COAGULATION FACTOR XIIIB PHENOTYPING IN A JAPANESE POPULATION AND IN BLOODSTAINS

N. Komatsu, A. Kido, Y. Kimura and M. Oya

Department of Legal Medicine, Yamanashi Medical University, Yamanashi-ken, Japan

Introduction

Human coagulation factor XIII B subunit (FXIIIB) exhibits genetic polymorphism with 3 codominant alleles FXIIIB*1, FXIIIB*2 and FXIIIB*3 (Board 1980). Up to the present time, a number of rare alleles have been identified, and in Japanese FXIIIB*13, FXIIIB*14 and FXIIIB*15 have been described (Nakamura et al. 1986). In the present study the distribution of FXIIIB types in a Japanese population was investigated and the phenotyping was attempted from bloodstains for medicolegal purpose.

Materials and Methods

Blood samples were collected from 555 unrelated Japanese individuals. Ten µl plasma was treated with 4 µl 40 U/ml neuraminidase (Sigma, USA) in 1 M potassium phosphate buffer (pH 7.0). After incubation for 18 h, the mixture was absorbed on 5 x 6 mm filter paper strips (Toyoroshi No. 2, Japan).

Twenty bloodstains with known phenotypes were made on filter paper (Toyoroshi No. 2) and stored at 37°C, room temperature and 4°C for different time intervals. The stains were cut in 5 x 6 mm pieces, treated with 10 µl 20 U/ml neuraminidase (Sigma) in 1 M potassium phosphate buffer (pH 7.0) for 18 h and directly applied onto the gel surface.

Isoelectric focusing was performed by the method of Sebetan and Azadeh (1989) with slight modifications. Following electrofocusing, proteins were transferred onto a sheet of nitrocellulose membrane (Bio-Rad, USA) using a Trans-Blot Cell (Bio-Rad) as described by Kido et al. (1991).

Immunologic detection of FXIIIB was performed as follows:
-brief rinse in 20 mM tris/500 mM sodium chloride buffer, pH 7.5 (TBS).
-immersion in TBS containing 3 % gelatine for 20 min.
-wash in TBS for 15 min.
-incubation in rabbit anti-human factor XIII-S serum (Behringwerke, Germany) diluted 1:500 in TBS containing 0.05 % Tween 20

Advances in Forensic Haemogenetics 4
Edited by Ch. Rittner and P. M. Schneider

(TTBS) for 120 min.
-2 washes in TTBS for 15 min.
-incubation in goat anti-rabbit IgG serum conjugated with alkaline phosphatase (Sigma) diluted 1:750 in TTBS for 120 min.
-2 washes in TTBS for 15 min.
-development with a freshly-prepared reaction mixture (125 mg ß-naphtyl phosphate, 12.5 mg Fast Blue BB salt and 30 mg magnesium sulfate dissolved in 25 ml 60 mM boric acid/45 mM sodium hydroxide buffer, pH 10.4) at 37°C for 30 min.

Results and Discussion

Figure 1 shows FXIIIB types in desialylated plasma samples from 555 Japanese. In our sample 5 common phenotypes FXIIIB 1-1, 2-1, 3-1, 3-2, 3-3 and a rare variant type FXIIIB 15-3 were observed. As the FXIIIB*15 allele has been detected exclusively in the Japanese population (Nakamura et al. 1986, Sebetan et al. 1990), it may be unique to Japanese.

The results for the distribution are given in Table 1. The population data fitted the Hardy-Weinberg equilibrium.

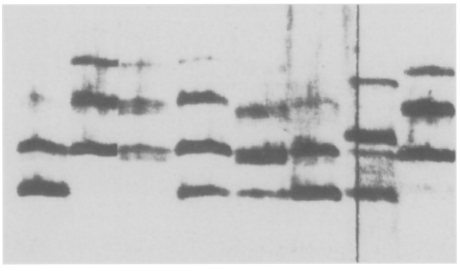

Fig. 1. Isoelectric focusing pattern of FXIIIB

Table 1. Distribution of FXIIIB types in a Japanese population

Phenotype	Observed	%	Expected
1-1	51	9.19	52.07
2-1	6	1.08	5.51
2-2	0	0.00	0.15
3-1	232	41.80	230.04
3-2	12	2.16	12.17
3-3	253	45.59	254.07
15-3	1	0.18	0.68
Others	0	0.00	0.33
Total	555	100.00	555.02

Allele frequency: FXIIIB*1 = 0.3063, FXIIIB*2 = 0.0162, FXIIIB*3 = 0.6766, FXIIIB*15 = 0.0009.
$\chi^2 = 0.71$, d.f. = 6, $P > 0.99$

The FXIIIB types were well demonstrated also from dried and stored bloodstains (Fig. 2). Our sample included 4 FXIIIB 1-1, 2 FXIIIB 2-1, 6 FXIIIB 3-1, 2 FXIIIB 3-2 and 6 FXIIIB 3-3. Reliable phenotyping was possible from bloodstains stored at 37°C for up to 4 months and from bloodstains stored at room temperature and at 4°C for over 6 months. The above results for the determination limits indicate that this system is stable enough to be used in medicolegal practice and quite suitable to be used even in summer or under tropical conditions. The FXIIIB system can be a new powerful genetic marker for the grouping of bloodstains.

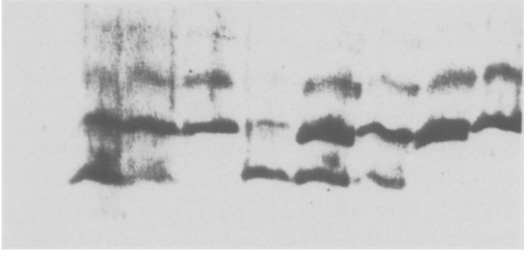

Fig. 2. Isoelectric focusing pattern of FXIIIB in bloodstains stored at room temperature for 3 months

REFERENCES

Board PG (1980) Genetic polymorphism of the B subunit of human coagulation factor XIII. Am J Hum Genet 32: 348-353

Kido A, Komatsu N, Kimura Y, Oya M (1991) C1R subcomponent polymorphism in Japanese: Description of a new allele. Hum Hered 41: 129-133

Nakamura S, Ohue O, Abe K (1986) Genetic polymorphism of coagulation factor XIII B subunit in the Japanese population: Description of the three new rare alleles. Hum Genet 73: 183-185

Sebetan IM, Azadeh B (1989) Genetic polymorphism of the B subunit of coagulation factor XIII in Libyans: Occurrence of a fourth common allele, FXIIIB*6. Z Rechtsmed 103: 125-128

Sebetan IM, Aoki Y, Sagisaka K, Oshida S (1990) Genetic polymorphism of the B subunit of coagulation factor XIII in a Japanese population. Res Pract Forensic Med 33: 55-57