DNA TYPING OF COAGULATION FACTOR XIII "a" SUBUNIT BY PCR-RFLP AND SSCP

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INTRODUCTION

Data on nucleotide sequences of conventional polymorphic markers leads to development of PCR-based typing methods such as PCR-RFLP, PCR-ASO, or SSCP. RFLP usually requires loss or gain of a recognition sequence of a restriction enzyme in which a polymorphic site is included, but artificially introduced mismatch of a single nucleotide in a PCR primer can produce a recognition site of a restriction enzyme (Kumar and Dunn 1989).

Polymorphism of the "a" subunit of the coagulation factor XIII is defined by four alleles, *F13A*1A, *1B, *2A,* and **2B* (Suzuki et al. 1988). Sequence analysis demonstrated that a single nucleotide change in exon 12 specified the "A"/"B" difference and two nucleotide changes at contiguous codons in exon 14 determined the "1"/"2" difference as described in this volume by the same authors. Thus four combinations of the two antithetic versions of the two exons were found to correspond to the four respective alleles. In this study, we present PCR-RFLP and SSCP analyses for specifying F13A alleles.

MATERIALS AND METHODS

Three primers with a single mismatch were designed so that recognition sequences were introduced through amplification of sequences of two polymorphic exons by PCR (fig.1).

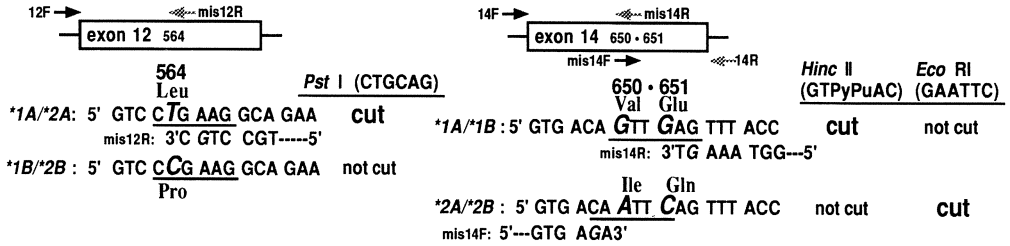

Fig.1. Schematic presentation of regions amplified by PCR with three sets of primers. Italicized nucleotides in the allelic sequences and in the three mismatched primer sequences indicate polymorphic sites and mismatched nucleotides, respectively.

After digestion of PCR products with appropriate restriction enzymes, RFLPs were detected by polyacrylamide gel electrophoresis and ethidium bromide stain. SSCP analysis was performed in polyacrylamide gels (12x10x0.1 cm) that were continuously cooled at 15℃. DNA bands were visualized by silver stain.

RESULTS AND DISCUSSION

Pst I, *Hinc* II and *Eco*R I recognition sites were artificially introduced in the PCR products

harboring type "A" exon 12, type "1" exon 14 and type "2" exon 14, respectively (fig.1). Figure 2 presents *Pst* I and *Eco*R I RFLPs that define allelic versions of exon 12 and 14, respectively. Ten F13A genotypes deduced from the four alleles were defined by the sum of the two RFLPs but F13A 1A2B and 1B2A could not be discriminated from each other.

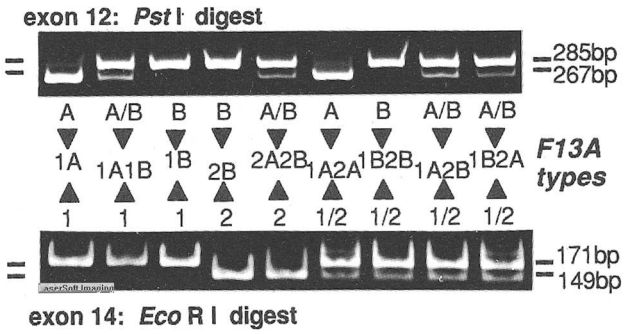

Fig.2. RFLPs of exons 12 and 14. Note that F13A 1A2B and 1B2A show the same RFLPs for both of the two exons.

