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GENETIC POLYMORPHISM OF THE A SUBUNIT OF HUMAN COAGULATION FACTOR XIII IN JAPANESE

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Summary Polymorphism of the A subunit of human coagulation factor XIII was investigated in a total of 561 plasma samples from unrelated adult Japanese using an agarose gel electrophoresis followed by a fluorescent technique for the localization of transglutaminase activity. Three common phenotypes were observed, which corresponded to the types 1, 2-1 and 2 reported by Board (1979) in Australians. The family material from 23 matings with 60 children indicated that these phenotypes are controlled by a pair of autosomal, codominant alleles. The estimated allele frequencies of *F13A*1* and *F13A*2* were 0.887 and 0.113, respectively.

INTRODUCTION

Factor XIII is the precursor of the transamidating enzyme, fibrinoligase, which is responsible for the covalent crosslinking of fibrin molecules in the final stage of the blood coagulation process (Folk and Chung, 1973). It is known that the factor XIII protein from human plasma is a tetramer composed of two different subunits, A and B. In the presence of thrombin and Ca^{2+}, the activated protein is cleaved into the enzymatic A subunit and the non-enzymatic B subunit (Schwartz *et al.*, 1973).

Genetic polymorphism of the A subunit of factor XIII was recently demonstrated in Australian Caucasians (Board, 1979). Three phenotypes (1, 2-1 and 2) were distinguishable by an agarose gel electrophoresis followed by a fluorescent technique for localization of transglutaminase activity. The phenotypes were shown to be controlled by a pair of autosomal, codominant alleles.

Few studies have been reported as to this polymorphism in population groups other than Caucasians. Recently, Kera and Nishimukai (in press) investigated a sample of Japanese using isoelectric focusing followed by immunofixation. They also reported three phenotypes which they referred to as type 1, 2-1 and 2. In this paper, we report the results of our investigation in which the Board's original method fo rtyping was adopted.

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MATERIALS AND METHODS

The family material consisting of 23 matings with 60 children was obtained in Tokyo. A total of 561 plasma samples from 515 unrelated blood donors and 46 parents of the family material were used for population study. Samples for typing were either heparinized or ACD plasma, and were stored at −30°C until use.

An agarose gel electrophoresis was carried out using the method of Board (1979) with slight modifications. A glass plate (180×180×1.2 mm) was covered with a 1.4 mm thick layer of 1% (w/v) agarose gel made with the gel buffer. The electrode buffer contained 29 mM lithium hydroxide and 191 mM boric acid, adjusted to pH 8.45. The gel buffer was prepared by combining 90 vol. solution containing 5.71 mM citric acid and 52 mM tris(hydroxymethyl)aminomethane with 10 vol. electrode buffer, adjusted to pH 8.65 (Ashton and Braden, 1961). Electrophoresis was carried out between the cooling plates at 18 V/cm until a hemoglobin marker migrated at least 6.5 cm from the origin.

Detection of the location of transglutaminase activity was essentially following the method described by Board (1979). The gel was overlaid with a filter paper (Toyo No. 1) soaked in the following reaction mixture: 1 ml 1 M Tris-HCl (pH 8.0); 1 ml 0.05 M $CaCl_2$; 3 ml casein (acc. to Hammarsten, Wako Pure Chemical, Osaka, Japan) 10 mg/ml dissolved in 0.2 M Tris-HCl (pH 8.0); 1 ml 0.025 M monodansyl cadaverine; 20 μl β-mercaptoethanol; *ca.* 10 U human thrombin (Green Cross, Osaka, Japan).

Monodansyl cadaverine was initially dissolved in a small amount of 0.1 M HCl and then the volume was made up with 0.1 M Tris-HCl (pH 8.0). The gel was then incubated at 37°C for 12–15 hr in a moist chamber. After incubation, the filter paper was removed and the gel was fixed for 5 min using a sheet of filter paper soaked in 7.5% (w/v) trichloroacetic acid. After fixing, the gel was again overlaid for 5 min with another filter paper soaked in 0.3 M Na_2HPO_4. The gel was subsequently washed under running tap water for 1–2 hr to remove the unincorporated monodansyl cadaverine, and then the location of transglutaminase activity was detected under UV light (long wave).

RESULTS AND DISCUSSION

Three different patterns were observed by the electrophoretic screening of plasma samples for population study (Fig. 1). Those patterns are characteristic of the electrophoretic variation of a dimeric protein with two alleles. For example, the pattern of type 2-1 consists of three bands with the intermediate, presumably heterodimeric band stained stronger than the others.

