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Erroneous Gender Identification by the Amelogenin Sex Test

ABSTRACT: Human gender identification, based on the amelogenin gene, has important applications in forensic casework, prenatal diagnosis, DNA databasing, and blood sample storage. However, we report on the first known case, in the Israeli population, of an amelogenin sex test failure on a phenotypically normal male. He was typed as a female by both the AmpFℓSTR® SGM plus™ and GenePrint™ kits. Subsequent, karyotyping of the soldier's blood sample showed no abnormalities. These results suggest that the determination of sex, based on the amelogenin test, should be interpreted cautiously.

KEYWORDS: forensic science, amelogenin, SGM plus, GenePrint, FTA, short tandem repeats, polymerase chain reaction

The amelogenin gene, located on the X and Y chromosomes in humans (1), produces a protein important in the development of the tooth enamel matrix (2). Using specific amelogenin PCR primers, different bp fragments are amplifiable from the X and Y chromosomes, respectively (3). Hence, it has been a central system to differentiate males from females especially in forensic casework and prenatal diagnosis. In the forensic field, the sex test is part of most commercially available PCR kits such as the AmpFℓSTR® SGM plus™ kit (PE Applied Biosystems) and the GenePrint™ (Promega) kit.

For the past few years, the IDF (Israel Defense Force) has been storing soldiers' blood samples on FTA® papers. Samples from this repository are solely for DNA profiling to identify a soldier's body or body parts in cases in which other methods, such as fingerprinting or dental comparisons, are not possible and for quality control of the bloodstain pool.

During the course of one of these quality control checks, a sample taken from a male soldier was genotyped as having originated from a female. Deoxyribonucleic acid profiling of a second bloodstain taken from the soldier confirmed that the amelogenin anomoly was not the result of a sampling error. Furthermore, the soldier's karyotype showed that of a normal male, i.e., containing both the X and Y chromosomes.

Materials and Methods

Sample

All the samples in the IDF repository were stored as dried bloodstains on FTA® paper. Deoxyribonucleic acid was purified by a modified method (4) from 1-mm diameter circles of bloodstained FTA® papers (including that of the soldier in this case). Distilled water was used to purify the papers instead of the buffer recommended by the manufacturer. Subsequently, DNA was extracted from a second blood sample of the soldier using a phenol/chloroform extraction method (5).

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Amelogenin Multiplex Typing

Deoxyribonucleic acid from the 1-mm diameter circle FTA® paper was amplified using the AmpFℓSTR® SGMplus™ kit (PE Applied BioSystems, Foster City, CA) in accordance with the manufacturer's recommendations (6). Amplified PCR products were analyzed by ABI PRISM® 310 Genetic Analyzer (PE Applied BioSystems). Electrophoresis separation was carried out using a 50-μm capillary, 47 cm in length, 30 min. running time, 5 sec. injection time, 60°C running temperature and loaded with POP-4 polymer. Allele size was calculated using the GeneScan® Analysis 3.7 software (PE Applied BioSystems) calibrated by a known internal lane size standard, ROX-500 (PE Applied BioSystems). Genotyping was implemented by using Genotyper® 3.7 software (PE Applied BioSystems).

Amelogenin Monoplex Typing

An estimated 1 to 3 ng of DNA, extracted by the organic method, was amplified using Gene Print™ silver stain detection kit (Promega, Madison, WI) according to the manufacturer's protocol (7). The PCR products were separated on a 4% polyacrylamide gel, visualized by silver staining (8) and compared with a ladder that included both amelogenin X and Y fragments.

Results and Discussion

The amelogenin standard (ladder), supplied with the SGMplus kit, yielded peaks of 103bp and 109bp (Fig. 1, I). The former peak was from the X chromosome and the latter was from the Y chromosome. As expected, DNA from a known male contained two peaks (Fig. 1, II) and that from a known female contained one peak (Fig. 1, III), corresponding to the X-Y and X chromosomes, respectively.

In contrast, the DNA of the known male soldier (Fig. 1, IV) amplified at 103bp (X) but failed to amplify at 109bp (Y). Moreover, the height and area of this 103bp peak (Fig. 1, IV) are similar to that of the female sample (Fig. 1, III) precluding the correct sex designation of this sample.

For the silver staining method, the PCR primer set differed from that used in the capillary electrophoresis method. The flanking region for the X and Y chromosomes was extended by 109bp. Despite this difference, the soldier's Y chromosome, the 218bp

