Identification of F13A*7 as a Population Specific Marker

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INTRODUCTION

Factor XIII(F13), a precursor of fibrinoligase, is a tetramer with 2A and 2B subunits. F13B functions as a carrier and regulator of the enzymatically active F13A subunit. The two most common alleles are F13A*1 and F13A*2 with all other alleles appearing as rare or uncommon in most population groups reported in the literature.(Board, 1979; Olaissen et al., 1983; Dykes et al., 1986). Ten different genetic variants of F13A have been reported using isoelectric focusing (IEF) techniques (Dykes et al, 1988). F13A can only be detectable in plasma samples, therefore it cannot be used in blood stain analysis. In parentage testing the exclusion probability of F13A=0.15. For this study we tested F13A on Whites and Blacks using IEF separation detected by immunoblotting. This report identifies the allele F13A*7 to be more specific for Black populations.

METHODS

Blood samples for parentage testing submitted to Analytical Genetic Testing Center were tested for GM, KM, ACP1, PGM1, ESD, GLO1, GC, PLG, F13A, F13B and 3-4 DNA probes. Plasma samples for F13A phenotyping were tested by isoelectric focusing on agarose gels at a pH range 4-7. Nytran was used for blotting gels and bands were detected by ALP conjugated antibodies, Table 1.

RESULTS

F13A phenotyping by IEF followed by ALP immunoblotting, in our study, permitted us to identify seven of the ten documented variants. Figure 1 shows a membrane after detection. As can be seen the F13A*7 gene product focuses slightly anodal to F13A 2. Figure 2 shows a diagram of the ten variants. The phenotyping results on 1,527 Whites and 975 Blacks are seen in Table 2. Except for F13A*7, all rare variants were simply classified as "V" for statistical purposes.

Gene frequency distribution of F13A demonstrated very similiar frequencies for both F13A*1 and F13A*2. In contrast the frequency for F13A*7 was 0.0067 in Blacks and 0.0003 in whites. We also identified more rare variants in Blacks (0.0026) versus Whites (0.0003). Although the data indicates a similiar exclusion probability for both populations, the gene frequency data tends to support the presence of F13A*7 as a Black marker.

Advances in Forensic Haemogenetics 3
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TABLE 1. IEF TECHNIQUE FOR F13A PHENOTYPING

Agarose IEF (Pharmacia)		0.225 gm
Sucrose		2.7 gm
LKB Ampholyte 5-7		0.8 ml
LKB Ampholyte 4-6.5		0.8 ml
Electrolytes:	Anode	1.0 M H_3PO_4
	Cathode	0.2 N NaOH
Sample Wicks		Whatman # 1 (4X4mm)
Focusing:	Volts:	2000
	Milliamps	unlimited
	Watts	5 (15 minutes) remove wicks
		7 (15 minutes)
		9 (15 minutes)
		10 (15 minutes)

1. Passively blot to Nytran, 5 X 20 cm, presoaked in TBS (20mM Tris-HCl, pH 7.5, 0.5 M NaCl). Place Nytran on gel, cover with piece of filter paper presoaked in TBS and 1 cm of paper towels. Blot for 30 minutes.

2. Air dry

3. Block membrane 20-30 minutes with 1% HiPure Gelatine, 5% Tween-20 and 0.25% Hammarsteen Casein in TBS.

4. To membrane add 10-15 ml of 1:500 diluted antibody 1(rabbit anti-human F13A) in block solution. Gently agitate for 60 minutes at room temperature.

5. Wash membrane 3 X 5 minutes in TBS.

6. To membrane add 10-15 ml of 1:1000 diluted antibody 2 (ALP conjugated goat anti-rabbit IgG) in block solution. Gently agitate for 60 minutes at room temperature.

7. Wash membrane 3 X 5 minutes in TBS.

8. Place membrane in clean dish with ALP substrate mixture:

   50 ml TBS (pH 9.1)
   25 ul NBT (75 mg in 700 ul DMF and 300 ul H_2O)
   75 ul BCIP (50 mg in 1 ml DMF)

   Place in dark at room temperature for 10-30 minutes.

9. Wash membrane briefly in tap water and blot dry in dark.

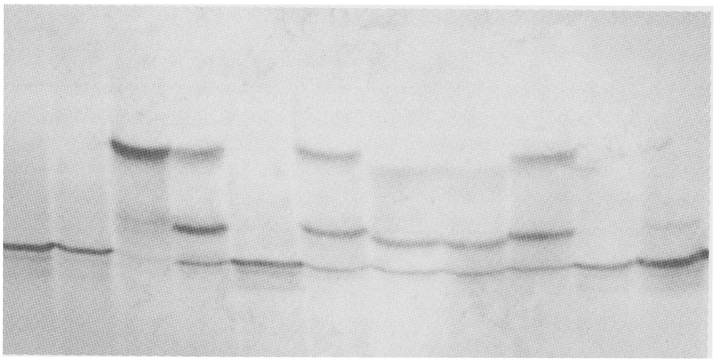

Fig. 1. F13A phenotypes blotted onto Nytran membrane. The cathode is on the top. From left to right: F13A 1, 1, 2, 1-2, 1, 1-2, 1-7, 1-7, 1-2, 1, 1.

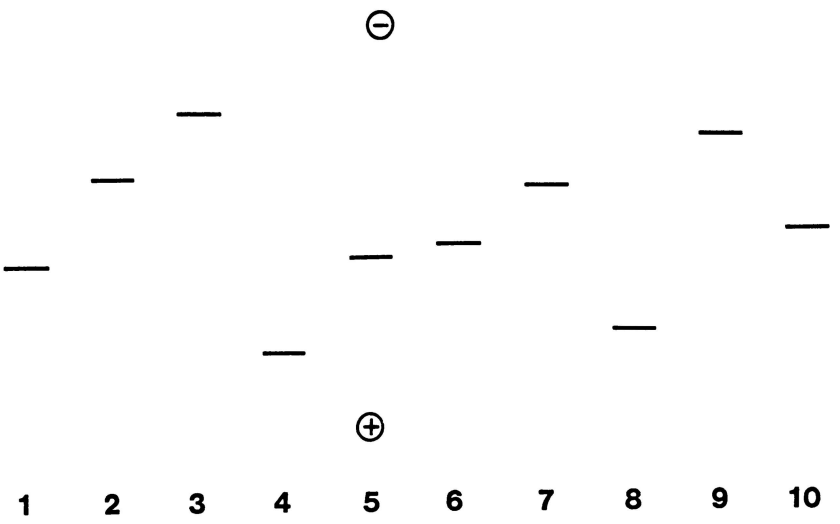

Fig. 2. Positions of F13A variants on IEF. The F13A 7 band is slightly anodal to the F13A 2 band.

TABLE 2. F13A PHENOTYPE AND GENE FREQUENCY DISTRIBUTION

Phenotypes			Gene Frequencies		
	White (No. 1527)	Black (No. 975)		White	Black
1	1001	636	F13A*1 =	0.8098	0.8062
1,2	469	285	F13A*2 =	0.1896	0.1846
2	55	37	F13A*7 =	0.0003	0.0067
1,7	1	11	V =	0.0003	0.0026
2,7	0	1			
1,V	1	5			
7,V	0	1			

Chi Square			
White	0.456	p >.99	(df6)
Black	1.379	.99 >p >.90	(df6)

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