GENETIC FXIIIB-VARIANTS DEMONSTRATED BY ISOELECTRIC FOCUSING ON AGAROSE GELS

Leifheit H-J[1], Gathof AG[1], Cleve H[2]

1 Blood Service Center, Bavarian Red Cross, Munich, FRG *

2 Institute of Anthropology and Human Genetics, University of Munich, FRG

INTRODUCTION

The polymorphism of the B-subunit of coagulation factor XIII was first described by Board in 1981 who used agarose gel electrophoresis with subsequent immunofixation (1). He proposed a three-allele model which has been confirmed by other authors (4, 5, 7, 9, 10).

Isoelectric focusing (IEF) of FXIIIB is an improvement over electrophoresis because a clearer separation of the individual phenotypes is obtained with this method.

Agarose is used as a carrier medium due to the following advantages. It is a non-toxic medium, the gels are easier to prepare and less expensive, the separation time is short because the pH-gradient is established within 20 min, immunofixation can be done directly on the gel and there is no limitation by gel pore size for large proteins to penetrate the gel.

The analysis of FXIIIB by IEF on agarose we described earlier (5). In this communication further results are presented: The phenotype of FXIIIB 2 is demonstrated which corresponds to the phenotype predicted by us (5). In addition, the rare variants FXIIIB 4-1 and 4-3 are shown as well as an unusual phenotype named FXIIIB 1-D (D = deficiency).

* Herzog-Heinrich-Straße 2, D-8000 München 2

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MATERIAL AND METHODS

The sera from 633 unrelated healthy blood donors from Southern Germany were examined, whereby the sample described previously (5) is included. Neuraminidase was used to treat the sera. Each 50 µl sample was incubated with 0.04 U enzyme for 2 hrs at 37 °C.

The agarose gels have a thickness of 0.5 mm and contain 0.8 % agarose IEF (Pharmacia), 0.8 % ACES and 10 % sorbitol dissolved in 18.8 ml distilled water. After degasing the gel solution were cooled to 75 °C then the ampholytes (LKB) were added (10 ml pH 5 - 8 and 0.2 ml pH 4 - 6.5). At a cooling temperature of 8 °C prefocusing is carried out for 30 min. 8 µl of the sample are applied to the cathodal side. The salts are then removed at 250 V for 30 min. Focusing is performed for 150 min at a setting of 1400 V, 20 mA, 10 W.

Immunofixation is done with 0.5 ml of a 1 : 2 diluted FXIIIB-antiserum (Behring) for 90 min at 37 °C.

After pressing, washing and drying the gel was stained with 0.5 % coomassie blue solution.

RESULTS AND DISCUSSION

Fig. 1 and Fig. 2 show the subunit B phenotyping of neuraminidase treated sera after isoelectric focusing and subsequent immunofixation.

In the homozygous phenotypes FXIIIB 1 and B 2 two main bands are observed. The bands of B 2 are slightly displaced towards the cathode. The phenotype B 3 is characterized by a single cathodal main band.

The heterozygous phenotypes are combinations of the homozygous phenotypes. B 2-1 is resolved in to a clear double

band pattern. This resolution can be used also as an indicator for the quality of the separation.

The rare phenotypes B 4-1 shows one band in the corridor between the type 1 and 3, the other band between the two B 1 bands.

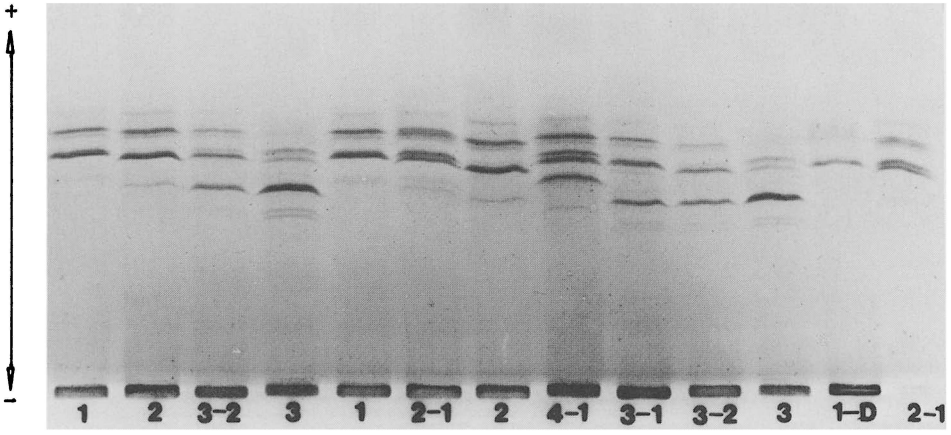

Fig. 1: Phenotypes of FXIIIB as attained by IEF on agarose followed by immunofixation. The alleles B*1, B*2 and B*3 determine six phenotypes; in addition B 1-D and B 4-1 are shown.

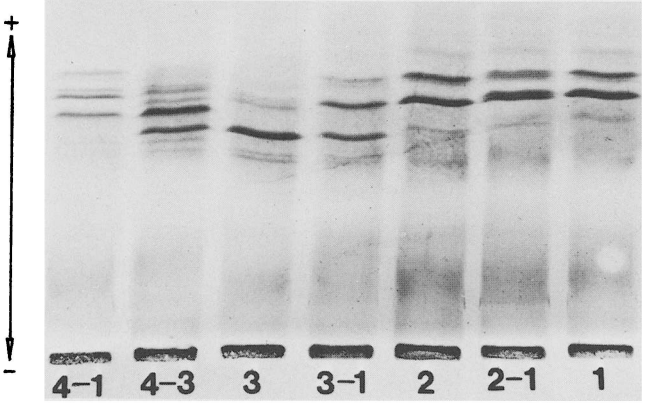

Fig. 2: Presentation of the rare phenotype FXIIIB 4-3 as resolved by IEF on agarose.

The phenotype B 4-3 demonstrated in Fig. 2 shows a combination of the B 3- and the B 4-pattern.

The incomplete phenotype we named provisionally FXIIIB 1-D shows a deficient anodal band.

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

Phenotypes	observed		expected		Allele frequencies
	n	%	n	%	
FXIIIB 1	371	58.61	370.2	58.48	FXIIIB 1 = 0.7647
2-1	94	14.85	90.2	14.25	
2	5	0.79	5.5	0.87	FXIIIB 2 = 0.0932
3-1	131	20.70	136.0	21.49	
3-2	14	2.21	16.6	2.63	FXIIIB 3 = 0.1405
3	16	2.52	12.5	1.98	
4-1	1	0.16	1.5	0.25	FXIIIB 4 = 0.0016
4-3	1	0.16	0.3	0.05	
Total	633	100.00	632.8	100.00	

$\Sigma X^2 = 0.0793$, df = 2; $0.3 > p > 0.2$

Table 1 presents the distribution of FXIIIB-phenotypes and the allele frequencies of 633 unrelated blood donors from Southern Germany. The theoretical exclusion rate is calculated to be 18.07 %.

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