TECHNICAL NOTE

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Analysis of the VNTR Locus DXS52 by the Amp-FLP Technique

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ABSTRACT: The locus DXS52 is useful genetic marker system for forensic analysis. It consists of a variable number of tandem repeats (VNTR) and can be analyzed by the Amp-FLP technique. As accurate data about the distribution of the alleles are among the most important prerequisites for the application in forensic biology, we studied the allele distribution of DXS52 locus in a Chinese population and applied the established technique to paternity testing.

KEYWORDS: forensic science, DNA, DXS52, genetic polymorphism, China

The human genome contains a class of tandemly repeated sequence elements (1). In general, they are scattered throughout the genome. In many cases, the number of tandem repeats is highly variable that results in the so-called variable number of tandem repeat (VNTR) polymorphism. These loci are the most informative genetic markers. Several of these VNTRs occur in association with various genes of clinical interest (2). In forensic science practice, identity tests including both paternity testing and personnel identification also rely on the detection of genetic differences among individuals.

VNTR loci can be located either on autosomes or on the sex chromosomes. Both DXYS14 and DXS52 loci are distributed only on the sex chromosomes. We investigated the DXS52 phenotype and allele distribution in 114 unrelated Chinese individuals, and the mode of inheritance, using an Amp-FLP technique that was also used in paternity testing.

Material and Methods

Sample Collection

Whole blood was obtained in EDTA vacutainer tubes by venipuncture from 114 unrelated Chinese individuals and from two two-generation families (six individuals) at the West China University of Medical Sciences in China.

DNA Extraction

DNA was isolated from leucocytes collected from blood anticoagulated with EDTA using standard methods (3). Following proteinase K digestion, phenol extraction, and ethanol precipitation, the DNA was resuspended in Tris-EDTA buffer for Amp-FLP analysis. Aliquots containing genomic DNA were resuspended as a substrate for the Amp-FLP analysis.

Amplification of DXS52 was achieved using the primers described by Richards and his colleagues (4). The primers were 5′-GGCATGTCATCACTTCTCTCATGTT-3′ and 5′-CACCACT-GCCCTCACGTCACTT-3′. Each sample that was amplified contained 10 to 200 ng of genomic DNA, 1.5 *M* Mg^{++}, 5% formamide, 0.5 units of Taq polymerase (Genetic Institute of Fu Dan University in Shanghai, China), 20 pmol of each primer, and 200 μM of each dNTP (Pharmacia). The total volume of each sample was 20 μL. Each reaction mixture was overlaid with 50 μL mineral oil. The PCR was carried out in a thermocycler (PCR 90-AD, Genetic Institute of China Academy of Sciences) for 30 cycles. Each cycle consisted of 1 min at 94°C for denaturation, 2 min at 58°C for primer annealing, and 3 min at 74°C for primer extension. The size of the generated fragments was determined by 2% agarose gel electrophoresis stained with ethidium bromide using a 123 bp ladder (GIBCO/BRL) and a mixture of λ-*Hind* III and $\phi \times 174$-*Hae* III DNA as size markers. The electrophoresis was run at 10 V/cm for 3 to 4 h. Amplification products were visualized under UV light.

Results

Testing by this Amp-FLP method proved to be adequately sensitive. It was possible to type DXS52 from approximately 5 to 500 ng of genomic DNA derived from liquid blood.

The DXS52 genes are X-linked. Two amplified product bands could be seen in a female's blood sample, whereas only one band could be found in a male's sample. In case of the female, one band indicates the homozygote, whereas two bands indicate the heterozygote.

Population Study

To determine if the Amp-FLP analysis was producing acceptable results, a population study on 114 unrelated Chinese individuals was performed. Fourteen alleles were found (Table 1, Fig. 1). The size of the amplified fragments were 695 to 2400 bp. The designation of 14 alleles was X1, X4, X5, X8, X9, X10, X11, X12, X13, X14, X15, X16, X18, and X29. The repeat unit of DXS52 was 60 bp, whereas the flanking sequence was 650 bp (4). The smallest gene was X1, containing one repeat unit. The largest gene was X29, containing 29 repeat units. The frequencies for 14 alleles are given in Table 1. It was found that the commonest alleles were X11 (frequency 0.221), X1 (frequency 0.171), X10

