

Study of Genetic Polymorphism of Coagulation Factor XIII B in a Italian Population Sample using PAGIEF and Semi-dry Electroblothing

R. Giorgetti, A. Tagliabracci

Istituto di Medicina Legale e delle Assicurazioni dell'Università di Ancona, Ospedale Regionale, I-60020 Torrette, Italy

INTRODUCTION

Coagulation factor XIII (fibrin-stabilizing factor) shows a quadrimeric structure (2A and 2B subunits) in plasma. No enzymatic activity has been described for the B subunit, which seems to act only as a plasma carrier.

Genetic polymorphism of both subunits were investigated first in Australians by Board (1979, 1980) with electrophoresis in agarose gel and immunofixation. Later the B subunit polymorphism was also investigated in other population samples, while new methods were progressively introduced (Mauff et al. 1983; Olaisen et al. 1983; Pytlik et al. 1984; Leifheit et al. 1985; Kuhl and Spielmann 1985; Hjalmarsson 1985; Dykes et al. 1985; Nakamura and Abe 1985; Nakamura et al. 1986; Leifheit et al. 1987). These investigations have provided further information about the factor XIII B system that was suggested as being determined by three common alleles in a single autosomal locus: F-XIII B 1, F-XIII B 2 and F-XIII B 3. Some rare variants have also been described.

This study is the first conducted on a Italian sample and uses some technical modifications to current methods in order to display the F-XIII B bands more rapidly and clearly.

MATERIALS AND METHODS

297 EDTA plasma samples were obtained from healthy blood donors living in Ancona. Neuraminidase treatment and isoelectric focusing were performed as described by Nakamura and Abe (1985) with slight modifications (for example, a 10 U/ml concentration of neuraminidase was used). Proteins were transferred to nitrocellulose sheets (0,45 µm LKB) by semi-dry electroblotting (nova blot LKB). The transfer was carried out, as suggested in the LKB technical note, for 45 min. at 0.8 mA/cm² without refrigeration.

The enzyme immunoassay was performed as follows:

- blocking 120 min. in BSA-TBS 3% + Tween 20 0.05%.
- washing 2 x 30 min. in TBS-Tween 20 0.15% for 60 min.
- rabbit antihuman factor XIII subunit S (Behring) diluted 1:1000 in TBS-Tween 20 0.15%.
- washing as above.
- goat antirabbit immunoglobulin, heavy and light chain specific, horseradish peroxidase conjugated (Calbiochem) diluted 1:2000 in TBS-Tween 20 0.15% for 60 min.
- washing as above.
- development with a solution of 10 mg of 3-3' diaminobenzidine in 40 ml TBS and 40 µl 10% H₂O₂. The reaction was stopped with running tap water.

RESULTS

The revealed genic frequencies are shown in Table 1. F-XIII B phenotype pattern obtained by PAGIEF and semi-dry electroblotting is shown in Fig. 1.

Table 1. Distribution of F-XIII B phenotypes in Ancona

Phenotype	Observed	%	Expected	Gene frequency
1 - 1	172	57.91	171.23	F-XIII B*1= .7593
2 - 2	2	0.67	1.56	
3 - 3	8	2.70	8.41	F-XIII B*2= .0724
1 - 3	76	25.59	75.91	
2 - 3	8	2.69	7.24	F-XIII B*3= .1683
1 - 2	31	10.44	32.65	
Total	297	100.00	297.00	

$$\Sigma\chi^2 = 0.311 \quad df = 3 \quad P > 0.95$$

Rare allotypes were not seen. The observed phenotypes do not present any meaningful deviation from the Hardy-Weinberg equilibrium.

The Italian population sample shows some significant differences from other European countries. In particular Italians have a higher frequency of F-XIII B 1 and a lower frequency of F-XIII B 2 while F-XIII B 3 seems almost identical to other Caucasian populations.

