Coagulation Factor XIIIA and XIIIB polymorphisms in the North of Germany (Mecklenburg area)

H.Schmidt, J.Rummel and R.Wegener

Institut für Gerichtliche Medizin der Wilhelm-Pieck-Universität Rostock, Friedrich-Engels-Straße 108, 2500 Rostock, DDR

INTRODUCTION

Coagulation factor XIII consists of two A and two B subunits and circulates in plasma and thrombocytes. The A subunit has transglutaminase activity forming cross links between fibrin monomers in the final step of coagulation. Both subunits show geneticly determined polymorphisms described first by Board (1979,1980). Subsequent investigations in Japanese,European and American populations identified two common alleles in the FXIIIA and three common alleles in the FXIIIB-system.

MATERIALS AND METHODS

FXIIIA polymorphism was studied on plasma or thrombolysates. For FXIIIB phenotyping plasma or serum samples were treated with Neuraminidase. Agarose gel electrophoresis (AGE) with subsequent passiv immunoblotting was carried out mainly according to the method of Olaisen et al. (1983) and Hjalmarson (1986). Polyacrylamide gel isoelectric focusing (PAGIF) was conducted on 0.35 mm thin gels (T=4,6%,C=3%) with 3% carrier ampholytes and 12% glycerine. For FXIIIA typing a mixture of 0.4 ml Servalyt 5-7 and 0.4 ml Servalyt 5-8 was used. The anode- and cathode solutions were Anode fluid 3 and Cathode fluid 10 (Serva). Samples were applied on 8x5 mm Whatman 3 papers at the cathodal side. For FXIIIB typing a mixture of 0.6 ml Pharmalyte 4-6.5 and 0.2 ml Servalyt 5-7 was employed. Samples were applied on 10x5 mm Whatman 3 paper strips at the anodal side. Focusing was carried out at 2000V, 8 W and 10 mA at 10°C for 4 hrs. including 40 min. prefocusing.

Transfer to NC-membranes (Schleicher & Schuell) was conducted by semidry electroblotting in 25 mM Tris, 192 mM Glycine, 20% Methanol buffer for 1 hr. at 0.8 mA/cm². After transfer NC-membranes were blocked for 2 hrs. in PBS-Tween. Clotimmun FXIIIA/S (Behring Werke) was used in a 1:400 dilution as first antibody. After a washing procedure in PBS-Tween the membranes were soaked in a 1:800 dilution of POD conjugated anti rabbit globulin for 4 hrs. Visualisation of banding patterns was performed in a mixture of 20 mg 3 Amino 9 ethylcarbazole (Sigma), 50 ml PBS and 30 µl 30% H_2O_2.

Advances in Forensic Haemogenetics 3
Edited by H. F. Polesky and W. R. Mayr

RESULTS AND DISCUSSION

Both AGE followed by immunoblotting technique and PAGIF followed by semidry electroblotting are useful for FXIIIA and FXIIIB phenotyping. There are no differences in banding pattern of FXIIIA detectable by these methods. In table 1 the FXIIIA phenotype distribution and gene frequencies are demonstrated.

Table 1: FXIIIA phenotypes and allele frequencies in Northern Germany (Mecklenburg area)

Phenotypes	observed	expected	allele frequencies
FXIIIA 1	201	200.82	FXIIIA*1 = 0.7803
2-1	111	111.55	FXIIIA*2 = 0.2166
2	16	15.50	FXIIIA*3 = 0.0015
3-1	1	0.77	FXIIIA*4 = 0.0015
4-1	1	0.77	
Total	330	329.51	Chi^2=0.193 0.9<p<0.95 2df

In table 2 the FXIIIB phenotype distribution and gene frequencies in a population sample of Northern Germany are presented.

Table 2: FXIIIB phenotypes and allele frequencies in Northern Germany (Mecklenburg area)

Phenotypes	observed	expected	allele frequencies
FXIIIB 1	209	209.17	FXIIIB*1 = 0.7676
2-1	47	42.18	FXIIIB*2 = 0.0774
3-1	80	84.40	FXIIIB*3 = 0.1594
2	0	2.12	
3-2	8	8.51	
3	11	8.52	
Total	355	354.90	Chi^2=3.430 0.5<p<0.7 5df

Gene frequencies of both systems are in accordance to other Caucasoid populations. The single exclusion chance for nonfathers is calculated to be 14.0% in FXIIIA- and 19.5% in FXIIIB-system.

In Fig. 1 the FXIIIB banding patterns by PAGIF and subsequent semidry electroblotting are illustrated. From left to right:

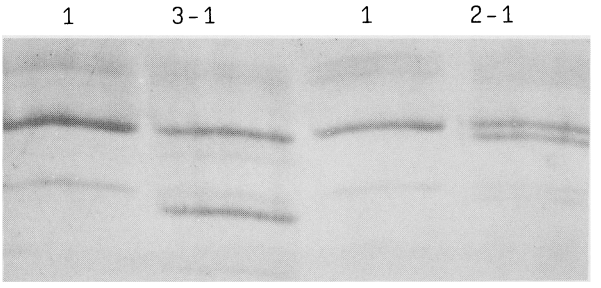

Fig. 1: FXIIIB phenotypes by PAGIF and semidry electroblotting, visualisation by POD development.

REFERENCES

Board PG (1979) Genetic polymorphism of the subunit A of human coagulation factor XIII. Am J Hum Genet 31: 116-124

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

Olaisen B, Siverts A, Gedde-Dahl T, Jonassen R, Teisberg P (1983) Coagulation factor XIIIA and -B polymorphisms. Proc 10th Intern Congr Soc Forensic Haemogenetics München 1983 p 459-465

Hjalmarsson K (1986) Coagulation factor XIIIB polymorphism in Sweden. In: Mayr WR (ed) Advances in Forensic Haemogenetics 2. Springer, Berlin Heidelberg New York, p 179-183