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Polymorphism of coagulation factor XIII B subunit: further occurrence of FXIIIB*15 in Japanese and phenotyping in bloodstains

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Summary. The polymorphism of FXIIIB was investigated in 555 unrelated Japanese individuals using isoelectric focusing and immunoblotting. Five common phenotypes and a rare variant type FXIIIB 15–3 were observed. The allele frequencies were FXIIIB*1 = 0.3063, FXIIIB*2 = 0.0162, FXIIIB*3 = 0.6766 and FXIIIB*15 = 0.0009. Phenotyping was also 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 FXIIIB system can provide a new powerful genetic marker for the medicolegal grouping of bloodstains.

Key words: Polymorphism FXIIIB – Population genetics – Bloodstains

Zusammenfassung. Der Polymorphismus des FXIIIB wurde bei 555 unverwandten Japanern mit Hilfe der isoelektrischen Fokussierung und anschließendem Immunoblotting untersucht. Fünf allgemein vorkommende Phänotypen und die seltene Variante FXIIIB 15–3 wurden beobachtet. Die Allel-Frequenzen waren FXIIIB = 0,3063, FXIIIB2 = 0,0162, FXIIIB3 = 0,6766 und FXIIIB15 = 0,0009. Die Bestimmung der Phänotypen war auch an Blutspuren mit folgenden Lagerungsbedingungen möglich: Bei 37°C über einen Zeitraum bis zu 4 Monate, bei Raumtemperatur und bei 4°C länger als 6 Monate. Das FXIIIB-System kann einen neuen, aussagekräftigen genetischen Marker für gerichtsmedizinische Blutspurenuntersuchungen darstellen.

Schlüsselwörter: Polymorphismus FXIIIB – Populationsgenetik – Blutspuren

Introduction

Coagulation factor XIII B subunit (FXIIIB) exhibits genetic polymorphism with three common alleles FXIIIB*1, FXIIIB*2 and FXIIIB*3 [1] and a number of rare alleles [2–7]. In the present study the polymorphism of FXIIIB was investigated in a Japanese population and phenotyping was attempted from bloodstains of various ages.

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Materials and methods

Blood samples were collected from 555 unrelated Japanese individuals living in Yamanashi Prefecture. Ten µl of the plasma was treated with 4 µl neuraminidase (40 U/ml type V, Sigma) for 18 h.

Blood samples with known phenotypes were dropped onto filter paper (Toyoroshi No. 2) and allowed to dry for a few hours. The bloodstains were stored at 37°C, room temperature and 4°C, and examined after different time intervals. The stains were cut in 5 × 6 mm pieces, treated with 10 µl neuraminidase (20 U/ml, Sigma) for 18 h and applied directly onto the gel surface.

Isoelectric focusing was performed by the method of Sebetan and Azadeh [8]. Following electrofocusing, proteins were transferred onto a nitrocellulose membrane (Bio-Rad) by electroblotting. The membrane was stained for FXIIIB by the immunological detection method of Kamboh and Ferrell [7].

Results and discussion

Population study

Five common phenotypes and a rare variant FXIIIB 15–3 (Fig. 1) which seems to be unique to Japanese [5, 9] were

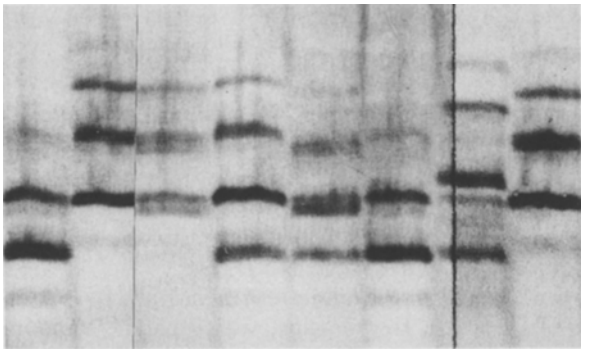

Fig. 1. Isoelectric focusing pattern of FXIIIB observed in the present study. The anode is at the top

