

## The Prevalence Study on Restriction Fragment Length Polymorphism Analysis for the Detection of Hemophilia A Carrier

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*We have analyzed two (BclI and XbaI) intragenic restriction fragment length polymorphisms (RFLPs) and St14 (DXS52) variable number of tandem repeats (VNTR) by rapid PCR method in 97 unrelated normal subjects. The incidences for positive BclI and XbaI polymorphic sites in the Koreans were 81% and 72%, respectively, which were higher than other ethnic groups but similar to that reported in the Chinese or Japanese, giving the heterozygosity rate of 0.32 and 0.40, respectively. The amplified allele size was 880 bp with no other polymorphism in the analysis of St14 (DXS52) VNTR. This finding should be taken into account in the planning of a prenatal diagnosis program for ethnic Koreans.*

**Key Words:** Hemophilia A, carrier detection, restriction fragment length polymorphism

Hemophilia A (classic hemophilia) is the most common inherited disease of blood coagulation. The disease is caused by a deficiency of factor VIII, an essential protein cofactor in the intrinsic coagulation pathway. The locus for factor VIII: C has been assigned to the long arm of the human X chromosome at Xq28-qter (Gitschier *et al.* 1984). Due to the broad range of clotting activity in normal and heterozygous females, it is often difficult to confirm the status of women at risk for carrying the disease (Elston *et al.* 1979; Klein *et al.* 1977). With the recent molecular cloning of the human gene for factor VIII, several polymorphic sites have been identified in various ethnic groups and used for carrier detection and prenatal diagnosis

(Gitschier *et al.* 1985; Wion *et al.* 1986; Harper *et al.* 1984; Oberle *et al.* 1985). Recently, Kogan *et al.* (1987) described the use of PCR (polymerase chain reaction) amplified gene products for the direct assessment of these RFLPs (restriction fragment length polymorphisms) within the factor VIII gene. Because of the clinical usefulness of RFLP when adopted to PCR technology, we established the allelic frequencies of the BclI (intron 18), XbaI (intron 22) RFLP and St14 VNTR to estimate the diagnostic values which depend in large on the relative frequencies of alleles at the polymorphic sites before a prenatal diagnosis program can be instituted in Korea.

### MATERIALS AND METHODS

DNA was extracted from peripheral blood leukocytes from each of the 97 normal subjects as described elsewhere (Kunkel *et al.* 1977).

Oligonucleotide primers (Table 1) were synthesized on an Applied Biosystem 380A DNA

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**Table 1.** Sequence of oligonucleotide primers according to the protocols of Kogan *et al.* (1987) and Richards *et al.* (1991).

	Region	Primer
BclI	8.1	5'-TAAAAGCTTTAAATGGTCTAGGC
	8.2	5'-TTCGAATTCTGAAATTATCTTGTTTC
XbaI	7.1	5'-CACGAGCTCTCCATCTGAACATG
	7.10	5'-GGGCTGCAGGGGGGGGGGACAACAG
St14 (VNTR)		5'-GGCATGTCATCACTTCTCTCATGTT
		5'-CACCACTGCCCTCACGTCCTT

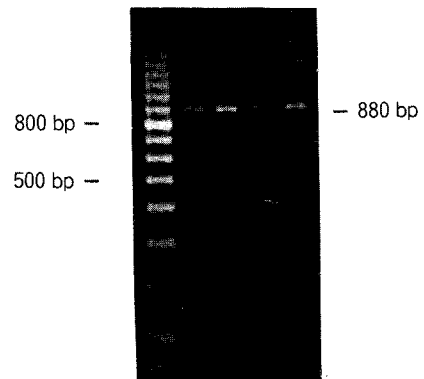
**Table 2.** Frequencies of genes and heterozygotes

	No. of Chromosomes	Alleles		Heterozygotes Expected
		(+)	(-)	
BclI	157	0.81	0.19	0.32
XbaI	116	0.72	0.28	0.40

