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Genetic Polymorphism of the B Subunit of Human Coagulation Factor XIII

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SUMMARY

Genetic variation of the B subunit of human coagulation factor XIII has been observed after electrophoresis of plasma or serum samples on thin layer agarose plates and subsequent immunofixation with a specific antiserum. The *F-XIIIB* locus is autosomal and has three alleles. In Australian blood donors, the *F-XIIIB*[1], *F-XIIIB*[2] and *F-XIIIB*[3] alleles have frequencies of .747, .084, and .169, respectively.

INTRODUCTION

Coagulation factor XIII from platelets and plasma is a zymogen which, when activated by thrombin and Ca^{++}, produces γ-glutamyl-ϵ-lysine cross links between fibrin molecules [1]. Cross linking of fibrin stabilizes clot structure and plays a significant role in hemostasis.

Factor XIII from plasma is considered to be comprised of two A subunits and two B subunits (A_2B_2) [2]. The B subunits apparently have no enzymatic activity and may serve as a plasma carrier molecule, since factor XIII from platelets is entirely comprised of catalytic A subunits [2].

We have previously described a polymorphism of the A subunits of factor XIII [3]. The A subunit locus was found to be autosomal and has two alleles. Further study of the plasma and serum factor XIII molecule has revealed significant genetic variation in the B subunit.

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MATERIALS AND METHODS

Sample Collection and Preparation

Blood samples were drawn onto heparin (15 IU/ml) or EDTA (1.5 mg/ml) as anticoagulants. Plasma was obtained within 3 hrs by centrifugation and used immediately or stored frozen in liquid nitrogen.

Electrophoresis was carried out on thin layer agarose gels prepared as described [3]. The buffer system was the discontinuous combination described by Ashton and Braden [4]. The electrode buffer contained 29 mM lithium hydroxide and 191 mM boric acid. The gel buffer was prepared by combining 90 vol solution containing 7.62 mM citric acid and 52 mM Tris with 10 vol electrode buffer. This buffer was always prepared immediately before use. Electrophoresis was carried out at 18V/cm until a hemoglobin marker migrated at least 6.5 cm down the gel.

Immunofixation

Factor XIII B subunits were specifically localized by immunofixation with a 15% (v/v in 150 mM NaCl) solution of rabbit antihuman factor XIII subunit S antiserum (lot 5902F, Behringwerke AG, Marburg, West Germany). "S" and "B" refer to the same subunit which will be known in this paper as the B subunit, in accordance with recent nomenclature. Procedure for the antiserum application and staining of the immunoprecipitates has been described [3].

RESULTS

An electrophoretic survey of plasma from normal Australian blood donors revealed five different patterns of immunoreaction comprised primarily of three equally spaced pairs of bands. The most anodal pair migrated about 2 cm, while the electrophoretically slowest pair migrated about 4 cm behind the hemoglobin marker. Three of the five observed patterns are shown in figure 1. The most anodal pair of bands are associated with three minor bands which migrate just in front of the major components. Similar minor bands were observed occasionally in association with the two slower pairs of bands. Phenotypes were not altered during storage in liquid nitrogen, and individuals sampled repeatedly over several months consistently presented the same phenotypes.

The electrophoretic patterns observed suggest the presence of three alternate forms of the B subunit of factor XIII. We, therefore, propose to refer to this system as the

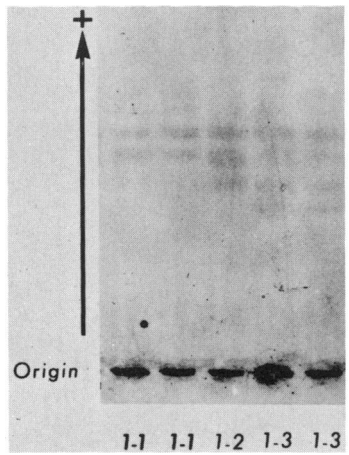

FIG. 1.—Three of the five observed phenotypes of B subunit of factor XIII

F-XIIIB locus with three alleles: *F-XIIIB*[1] controlling the most anodal components, *F-XIIIB*[2] controlling the intermediate components, and *F-XIIIB*[3] controlling the electrophoretically slowest pair of bands. Because of the weak staining of these bands, photographic reproduction is difficult. Figure 2 indicates schematically the relative position of the different phenotypic patterns. This figure includes the 2-2 phenotype, which we have not observed but predict from the combinations of other alleles.