Table 1. Distribution of FXIIIB types in a Japanese population

Phenotype	Observed	Expected
1–1	51	52
2–1	6	6
2–2	0	0
3–1	232	230
3–2	12	12
3–3	253	254
15–3	1	1
Others	0	0
Total	555	555

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

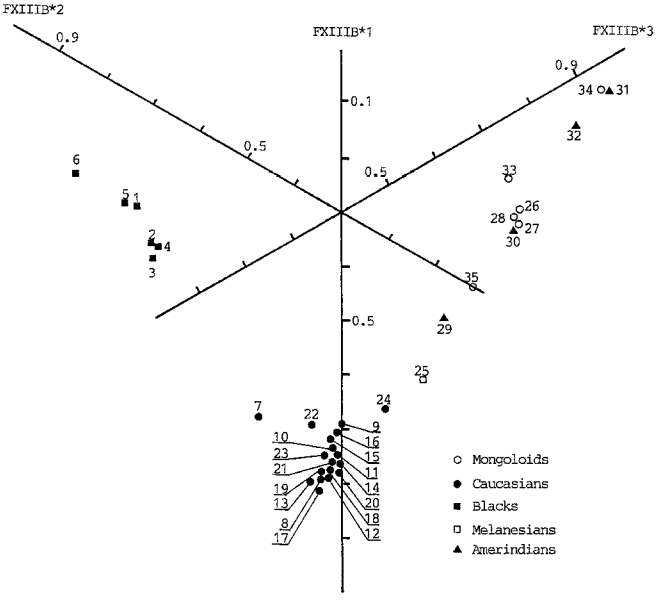

Fig. 2. Graphic representation of three common FXIIIB allele frequencies in various racial groups. The allele frequencies for each population (symbol) are obtained by the numerically cited literature. *1:* US Blacks [10], *2:* US Blacks [5], *3–5:* US Blacks [7], *6:* Nigerian Blacks [7], *7:* Libyan [8], *8:* US Whites [10], *9:* US Whites [11], *10:* US Whites [6], *11:* Australian Whites [1], *12:* Swiss [12], *13:* Swiss [13], *14:* Italians [14], *15:* Germans [15], *16:* Germans [16], *17:* Germans [3], *18:* Germans [17], *19:* Germans [18], *20:* Germans [19], *21:* Germans [20], *22:* Norwegians [21], *23:* Swedish [22], *24:* Indians [23], *25:* Melanesians [23], *26:* Japanese [5], *27:* Japanese [9], *28:* Japanese (present study), *29:* Minnesota Amerindians [10], *30:* Mayan Indians [11], *31:* Dogrib Indians [11], *32:* Vancouver Island Indians [11], *33:* Kodiak Island Eskimos [11], *34:* St. Lawrence Island Eskimos [11], *35:* Aleuts [11]

observed. Our population data conform to the Hardy-Weinberg equilibrium (Table 1).

Figure 2 displays a graphic representation of 3 common FXIIIB allele frequencies, which clearly demonstrates that Caucasian, Black and Mongoloid populations can be separated into 3 distinct racial clusters. Without exception the 3 common alleles occur with the following order of frequency: (1) in Caucasians FXIIIB*1 > FXIIIB*3 > FXIIIB*2, (2) in Blacks FXIIIB*2 > FXIIIB*1 > FXIIIB*3, (3) in Mongoloids FXIIIB*3 > FXIIIB*1 > FXIIIB*2. Our results are similar to those for Eskimos, Aleuts and Amerindians as well as other Japanese subpopulations, which strongly supports the generally accepted view that the Mongoloid race migrated via Siberia and Alaska to North and South America. The dominant frequency of FXIIIB*1 (0.500) [10] in Amerindians from Minnesota (No. 29 in Fig. 2) could have been produced by interbreeding with Caucasians.

Thus, FXIIIB has been confirmed to be a useful genetic marker for further population and anthropological studies.

