Electrophoretic Variation of Human Serum α_1 -antitrypsin in Japan

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Abstract Individual variation of serum α_1 -antitrypsin, a protease inhibiting protein, was studied by starch gel electrophoresis. A total of 1,475 serum and plasma samples taken from three racial groups, namely Japanese, Ainu and U. S. A. White, were examined. Seven phenotypes altogether found were classified according to FAGERHOL's nomenclature of Pi-system. In each population group the type MM was by far the most common, while other variants had low incidence. A similar frequency distribution was found in the Japanese population to that in U. S. A. White, except that type MF was commoner and type MS rarer in the former than in the latter. A reduced heterogeneity in the Ainu population was indicated. Gene frequencies were estimated on the basis of the postulated genetic mode of codominant alleles at a locus. It is found that the α_1 -antitrypsin deficient type occurs also in Japan, although there is still uncertainty as to whether this is genetically a homogeneous phenotype. No difference in the trypsin inhibiting capacity was found among the three racial groups.

INTRODUCTION

The serum α_1 -antitrypsin is the protein recognized in α_1 -globulin region by agar or filter paper electrophoresis of human serum. A slowly migrating variant and an α_1 -antitrypsin deficient type both of which were under genetic control were first found by agarose gel electrophoresis (AXELSSON *et al.*, 1965).

About the same time FAGERHOL and BRAEND (1965) found five electrophoretic phenotypes migrating in front of the albumin zone on the starch gel

using a modified POULIK's discontinuous buffer system (pH 4.95) and called them prealbumin phenotypes indicating three codominant alleles at an autosomal locus. It was soon proved, however, that these components corresponded to α_1 -antitrypsin (KUEPERS and BEARN, 1966; FAGERHOL and LAURELL, 1967). Thereafter, fifteen phenotypes controlled by seven alleles have been observed in 2,830 Norwegian blood samples by FAGERHOL *et al.* (1967, 1968) and, recently, two further alleles have been reported in an additional study by the same authors (1968).

They proposed to use the term Pi (Protease inhibitor) for the system of these genetic variants. Although the

* This study was reported at the 14th General Meeting of the Japan Society of Human Genetics (Sapporo, 1969)

distribution of Pi phenotypes in some population groups other than Norwegian were reported by FAGERHOL and TENFJORD (1968), the data of Asian samples were insufficient and the Japanese population has not thus far been studied as to this system.

In this paper the distribution of Pi phenotypes in Japanese is presented including that of the Ainu. Samples of U. S. A. White obtained in Japan were also examined as controls. Additionally, the trypsin inhibiting capacity (T. I. C.) of sera was measured in order to distinguish the deficient type of α_1 -antitrypsin and to compare the mean values of T. I. C. among the three racial groups.

MATERIALS AND METHODS

A total of 973 serum or plasma samples were taken from 180 junior high school children in Hidaka district, Hokkaido, 659 adult inhabitants of Shibukawa City area in the northern parts of Kanto and 134 blood donors in Tokyo. The samples of 239 Ainus* were collected from adults and junior high school children in Hidaka district, Hokkaido. The samples of 263 U. S. A. Whites were supplied by the U. S. Naval Hospital, Yokosuka. Most subjects were healthy except some Ainus and U. S. A. Whites who were convalescent inpatients.

Sera collected in Hokkaido and plasma collected in the northern parts of Kanto area were stored at -20°C and examined within one month after collection. Most samples from Tokyo and the U. S. Naval Hospital have been stored at 4°C and tested within a few days after collection, while others have been stored at -20°C for up to ten months before analysis.

The Pi types were determined according to the original method of FAGERHOL (1968), which is a horizontal starch gel electrophoresis using a discontinuous buffer system with tris-citric acid buffer of pH 4.95 in the starch gel, borate buffer of pH 8.9 in the cathodal bridge and citrate-phosphate buffer of pH 4.5 in the anodal bridge. The hydrolysed starch of the Connaught Laboratory was used in a concentration of 15%.

The bottom part of the sliced gel was stained with Amido Black 10B.

Antigen-antibody crossed electrophoresis (LAURELL *et al.*, 1965, 1967) was performed using rabbit anti- α_1 -antitrypsin (Hoechst) as antibody. This test was carried out as follows: after serum protein was separated by starch gel electrophoresis, a 2mm wide strip with separated α_1 -antitrypsin was sliced from the bottom part of 2mm thick starch gel and was inserted into an agar gel containing 1% agar (Difco Special Noble), barbital buffer (pH 8.6) and 6% antiserum. When electrophoresis was carried out for a short time (10 V/cm, 1.5 hours) in the perpendicular

* The degree of admixture of non-Ainu Japanese in this population has been estimated to be about 30-40% (OMOTO & HARADA, 1968).

direction to the first run, the α_1 -anti-trypsin moved from the starch gel into the agar to form a precipitation line indicating several peaks corresponding to the α_1 -antitrypsin components.

