TECHNICAL NOTE

David R. Young,[1] B.S.; Zaw Tun,[2] Ph.D.; Katsuya Honda,[2] Ph.D.; and Ryoji Matoba,[2] Ph.D.

Identifying Sex Chromosome Abnormalities in Forensic DNA Testing Using Amelogenin and Sex Chromosome Short Tandem Repeats

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ABSTRACT: Forensic DNA laboratories worldwide have begun using multiplexed STR systems to decrease analysis time and increase sample throughput. The loci used in these systems are basically "nonsense" regions of human DNA. However, due to the chromosome on which some of these loci are located, various genetic abnormalities can sometimes be detected. This paper will show one such abnormality—Klinefelter's Syndrome—and the process used to show the possibility of this defect in two undiagnosed males using peak height ratios at the Amelogenin locus, and X-Y STRs.

KEYWORDS: forensic science, DNA typing, short tandem repeat, amelogenin, X chromosome, Y chromosome, polymerase chain reaction, Klinefelter's Syndrome

The use of PCR (Polymerase Chain Reaction) and STR (Short Tandem Repeat) loci has, in a very short time period, taken the place of RFLP and AmpFLP testing as the mainstay of forensic laboratories around the world. By creating multiplexes capable of amplifying numerous loci in a single amplification, a forensic DNA analyst may gain an enormous amount of information in a relatively short amount of time. There are times, however, when additional information about a biological stain may prove to be very beneficial.

Genetically "normal" individuals have 23 pairs of chromosomes, one from each parent. This includes one X chromosome from each parent in females, and one X and one Y chromosome from each parent in males. Sometimes, however, partial nondisjunction of chromosome pairs during cell division results in gametes with more or less genetic material than normal. This can result in the appearance of any of a variety of genetic abnormalities.

All forensic laboratories which have been conducting DNA testing for even a short amount of time are aware of the possibility of coming across various genetic abnormalities. These abnormalities could include such things as Down's Syndrome, which exhibits three chromosome 21 alleles and can be seen at the STR locus D21S11, and various sex chromosome abnormalities such as Klinefelter's Syndrome which exhibits more than one X allele in affected males and XYY Syndrome which exhibits two Y alleles in affected males. Klinefelter's males are typically tall and have a high occurrence of testicular atrophy; otherwise they are normal in appearance. Many individuals with Klinefelter's are diagnosed because of problems with infertility. By using peak height ratios at the Amelogenin locus and X-Y STRs, forensic DNA analysts have the capacity to visualize sex chromosome anomalies such as Klinefelter's and XYY Syndromes. Down's Syndrome can occur as frequently as 1 in every 700 births (1), and Klinefelter's and XYY Syndromes can occur as frequently as 1 in every 700 males (2). While most forensic laboratories do not have the capability of karyotyping samples to determine the presence of these anomalies, current STR methods do give the forensic DNA analyst the ability to observe possible genetic defects in a sample.

Materials and Methods

Sample Preparation

Whole blood was obtained from two suspected Klinefelter males (adjudicated case samples) and five diagnosed Klinefelter males (graciously provided by LABCORP, North Carolina), dried onto FTA paper™ (Life Technologies, Rockville, MD), and frozen prior to use. DNA was extracted from all seven samples organically using the phenol chloroform method followed by concentration in Centricon 100 tubes (Millipore, Bedford, MA) (3). The DNA was then quantitated using the Quantiblot Human DNA Quantitation Kit (PE Biosystems, Foster City, CA) (4).

STR Typing and DNA Analysis

DNA amplification was performed using the AmpFℓSTR Profiler Plus kit (PE Biosystems, Foster City, CA), which coamplifies D3S1358, vWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820. Amplification was carried out as per manufacturer's recommendations (5) except that 25 μL reaction volumes containing 1 ng of template DNA were used. PCR conditions were as per manufacturer's recommendations (5) using a Perkin Elmer Thermalcycler 480.

[1] Forensic DNA analyst, Texas Department of Public Safety Crime Laboratory, Lubbock, Texas.

[2] Research assistant, associate professor, professor, respectively, Department of Legal Medicine, Osaka University Graduate School of Medicine, Osaka, Japan.

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Electrophoresis of the amplified products was carried out on an ABI PRISM 310 Genetic Analyzer. The data was collected using the ABI PRISM 310 Collection software (PE Biosystems, Foster City, CA), analyzed using Genescan 3.1 (PE Biosystems, Foster City, CA) software, and genotyped using Genotyper 2.0 (PE Biosystems, Foster City, CA) software. Peak height ratios were calculated using simple ratios of the X and Y peaks at the Amelogenin locus.

X-Y STR Typing and DNA Analysis

For corroboration of a possible genetic abnormality (i.e., Klinefelter's Syndrome), additional testing of these sample extracts was conducted using X-Y specific STRs. Amplification and typing of three X and two Y chromosomal STRs (STRX1/HPRTB/ARA and DYS390/DYS393 respectively) was accomplished as previously described by Tun et al. (6).

Results and Discussion

During the course of routine casework, samples from two males—referred to later as samples A and B—were shown to contain abnormal peak height distribution at the Amelogenin locus (Fig. 1). Upon further investigation, it was determined that the Y:X peak height ratios were less than 50%. Normal male samples exhibit peak height ratios of 70% or greater within a locus. This abnormality was also seen in questioned stains. It was hypothesized that these two individual males might have an extra X chromosome—also known as Klinefelter's Syndrome. Since these cases were adjudicated and only being analyzed for CODIS (COmbined DNA Index System) databasing, permission was obtained to further investigate these samples. Samples of known Klinefelter males were obtained for a comparison study with the two suspected samples. All seven samples presented similar results at the Amelogenin locus. The average Y:X peak height ratio of the five known Klinefelter samples is 54% (Fig. 1). The Y:X peak height ratio for samples A and B, however, are approximately 15% lower at 36% and 39% respectively. This could indicate the possibility of XXXY individuals; however, this was not proven in subsequent X-Y STR analysis. In each sample, the data collected at the Amelogenin locus shows the X peak heights to be consistently higher than the Y peak heights. By using simple peak height ratios, one can see the amount of variation between the X and Y peaks present in each sample (Fig. 1). While this alone is not definitive proof of a genetic abnormality, it does provide evidence that these two individuals—A and B—carry a sex chromosome anomaly in the form of XXY (or XXXY).

