MOLECULAR BASIS FOR POLYMORPHISM OF THE "a" SUBUNIT OF COAGULATION FACTOR XIII

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INTRODUCTION

Two major alleles of the "a" subunit of coagulation factor XIII (F13A), *F13A*1* and **2* (Board 1979) were found to split into four alleles, *F13A*1A, *1B, *2A,* and **2B* (fig.1, Suzuki et al. 1988). We hypothesized on the basis of the differences in isoelectric points between the four allele products that two point mutations and one intragenic crossing over event might generate the four alleles (fig 2). In this study the 14 coding exons (Ichinose and Davie 1988) of the four F13A alleles were amplified by using polymerase chain reaction (PCR) and sequenced to reveal nucleotide differences between the alleles and to provide support for the hypothetic model, and nucleotide changes in two rare variant alleles were also presented.

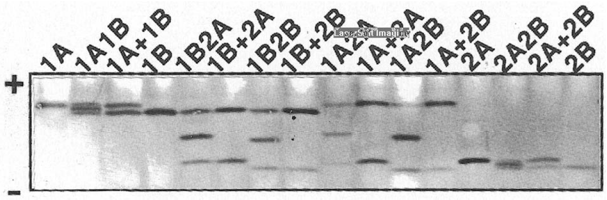
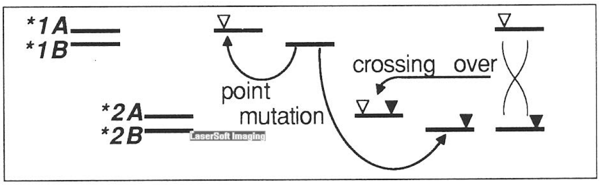


Fig.1. Separation of ten F13A phentypes deduced from four common alleles. Plasma samples were subjected to agarose gel (1%) isoelectric focusing with carrier ampholyte mixture of pH5-6 and 5-6.5 and 2M urea.

Fig.2. Hypothetic model for the genesis of F13A polymorphism. Although the most frequent *F13A*1B* allele is assumed here as an ancestral monomorphic gene, any other alleles are compatible as an ancestor with the hypothesis.



MATERIALS AND METHODS

Fourteen pairs of oligonucleotide primers, each of which corresponds to the flanking sequences of the 14 coding exons were synthesized according to the genomic sequence reported by Ichinose and Davie (1988). Single-stranded DNAs of sense and antisense sequences of each coding exon were prepared from double-stranded PCR products and then sequenced by dideoxy sequencing.

Single strand conformational polymorphism (SSCP) was analyzed by using polyacrylamide gel electrophoresis and silver stain.

RESULTS AND DISCUSSION

Genomic DNAs extracted from three homozygetes, 1A, 1B and 2B, and one heterozygote 1A2A were amplified by PCR and sequenced. PCR using each primer pair was demonstrated by agarose gel electrophoresis to produce a single band under appropriate amplification conditions. Two exons were found to be responsible for the allelic differences. A C/T transition of the second nucleotide of the codon encoding the amino acid residue 564 in exon 12 was identified as a nucleotide difference between the *F13A*1A* and **1B* allele, and between the *F13A*2A* and **2B* allele (fig.3A). This transition leads to substitution of Leu564 in *F13A*1A* and **2A* for Pro564 in *F13A*1B* and **2B*, resulting in subtypic splits of the former diallelism (*F13A*1 and *2*) into the present four alleles. Exon 14 was found to harbor two changes at the first nucleotides of the contiguous codons encoding amino acid residues 650 and 651 (fig.3B). These two mutations in exon 14 were found to define the former diallelism. Nucleotide changes detected in this study and deduced amino acids were summarized in table 1.

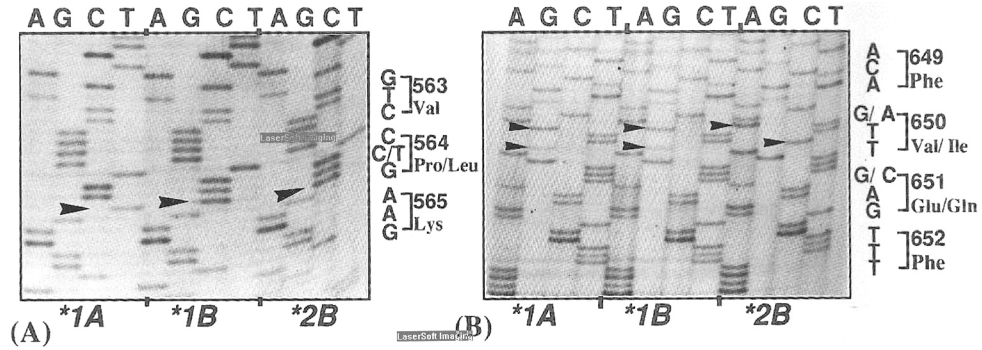


Fig.3. Sequences in the regions of the codon for the amino acid resideu 564 in exon 12 (A) and of the codons for the amino acid residues 650 and 651 in exon 14 (B). Arrow heads indicate substitutinal bands.

