NUCLEOTIDE CHANGES IN VARIOUS VARIANTS OF THE COAGULATION FACTOR XIII A-SUBUNIT

Koichi Suzuki, Jürgen Henke*, Misa Iwata, Lotte Henke**, Maria Szekelyi*, Shigenori Ito, Atsuko Uchida

Department of Legal Medicine, Osaka Medical College, 2-7 Daigakumachi Takatsuki 569, Japan
*Institut für Blutgruppenforschung, Hohenzollernring 57 Postfach 19 04 20, D-50501 Köln, Germany
**Institut für Blutgruppenforschung, Abtl. forensische Blutgruppenkunde und Molekulargenetik, Düsseldorf, Germany

INTRODUCTION

Blood coagulation factor XIIIa (F13A) is reported to be a polymorphic protein defined by two alleles (Board 1979), *F13A*1* and **2*, both of which are further classified into two suballeles (Suzuki 1988), *F13A*1A, *1B, *2A,* and **2B,* by subtyping IEF. We have recently reported the molecular basis of the differences between the four suballeles (Suzuki 1994) and disclosed novel polymorphisms at five nucleotide sites in Caucasians (presented in this volume). These sites are located in codon 34 of exon 2, codon 204 of exon 5, codon 331 of exon 8, codon 567 of exon 12, and codon 651 of exon 14. These polymorphic sites including the protein allele-determining sites (codon 564 of exon 12 and codons 650-651 of exon 14) are segregated as sequence haplotypes.

This study presents molecular characterization of 16 rare F13A variant alleles and one silent allele sampled mainly from German and Japanese, together with the sequence polymorphisms underlying those variant alleles.

MATERIALS AND METHODS

Plasma and/or genomic DNA samples of various F13A variant alleles were derived from Buryat, Finnish, German, and Japanese individuals. The F13A genotype was determined for plasma samples by subtyping isoelectric focusing (IEF) (Suzuki 1988) and conventional IEF (Henke 1994). Each coding exon of the F13A genes was amplified by the polymerase chain reaction (PCR) (Suzuki 1994). Single strand conformational polymorphism (SSCP) was analyzed in mini-polyacrylamide gels followed by silver staining. PCR products showing mobility alterations in SSCP were subjected to sequencing by the Sanger dideoxy method.

RESULTS AND DISCUSSION

Because to the best of my knowledge at present there are no reference laboratories for identifying rare F13A alleles by comparing unknown samples with registered controls, we tentatively named the variant samples analyzed here according to the scheme presented by Dykes et al. (1988). The relative positions of the variants are presented in Fig. 1.

To detect mutations which cause changes in electrophoretic mobilities in IEF, the genomic DNAs from the individuals with F13A variant proteins were analyzed by PCR-SSCP. PCR products amplified from each of all the coding exons of variant alleles were indistinguishable from those amplified from the common four suballeles in agarose gel electrophoresis, indicating that no major alterations in length of coding regions were associated with the variants. PCR products were then subjected to SSCP electrophoresis.

Exons of which PCR products showed mobility shifts in SSCP analysis were then sequenced. The sequence changes of the variant alleles are listed in Table 1. For 16 variant alleles and one silent allele, we disclosed point mutations at 13 different sites in the coding exons, a deletion of a single codon, and a point mutation at a splice acceptor site, and at the same time, we determined their underlying sequence haplotypes. The amino acid changes inferred from those point mutations in the coding exons and from that single codon deletion seemed to be consistent with the mobility shifts of the corresponding variant proteins in subtyping IEF; but unfortunately we have had no chance to type the plasma sample of Fi.15 by IEF.

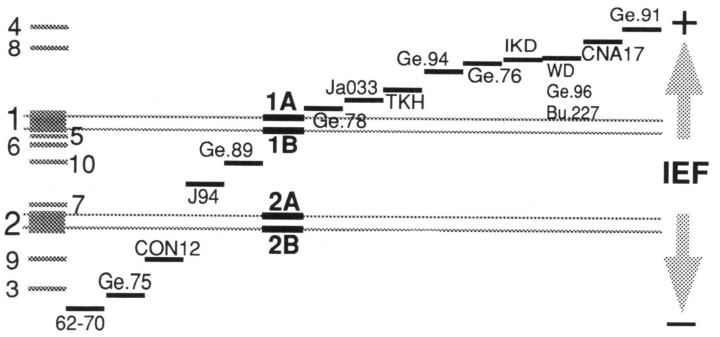

Fig.1 Relative positions of rare variant proteins on subtyping IEF. Genomic DNAs of CNA17, J94, CON12, 62-70 were unavailable and thus sequence changes of the variants were not delineated. Plasma of Ge.74, Ge.86, and Ge.79 were typed by conventional IEF and not depicted here. On the left lane are the relative positions of the two conventional alleles, *F13A*1* and **2*, and eight rare alleles presented by Dykes (1988).