Board (1979) proposed to refer to this system as the *F-XIII* locus with two alleles termed *F-XIII*^{1} and *F-XIII*^{2}. Later, however, he demonstrated a genetic

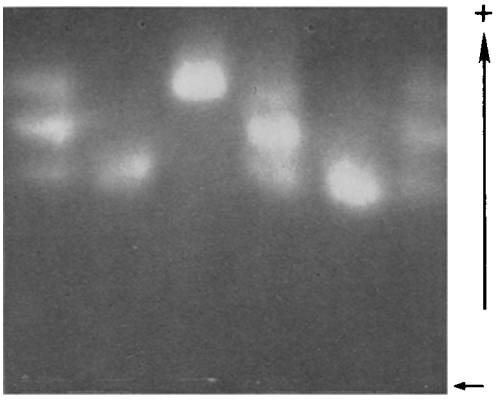

Fig. 1. Photograph showing electrophoretic patterns of three phenotypes of the A subunit of factor XIII. The small arrow indicates the place of sample application.

polymorphism of the B subunit of factor XIII, which was controlled by *F-XIIIB* locus with three alleles (Board, 1980). To avoid confusion, we adopt a symbol, *F13A* to refer to the structural locus controlling the A subunit of factor XIII, *F13A*1* and *F13A*2* standing for two alleles according to the International System for Human Gene Nomenclature (Shows *et al.*, 1979). *F13A*1* controls the fast component and *F13A*2* controls the slow component. In order to identify the phenotypes with those reported by Board (1979), plasma samples from Caucasoid individuals living in Tokyo were used in comparison runs. The three phenotypic patterns observed in Japanese and Caucasians were identical. No rare variant was detected in the present Japanese sample.

Investigation was also carried out in a family material (Table 1, Fig. 2). The result was consistent with the hypothesis that this polymorphism is controlled by two codominant alleles, *F13A*1* and *F13A*2*, at an autosomal locus.

The mode of inheritance of a congenital factor XIII deficiency has been reported. Possibility of a sex-linkage was suggested (Hampton *et al.*, 1966; Ratnoff and Steinberg, 1968), although recent reports indicated an autosomal ressesive inheritance (MacDonagh *et al.*, 1971; Losowsky and Miloszewski, 1977; Barbui *et al.*, 1978). The result of the present family study obviously indicates that the structural gene of the A subunit is on an autosomal chromosome.

The distribution of phenotypes and allele frequencies in the present study are shown in Table 2. The observed numbers of phenotypes were in good agreement with the numbers expected on the basis of Hardy-Weinberg's law. The allele frequencies of *F13A*1* and *F13A*2* were estimated at 0.887 and 0.113, respectively. These allele frequencies do not differ significantly from those observed in another study on Japanese population (Kyoto City) using the method quite different from

Table 1. Factor XIII-A phenotypes in 23 families with 60 children.

Parental types	No. families	Offspring types		
		1-1	2-1	2-2
1-1×1-1	12	30	0	0
1-1×2-1	9	15	9	0
2-1×2-1	2	1	4	1

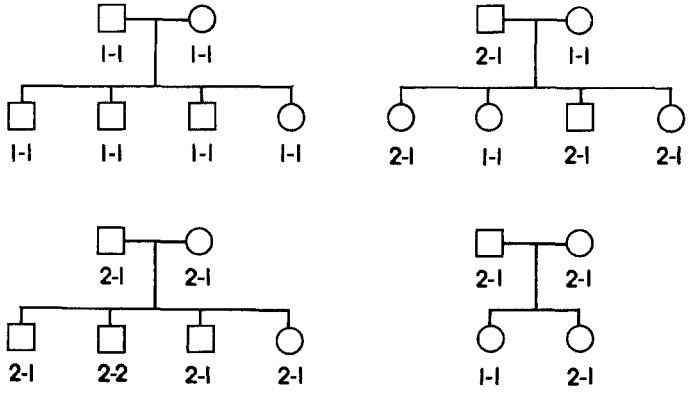

Fig. 2. Examples of families showing inheritance of factor XIII-A.

Table 2. Distribution of factor XIII-A phenotypes and allele frequencies in a Japanese population.

	Phenotypes			Total
	1	2-1	2	
Obs. No.	441	113	7	561
Exp. No.	441.4	112.5	7.2	

$\chi^2=0.008$, d.f.$=1$, $p>0.9$.
Allele frequencies: *F13A*1*$=0.887$, *F13A*2*$=0.113$.

Table 3. Allele frequencies of factor XIII-A in various populations.

Population	N	*F13A*1*	*F13A*2*	Authors
Australian	179[a]	0.79	0.21	Board (1979)
Australian	204[b]	0.80	0.20	Board (1979)
Japanese	250[a]	0.90	0.10	Kera *et al.* (in press)
Japanese	561[a]	0.89	0.11	present study

[a] Determined from plasma, [b] determined from expired platelets.

that of Board (1979), namely, the agarose gel isoelectric focusing followed by immunofixation (Kera and Nishimukai, in press). The allele frequency of *F13A*1* in the Japanese population appears to be higher than that in the Caucasian population (Table 3), so far as the limited data available now are concerned.

Since the technique for determining phenotypes is relatively easy and inexpensive, genetic polymorphism of the A subunit of factor XIII is a useful marker in human genetics for population studies.

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