Table 1. Allele specific base changes in F13A polymorophism

alleles	exon12	exon14		abbreviation
	codon 564	codon 650	codon 651	
*F13A*1A*	CTG(Leu)	GTT(Val)	GAG(Glu)	T-GG
*F13A*1B*	CCG(Pro)	GTT(Val)	GAG(Glu)	C-GG
*F13A*2A*	CTG(Leu)	ATT(Ile)	CAG(Gln)	T-AC
*F13A*2B*	CCG(Pro)	ATT(Ile)	CAG(Gln)	C-AC

The Glu/Gln substitution at residue 651 inferred from the nucleotide change in exon 14 seemed to be consistent with the mobility difference between *F13A*1A/*1B* and *F13A*2A/*2B*. The substitution at residue 564 is a neutral one and thus undetectable as a mobility shift by usual zone electrophoresis. According to the hypothesis by Righetti and Gianazza (1980) based on the data showing IEF separation of hemoglobin variants with neutral mutations (Whitney III et al. 1979), increase in hydrophobicity of polypeptide chains in general leads to decrease in isoelectric point via loss of positive charge and vice versa. This hypothesis predicts that F13A 1B and 2B carrying proline at position 564 are likely to exhibit a little higher isoelectric points, i.e., more cathodal mobilities than their point mutation counterparts with leucine, F13A 1A and 2A since proline is more amenable to be exposed to water and thus less hydrophobic than leucine. The separation by IEF of each subtype of *F13A*1* and **2* was in agreement with this consideration.

Thus, there are two antithetic versions in each exon , "A" or "B" in exon 12 and "1" or "2" in exon 14, and the resulting four combinations of them were shown to correspond to the four alleles (*F13A*1A, *1B, *2A, *2B*). When assumed that one of the four alleles was an ancestral monomorphic gene, the point mutations in exon 12 or 14 brought about two new alleles and the fourth one was likely to result from intragenic crossing over between the two of the pre-existing three alleles. Although we have not obtained direct evidence for crossing over event yet, relatively long intronic sequences intervening between the exons of the F13A gene seem to favor intragenic recombination.

Besides the four common alleles, several rare variant alleles have been reported thus far. A rare variant named as *F13A*4* was analyzed by SSCP and a putative exon harboring mutation was sequenced. Two samples of F13A 1B-4 showed different SSCP patterns, one in exon 11 and the other in exon 12. Careful IEF analysis revealed a very small difference in mobility between the F13A 4 bands of the two samples and the two *F13A*4* alleles were then tentatively renamed as *F13A*4I* and **4W* (fig.4A). Polymorphic bands were demonstrated by SSCP in exon 11 for *F13A*4I* and in exon 12 for *F13A*4W* as presented elsewhere in this volume by the same authors. A C to G transversion in the codon (CAA to GAA) encoding residue 468 and a G to A transition in the codon (CGG to CAG) for residue 540 were found to specify *F13A*4I* and **4W*, respectively. Amino acids inferred from the nucleotide sequences were Glu from Gln for **4I* and Gln from Arg for **4W*, being compatible with IEF separation pattern of both bands. Codons for residue 564 ("A"/"B") and for residues 650-651 ("1"/"2") are CTG564-GTT650-GAG651 for *F13A*4I* and CCG564-GTT650-GAG651 for *F13A*4W*. *F13A*4W* was previously identified as *F13A*4* by comparing the mobility with original *F13A*4* (Board and Coggan, 1981) on agarose gel electrophoresis (Suzuki et al. 1986). *F13A*4I* and **4W* were discriminated from each other only by comparing them side-by-side in "subtyping" gels. These findings therefore suggests that sequence data is finally required to discriminate variant alleles that have been defined only by electrophoretic procedures.

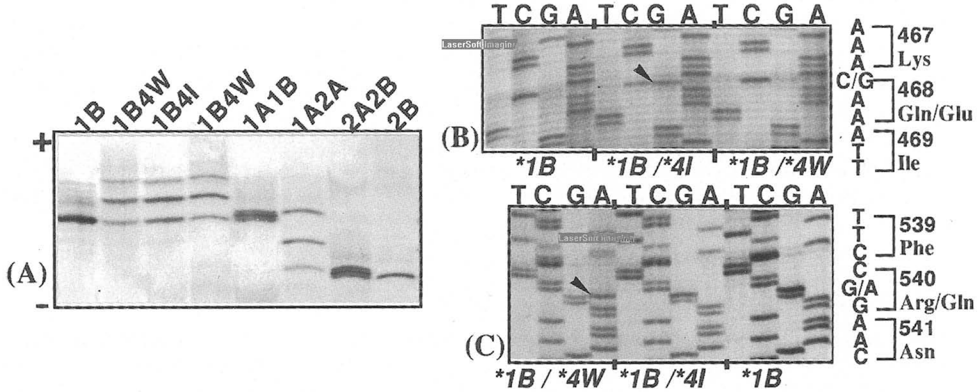


Fig.4. Separation of F13A*4I and *4W by AGIF (A). Sequences in the regions of the codon for the amino acid residue 468 in exon 11 (B) and of the codon for the amino acid residue 540 in exon 12 (C). Arrow heads indicate substitutinal bands.

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