Coagulation Factor 13B Polymorphism in Sweden.

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Introduction

Human blood coagulation factor 13, the fibrin stabilizing factor, is a zymogen which after activation by thrombin and calcium ions catalyses the formation of γ-glutamyl-ε-lysine cross links between fibrin molecules (Losowsky and Miloszewski, 1977). Factor 13 consists of two A subunits and two B subunits (A_2B_2) (Schwartz et al 1973). Both A and B subunits are found in plasma but only B subunits in serum. The B subunit has no enzymatic activity and may serve as a carrier molecule in plasma (Schwartz et al 1973).

Electrophoretic polymorphisms of both A and B subunits have been described by Board (1979, 1980). Recently Olaisen et al (1983) have presented an enzyme-linked immunoblotting technique which improves the method for phenotyping products of the factor 13B locus after high voltage agarose electrophoresis.

The purpose of this investigation is to present the distribution of factor 13B phenotypes and gene frequencies from unrelated adult Swedes. Two rare alleles have been observed. A Swedish mother/child material is also presented.

Material and methods

Serum samples from 1500 unrelated Swedish adults and a mother/child material consisting of 455 pairs were investigated. All persons were involved in paternity cases.

After arrival at the laboratory the serum was separated from the blood cells by centrifugation and stored at +4°C. Factor 13B was typed in desialized serum samples (neuraminidase treatment) and in some cases in untreated serum samples. The phenotypes were determined twice or more.

The determination of factor 13B phenotypes was carried out by high voltage agarose electrophoresis followed by enzyme-linked immunoblotting technique mainly according to Olaisen et al (1983). 1.6 mm thick gels 0.5 % in agarose (Seakem ME), were cast onto glass plates 12 x 26 cm. The electrode buffer consisted of 186.6 mM Tris/374.3 mM glycine/ 31.8 mM Na-barbiturate/5.6 mM barbituric acid with pH 8.9. The gel buffer was diluted 1:4 with the electrode buffer. 10 ul serum or neuraminidase treated serum was applied on the cathodic side of the gel. The electrophoresis was performed for 2 h at maximum settings of 600V, 80 mA and 40W. The proteins were then transferred to nitrocellulose sheets (BIORAD) by passive blot for about 20 minutes.

Visualization of factor 13B bands was achieved by soaking the nitrocellulose sheets in rabbit anti factor 13B (Clotimmun-factor XIIIS, Behringwerke, Marburg) diluted 1:500 with 0.15 % Tween in PBS overnight washed 2 h with 0.15 % Tween in PBS, followed by a peroxidase conjugated goat anti rabbit antiserum (Behringwerke, Marburg) diluted 1:700 with 0.15 % Tween in PBS for 4 h and finally washed 1 h with 0.15 % Tween in PBS.

The development was performed in 50 ml of 0.1 M Tris-HCl, pH 7.5 with 35 mg 4 chloro-1-naphtol (BIORAD), 10 ml ethanol and 20 μl H_2O_2. The reaction was stopped with water.

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Results and discussion

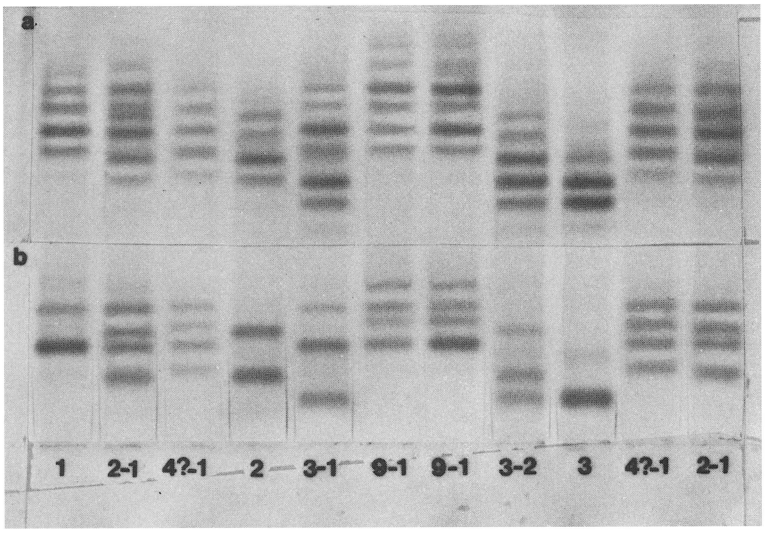

Fig. 1. Coagulation factor 13B patterns in serum revealed by high voltage electrophoresis and immunoblotting procedures. a and b show untreated and neuraminidase treated samples, respectively.