To test the hypothesis that the different phenotypes are products of a single locus with three alleles, we compared the observed and expected phenotype numbers in a population of unrelated Australian blood donors (table 1). Observed phenotype numbers did not differ significantly from those expected if a Hardy-Weinberg equilibrium exists. Family data, table 2, are also consistent with the hypothesis that the observed phenotypes are the product of a single locus with three alleles.

To evaluate the possible interaction between the electrophoretic variation of both B and A subunits, we examined the electrophoretic mobility of factor XIII in plasma from individuals homozygous for the same *F-XIIIA* allele but differing at the *F-XIIIB* locus. Figure 3 shows an example of the electrophoretic separation of the A subunit (identified by anti-A antiserum [3]) from two individuals with F-XIIIA 1-1 phenotypes but who had previously been shown to have F-XIIIB 1-1 and F-XIIIB 3-3 phenotypes. We were unable to show any difference in the migration rate of the A subunits which could be attributed to the differences observed in the B subunit.

Electrophoretic studies of the A subunit of factor XIII are restricted to the use of plasma [3]. We have found, however, that the B subunit can easily be detected in either serum or plasma.

DISCUSSION

The data clearly support the hypothesis that the B subunits of coagulation factor XIII are the product of an autosomal locus with three alleles. It is not surprising that a homozygote for the proposed *F-XIIIB*[2] allele has not been observed, since the expected frequency of this phenotype is very low.

Heterogeneity of purified B subunits from pooled plasma has previously been reported after isoelectric focusing [5]. This heterogeneity was partially removed by

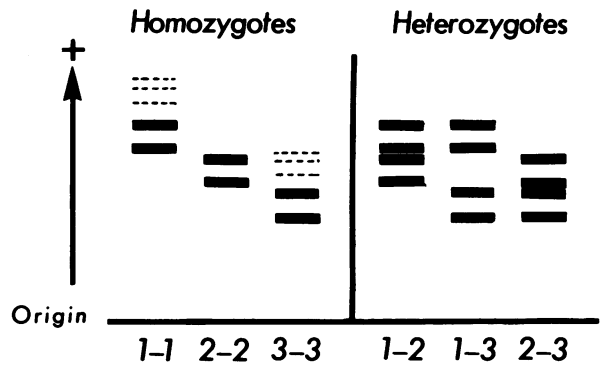

FIG. 2.—Diagram of F-XIIIB phenotypes. *F-XIIIB*[2] allele has only been observed in the heterozygous state. *Dotted lines* indicate position of occasionally observed minor components.

TABLE 1

F-XIIIB PHENOTYPES OF UNRELATED AUSTRALIAN BLOOD DONORS

	PHENOTYPES						
	1-1	2-2	3-3	1-2	1-3	2-3	
Observed	138	0	6	30	60	11	
Expected	136.68	1.71	7.02	30.59	61.96	6.9	$\chi^2 = 4.377$

NOTE.—Expected values calculated from gene frequencies: *F-XIIIB*[1] = .7469, *F-XIIIB*[2] = .0836, and *F-XIIIB*[3] = .1693. No. = 245.

desialation with neuraminidase, but the remaining variation could not be explained. The present findings suggest that the residual heterogeneity was due to genetic variation between the pooled plasma samples. The variation we observed is possibly the product of an in vivo or in vitro posttranslational event, such as deamidation or disulphide formation. However, a genetic explanation is more likely since the band pairs are always of equal intensity and equally spaced and the phenotypes are not altered by repeated freeze and thaw treatment or long-term frozen storage. In addition, in vitro combination of plasma from individuals with the 1-1 and 3-3 phenotypes gives a phenotype indistinguishable from that of a 1-3 heterozygote. In contrast, the minor components observed primarily with the more strongly staining 1-1 phenotype, but occasionally with the other phenotypes, are more variable and more probably the product of varying degrees of posttranslational modification.