Synthesizer by the methoxyphosphoramidite method (Beaucage and Caruthers 1981). Amplification with Taq polymerase (Pharmacia) were performed according to a modification of the procedure described by Kogan and Gitschier (1990) for BclI/XbaI RFLP and by Richards *et al.* (1991) for St14 (DXS52) VNTR. Typically, 10ul of amplified samples for BclI and XbaI RFLP was digested with the BclI and XbaI restriction enzyme for two hours, subjected to electrophoresis on 12% polyacrylamide mini-gels, and visualized by ultraviolet fluorescence after staining with ethidium bromide. The amplified products from the St14 VNTR locus were analyzed on an ethidium bromide stained 1% agarose gel run in TBE buffer at 10 V/cm.

## RESULTS

The 142 bp BclI fragment was cleaved to 99 and 43 bp in persons who have (+) allele for the presence of the site and the 96 bp XbaI fragment was cleaved to 68 and 28 bp persons who have (+) allele for the presence of the site. The women who are heterozygous for the BclI site had 142, 99 and 43 bp.

**Fig. 1.** Agarose gel electrophoresis of allelic products from the St 14 VNTR locus. Lane 1: size marker (100 Base-Pair Ladder from Pharmacia); Lane 2, 3, 5: unrelated three normal females; Lane 4: a normal male.

The amplified allele size (frequency) of the St 14 (DXS52) VNTR observed was 880 bp (100%) without any other allele size frequency (figure 1). A faint band sizing between 600 bp and 700 bp was seen randomly both in males and females, which was considered a nonspecific product. Gene frequencies of (+) allele and (-) allele for the BclI/XbaI site are summarized in Table 2.

## DISCUSSION

Although PCR technique is especially valuable for prenatal diagnosis of hemophilia because data on informative loci can be gath-

ered extremely rapidly, it suffers from the same limitation as a Southern blot, namely, that approximately 30 percent of women will not be heterozygous for the BclI or XbaI polymorphisms within the white populations of Europe and North America (Wion *et al.* 1986; Janco *et al.* 1987). In Koreans, the incidence of positive site was 81% for BclI and 72% for XbaI. These incidences for BclI and XbaI were both higher in Koreans than in Caucasians, Mediterraneans, Asian Indians, and American blacks (Gitschier *et al.* 1985; Wion *et al.* 1986; Antonarakis *et al.* 1985). However, the incidence of positive site for BclI was similar to that (82%) reported in Chinese (Chan *et al.* 1988) and to those (85~86%) reported in Japanese (Nishino *et al.* 1987; Mikami 1988). The heterozygote rates for BclI and XbaI polymorphism were 0.32 and 0.40, respectively, which were both lower in Koreans than in Caucasians (Peake 1992). The amplified allele size and frequency by a PCR-based method for analysis of St14 VNTR was 880 bp (100%).

According to Richards *et al.* (1991), 880 bp size was observed as one of the rare allelic products. An extraband of size between 600 bp and 700 bp was occasionally seen in samples both from males or females. Although the significance of this band is not exactly explained, it was considered a nonspecific product because it was seen even in males and was not a constant finding. The product of 880 bp appears to contain 4 tandem repeats. The St14 VNTR specifically maps to Xq28 and is about 2 cM from the hemophilia A locus but its lack of polymorphism makes it useless in the diagnosis of hemophilia A in Korea. This finding is very different from the usefulness based on the large number of alleles in Caucasians and should be useful for the planning of a prenatal diagnosis program for ethnic Koreans.

## SUMMARY

The incidences for positive BclI (intron 18) and XbaI (intron 22) polymorphic sites in the Koreans were 81% and 72%, giving the expected heterozygosity rate of 0.32 and 0.40, respectively. The amplified allele size was

880bp with no other polymorphism in the analysis of St 14 (DXS 52) VNTR.

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