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Improved FXIIIB-Phenotyping by Isoelectric Focusing with Immobilized pH-Gradients

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Introduction

A genetic polymorphism of the B-subunit of the coagulation factor XIII (FXIIIB) was first described by Board in 1980 by agarose gel electrophoresis. By isoelectric focusing on agarose gels we found the four alleles FXIIIB*1, B*2, B*3 and B*4.

As a very sensitive staining procedure we employed the silver nitrate staining method of Willoughby and Lambert (1983). For improved resolution we adapted hybrid isoelectric focusing (HIEF) with an immobilized pH-gradient (IPG) and added carrier ampholytes (CA) (Altland et al. 1986).

Material and methods

Neuraminidase treated serum from 1496 healthy unrelated blood donors from Southern Germany and from 31 families were investigated. All samples were typed first by agarose isoelectric focusing and immunofixation as previously described (Leifheit et al. 1985). Gels were stained with silver nitrate. Questionable phenotypes were examined by IEF with immobilized pH-gradients and subsequent detection by immunoblotting.

Gel preparation

Polyacrylamide gels (T = 5%; C=3%) of 0.5 mm thickness were prepared with an immobilized pH-gradient from pH 5.25 - 6.75 (LKB Appl. Note 324, 1984). After washing and drying the gels were reswelled in destilled water containing 0.5 % carrier ampholytes (pH 5-8).

Isoelectric focusing (IEF)

IEF was carried out for 5 hrs as follows:
1 hr at 600 V; 2 hrs at 5 W; 2 hrs at 25 W. 10 µl samples were applied with a silicon application strip to the cathodal side of the gel. As electrode solutions destilled water was used. The cooling temperature was 10 °C.

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Blotting

For protein transfer nitrocellulose membrane filter (0.45 µm, Schleicher and Schüll) was placed on the gel surface followed by 5 layers of filterpaper (Whatman N° 1), a glass plate and 2 kg weight for 30 min. Subsequently the NC-membrane was washed three times for 10 min in 5 % skim milk-powder in 0.1 m PBS, pH 7.3.

As first antibody a monospecific FXIIIB-antiserum from rabbit (Behring) was used at a dilution of 1 : 1000, incubation for 5 hrs at room temperature. The subsequent washing procedure was as described above.

As second antibody a peroxidase conjugated anti-rabbit IgG antiserum (Sigma) was applied. At a dilution of 1 : 2.000 the incubation time at room temperature was 2 hrs, again followed by the washing procedure.

For the development of the bands with chlornaphthol reaction the NC- membrane was soaked in a solution containing 49 ml 0.1 m PBS, pH 7.3; 30 mg 4-chlor-1-naphthol in 10 ml methanol and 1 ml of 0.06 % H_2O_2.

Results and discussion

Figure 1 presents FXIIIB-phenotypes by agarose IEF and immunofixation. The dried Coomassie stained gel was subsequently silver stained. This very sensitive staining procedure is recommanded in particular for FXIIIB typing of older serum or unconcentrated plasma samples.

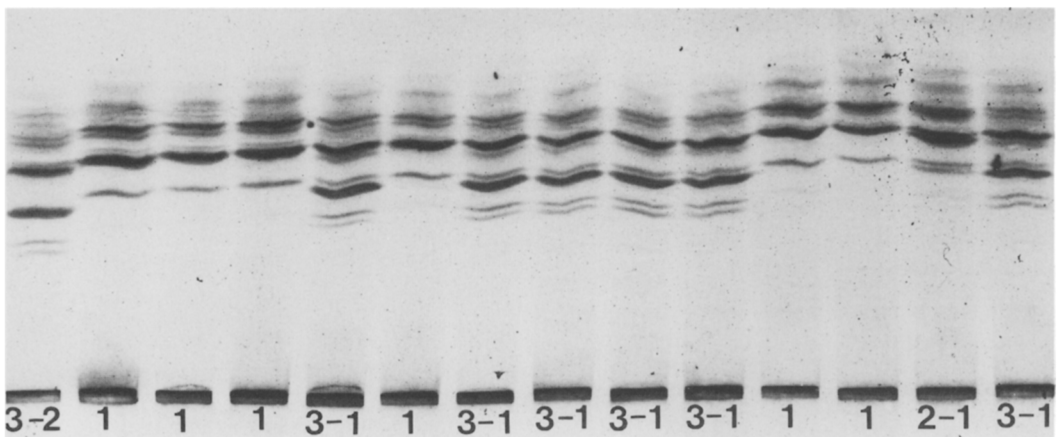

Fig. 1: Immunofixation agarose IEF and visualisation by combined Coomassie and silver stain. Anode on top.

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An improved resolution of FXIIIB is obtained by HIEF with IPG and CA. After immunoblotting the bands were obtained by chlornaphtol reaction.

Figure 2 shows the separation with a high degree of resolution. The method of visualisation is very sensitive; the consumption of monospecific antiserum is economical.