Earlier reports [6, 7] suggest that coagulation factor XIII deficiency may be inherited as an X-linked character, whereas more recent reports [8– 10] indicate an autosomal recessive mode of inheritance. Our previous studies [3] clearly show that the A subunit is the product of an autosomal locus. The observation of several male heterozygotes in the present data eliminates the possibility of the B subunit being located on the X chromosome. The previously suggested X-linked inheritance of coagulation factor XIII deficiency is, therefore, not supported by our findings. Some factor XIII deficiencies could still result from an abnormal control or regulator X-linked gene, separate from the A and B subunit structural loci.

Deficiency of the A subunit of factor XIII has been described [11, 12]. In some cases [10, 13], the level of the B subunit is also decreased or absent, suggesting that B subunit synthesis may be influenced by the A subunit. At present, we have no evidence

TABLE 2

INHERITANCE OF *F-XIIIB* ALLELES

	OFFSPRING TYPES					
PARENTAL TYPES	1-1	2-2	3-3	1-2	1-3	2-3
1-1 × 1-1 (7)*	27	0	0	0	0	0
1-3 × 1-1 (4)	11	0	0	0	9	0
3-2 × 1-1 (2)	0	0	0	3	2	0

* No. families in parentheses.

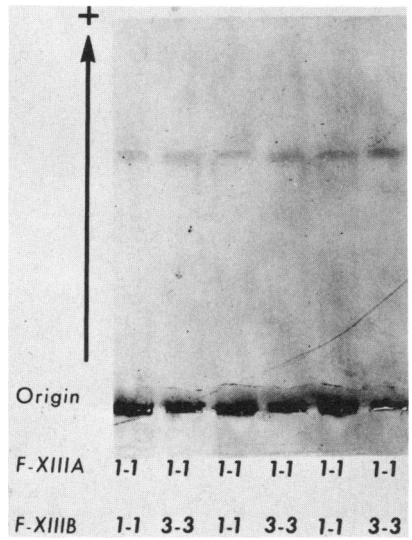

FIG. 3.—Plasma coagulation factor XIII as identified by anti-A subunit antiserum, from F-XIIIA 1-1 individuals with differing F-XIIIB phenotypes.

to suggest that the different allelic combinations of the A and B subunits have any significant differences in their in vivo activity. We have, however, noted that the products of the two least common alleles of the *F-XIIIB* locus (*F-XIIIB*^{2} and *F-XIIIB*^{3}) have less staining intensity than the *F-XIIIB*^{1} product after electrophoresis and immunofixation. Further studies are in progress to evaluate the significance of this difference.

Our failure to find any interaction between the migration rates of A subunits in individuals who differ in their B subunits is of interest. The bond between the A and B subunits is probably broken during electrophoresis, thus allowing the independent recognition of the polymorphism at each locus. We found identical electrophoretic B subunit phenotypes in both plasma and serum from the same individuals. In serum, the B subunits would be expected to be in the free form after thrombin and Ca^{2+} mediated activation and dissociation of the A_2B_2 complex [14]. This indicates the plasma immunofixation phenotypes observed represent B subunits and not the A_2B_2 complex. The possibility that A and B subunits are dissociated during electrophoresis is supported by the previous observations that they are weakly associated and can be separated by freezing and thawing in Tris-EDTA buffers [2] and during immunodiffusion [11].

Further investigation of the A and B subunits by electrophoresis and immunofixation, as described here, may provide further information about the nature of factor XIII deficiencies and the structure of the factor XIII molecule. The F-XIIIB system provides a new marker for population genetic studies and gene mapping by pedigree analysis.

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