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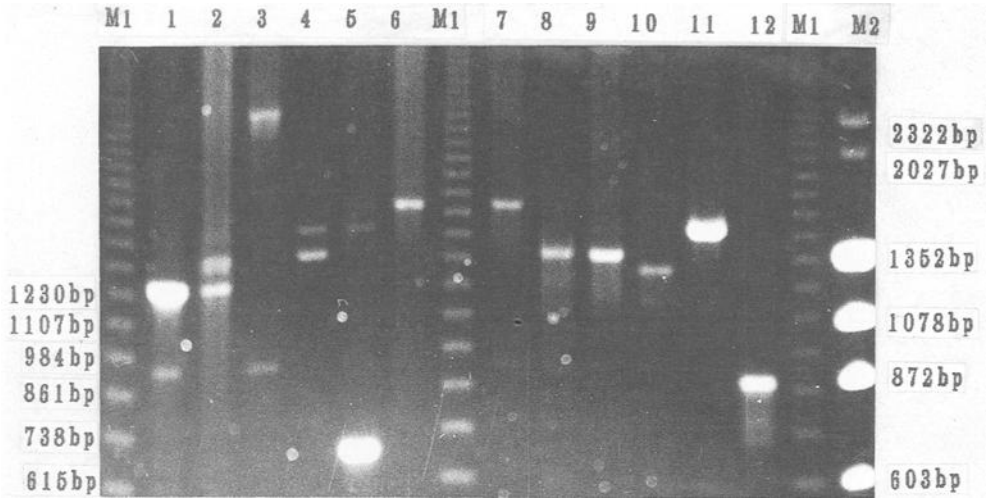

FIG. 1—*Genotypes of DXS52 locus. From left to right: X5-X10, X10-X12, X5-X28, X12-X15, X1-X15, X16-X16, X16-Y, X12-Y, X12-Y, X11-Y, X15-Y, X4-Y. M1 is the 123bp ladder. M2 is a combination of ϕ X174 DNA/Hae III and λ DNA/Hind III. Lane 1–6 are from female; 7–12 from male.*

TABLE 1—*The alleles of DXS52 locus on 158X chromosomes in 114 Chinese of Han population (70 males and 44 females).*

Allele	Number of Alleles: 70X Chromosomes (in 70 males)	Number of Alleles: 88X Chromosomes (in 44 females)	Total	Allele Frequencies
1	11	16	27	0.171
4	3	5	8	0.051
5	1	3	4	0.025
8	0	1	1	0.006
9	5	5	10	0.063
10	7	12	19	0.120
11	15	20	35	0.221
12	7	7	14	0.089
13	4	4	8	0.051
14	3	8	11	0.070
15	3	1	4	0.025
16	6	2	8	0.051
18	3	1	4	0.025
29	2	3	5	0.032

(frequency 0.120), and X12 (frequency 0.089). The frequencies of the rest of the genes were in the range from 0.006 to 0.070. The frequency of X8 gene was the lowest (0.006).

Thirty-five different genotypes (13 in males and 22 in females) were detected in this study. It was found that the genotypes comprising the X1, X10, X11, and X12 alleles were the commonest (X1-Y, X10-Y, X11-Y, and X12-Y in males and X1-X11, X11-X11, and X10-X12 in females). In females, the expected heterozygosity was 0.90 and the observed heterozygosity was 0.77. The discriminating power (DP) was 0.93 in females and 0.89 in males.

Mendelian inheritance of the allelic products and the codominant segregation were observed in two two-generation families (Fig. 2).

Paternity Testing

One couple married in November 1988. They have a boy and a girl and are accused of going against the family planning policy in China. The couple claimed that the girl was adopted by them. The results of the Amp-FLP analysis of DXS52 locus indicated

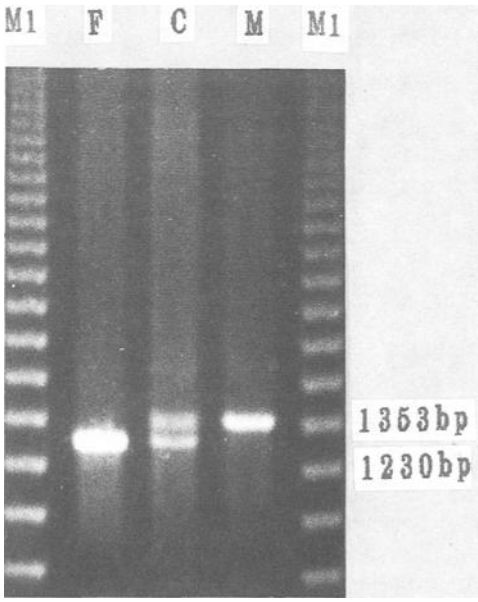

FIG. 2—*Inheritance of DXS52 alleles of one family. DXS52 genotypes of individuals are shown directly above the gel lanes. M1 123bp ladder. F = father, X11-Y; M = mother, X12-X12; C = child; X11-X12.*

that the genotypes of the alleged father, mother, and the girl were X12-Y, X12-X14, and X12-X12, respectively (Fig. 3). It indicated that both the alleged father and mother can provide the obligatory gene of X12 to their girl. So they cannot be excluded as the girl's biological parents. In combination with other genetic markers, the calculated probability of paternity was 99.75%.

