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Genetic polymorphism of the B subunit of coagulation factor XIII in Libyans: Occurrence of a fourth common allele, FXIIIB*6

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Summary. FXIIIB phenotypes were determined in neuraminidase-pretreated serum samples by using isoelectric focusing in ultrathin-layer polyacrylamide gels containing 1 M urea and subsequent immunoblotting. In a Libyan population sample from Tripoli, ($n = 108$) nine different phenotypes as products of four common alleles were recognized, with frequencies as follows: FXIIIB*1 = 0.6574, FXIIIB*2 = 0.2454, FXIIIB*3 = 0.0741 and FXIIIB*6 = 0.0231. It is suggested that FXIIIB*6 is the fourth common allele of the FXIIIB system in this population.

Key words: Coagulation factor XIIIB, Genetic polymorphism in Libyans – Allele FXIIIB*6

Zusammenfassung. Der genetisch determinierte Polymorphismus des FXIIIB wurde durch isoelektrische Fokussierung in ultradünner Schicht eines Harnstoff-haltigen Polyacrylamidgels mit anschließendem Immunoblott dargestellt. Die Serumproben wurden vor der Verimpfung mit Neuraminidase behandelt. Bei Libyern aus Tripoli (n = 108) wurden die neun häufigen Phänotypen FXIIIB 1–1, 2–1, 3–1, 2–2, 3–2, 3–3, 6–1, 6–3 und 6–6 beobachtet. Folgende Allelfrequenzen wurden ermittelt: FXIII*1 = 0,6574, FXIIIB*2 = 0,2454, FXIIIB*3 = 0,0741 und FXIIIB*6 = 0,0231. Es wird vermutet, daß das Allel FXIIIB*6 das vierte, häufige (allgemeine) Allel in dieser Population darstellt.

Schlüsselwörter: Gerinnungsfaktor XIIIB, genetischer Polymorphismus bei Libyern – Allel FXIIIB*6

Introduction

The genetic polymorphism of the B subunit of human coagulation factor XIII (FXIIIB), which was originally described by Board [1] is known to be represented by three common autosomal codominant alleles FXIIIB*1, FXIIIB*2 and FXIIIB*3. Several rare alleles have been reported since Board's original publication: FXIIIB*4 [2], FXIIIB*5 [3], FXIIIB*6, FXIIIB*7, FXIIIB*8,

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FXIIIB*9 and FXIIIB*10 [4], FXIIIB*13, FXIIIB*14 and FXIIIB*15 [5], FXIIIB*16, FXIIIB*17, FXIIIB*18, FXIIIB*19, FXIIIB*20, FXIIIB*21 and FXIIIB*22 [6]. To the authors knowledge, the alleles FXIIIB*5, FXIIIB*7, FXIIIB*8, FXIIIB*9 and FXIIIB*10 have been reported, but the ethnic origin and gene frequencies were not given. Also, none of the reported alleles have been designated 11 or 12.

The present study deals with FXIIIB polymorphism in Libyans. The occurrence of a fourth common allele in this population is described.

Materials and methods

Samples

Sera used in this investigation ($n = 108$) were taken from the same population sample as was previously analysed for the genetic polymorphisms of alpha$_2$ HS-glycoprotein and orosomucoid [7, 8]. Neuraminidase treatment was performed by adding 10 µl enzyme 1 unit/ml to 20 µl serum, and the mixture was incubated at 37°C for about 18–24 h before analysis.

Isoelectric focusing

IEF was carried out using ultrathin-layer polyacrylamide gels of 0.2 mm thickness, containing 1 M urea, 12.5% sucrose, 3% ampholine, pH range 5–7, gel concentration (T) = 5%, and degree of cross-linkage (C) = 3%. Riboflavin was used for the polymerization. The electrode solutions were 1 M phosphoric acid for the anode and 0.2 M sodium hydroxide for the cathode. The maximum voltage was 1800 V with unlimited mA. After 40 min prefocusing, 5 µl neuraminidase-treated serum was applied near the cathode. The sample strips were removed after 30 min and the total focusing time was 3.5 h at 2°C.

Immunoblotting

This was done using a nitrocellulose membrane (Bio-Rad, 0.45 µm) for passive transfer of proteins, a 1:500 dilution in TRIS-buffered saline (TBS) for the first antibody; rabbit anti-human FXIIIB (Behring), and 1:800 dilution in TBS for the second antibody. The second antibody was peroxidase-conjugated goat anti-rabbit IgG (Cappel). Finally, the FXIIIB pattern was visualized using the following solution: 15 mg 4-chloro-l-naphthol dissolved in 1 ml acetone then mixed with 40 ml TBS and 30 µl 20% hydrogen peroxide.