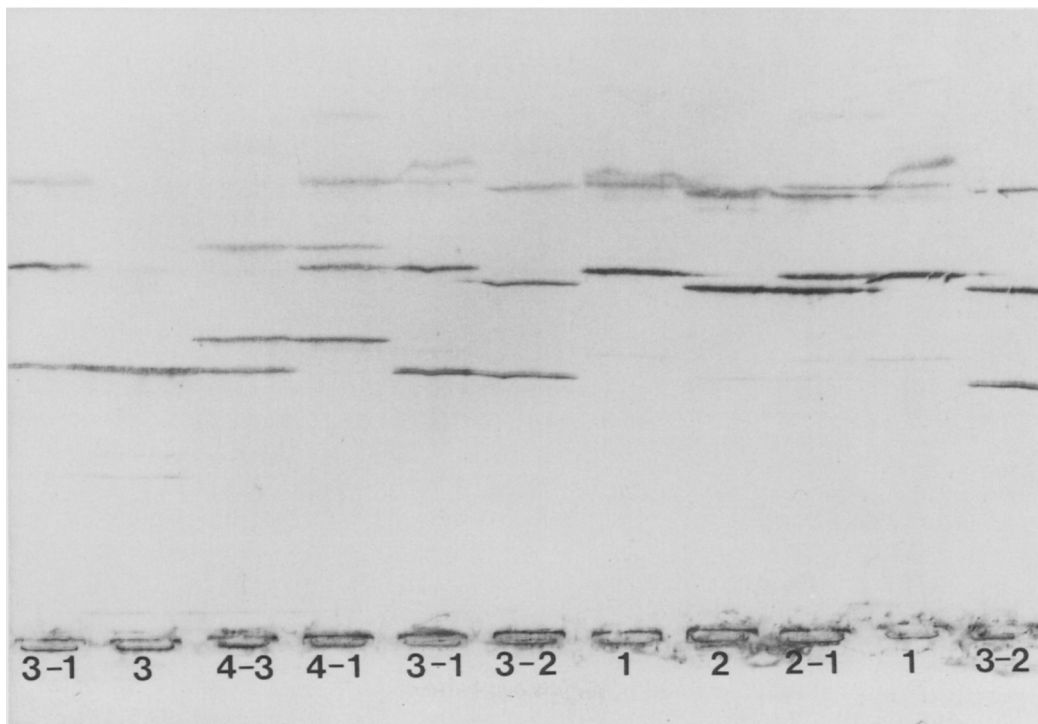

Fig. 2: Hybrid isoelectric focusing with IPG and CA and visualisation by immunoblotting. Anode on top.

Table 1 shows the distribution of FXIIIB-phenotypes and allele frequencies found in a sample of 1496 blood donors of the Blood Transfusion Center of the Bavarian Red Cross. The gene product of FXIIIB*4 was found repeatedly in the combination of B 4-1 and B 4-3. The expected types FXIIIB 4-2 and the homozygous type FXIIIB 4 were not observed yet.

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Table 1: Distribution of FXIIIB phenotypes and allele frequencies in Southern Germany

Phenotypes	observed		expected		allele frequencies
	n	%	n	%	
FXIIIB 1	861	57.55	864.8	57.81	FXIIIB*1 = 0.7603
2-1	202	13.50	191.8	12.82	
2	9	0.60	10.6	0.71	FXIIIB*2 = 0.0843
3-1	347	23.20	348.9	23.33	
3-2	32	2.14	38.7	2.59	FXIIIB*3 = 0.1534
3	39	2.61	35.2	2.35	
4-1	4	0.27	4.6	0.30	FXIIIB*4 = 0.0020
4-3	2	0.13	0.9	0.06	
Total	1496	100.0	1495.5	99.97	

$Chi^2 = 3.8036$

The value of theoretical exclusion rate for non-fathers is calculated to be 22.7 %.

In table 2 the FXIIIB-allele frequencies in various populations are demonstrated. The allele frequencies of the caucasien populations (Europe, USA, Australia) shows close agreement. In comparison the allele frequencies in Blacks and Mongoloids are extremely different. The frequencies for B*1 are high in Whites and low in Blacks, Asians and American Indians. In Japanese populations (Nakamura et al. 1986) the value of FXIIIB*3 is elevated. The same tendency is observed in Amerindians (Miller et al. 1985). A high FXIIIB*2 frequency is obtained in US- Blacks from Minnesota (Miller et al. 1985).

The limited data available at the present time permit already the conclusion that this system should be very useful for anthropological studies.

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Table 2: Comparison of FXIIIB allele frequencies in several populations

Population (author)	n	B*1	B*2	B*3	B*4	Var
			allele frequencies			
Australia (Board 1980)	245	0.7469	0,0836	0,1693	-	-
Japan						
(Nakamura et al. 1986)	435	0.2977	0.0184	0.6805	-	++
USA						
(Miller et al. 1985)						
Whites	328	0.776	0.088	0.136	-	-
Blacks	178	0.286	0.635	0.079	-	-
Amerindians	88	0.500	0.034	0.466	-	-
Norway						
(Olaisen et al. 1983)	283	0.69	0.15	0.16	-	-
Germany						
NRW (Mauff et al.1983)	334	0.7290	0.1033	0.1632	-	+
Hessen						
(Kühnl et al. 1983)	500	0.73	0.09	0.17	+	+
Bavaria						
(this study 1987)	1496	0.7603	0.0843	0.1534	0.002	-

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