NOVEL POLYMORPHISMS IN THE CODING SEQUENCE OF THE COAGULATION FACTOR XIII A-SUBUNIT AND THEIR HAPLOTYPE DIVERSITY

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INTRODUCTION

Genetic polymorphism of coagulation factor XIII A-subunit (F13A) is defined by four suballeles, *F13A*1A, *1B, *2A*, and **2B* (Suzuki 1988). Some of the authors have recently determined nucleotide substitutions responsible for the allelic differences of the F13A protein by using the polymerase chain reaction (PCR) and direct sequencing, and have reported PCR-mediated typing procedure for the four alleles (Suzuki 1994). Further analysis of the coding sequences of the F13A gene has demonstrated several novel polymorphisms based on nucleotide substitutions in the coding sequences. Here, we present these nucleotide site polymorphisms and haplotypic combinations of them.

MATERIALS AND METHODS

Genomic DNA and/or plasma samples were obtained from 53 Finnish, 39 Russian, 16 German, and 50 Japanese individuals. Plasma samples were unfortunately unavailable from all of the Finnish individuals and some of the German individuals. Plasma samples were subjected to subtyping isoelectric focusing (IEF) (Suzuki 1988) and conventional IEF (Henke 1994). Genomic DNAs were PCR-amplified by using oligonucleotide primers (Suzuki 1994). Single strand conformational polymorphism (SSCP) was analyzed in mini-polyacrylamide gels. Single strands with altered mobilities were sequenced by the Sanger dideoxy method.

RESULTS AND DISCUSSION

The entire coding regions of each exon of the F13A gene were analyzed by SSCP after PCR amplification. Shifted band patterns were observed in three exons in addition to exons 12 and 14 where the nucleotide changes responsible for the differences between the four suballeles are located. Furthermore, a novel band was also detected for each of exons 12 and 14.

Direct sequence analysis showed nucleotide changes conferring each shifted band pattern. A novel band for exon 12 was based on an A to G transition at the third nucleotide of codon 567. Thus, three kinds of sequences occurred for exon 12 in the three Caucasian populations, corresponding to CTG·GAA, CCG·GAA, and CCG·GAG at positions 564 and 567. Interestingly, a novel band for exon 14 was found to correspond to GTT·CAG sequence at positions 650 and 651, where only two antithetic sequences, GTT·GAG (*F13A*1A* and **1B*) and ATT·CAG (*F13A*2A* and **2B*) , were detected in a Japanese population. In addition, the GTT·CAG sequence was found to occur a little more frequently than the ATT·CAG sequence in the three Caucasian groups. Our previous prediction (Suzuki 1994) that the ATT·CAG mutated from the GTT·GAG via either of the GTT·CAG or the ATT·GAG was confirmed in this study.

Polymorphic nucleotide changes in exon 2, 5, 8, 12 and 14 were listed in Table 1. A G to T transversion in exon 2 and a A to T transversion in exon 5 result in amino acid changes without any detectable mobility shift in IEF. Nucleotide changes in exon 8 and 12 (codon 567) were found to be synonymous.

Next, we defined haplotypic combinations of the five polymorphic exons. For haplotype description, we gave numerical codes for each of allelic versions of the five exons as shown in Table 1. For instance, a single-heterozygotes such as 2/1-1/1-1/1-1/1-1/1 were able to be

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unambiguously divided into the haplotypes, 21111 and 11111, and haplotypic combinations of some multiple-heterozygotes were also determined with the aid of pedigree data.

Table 1. Novel polymorphisms in the F13A coding sequences and nucleotide substitutions responsible for the allelic differences of the F13A protein.

exon	number of amino acid residue	codon	amino acid	corresponding alleles of protein	numerical code
2	34*	GTC	Val		1
		TTC	Leu		2
5	204	TAT	Phe		1
		TTT	Tyr		2
8	331	CCA	Pro		1
		CCC	Pro		2
12	564·567	CTG·GAA	Leu·Glu	A	2
		CCG·GAA	Pro·Glu	B	1
		CCG·GAG	Pro·Glu	B	3
14	650·651	GTT·GAG	Val·Glu	1	1
		GTT·CAG	Val·Gln	2	2
		ATT·CAG	Ile·Gln	2	3

*) Polymorphism at this site has been already reported in a Finnish population by Mikkola et al. (1994).