Agar immunodiffusion test was carried out following the OUCHTERLONY's method (1953) and DARCY's method (1968). The latter method is carried out as follows: after electrophoresis the upper half of the starch gel strips are put on the agar layer containing 1% agar and barbital buffer (pH 8.6). The troughs are filled with anti- α_1 -antitrypsin. The precipitation lines are formed in the agar layer corresponding to the α_1 -antitrypsin zone after incubation for overnight.

The trypsin inhibiting capacity of serum was measured by the method of BUNDY and MEHL (1958). Trypsin inhibitor (3 times crystallized) and soybean trypsin inhibitor (3 times crystallized) of Worthington Biochem. Corp. and casein according to Hammersten (Wako Pure Chemical Tokyo) as substrate were used throughout. Optical density was measured at 280 m μ . Materials stored at -20°C for not longer than several weeks and at 4°C for not longer than several days were used to measure T. I. C.*

RESULTS

As shown in Table 1, six phenotypes in 973 Japanese as well as in 263 White samples were observed, while there were only three in the Ainu samples. It was supposed that these different phenotypes on the starch gel were identical to those reported by FAGERHOL *et al.* by comparison of electrophoretic patterns. We followed FAGERHOL and assumed that six phenotypes observed, namely FF, FM, MM, MS, FS, MX are controlled by 4 codominant alleles: Pi^F , Pi^M , Pi^S and Pi^X .

The gene frequencies estimated for three racial groups mentioned above were: in the Japanese population $Pi^F=0.014$, $Pi^M=0.983$, $Pi^S=0.003$, $Pi^X=0.0005$, in the Ainu population $Pi^F=0.019$, $Pi^M=0.979$, $Pi^S=0.002$ and in the White population $Pi^F=0.029$, $Pi^M=0.950$, $Pi^S=0.017$, $Pi^X=0.002$ (Table 2).

In each population the gene Pi^M had the highest frequency while other genes appeared to be rather rare. This result agreed well with that of FAGERHOL (1968).

In Table 3 are given the mean values of trypsin inhibiting capacity of normal samples measured by the method of BUNDY and MEHL (1958). The mean values of trypsin inhibiting capacity are not significantly different from each other among three racial groups, namely:

1) Japanese and Ainu:

$$T=0.951 \quad D.F.=204 \quad P>0.5$$

2) Japanese and U. S. A. White:

* JACOBSSON (1955) and ERIKSSON (1965) have reported that T. I. C. is not altered significantly after storage up to one year at -20°C .

Table 1. Distribution of Pi phenotypes.

		MM	MF	MS	MX	FF	FS	χ^2
Japanese N=965	Obs. No.	934	24	5	1	1	—	$\chi^2=3.806$
	%	96.8	2.5	0.5	0.1	0.1	—	D. F. =6
	Exp. No.	932.46	26.26	5.69	0.98	0.19	0.08	$.50 < P < .75$
Ainu N=238	Obs. No.	228	9	1	—	—	—	$\chi^2=0.113$
	%	95.8	3.8	0.4	—	—	—	D. F. =3
	Exp. No.	228.1	8.85	0.93	—	0.086	0.018	$.99 < P < .995$
U. S. A. White N=261	Obs. No.	238	12	8	1	1	1	$\chi^2=5.415$
	%	91.2	4.5	3.1	0.4	0.4	0.4	D. F. =6
	Exp. No.	236.55	14.41	8.45	0.99	0.22	0.26	$.25 < P < .50$

Table 2. Pi gene frequency in various populations.

Population	No.	Pi^M	Pi^S	Pi^Z	Pi^F	Pi^I	Pi^V	Pi^W	Pi^X	Pi^P	authors
Norwegian	2830	.9460	.023	.0157	.0133	.0012	.0004	*	*	*	FAGERHOL (1967) FAGERHOL & HAUGE (1968)
Spanish	378	.8664	.1124	.0119	.0026	.0013	.0026	.0026	—	—	FAGERHOL & TENFJORD (1968)
Asian	68	1.000	—	—	—	—	—	—	—	—	FAGERHOL & TENFJORD (1968)
Norwegian Lapps	302	.9917	—	.0083	—	—	—	—	—	—	FAGERHOL & HAUGE (1968)
Finnish Lapps	468	.9957	.0032	.0011	—	—	—	—	—	—	FAGERHOL & HAUGE (1968)
Finns	223	.9955	—	.0045	—	—	—	—	—	—	FAGERHOL & HAUGE (1968)
Aland Islanders	450	.9956	.0044	—	—	—	—	—	—	—	FAGERHOL & HAUGE (1968)
Japanese	965	.983	.003	—	.014	—	—	—	.0025	—	Present authors (1969)
Ainu	238	.979	.002	—	.019	—	—	—	—	—	Present authors (1969)
U. S. A. White	261	.952	.017	—	.029	—	—	—	.002	—	Present authors (1969)

* According to FAGERHOL and HAUGE (1968), frequencies for these alleles in Caucasians are approximately 0.0002.

