. 1. Photograph showing various Pi patterns detected by isoelectric focusing. 1: M_1, 2,9: M_1M_2, 3: M_2, 4: E_{Tokyo} M_1, 5: IM_1, 6: M_1N, 7: M_1S, 8: M_2P.

The α_1-antitrypsin concentration of samples in family studies was examined by single radial immunodiffusion (M-Partigen, Behringwerke, Marburg). The normal range of serum α_1-antitrypsin in our sample was 200–350 mg/100 ml.

RESULTS AND DISCUSSION

We previously reported the distribution of Pi phenotypes detected by isoelectric focusing in Japanese (Harada *et al.*, 1977). However, we found upon careful observation of Pi patterns that a modification of the method by using extended focusing time produced an essentially increased resolution of the phenotypes. The α_1-antitrypsin bands on the gel, minor bands (M_7 and M_8) as well as main bands (M_4 and M_6), are definitely sharper than in the previous examination, resulting in the discrimination for Pi subtyping. The bands of Pi M_1M_2 are well separated each other, and this is more apparent in the region of M_6 and M_8 than M_4 and M_7.

In Table 1 is given the distribution of different Pi phenotypes and alleles obtained on the 1,271 sera of Japanese. Figure 1 shows the patterns of various Pi phenotypes detected in this investigation. 99.5% of Japanese tested is so-called Pi MM type in agreement with the previous reports which were based on acid starch gel electrophoresis (Harada and Omoto, 1970; Roberts *et al.*, 1977). The three Pi M subtypes, manely, M_1M_1, M_1M_2, M_2M_2, were detected in 70.1, 25,0, 4.9%, respectively of 1,264 samples, excluding those of rare variants. The gene frequencies of the subtypes ($Pi^{M1}=0.83$, $Pi^{M2}=0.17$) are close to those reported for Caucasians (Kueppers, 1976; Frants and Eriksson, 1976; van den Broek *et al.*, 1976). In our previous report, the gene frequencies for Pi^{M1} and Pi^{M2} were 0.89 and 0.11, respectively. However, the distribution of the Pi M subtypes in this study was found to deviate significantly from the Hardy-Weinberg equilibrium ($\chi^2=21.5$, df=1, $p<0.001$). This is mainly due to an excess of homozygotes, Pi M_2M_2. We suggest that there is the possibility of further microheterogeneity in the M_2 region, as discussed below.

Although there is a great contrast in the frequencies of the rare variants between European populations (5–25%) (Fagerhol, 1976) and Japanese (0.5%), a few characteristics in the rare variants in Japanese seem to be worthy of mention. Firstly, no Z variants were detected in the present study. Two examples of Pi M_1Z which