Results and Discussion

Figure 1 illustrates the nine FXIIIB phenotypes observed, as revealed by isoelectric focusing in 1 M urea of desialysed serum with subsequent immunoblotting. The identified phenotypes corresponded to the three common alleles FXIIIB*1, FXIIIB*2, FXIIIB*3, and a fourth allele electrofocused slightly cathodal to the FXIIIB*2 allele. Comparative testing with reference samples in-

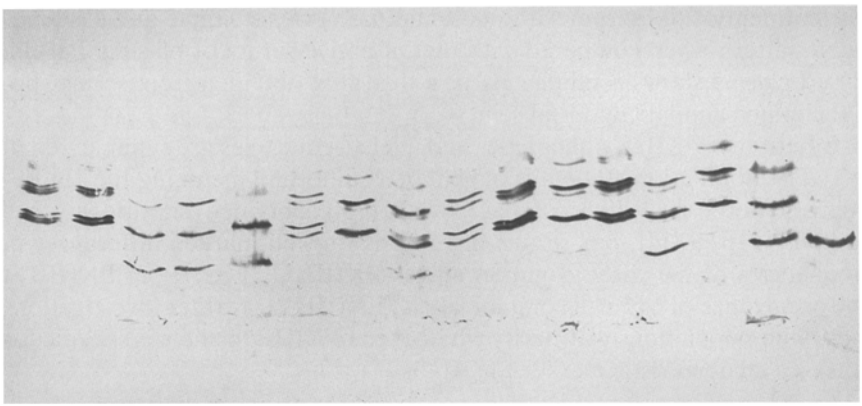

Fig. 1. Electrofocusing and immunoblotting pattern of various FXIIIB phenotypes. Anode at the *top*. *Left* to *right*: 1-1/2-2/6-6 (mixture), 2-1, 3-2, 3-2, 3-1, 6-1, 6-6, 6-3, 6-1, 2-1, 2-2, 2-1, 3-2, 1-1, 3-1 and 3-3

Table 1. Distribution of FXIIIB phenotypes and allele frequencies in Libyans

Phenotypes	Observed		Expected		χ^2	Allele frequencies
	n	%	*n*			
1–1	48	44.44	46.68		0.037	FXIIIB*1 = 0.6574
2–1	35	32.41	34.85		0.001	FXIIIB*2 = 0.2454
3–1	9	8.33	10.52		0.220	FXIIIB*3 = 0.0741
2–2	7	6.48	6.50		0.038	FXIIIB*6 = 0.0231
3–2	4	3.70	3.93		0.001	($0.80 < P < 0.90, 3\,df$)
3–3	1	0.93	0.59		0.285	
6–1	2	1.85	3.28			
6–2	0	0.00	1.22			
6–3	1	0.93	0.37			
6–6	1	0.93	0.06			
	4			4.93	0.175	
Total	108	100.00	108.00		0.757	

dicated that this is identical to the FXIIIB*6 allele first reported by Kühnl and Spielmann [4]. These authors described a single example of the phenotype FXIIIB 6–1, but the details of the population studied and the gene frequency were not mentioned. Since then, the FXIIIB*6 allele has also been reported in US whites and blacks at frequencies of 0.00013 and 0.01807, respectively [6]. In the present population sample this allele was observed in homozygote form and in heterozygote combination with FXIIIB*1 and FXIIIB*3 alleles. The heterozygote combination of FXIII*6 and FXIII*2 was not encountered in our population and has not been described previously. Since the isoelectric points of these two alleles are very close, the detectability of FXIIIB 6–2 phenotype was tested and confirmed. As shown in Fig. 1, three bands were clearly demonstrable when a mixture of FXIIIB 1–1, FXIIIB 2–2 and FXIIIB 6–6 phenotypes was

used. Treatment of the serum with neuraminidase gives a simple and easily interpreted pattern when compared with that of native serum or plasma, but the desialysed plasma shows a simpler pattern than that obtained from serum, because the minor anodal band is absent.

Distribution of FXIIIB phenotypes and allele frequencies are summarized in Table 1. The population studied is in genetic equilibrium assuming the Hardy-Weinberg hypothesis. When compared with those obtained in various other populations [1–6, 9–12], our results in Libyans showed marked differences in the frequencies of the three common alleles FXIIIB*1, FXIIIB*2, FXIIIB*3 and the occurrence of a fourth common allele, FXIIIB*6. Further investigations in other Arab populations will clarify whether the FXIIIB*6 allele is specific for Libyans or a characteristic marker for Arabs.

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