Table 3. Mean values for T. I. C. (mg trypsin inhibited/ml serum)

Japanese	1.082 $\pm$ 0.302	N=154
Ainu	1.056 $\pm$ 0.233	N=82
U. S. A. White	1.072 $\pm$ 0.287	N=77

$T=0.122$ $D. F.=231$ $P>0.5$

3) Ainu and U. S. A. White:

$T=0.385$ $D. F.=153$ $P>0.5$.

On the other hand, 8 in 973 Japanese (0.8%), 1 in 239 Ainus (0.4%) and

2 in 263 U. S. A. Whites (0.8%) had no distinct zone in the region of α_1 -antitrypsin, thus they were classified as "deficient type". These types were omitted in calculating phenotypic in-

Fig. 1. Seven phenotypes of P_i system on the starch gel stained by amido black 10 B. Japanese and U. S. A. White samples. DEF. means deficient type.

Fig. 2. Schema of P_i phenotypes observed in Japanese, Ainu and U.S.A. White Samples. D.L. means discontinuous line.

Fig. 3. Photograph of the antigen-antibody crossed electrophoresis (phenotype MM). The precipitation lines formed by antigen-antibody (anti- α_1 -antitrypsin) crossed electrophoresis is shown above the starch gel strip. The arrows indicate the direction of electrophoretic

Fig. 4. Photograph of the immunodiffusion test. The upper half of starch gel strips (5 mm wide) are put on the agar layer and the troughs are filled with anti- α_1 -antitrypsin. The precipitation lines are formed corresponding to the α_1 -antitrypsin zone after incubation. On the photograph the bottom half of starch gel strips stained by amido black 10 B are put on the agar layer.

cidences and gene frequencies.

DISCUSSION

Fig. 1 presents the different phenotypes observed in the present study on the starch gel stained with Amido Black 10 B, and Fig. 2 is the schema of them. FAGERHOL and his coworkers have reported mainly in Scandinavians and Spaniards the following phenotypes: FF, FM, FS, FZ, IM, IS, MP, MM, MS, MV, MW, MX, MZ, SS, SZ, XZ, and ZZ, which are believed to be controlled by a number of autosomal alleles called in order of decreasing electrophoretic mobility of the protein zone: Pi^F , Pi^I , Pi^M , Pi^P , Pi^S , Pi^V , Pi^W , Pi^X and Pi^Z .

Several studies support that the Pi types are determined by simple Mendelian codominant inheritance. So far no contradiction to the hypothesis that all Pi genes are alleles has been reported (FAGERHOL *et al*, 1965, 1967, 1968 and 1969).

The phenotype MM is by far the most frequent one and shows two clearly stained zones with one weak zone in front of them on starch gel. It is shown in Fig. 3 that two main and one minor zones of type MM on the starch gel are identified as three peaks on antigen-antibody crossed electrophoretic pattern. Furthermore, type MM has two faint zones toward the cathode.

It was demonstrated by the immunodiffusion test of the starch gel strip, that the precipitation lines appear corresponding to the α_1 -antitrypsin zones of the starch gel (Fig. 4). When

samples of the apparently deficient type, in which no zone was detected in the usual region of α_1 -antitrypsin on the starch gel, are subjected to the same test, precipitation also occurred, indicating that in these phenotypes the main α_1 -antitrypsin zones had much slower electrophoretic mobility in the starch gel than the other types and were covered by the heavily stained albumin zone. According to FAGERHOL (1968) this condition is a homozygote for the rare allele Pi^Z , and has only about 15% concentration of α_1 -antitrypsin.

As mentioned above, we have typed 8 in 973 Japanese, 1 in 239 Ainu and 2 in 263 U. S. A. White samples as deficient in α_1 -antitrypsin zone. Measurements of trypsin inhibiting capacity on these samples showed much decreased values indicating that these correspond to the ERIKSSON's deficient type. However, we have no indication that all these samples can be typed as FAGERHOL's ZZ type. Since no clear case of heterozygous MZ was encountered, it is unlikely that such is the case. If one postulates that all of the observed "deficient" type are of homozygous ZZ, one must expect an unusually high gene frequency for Pi^Z in the Japanese samples, namely roughly 0.09, which in turn indicates an expected number of the heterozygous type MZ to be approximately 160. The conclusion as to the incidence should be drawn after we have more information on the genetic basis of the

deficient phenotype. Family studies are now under way on some of the deficient phenotypes observed in the present study.