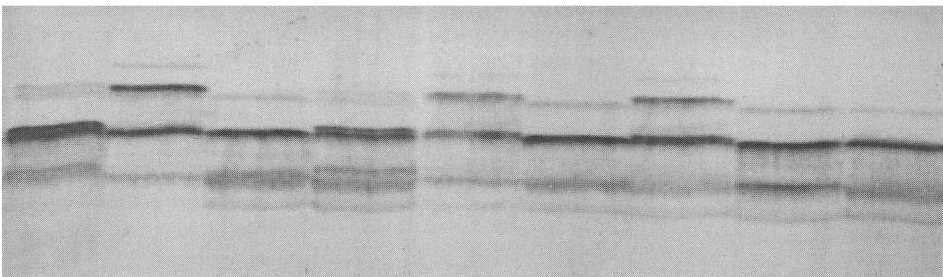


Fig. 1. F-XIII B phenotypes after PAGIEF in the pH range 3.5-9.5 and semi-dry electroblotting with enzyme immunoassay. Cathode is at the top. From left to right: 1-2, 1-3, 1-1, 1-2, 3-3, 1-1, 1-3, 1-1, 1-1.

REFERENCES

- Board P G (1979) Genetic polymorphism of the A subunit of human coagulation factor XIII. *Am J Hum Genet* 31: 116-124
- Board P G (1980) Genetic polymorphism of the B subunit of human coagulation factor XIII. *Am J Hum Genet* 32: 348-353
- Dykes D D, Miller S, Polesky H (1985) Gene frequency distribution of 13A and 13B in U.S. Whites, Blacks, Amerindians and Mexicans-Americans. In: Brinkmann B and

- Henningsen K Proc 11th Int Congress of the Society for Forensic Haemogenetics, Springer-Verlag, Berlin Heidelberg New York Tokyo, p 261
- Hjalmarsson K (1985) Coagulation Factor 13B in Sweden. In: Brinkmann B and Henningsen K Proc 11th Int Congress of the Society for Forensic Haemogenetics, Springer-Verlag, Berlin Heidelberg New York Tokyo, p 257
- Kuhnl P, Spielmann W (1985) F 13 B*10, ein neues Allel im System der Untereinheit B des Gerinnungsfaktors 13. In: Brinkmann B and Henningsen K Proc 11th Int Congress of the Society for Forensic Haemogenetics, Springer-Verlag, Berlin Heidelberg New York Tokyo, p 151
- Leifheit H J, Gathof A G, Cleve H (1985) Genetic F-XIII B - Variants demonstrated by isoelectric focusing on agarose gels. In: Brinkmann B and Henningsen K Proc 11th Int Congress of the Society for Forensic Haemogenetics, Springer-Verlag, Berlin Heidelberg New York Tokyo, p 146
- Leifheit H J, Gathof A G, Cleve H (1987) Improved F XIII B phenotyping by isoelectric focusing with immobilized pH gradients. In: Mayr W R Proc 12th Int Congress of the Society for Forensic Haemogenetics, Springer-Verlag, Berlin Heidelberg, New York London Paris Tokyo, p 207
- Mauff G, Schilling A, Pulverer G (1983) Coagulation factor XIII: determination of the genetic polymorphism of subunits B by immunoblotting in a west German population sample. In: Proc 10th Int Congress of the Society for Forensic Haemogenetics, Schmitt & Meyer, Wurzburg, p 453
- Nakamura S, Abe K (1985) Genetic polymorphism of human factor I (C3b inactivator) . Hum Genet 71:45-48
- Nakamura S, Ohue O, Abe K (1986) Genetic polymorphism of coagulation factor XIII B subunit in the Japanese population: description of three new alleles. Hum Genet 73: 183-185
- Olaisen B, Sieverts A, Gedde-Dahl T, Jonassen R, Teisberg P (1983) Coagulation factor 13 A and B polymorphisms. In: Proc 10th Int Congress of the Society for Forensic Haemogenetics, Schmitt & Meyer, Wurzburg, p 459
- Pytlík Ch, Gansz A, Brinkmann B (1984) Zur forensischen Anwendung des Polymorphismus des Gerinnungsfaktors XIII B. Z. Rechtsmed, 93: 175-180