F13A types of randomly collected blood samples from unrelated 134 individuals were defined by both AGIF for plasma and PCR-RFLPs for genomic DNA. Phenotype distribution and allele frequencies determined from the samples were listed in table 1. F13A type of each individual determined by the two methods was completely coincide with each other except for the two types, 1A2B and 1B2A, both of which were defined as heterozygote for each exon by PCR-RFLPs.

Table 1. Phenotype distribution and allele frequencies calculated from the results by plasma typing and by DNA typing.

phenotypes	no.obs.	no.exp.	allele frequencies
1B	49	47.76	*F13A*1A* = .2948
1A1B	43	47.17	*F13A*1B* = .5970
1B2B	18	16.11	*F13A*2A* = .0075
1A	14	11.65	*F13A*2B* = .1007
1A2B	8	7.96	
1B2A	1-------		
2A2B	1		
2B	0	3.35	
1A2A	0		
2A	0-------		

$\chi^2 = 1.641$, $.30 < p < .50$ (d.f. = 2)

Hinc II RFLP defined by cleaving the "1" version of exon 14 showed together with *Eco*R I RFLP that the contiguous two codons specifying the two antithetic versions, GTTGAG and ATTCAG, are homogeneous in their sequences because all of the F13A*1A and *1B alleles were cleaved by *Hinc* II and all of the F13A*2A and *2B were cut by *Eco*RI. Thus, the two nucleotide

changes at the contiguous codons encoding the amino acid residues 650 and 651 were found not to result from artifact during sequencing reaction.

Polymorphic single strands of exon 11, 12 and 14 were successfully detected by SSCP analyses under the conditions as follows; electrophoresis at 150V overnight in 20% polyacrylamide gels without glycerol for exons 11 and 12, and with 5% glycerol for exon 14.

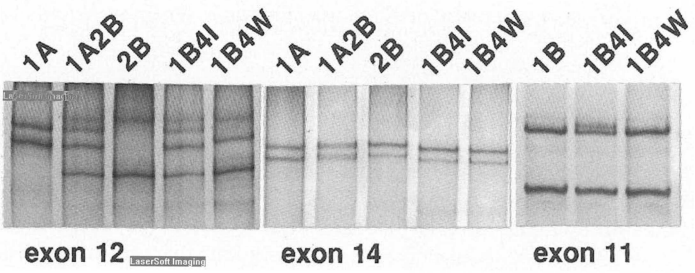

Fig.3. SSCP analysis of three exons of the F13A gene.

Figure 3 shows SSCP patterns of the three exons. SSCP analysis is a powerful technique to reveal a base substitution harbored in a short DNA stretch of a few hundreds bases but is not a completely potent one because monomorphic separation of single-stranded DNAs by SSCP analysis does not always mean that there are no base substitutions along the sequence tested. To separate polymorphic single strands in this study, three parameters, acrylamide/*N-N'*-methylenebisacrylamide ratio, gel concentration and temperature during electrophoresis, were tested. Then the two selected conditions described above were applied to SSCP analysis of all the coding exons of F13A alleles. There were no SSCPs in the 12 exons other than exon 12 and 14 for the four common alleles, the findings which are compatible with sequence data showing allelic base changes only in the 2 exons. Fortunately, SSCP analysis under the same conditions could separate polymorphic bands in exon 11 for *F13A*4I* and in exon 12 for *F13A*4W,* the sequences of which were presented elsewhere in this volume by the same authors.

DNA typing of conventional markers by PCR-based methods is one of potent tools for genetic characterization of forensic materials owing to the feasibility of their detection, ample accumulation of population data of many ethnic groups and stability of allelic sequences of a gene against de novo mutations although DNA typing of conventional markers is not so powerfully discriminative as tandem repeat DNA markers such as VNTR, STR or DNA fingerprint. Thus, DNA typing of the F13A gene provides an additional chance for forensic identification of individuals.

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