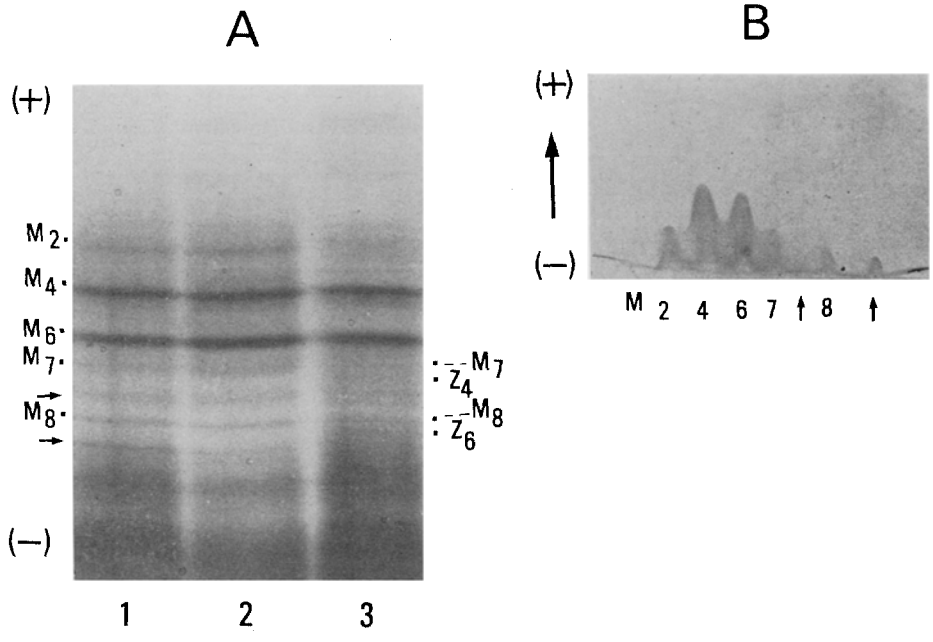

Fig. 2. Photographs showing the isoelectric focusing pattern (A) and antigen-antibody crossed electrophoretic pattern after isoelectric focusing (B) of the α_1-antitrypsin found in the alcoholic. 1: M_1M_1 with extrabands (arrows), 2: M_1M_1 (1 month after cessation of alcohol intake), 3: MZ (control).

we reported in the previous report (Harada *et al.*, 1977) have to be corrected as Pi M_1M_1. As shown in Fig. 2, two minor bands which we first misstook for Z bands are located between M_7 and M_8 and more cathodally than M_8 and Z_6. Family studies did not prove these bands to be genetically determined. These minor bands were observed in sera of alcoholics, which disappeared after cessation of the alcohol intake.

At least five different Pi variants were discovered. One of these has the variant Pi bands which are located more anodal than Pi E bands on isoelectric focusing (Fig. 3). This variant was compared with the reference samples of Pi EM and Pi

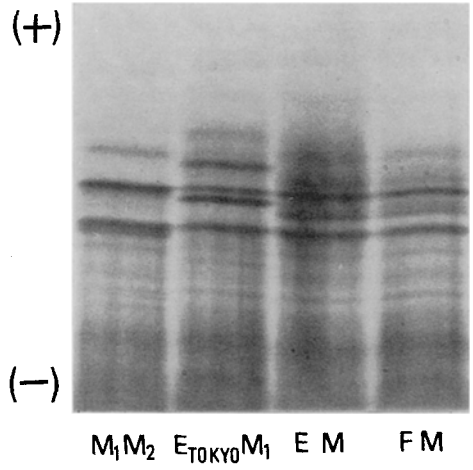

Fig. 3. Comparison of Pi E_{Tokyo} M_1 to the reference sera Pi EM and Pi FM on the gel.

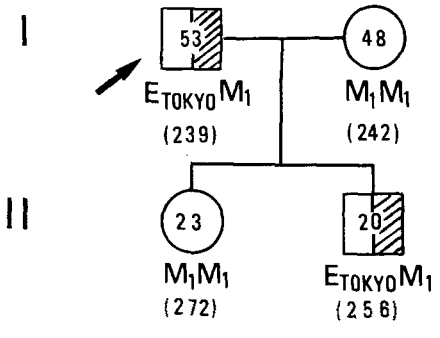

Fig. 4. Family pedigree of propositus Pi E_{Tokyo} M_1. Propositus Y. T. (↗). Age is within the square or circle. α_1-Antitrypsin phenotypes and concentration are underneath. ( ): α_1-antitrypsin mg/100 ml.

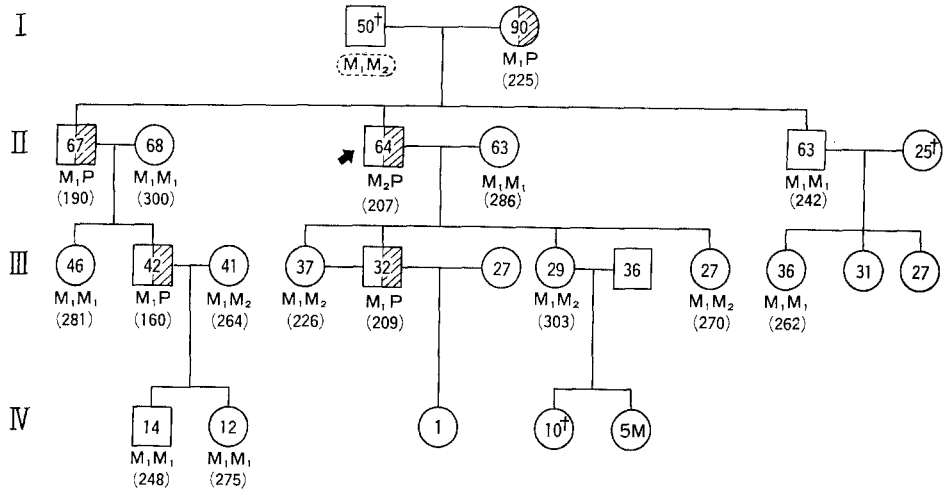

Fig. 5. Family pedigree of propositus Pi M_2P. Propositus T.N. (↗). Age is within the square or circle. α_1-Antitrypsin phenotypes and concentration are underneath. ( ): α_1-antitrypsin mg/100 ml, †: decreased.

