Int J Legal Med (2002) 116: 117–120

SHORT COMMUNICATION

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Rare failures in the amelogenin sex test

Received: 12 February 2001 / Accepted: 24 July 2001

Abstract Determination of sex using the amelogenin sex test is well established in the forensic field especially for casework and DNA databasing purposes. The sex test is part of commercially available PCR kits. Among 29,432 phenotypic male individuals stored in the Austrian National DNA database, 6 individuals were found to lack the amelogenin Y-specific PCR product which was confirmed using alternative amelogenin primers. The amplification of eight Y-chromosomal STR markers resulted in full profiles in five out of the six samples, one sample failed to amplify Y-STRs at all. The amplification of a fragment of the SRY gene gave positive results in all six samples, confirming the male phenotype of the individuals. The observed failure rate of the amelogenin sex test was 0.018% in this study.

Keywords DNA · PCR · Amelogenin · Sex test · SRY · Y-STRs

Introduction

The human amelogenin gene, usually typed for sex determination, is located on both the X- and Y-chromosomes as single copies in X and Y homologous regions [1, 2]. Several PCR primer sets have been developed [3, 4, 5, 6, 7, 8], and the most commonly used PCR-based sex test is the one described by Sullivan et al. [6], flanking a 6 bp deletion on the X homologue, resulting in 106 bp and 112 bp PCR products from the X and Y chromosomes, respectively. Amelogenin-based sex tests are part of various PCR multiplex reaction kits from different manufacturers which are widely used for DNA typing of both reference samples and casework samples in the forensic field [9, 10, 11, 12, 13], especially for DNA databasing purposes [14, 15, 16].

The Austrian National DNA Database, which currently has about 35,000 suspect profiles stored, is routinely quality-checked. One of the check routines consists of a comparison of the sex documented on the intelligence submission form and the sex determined by the amelogenin sex test. Discrepancies could be due to administrative errors or sex test failures. The phenomenon of failures has been described by Santos et al. [17], Roffey et al. [18] and Thangaraj et al. [19]. The forensic usefulness of SRY was described by Naito et al. [20].

This paper presents the strategies and results in order to solve problems with sex test reliability.

Material and methods

Buccal swabs were taken from 29,432 male individuals as reference samples for the Austrian National DNA database during the last 3 years. The sex of the sample donors was documented on an intelligence submission form by the police officer and additionally a photograph of the donor was taken.

DNA was Chelex-extracted and amplification was carried out applying a heptaplex PCR multiplex kit [15] or the AmpFlSTR SGM plus systems kit (PE Applied Biosystems) according to the manufacturer's recommendations and repeat amplification was carried out using the AmpFlSTR SGM plus kit.

An alternative amelogenin primer set was designed using Primer Express software (PE Applied Biosystems) and sequence data were obtained from the GenBank sequence database (accession numbers M55418 and M55419). These alternative primer sequences were 5′-(FAM)-ACCACCTCATCCTGGGCAC-3′ (position 194–212 on AmelX and on AmelY) for the upstream strand and 5′-ACACAGGCTTGAGGCCAACC-3′ (position 393–412 on AmelX and position 399–418 on AmelY) for the downstream strand resulting in 219 bp and 225 bp products from the X and Y chromosomes, respectively.

In order to amplify the SRY marker, primer sequences located in the non-coding region of the gene as described by Lo et al. [21] were used, and the forward primer was FAM-labelled.

Co-amplification of both amelogenin and SRYloci was carried out using 20 µl reaction volume containing 1 U AmpliTaqGold (PE Applied Biosystems), 0.1 µM of each primer and 200 µM of each dNTP. All samples were amplified through 28 cycles comprising 1 min at 94°C, 45 s at 60°C and 60 s at 72°C following initial denaturation at 95°C for 11 min and final incubation at 72°C for 60 min.