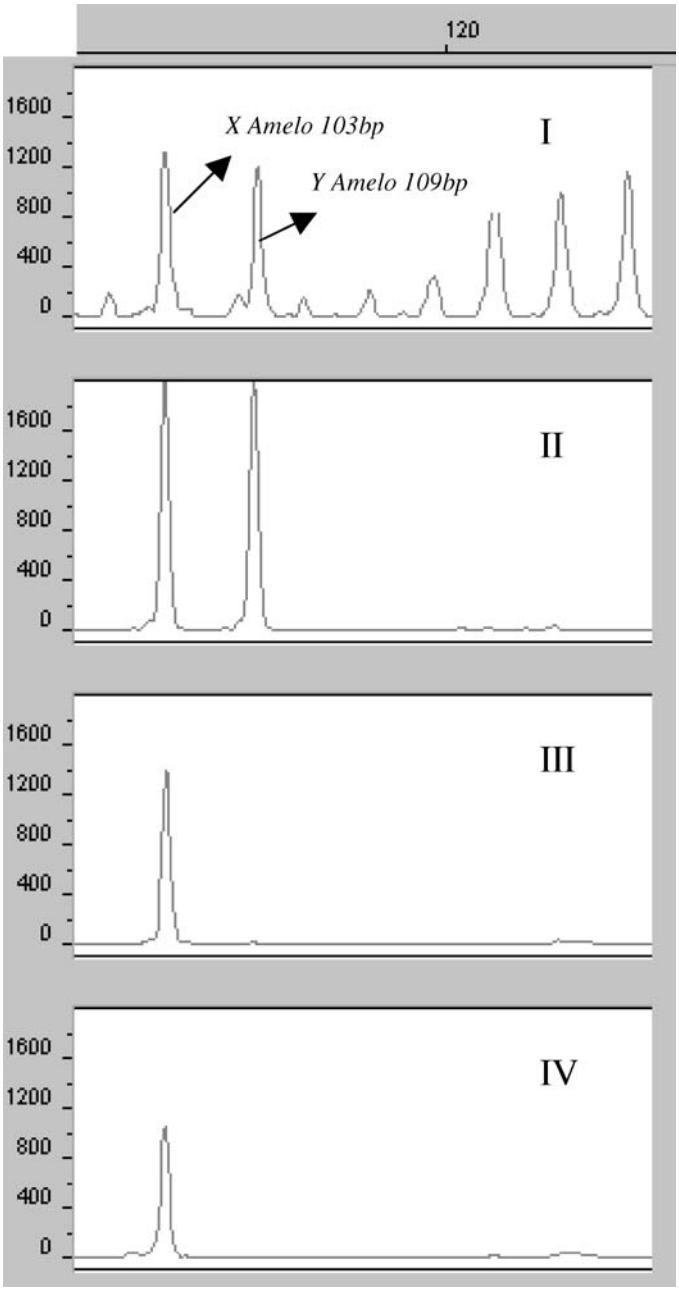

FIG. 1—*Capillary electrophoresis of the amelogenin. I – allelic ladder, II – known male sample, III – known female sample, IV – sample of the soldier.*

fragment, failed to amplify (Fig. 2, Lane C). Both the allelic ladder and the known male showed the expected amplification products of 212bp and 218bp (Fig. 2, Lanes A and B).

Finally, the karyotyping results on the soldier's blood sample confirmed that he possessed a normal Y chromosome (data not shown).

The occurrence of this phenomenon has been reported as an 0.018% observed sex test failure rate in the Austrian National DNA database (9), 1.85% observed sex test failure rate in Indian males (10), 0.6% frequency of sex test failure attributable to deletion from 350 specimens from all around the world (11), and 8% (2 out of 24) samples of unrelated Sri Lankan males (11).

With the finding of our first mistyped amelogenin result on a male out of a total of 96 samples, we can report the failure rate of this test as 1.04% in Israel. Moreover, the failure of two different primer sets, to amplify the Y chromosome DNA, suggests that this sample contains a deletion in the relevant area.

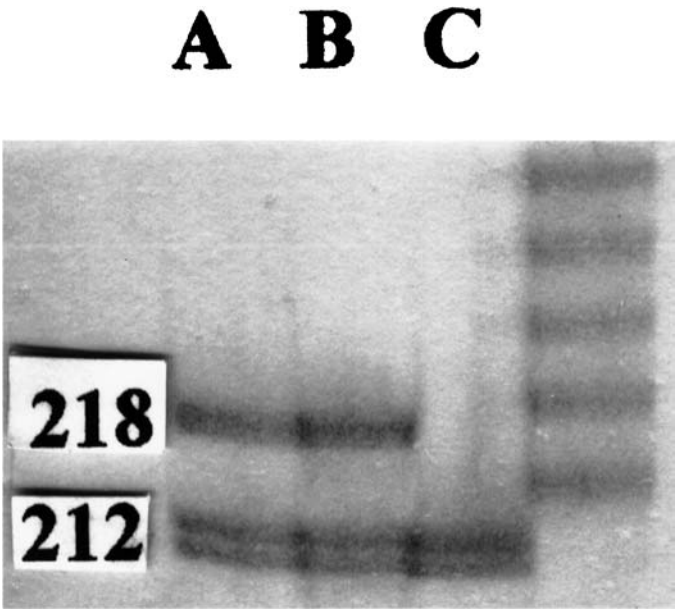

FIG. 2—*Polyacrylamide gel. Lane A – allelic ladder, Lane B – known male sample, Lane C – the soldier's sample.*

In the forensic field, a mistyped amelogenin test could have far reaching consequences. Based on such a result, a body fluid from a crime scene may be incorrectly reported as having a female origin thereby erroneously excluding the male perpetrator of the crime.

Therefore, our case and that of others strongly supports the suggestion that other Y-locus STR's should be checked routinely in sex crime cases to avoid incorrect conclusions as a result of possible amelogenin test failures.

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