Table 2. Subtyping of Pi M in various populations.

Population	Total No. subtypes, %				Genes		χ^2 (1)
	Pi M	M_1	M_1M_2	M_2	PiM_1	PiM_2	
Icelanders[1]	42	78.6	19.0	2.4	0.88	0.12	0.4
(Reykjavik)		(77.6)	(21.0)	(1.4)			
Aland Islanders[1]	47	83.0	14.9	2.1	0.90	0.10	0.9
		(81.8)	(17.3)	(0.9)			
Finns[1]	133	84.2	12.8	3.0	0.91	0.09	8.3
(Ristiina)		(82.1)	(17.0)	(0.9)			
Lapps[1]	48	91.7	8.3	0.0	0.96	0.04	0.1
(Utsjoki. Finland)		(91.8)	(8.0)	(0.2)			
Mari[1]	50	68.0	24.0	8.0	0.80	0.20	3.1
(USSR)		(64.0)	(32.0)	(4.0)			
Eskimos[1]	31	80.6	16.1	3.2	0.89	0.11	1.2
(N.W. Greenland)		(78.7)	(20.0)	(1.3)			
Bantus[1]	48	95.8	4.2	0.0	0.98	0.02	0.0
(Kenya)		(95.9)	(4.1)	(0.0)			
Dutch[2]	647	76.8	18.7	4.5	0.86	0.14	29.4
(Leiden)		(73.9)	(24.1)	(2.0)			
Japanese[3]	1,264	70.1	25.0	4.9	0.83	0.17	21.5
(Tokyo)		(68.2)	(28.8)	(3.0)			

Expected frequency shown in parentheses under observed frequency.
1: Frants and Eriksson (1976), 2: Klasen *et al.* (1977), partly revised, 3: present investigation.

FM, and found to be different from these. This protein migrates close to Pi E on acid starch gel electrophoresis (Miyake *et al.*, 1978). The new variant was designated Pi E_{Tokyo}. Pi E_{Tokyo} was also confirmed as a new variant by Dr. D. W. Cox, Tronto, and Dr. J. Constans, Toulouse (personal communications, 1977). It is evident from Fig. 4 that Pi E_{Tokyo} is inherited, and in the variant sample which is a heterozygote Pi E_{Tokyo} M_1 the protein concentration resulting from Pi^{ETokyo} is similar to that resulting from Pi^{M1}.

A sample with the phenotype Pi M_2P was also identified by the reference sample of Pi MP. It is inherited, as shown in Fig. 5. Pi MP, one of the deficient types of α_1-antitrypsin (Fagerhol and Hauge, 1968), has been reported to be lower in frequency than Z variants in European populations (Fagerhol, 1976). On the other hand, we discovered 4 cases of Pi MP among 652 unrelated persons of the independent group (preliminary observation). It is likely that Pi MP is commoner than Pi MZ among deficient types of α_1-antitrypsin in Japanese. Two cases of Pi M_1N were also detected, which were identical to the reference Pi MN which was kindly supplied by Dr. D. W. Cox.

Table 2 shows the Pi M subtypes in various populations thus far reported. It is interesting that there is a similarity in the frequencies of Pi M subtypes in different populations including Japanese, though in some instances the significant deviation from Hardy-Weinberg equilibrium is notable. Recently, some investigators suggested the presence of the third M subtype, Pi M_3 (Genz *et al.*, 1977; Frants and Eriksson, 1978), which is reported to have an intermediate position between Pi M_1 and Pi M_2 on isoelectric focusing patterns. If this is in fact the case, the reason why there is the difference between observed and expected values in the present investigation might be responsible for this subtype. However, it is also likely that there is another subtype which is hardly distinguishable by the present method of isoelectric focusing. Further study is needed to solve this question.

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