Table I. Distribution of F13B phenotypes in Sweden

Phenotype	Observed n	%	Expected n	%	Gene frequency
1	829	55.27	829.63	55.30	$F13B^1$ =0.7437
2-1	229	15.27	233.60	15.57	
2	20	1.33	16.44	1.10	$F13B^2$ =0.1047
3-1	336	22.40	332.43	22.16	
3-2	45	3.00	46.80	3.12	$F13B^3$ =0.1490
3	33	2.20	33.30	2.22	
1-rare	8*	0.53	6.02	0.40	$F13B^{rare}$=0.0027
2-rare	0	0	0.85	0.06	
3-rare	0	0	1.21	0.08	
rare	0	0	0.01	0.00	
Total	1500	100.00	1500.29	100.01	

χ^2 = 0.973 $0.80 < p < 0.90$ 3 d.f.

*Totally eight rare variants of which six probably are of the type 4-1. The other two are tentatively classified as 9-1.

The distribution of F13B phenotypes and gene frequencies in the population of Sweden are presented in table I and figure 1. The population was in Hardy-Weinberg equilibrium with a chi-square = 0.973, $0.80<p<0.90$ at 3 d.f.

Except the three common alleles with the six corresponding phenotypes eight rare phenotypes with two rare alleles involved were developed (see table I and figure 1). One of them is probably the type 4 variant which migrates between the type 2 and type 1 allele products. This type was reported by Kreckel et al (1983). The other rare allele has an electrophoretically migration in a position anodal to the type 1 allele product. This allele has been observed in a Swedish mother/child combination (see table II) as well as in another unrelated Swedish woman. To my knowledge this variant has not been described so far and the allele was therefore tentatively designated F13B*9.

F13B typing of 455 mother/child combinations is shown in table II. Unexpected combinations were not found. The data are in full accordance with the postulated genetic model of an autosomal locus with several codominant alleles. (χ^2=8.4, 0.90<p<0.95 15 d.f.)

The gene frequencies of F13B in Sweden are similar to those found in other European countries (Kreckel and Kühnl 1982; Mauff et al 1983; Olaisen et al 1983). The F13B-system is informative. The method of determination is sensitive and gives excellent band visualization. The classification results are highly reproducible. The system appears to be useful for both population studies and cases of disputed paternity.

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Table II. Distribution of 455 mother/child combinations in Sweden

Mother		Child									
		1	2-1	2	3-1	3-2	3	1-rare	2-rare	3-rare	rare
1	obs	203	22	-	37	-	-	0	-	-	-
	exp	187.16	26.35	-	37.50	-	-	0.70	-	-	-
2-1	obs	22	41	1	0	4	-	0	0	-	-
	exp	26.35	30.06	3.71	5.28	5.28	-	0.10	0.10	-	-
2	obs	-	5	0	-	1	-	-	0	-	-
	exp	-	3.71	0.52	-	0.74	-	-	0.01	-	-
3-1	obs	33	4	-	38	8	7	0	-	0	-
	exp	37.50	5.28	-	45.01	5.28	7.51	0.14	-	0.14	-
3-2	obs	-	6	0	4	1	4	-	0	0	-
	exp	-	5.28	0.74	5.28	1.80	1.06	-	0.02	0.02	-
3	obs	-	-	-	9	1	2	-	-	0	-
	exp	-	-	-	7.51	1.06	1.50	-	-	0.03	-
1-rare	obs	1	0	-	0	-	-	1	0	0	0
	exp	0.68	0.10	-	0.14	-	-	0.68	0.10	0.14	0.00
2-rare	obs	-	0	0	-	0	-	0	0	0	0
	exp	-	0.10	0.01	-	0.02	-	0.10	0.01	0.02	0.00
3-rare	obs	-	-	-	0	0	0	0	0	0	0
	exp	-	-	-	0.14	0.02	0.03	0.14	0.02	0.03	0.00
rare	obs	-	-	-	-	-	-	0	0	0	0
	exp	-	-	-	-	-	-	0.00	0.00	0.00	0.00

$\chi^2 = 8.4$ $0.90 < p < 0.95$ 15.d.f.