Of the predicted 72 haplotypic combinations, 18 haplotypes were delineated as shown in Table 2. When linkage disequilibrium between the five polymorphic exons was tested for the Finnish population, the frequencies of any two pairs were found not to deviate significantly from those

Table 2. Haplotypes of nucleotide polymorphisms in five exons.

sequence haplotype		number of observed haplotype							
		Finn (n=58)		Russian (n=42)		German (n=28)		total (n=128)	
P1	11111	31	.5345	20	.4762	10	.3571	61	.4766
P2	21111	7	.1207	7	.1667	2	.0714	16	.1250
P3	12111	-	-	-	-	1	.0357	1	.0078
P4	11211	7	.1207	6	.1429	1	.0357	14	.1094
P5	11121	8	.1379	4	.0952	6	.2143	18	.1406
P6	11131	1	.0172	1	.0238	-	-	2	.0156
P7	11112	-	-	-	-	4	.1429	4	.0313
P8	11113	2	.0345	-	-	-	-	2	.0156
R1	21121	1	.0172	-	-	-	-	1	.0078
R2	11133	1	.0172	-	-	-	-	1	.0078
R3	21211	-	-	1	.0238	-	-	1	.0078
R4	11221	-	-	1	.0238	-	-	1	.0078
R5	11123	-	-	-	-	1	.0357	1	.0078
R6	11132	-	-	-	-	1	.0357	1	.0078
R7	21131	-	-	1	.0238	-	-	1	.0078
R8	21132	-	-	1	.0238	-	-	1	.0078
R9	11222	-	-	-	-	1	.0357	1	.0078
R10	21222	-	-	-	-	1	-	1	.0078

calculated under the assumption of random combinations of those exons. These findings led us to the idea that frequent recombinational events might equilibrate the combination of the exons of the F13A gene.

When assumed that the most frequent 11111 haplotype (P1) is the ancestral sequence, seven haplotypes (P2-P8) with a nucleotide change at a single site must result from the ancestor through a point mutation, and recombination between the seven haplotypes seem to lead to seven haplotypes (R1-R7) with polymorphic sites in two separate exons. The remaining three haplotypes (R8-R10) with polymorphic sites in more than two exons may also originate from recombination (Watt 1972; Strobeck and Morgan 1978) between the existing haplotypes.

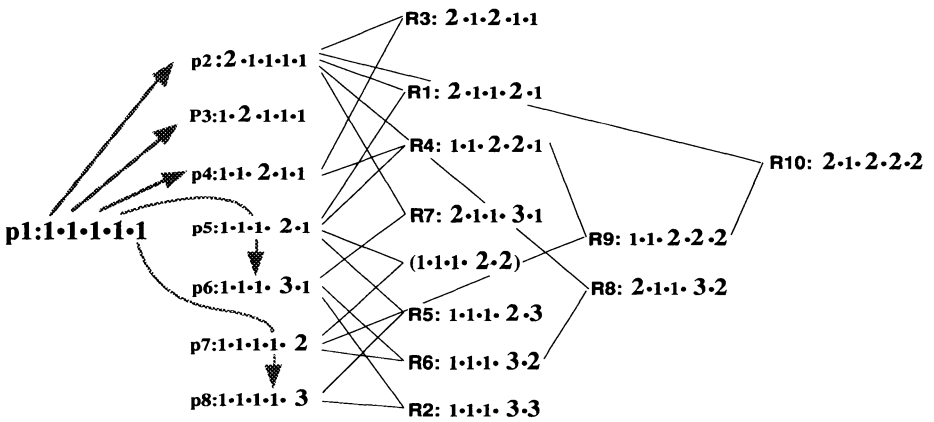

Figure 1. Hypothetic model for the generation of haplotypes. A pair of lines converging to the right indicate recombinations of any two of the existing haplotypes to produce novel haplotypes consisting of the existing polymorphic sites. A haplotype in parentheses is not defined in this study.

We detected the nucleotide site polymorphisms of the F13A gene in three Caucasian populations and only the substitution in exon 2 at a low frequency (1 in 50 individuals) in a Japanese population. Further investigations will be required for demonstrating occurrence of the site polymorphisms in other ethnic groups, especially in Negroid, and evidence of recombinational hot spots in the F13A gene region.

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