As given in Table 2, the Japanese and the Ainu populations have approximately the same frequency of Pi^M (0.983, 0.979), while that of U. S. A. Whites is somewhat lower (0.952). On the contrary the gene frequency of Pi^S is higher in U. S. A. Whites.

The number of phenotypes in Ainu is fewer than in Japanese, probably because of either the smaller sample size, or the decreased heterogeneity in the Ainu population suggested by the examinations of other polymorphic systems (OMOTO & HARADA, 1968).

The phenotypic and gene frequencies for Pi-system of different racial groups hitherto reported are summarized in Table 2. A gene frequency of Pi^S is high in Caucasoid groups especially in Spanish and Portuguese groups. In our studies, too, the U. S. A. White group shows a higher gene frequency of Pi^S than the Japanese population. On the other hand the frequency of $Pi^{F'}$ is much higher in the Japanese and in the Ainu populations than in the Spanish. The frequencies in the former two populations are similar to Norwegians and U.S.A. Whites. The rare genes Pi^Z , Pi^I , Pi^V , Pi^W which have been reported in Spanish and Norwegian populations were not observed in our materials, while the gene Pi^X was rarely found in Japanese (0.0025), and U. S. A. White samples (0.002).

The mean values of normal α_1 -antitrypsin activity were not significantly different among the three racial groups. In this examination both sexes were combined together, since it is reported that difference between sex was insignificant (ERIKSSON, 1965). It is surmised that the quantity of α_1 -antitrypsin is not influenced by the regional and racial differences.

ERIKSSON (1965) reported an association between α_1 -antitrypsin deficiency and the occurrence of chronic obstructive broncho-pulmonary disease. The individuals with the deficient type of α_1 -antitrypsin are exposed at the risk of chronic obstructive pulmonary disease which will frequently result in death below 50 years old. FAGERHOL and HAUGE (1969) have reported that in patients with chronic obstructive pulmonary disease the Pi types SS, SZ and ZZ were found more often than in healthy subjects. The low frequency of the deficient type as well as other variants in the Ainu samples may put on interesting question from the aspect of selection by disease, since the Ainu population suffered severe loss of population numbers after having been brought into contact with the Japanese immigrants in 18-19 century through the spread of several epidemic diseases such as measles, smallpox and tuberculosis (KODAMA, 1969). It is tempting to speculate that the individuals with the α_1 -antitrypsin deficient type has been selected against in the present Ainu population by tuberculosis, al-

though an alternative interpretation in terms of random disappearance of rare genes should also be considered.

SUMMARY

Sera and plasma samples from 973 Japanese, 239 Ainu and 263 U.S.A. Whites were examined by starch gel electrophoresis for the electrophoretic variation of α_1 -antitrypsin (Pi-system, FAGERHOL, 1967). Five phenotypes along with a deficient type were observed in the Japanese sample group, while only three were detected in the Ainu. Six phenotypes were found in the U.S.A. White samples. In accordance with the previous reports, the type MM was by far the most common phenotype. The type MF was found in 2.5% of the Japanese in 3.8% of the Ainu and in 4.5% of the U.S.A. White samples, while the type MS was found in 0.5%, 0.4% and 3.1% respectively. Furthermore, a single MX type was encountered in the Japanese sample. Gene frequencies estimated for the present material were compared with those previously reported.

Mean values of trypsin inhibiting capacity were not significantly different among the three racial groups.

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日本における血清 α_1 -antitrypsin の変異

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血清中の主要な protease-inhibitor である α_1 -antitrypsin には pH 4.95 の不連続 buffer system を用いた澱粉ゲル電気泳動により、多型性変異が検出される (Pi-system: FAGERHOL, 1967).

日本における各表現型の分布を調べる目的で、東京 (134名)、北関東 (659名)、北海道日高 (和人180名、アイヌ系 239名) の血清試料の他、比較のために白人 (263名) の試料を検査した。FAGERHOL (1968) は17種類の表現型を記載しているが、今回の調査では日本人集団で5種類 (MM 96.8%, MF 2.5%, MS 0.5%, MX 0.1%, FF 0.1%), アイヌ系集団で3種類 (MM 95.8%, MF 3.8%, MS 0.4%), 白人集団で6種類 (MM 91.1%, MF 4.2%, MS 3.1%, FF 0.8%, FS 0.4%, MX 0.4%) が同定された。尚、BUNDY et MEHL (1957) の方法に基き, trypsin inhibiting capacity を測定したところ、日本人、アイヌ系、及び白人の各集団間では有意の差は見られなかったが、日本人及び白人の集団で活性値が著るしく低いもので α_1 -antitrypsin deficient type と思われるものも数例みられた。