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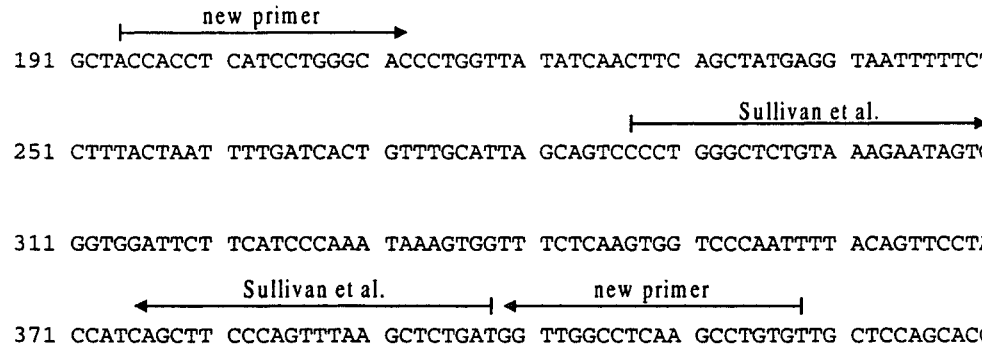
Fig. 1 Part of the nucleotide sequence of the human amelogenin gene on the Y-chromosome (GenBank accession number M55419) showing the location of the annealing regions of the two primer sets used (numbers indicate position according to GenBank)



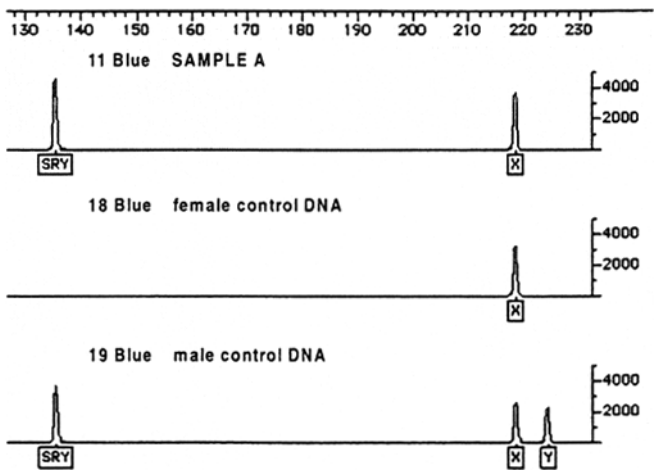


Fig. 2 Electropherograms of sample A, male and female controls, coamplified with amelogenin (newly designed primers) and SRY

The Y-chromosomal STR markers DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385 were amplified as described by Kayser et al. [22].

All PCR reactions were carried out in a Perkin Elmer 9600 thermal cycler and products were loaded on the CE310 Genetic Analyser (ABI), using Genescan-500 Tamra or Genescan-500 ROX (PE Applied Biosystems) as internal lane standards. GeneScan analysis was performed on the raw data and alleles were labelled according to the international nomenclature [23] using the Genotyper Software package (PE Applied Biosystems). Confidence intervals for the observed deletion frequency were calculated using bootstrapping as described by Evett and Weir [24].

Results and discussion

Routinely performed plausibility check procedures of the data in the Austrian National DNA Database revealed discrepancies between the sex documented on the intelligence submission form and the results of the amelogenin sex test in 6 out of 29,432 male individuals.

The male phenotype of all six persons lacking the amelogenin Y-specific PCR product was initially confirmed by the Austrian intelligence database and then confirmed through analysis of the stored digital photographs of the individuals. Therefore registration errors resulting from the admission of a new record at the DNA database site in the Austrian Ministry of the Interior were excluded.

Repeated PCR analysis with the SGM plus system of all six samples confirmed the original results (data not shown).

One possible explanation for the phenomenon could be a mutation within the annealing region of the primers [25]. A strategy to solve this problem was to amplify amelogenin using alternative primers. These were designed as depicted in Fig. 1, resulting in 219 bp and 225 bp amplification products, spanning the 6 bp deletion.