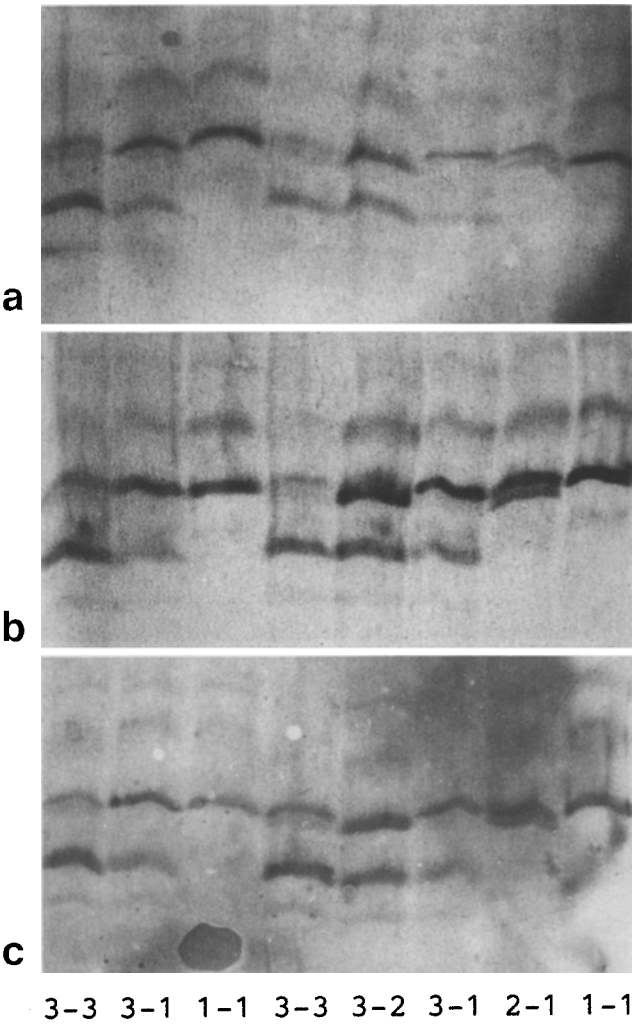

Fig. 3a–c. Isoelectric focusing patterns of FXIIIB in bloodstains stored at 37°C for 1 month **a**, at room temperature for 3 months **b** and at 4°C for 5 months **c**. The anode is at the top

Phenotyping in bloodstains

The FXIIIB types could also be demonstrated in dried and stored bloodstains although the bands were slightly indistinct and distorted as compared with plasma samples (Fig. 3). All the bloodstains examined were correctly typed after storage at 37°C for periods of up to 4 months,

Table 2. Positive results for the determination of FXIIIB types in bloodstains stored at different temperature

Phenotype	No. tested	Temperature	Age of bloodstains (months)					
			1	2	3	4	5	6
1–1	4	37°C	4	4	4	4	0	
2–1	2		2	2	2	2	0	
3–1	6		6	6	6	6	0	
3–2	2		2	2	2	2	0	
3–3	6		6	6	6	6	0	
1–1	4	Room temperature	4	4	4	4	4	4
2–1	2		2	2	2	2	2	2
3–1	6		6	6	6	6	6	6
3–2	2		2	2	2	2	2	2
3–3	6		6	6	6	6	6	6
1–1	4	4°C	4	4	4	4	4	4
2–1	2		2	2	2	2	2	2
3–1	6		6	6	6	6	6	6
3–2	2		2	2	2	2	2	2
3–3	6		6	6	6	6	6	6

and at room temperature and 4°C for periods over 6 months (Table 2).

The present isoelectric focusing method combined with immunoblotting permits reliable FXIIIB phenotyping from bloodstains after months of storage. The limits of determination indicate that this system is suitable for use in medicolegal practice. Furthermore, its relatively favourable phenotypic distribution gives an excellent discrimination probability (0.53 using our data). The FXIIIB phenotyping can therefore provide a new powerful means for the grouping of bloodstains in forensic investigations.

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