When coupled with X-Y STR typing, much stronger proof of a genetic abnormality is evident in all but one of the samples. The current trend in forensic and paternity DNA testing involves implementing the use of X-Y STRs (7–9). A special interest lies with Y STR typing in rape cases (10) where the process used to separate epithelial and sperm cells does not always allow for complete separation, and some "carry-over" can occur between male and female fractions. This "carry-over" is not a problem with Y chromosomal STRs. X-Y chromosomal STRs can also provide paternity testing labs with the ability to show maternal and paternal inheritance, including forensic paternity and reverse paternity samples.

A quadruplex PCR containing two Y (DYS393/DYS3909) and two X (HPRTB/STRX1) STR loci readily identifies one allele at each Y locus and two alleles at each X locus (Fig. 2). Additional testing utilizing the X STR locus ARA obtained similar heterozygous results. In six of the seven samples analyzed—including the two undiagnosed males—heterozygosity can be seen in at least

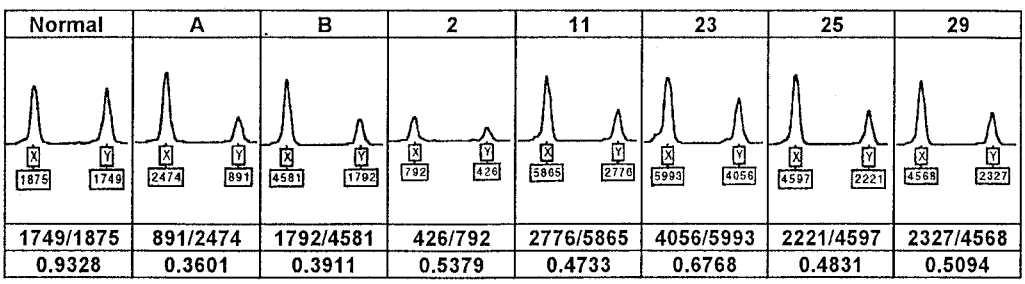

FIG. 1—*Graphical representation of the Amelogenin locus in a normal male; suspected Klinefelter males (A,B); diagnosed Klinefelter males (2,11,23,25,29); Corresponding Y/X peak heights and ratios.*

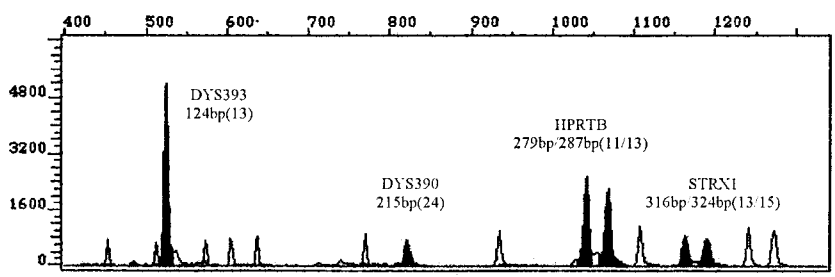

FIG. 2—*Electropherogram of quadruplex PCR of undiagnosed male sample B showing XXY pattern. Two peaks are detected in each of two X loci. Repeat units are in parenthesis.*

TABLE 1—*Typing results of X-Y STR loci.**

Sample	STRX1	HPRTB	ARA	DYS390	DYS393
A	13/15	12/12	25/26	23	13
B	13/15	11/13	19/24	24	13
2	14/14	11/11	25/26	26	13
11	12/13	12/16	19/21	24	13
23	13/13	12/14	22/22	24	13
25	15/15	15/15	18/18	24	13
29	14/14	12/12	26/28	25	12

* Shaded areas indicate heterozygosity of X STR loci.

one of the X STR loci (Table 1). The remaining sample, a diagnosed Klinefelter male, proved to be homozygous at all three X STR loci. Due to the occurrence of homozygotes, as was seen in this sample, caution should be urged when interpreting this type of data. It should also be noted that even though this sample showed to be homozygous in all three X STR loci, the peak height ratio seen at the Amelogenin locus is still consistent with the data seen in the other six samples tested. As previously mentioned, there was no data to substantiate the possibility of XXXY. No more than two alleles were seen at each X locus, and the alleles at each locus were in balanced proportions (Fig. 2). This data supports the XXY hypothesis.

This sort of analysis may seem somewhat limited in value, but it could be quite useful on very old or highly degraded samples. These types of samples may yield incomplete results at some of the larger base pair loci, but since the Amelogenin locus and some of the X-Y STR loci are considerably smaller, an anomaly such as Klinefelter's Syndrome could be the difference in obtaining limited results, and acquiring information that could prove to be indispensable. This same line of testing should also be useful on other sex chromosome disorders (i.e., XYY, XO, XXXX), as well as other genetic aberrations caused by chromosome nondisjunction. While this type of analysis cannot replace karyotyping in determining the presence of genetic abnormalities, it can offer very useful information during the course of routine forensic casework.

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Additional information and reprint requests:
David Young
Texas Department of Public Safety
Crime Laboratory
PO Box 420
Lubbock, Texas 79408
e-mail: david.young@txdps.state.tx.us