Amplification with the newly designed primers resulted in the same phenomenon – the lacking of the amelogenin Y-specific PCR product (data shown in Fig. 2 and Table 1). Our results suggest a deletion on the Y-encoded gene rather than a polymorphism at one of the priming sites of the two amelogenin primer sets used for all six cases.

The amplification of eight Y-chromosomal STR markers DYS19, DYS389I, DYS389II, DYS390, DYS391,

Table 1 Results of the amelogenin sex test (same for both primer sets used), Y-STR haplotypes and SRY results of six phenotypically male individuals lacking the amelogenin Y-specific PCR product

Sample	Sex phenotype	Amelogenin	Y-STR haplotypes								SRY
			DYS19	DYS389/I	DYS389/II	DYS390	DYS391	DYS392	DYS393	DYS385	
A	Male	X	16	12	28	22	10	12	13	13,14	+
B	Male	X	14	13	29	22	10	11	12	13,15	+
C	Male	X	13	13	30	23	10	11	12	15,18	+
D	Male	X	14	12	27	22	10	11	13	14,14	+
E	Male	X	13	13	29	24	10	13	13	11,14	+
F	Male	X	–	–	–	–	–	–	–	–	+

DYS392, DYS393 and DYS385 was successful in five of the six samples, one sample failed to amplify all eight loci (data shown in Table 1). The amplification of SRY, the male sex determining gene [26], was successful with all six samples, which is a confirmation of the male phenotype of all six individuals (Fig. 2 and Table 1). In conclusion the sex could be determined correctly in five of the six cases by typing Y-STR loci, in one case only typing of SRY led to the correct sex test result.

These results suggest that for the five samples showing amplifiable Y-STRs, the assumed deletion seems to be limited to the amelogenin-related region on the Y-chromosome. The Y-STR haplotypes of the five individuals are different which can be interpreted as being a relatively old single deletion event or that the deletion occurred several times.

For sample F, a deletion polymorphism spanning a major part of the Y-chromosome from Yp11.2 on the short arm up to Yq11.21 on the long arm or a translocation of SRY to the short arm of the X-chromosome can be surmised as possible explanations [27]. The second alternative could also mean that this phenotypic male individual does not posses any Y-chromosome at all. Our samples were taken as buccal swabs for databasing purposes, therefore routine karyotyping was not possible for further investigations.

Santos et al. [17] reported a 0.6% frequency of sex test failures due to deletion polymorphisms in their sample set (n=350, samples from all around the world). The two individuals showing the deletion both came from Sri Lanka. Santos et al. [17] observed a deletion frequency of 8% within their Sri Lankan population sample (n=24) and suggested warningly, that the frequency might be higher in some populations, than the aforementioned 0.6%.

Thangaraj et al. [19] investigated a sample of 270 Indian male individuals and found 5 individuals showing deletion polymorphisms and the observed deletion frequency was 1.85%.

The number of Caucasians in our sample of phenotypic male individuals (n=29,432) was 28,182 and the 6 individuals showing sex test failures were all Caucasians. Only the five consistent cases were taken for frequency calculations, the sixth case represents an additional phenomenon. The observed frequency of the ameleogenin sex test failures in our Caucasian sample is thus 0.018% with 95% confidence interval limits of 0.004–0.035%. This is significantly lower than that found by Santos et al. [17] and Thangaraj et al. [19] and could be explained by population-specific differences or by the large sample number of our data.

If the accuracy of sex determination is required for active criminal investigations, the observed amelogenin sex test failure rate and the presented strategy to avoid sex test failures should be considered.

Acknowledgements The authors would like to thank the database staff at the Ministry of the Interior, Vienna, Austria for the excellent cooperation. We would also like to thank the database staff at the Institute of Legal Medicine for their outstanding work and their valuable assistance. This study was partially supported by a grant of the Oesterreichische Nationalbank, Jubiläumsfondprojekt Nr.: S97/4 (Austrian National Bank).

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