Table 1. Nucleotide changes and sequence haplotypes in various variants.

sample code*	protein allele	exon involved	codon involved	nucleotide change (amino acid)	sequence haplotype exon 2	5	8	12	14
					codon 34	204	331	564 567	650 651
Ge.91	4	15	696	TGC CGG (Arg)→TGC TGG (Trp)	1	1	1	2	1
Ge.96 WD Bu.227	4	12	540	TTC CGG(Arg)→TTC CAG(Gln)	1	1	1	1	1
Ge.74	4	7	303	TAC CGG(Arg)→TAC CAG(Gln)	1	1	1	2	1
IKD	4	11	468	AAA CAA(Gln)→AAA GAA(Glu)	1	1	1	1	1
Ge.76	4	13	621	GCC AAG(Lys)→GGC GAG(Glu)	1	1	1	1	1
Ge.94	8	4	174	AGT CGA(Arg)→AGT CAA(Gln)	1or2	1	1	1	1
TKH	8	4	114	AAG GGA(Gly)→AAG GAA(Glu)	1	1	1	1	1
Ja.033	8	4	158	TTC CGC(Arg)→TTC TGC(Cys)	1	1	1	1	1
Ge.78	8	13	626	ACC GTG(Val)→ACC ATG(Met)	1	1	1	1	1
Ge.89	6or(5)	14	668	CTG GAT(Asp)→CTG GGT(Gly)	1	1	2	1	1
Ge.86	10or(6)	12	499	CTG ATG(Met)→CTG AAG(Lys)	2	1	1	1	1
Ge.79	7or(10)	12	509	ACA GAA(Glu)→ACA ---	1	1	1	1	1
Ge.75	3or(9)	13	593	GGC GAG(Glu)→GGC AAG(Lys)	1or2	1	1or2	2	1
Fi.15	1B?	8	353	ATC TTC(Phe)→ATC TAC(Tyr)	1	1	1	1	1
Ge.84	Q0	intron V		···aaatag TTT→···aaatag TTT	1	1	1	1	1

*)Samples with the following headings, "Ge.", "Fi.", and "Bu." are German, Finn, and Buryat, respectively. WD, IKD, TKH, and Ja.033 are Japanese.

Ge.96, Bu.227, and WD, all typed as F13A*4, were found to share an identical nucleotide change on the same background of a sequence haplotype (11111) although they were of different ethnic

origins. It is unknown now whether these three mutations occurred independently in the three groups or were due to historical interbreeding between them.
For a case in which a silent allele was transmitted from a mother (Ge.85:F13A 1) to her child (Ge.84:F13A 2), we have found a G to A transition at the splice acceptor site of intron V in both of them. An F13A deficiency resulting from the same mutation has been recently reported to be the resultant introduction of a premature stop codon due to frame shift that was caused by an alternative use of the first ApG dinucleotide downstream in exon 6 as a splice acceptor (Vreken 1995). Therefore, the homozygote of the silent allele must lead to the F13A deficiency.

The 14 different point mutations were shown to result from 11 transitions and 3 transversions. Seven (50%) of the mutations occurred in CpG dinucleotides. This high proportion is consistent with the relatively frequent occurrence of CpG dinucleotides in the coding region of the F13A gene, which has 60 (5.5%) CpG dinucleotides among 1098 dinucleotides as compared with 44 CpGs expected from an average G + C content (about 40%) in mammalian genomes. The high proportion of the mutations support the evidence that CpG dinucleotides act as a hot spot for mutation (Cooper and Youssoufian 1988).

All the mutants were further characterized for their haplotypic backgrounds by SSCP analysis of the sequence polymorphisms in exons 2, 5, 8, 12, and 14, 11 mutations (64.7%) occurring on the 11111 haplotype, 2 (11.8%) on the 11121 haplotype, 2 on the 21111 haplotype, 1 (5.9%) on the 11211 haplotype, and 1 on an undefined haplotype. This order of the frequencies agreed with that of the haplotype frequencies reported for Caucasian populations presented elsewhere in this volume.

In conclusion, much more polymorphisms can be latently harbored in the coding sequences of conventional marker genes than in their gene products, as shown in this study as well as in other genes. In addition to elucidating sequence differences between the alleles of the conventional markers, a full delineation of such hidden sites underlying common and rare alleles will serve as much more detailed characterization of human populations and also allow those rare alleles to be traced back to their founders. Thus, molecular analysis on the conventional marker genes will be required still in future.

REFERENCES

Board PG (1979) Genetic polymorphism of the A subunit of human coagulation factor XIII. Am J Hum Genet 31:116-124

Cooper DN, Youssoufian H (1988) The CpG dinucleotide and human genetic disease. Hum Genet 78:151-155

Dykes DD et al (1988) Incidence of rare variants among serum proteins and RBC enzymes in Us Whites and Blacks. In: Mayr WR (ed) Advances in Forensic Haemogenetics 2. Springer-Verlag, Berlin Heidelberg, p 125-131

Henke J et al (1994) On the occurrence of common and rare alleles in the coagulation FXIIIA system. Klin Lab 40:433-436

Suzuki K et al (1988) Polymorphism of the A subunit of coagulation factor XIII: Evidence for subtypes of the FXIIIA*1 and FXIIIA*2 alleles. Am J Hum Genet 43:170-174

Suzuki K et al (1994) Molecular basis for subtypic differences of the "a" subunit of coagulation factor XIII with description of the genesis of the subtypes. Hum Genet 94:129-135

Vreken P et al (1995) A point mutation in an invariant splice acceptor site results in a decreased mRNA level in a patient with severe coagulation factor XIII subunit A deficiency. Thromb Haemost 74:584-589