Discussion

Oberle and his colleagues (5) reported that the DXS52 locus maps to Xq26-28. The St14 VNTR specifically maps to Xq28 and is located at the distal end of the X chromosome long arm. Its large number of alleles make it useful in the diagnosis of hemophilia.

Analysis of the St14 VNTR by Southern blotting has revealed ten alleles ranging in size from 3.4 to 6.6 Kb (2,6). The region

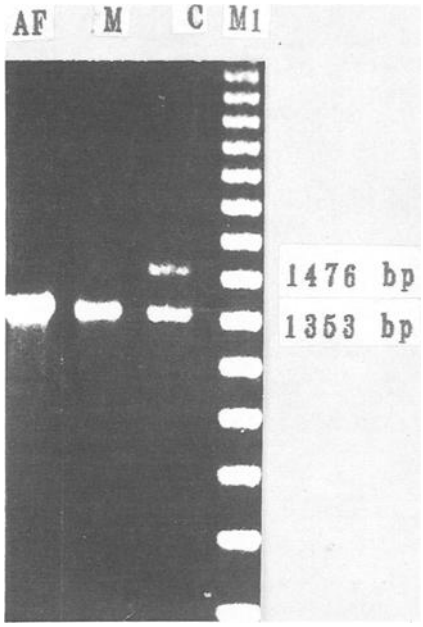

FIG. 3—*Amp-FLP analysis of DXS52 locus in one parentage dispute case. M1 123bp ladder. AF = alleged father, X12-Y; M = alleged mother; X12-X14; C = child, X12-X12.*

responsible for St14 VNTR polymorphism was sequenced from a cloned allele and was found to be a duplicated 60 bp repeat (2).

Amplification primers for the St14 VNTR were designed on either side of the repeat by Richards and his colleagues (4). Fifty unrelated Caucasian males were analyzed using above primers (Table 2). Alleles ranging in size from 700 to 3000 bp were observed. The allele frequencies observed were 0.02 to 0.36. Allele X17 had the highest frequency, 0.36.

We analyzed the polymorphism of DXS52 in a sample of 114 unrelated Chinese individuals by Amp-FLP technique and found 14 alleles ranging in size from 695 to 2400 bp, which is nearly the same as those reported by Richard and his colleagues (4). The amplified allele frequencies observed were 0.025 to 0.221. The difference in gene frequencies was obvious between Chinese individuals and Caucasians. The most frequent allele in Chinese individuals was X11 and, in Caucasians, was X17 (4). The alleles X17, X37, and X39 found in Caucasians were not observed in our study. On the other hand, alleles X4, X5, X8, X9, X13, X14, and X18 that were not found in Caucasians were observed in our study (Table 2).

TABLE 2—*Comparison of allele frequencies of DX52 locus between Chinese and US Caucasian Population.*

Alleles (Number of Repeat Units)	Observed Size, bp	Frequencies	
		Chinese of Han Population (70 males and 44 females) n = 114	US Caucasians (males) n = 50
1	695 (700)	0.171	0.120
4	880	0.051	···
5	930	0.025	···
8	1100	0.006	···
9	1170	0.063	···
10	(1220)*	0.120	0.020
11	1300	0.221	0.020
12	1370 (1390)	0.089	0.100
13	1420	0.051	···
14	1480	0.070	···
15	1540 (1570)	0.025	0.140
16	1630	0.051	0.020
17	··· (1690)*	···	0.360
18	1760	0.025	···
29	2400	0.032	0.120
37	··· (2900)*	···	0.080
39	··· (3000)*	···	0.020

*The size of alleles observed by Richards.

The DP value of this locus was 0.89 in Chinese males and was 0.93 in females. It is suggested that the Amp-FLP analysis of DXS52 is useful in paternity testing in China. This locus can be used to assist in identifying a girl's mother or father or a boy's mother.

This locus can also be used for sex determination. Two amplified product bands indicate the sample from female (heterzygote). One amplified product band indicates that the sample is from a homozygous female or from a male.

In a relatively small sample, not all genotypes can be observed. It might be the reason that a departure from the expected heterozygosity (0.90) versus the observed heterozygosity (0.77) was seen in this study. Compared with other studies, the sample in the present study is not too small. To meet the requirement of Hardy-Weinberg equilibrium test, the sample seems not large enough.

In general, amplified product bands of a large allele are weaker than those of a small allele. However, a peculiar phenomenon in which no regularity was demonstrated between the intensity of the allelic product bands and the size of allele for a female heterozygote. For example, the X10 was greater than X5 in the first sample of the figure, whereas the X5 was less than X12 in the fourth sample, and so forth. Once a weak amplified product band had been occurred in the case of a female heterozygote, adequate template DNA was added to the reaction mixture to guard against the occurrence of allelic loss.

The method presented here is simple and rapid, and no radioactivity is involved. It is a suitable tool for solving forensic parentage problems.

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