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SHORT COMMUNICATION

TaqI DIGESTION OF PCR PRODUCT INCREASES THE INFORMATIVITY OF St14 VNTR FOR THE DIAGNOSIS OF HEMOPHILIA A

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SUMMARY

Recently, a pair of PCR primers have been described that make it possible to amplify a highly polymorphic VNTR locus DX552 (St14). PCR products range in size from approximately 650 to 3000 bp. Ninety X chromosomes from unrelated Caucasian subjects were investigated. Digestion of the PCR products with TaqI revealed the presence of a polymorphic TaqI restriction site within the product 200 bp from the end. This restriction site is present on 60% and absent on 40% of all alleles, but the absence is confined solely to the alleles 1690 bp (39%) and 2100 bp (1%). Thus, there is a strong allelic association between the most frequent 1690 bp allele and the absence of the TaqI restriction site.
Determination of this polymorphisms within the St14 VNTR region increases the expected heterozygosity at the DXS52 locus from 72% to 80%. This increases the fraction of hemophilia A families where this marker is informative for indirect prenatal diagnosis and carrier identification.

KEY WORDS VNTRs RFLPs PCR St14 (DXS52) locus TaqI digestion hemophilia A

INTRODUCTION

When hybridized with the TaqI–digested DNA, the probe St14 detects at the DXS52 locus a VNTR polymorphism with over 10 alleles ranging in size from 3.4 to 6.6 kb (Oberle *et al.*, 1985). Because of its close linkage with factor VIII gene, and high PIC value, this VNTR is frequently used in indirect diagnosis of hemophilia A. Recently, a pair of primer sequences have been described (Richards *et al.*, 1991) that made it possible to detect this VNTR by PCR amplification. We compared the sizes of St14/TaqI fragments with PCR alleles obtained using the above pair of primers.

MATERIALS AND METHODS

Ninety X chromosomes from unrelated Caucasian subjects were investigated. Standard Southern hybridization with the St14 probe, and PCR amplification were used as described (Richards *et al*, 1991).

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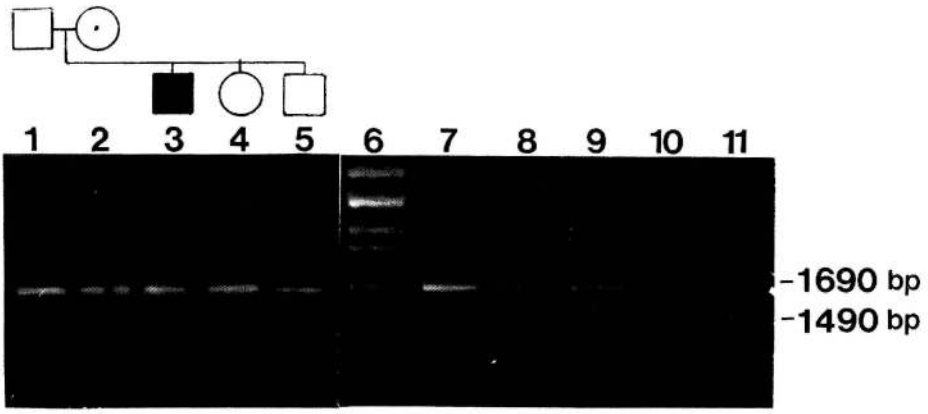

Figure 1. Ethidium bromide stained 1% agarose gel showing VNTR alleles obtained by PCR amplification using St14 primers published (Richards *et al.*, 1991), in a family affected with hemophilia A without TaqI digestion (lanes 1–5), and the same family after TaqI digestion of the PCR products (lanes 7–11). Lane 6-PstI digested phage λ DNA. Digestion reveals that mother and her daughter are heterozygous for the TaqI RFLP within the 1690 bp VNTR allele (the 200 bp fragment in lanes 8, 10 and 11 is not visible). TaqI digestion of the St14 PCR product renders this family informative.

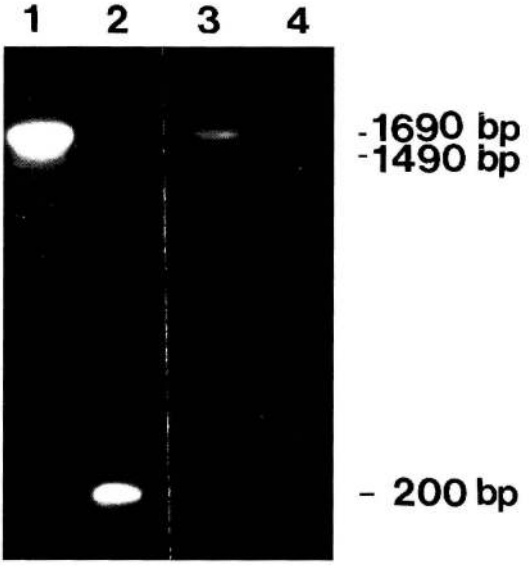

Figure 2. Autoradiogram of the agarose gel analysis of the 1690 bp long DXS52 allele obtained by PCR amplification using St14 primers published (Richards *et al.*, 1991). Lanes: 1) primer A (5'GGCATGTCATCACTTCTCTCATGTT3') was labelled with [^{32}p–γ–ATP]; 2) the same after TaqI digestion; 3) primer B (5'CACCACTGCCCTCACGTCACTT3') was labelled; 4) the same after TaqI digestion.

RESULTS

Lack of a one-to-one correspondence was observed in the PCR alleles sized 1690 bp. While 80% of them (35/44) correspond with the 4.8 kb TaqI fragment, 20% of them (9/44) correspond with 4.5 kb TaqI fragment. Digestion of the 1690 bp allele with TaqI revealed the presence of a polymorphic restriction site within the PCR product (Figure 1). The restriction site is present on all chromosomes with the shorter (4.5 kb) TaqI Southern fragment, and absent on chromosomes with the larger (4.8 kb) fragment. It lies 200 bp from the primer A on the PCR product (Figure 2), so that the 1690 bp allele is cut into 1490 bp and 200 bp fragments. While absent on 80% of the 1690 bp alleles, this restriction site was found absent only once on 46 non-1690 bp alleles investigated (on one of the two 2100 bp alleles) thus exhibiting strong allelic association with the 1690 bp allele. Mendelian inheritance of this RFLP has been verified. The allele sizes and frequencies observed are: 2900 bp (3.3%), 2700 bp (1.1%), 2400 bp (5.5%), 2100bp/TaqI+ (1.1%), 2100 bp/TaqI– (1.1%), 1690 bp/TaqI+ (10.0%), 1690 bp/TaqI– (38.9%), 1630 bp (1.1%), 1570 bp (13.3%), 1390 bp (8.9%), 1300 bp (6.8%), 1220 bp (2.2%), 650 bp (6.7%).

Determination of the TaqI RFLP on the PCR amplified St14 alleles increases the overall expected heterozygosity at the DXS52 locus from 72% to 80%, increasing correspondingly the informativity of this marker for indirect diagnosis of hemophilia A. Figure 1 shows a situation where the TaqI digestion of the 1690 bp allele makes the family informative for prenatal diagnosis and carrier detection.

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