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SHORT COMMUNICATION

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Is the amelogenin gene reliable for gender identification in forensic casework and prenatal diagnosis?

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Abstract In humans, the amelogenin gene is present on both the X and the Y chromosomes. However, there are size differences in this gene between these chromosomes, which have been utilised for sexing in forensic casework and prenatal diagnosis. Our study using the AmpF*l* STR Profiler Plus kit, showed a deletion of Y chromosome-specific amelogenin in five Indian males (1.85%). We propose to call them "*d*eleted-*a*melogenin *m*ales" (*DAMs*), who but for the detection of the presence of other Y-specific markers (e.g. *SRY*, STR and 50f2) would have been identified as females. Considering the consequences of the result obtained only using the amelogenin marker, we suggest the use of additional Y chromosome markers for unambiguous gender identification.

Keywords Amelogenin · Gender identification · Haplotype · STR · Y chromosome

Introduction

Human identification is an important forensic application and various strategies have been adopted to utilise the minimum quantity of sample to detect maximum variability in a single PCR reaction. The amelogenin gene present on the X (*AMEL X*) and the Y (*AMEL Y*) chromosomes of humans (Bailey et al. 1992) showed size differences between these two chromosomes and therefore, this gene has been used to differentiate males from females (Mannucci et al. 1994; Haas-Rochholz and Weiler 1997). Various companies manufacture multiplex STR kits containing the amelogenin system for individual and gender identification, respectively. Our study using one such kit (AmpF*l* STR Profiler Plus, Perkin Elmer) showed a deletion of the amelogenin gene on the Y chromosome in five Indian males.

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Materials and methods

Samples

We have studied a total of 270 male samples of which 20 were analysed for establishing parentage, 190 were random males belonging to various caste and tribal populations, analysed for genetic diversity and the remaining 60 samples were infertile males. DNA was extracted from the above samples using a standard protocol.

STR profiling

DNA samples were amplified using the AmpF*l* STR Profiler Plus kit (Perkin Elmer) as per the manufacturer's instructions. Y-STRs such as DYS19, DYS389 (amplifies two loci DYS389I and DYS389II), DYS390, DYS391 and DYS393 were amplified in a multiplex reaction and the amplified products were analysed in an ABI PRISM 377 (Thangaraj et al. 1999).

PCR assay of the *SRY* gene

The sex determining region on the Y chromosome (*SRY*) of the individuals, who showed the amelogenin deletion, were amplified using following primers:

- *SRY2* – CTG TAG CGG TCC CGT TGC TGC GGT G
- *SRY3* – CCC GAA TTC GAC AAT GCA ATC ATA TGC TTC TGC

Amplification was carried out in a GeneAmp 9600 thermal cycler employing the conditions: 30 cycles at 94 °C for 2 min, 60 °C for 2 min, and 72 °C for 2 min. Amplified products were separated in 2% agarose gels (Singh et al. 1999).

Southern hybridisation

All the five Y-amelogenin deleted male samples were hybridised with an α^{32}P-labeled 50f2 probe (Jobling 1994).

Results and discussion

Analysis of the 270 male samples with the AmpF*l* STR Profiler Plus kit revealed a deletion of Y-specific amelogenin (*AMEL Y*) in five males accounting for about 1.85%

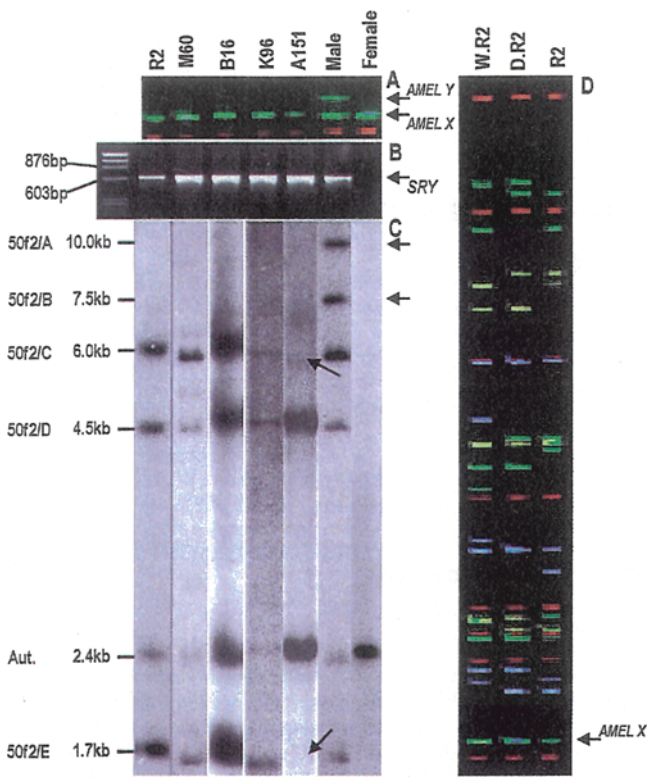

Fig. 1 **A** GeneScan analysis using the AmpF*l* STR Profiler Plus kit shows deletion of *AMEL* Y in five males (R2, M60, B16, K96 and A151). **B** PCR products of the *SRY* gene show the presence of *SRY* (609 bp) in all the five *d*eleted-*a*melogenin *m*ales (R2, M60, B16, K96 and A151). **C** Deletions of 50f2 A and B loci in all five males (R2, M60, B16, K96 and A151) confirm a large deletion in the p-arm of the Y chromosome in all the *DAM*s. Also note the deletion of the C and E loci (*arrow*) in an infertile male (A151). Aut, which is also present in females, is an autosomal loci whereas the rest are Y-specific markers. **D** STR profiles of a *DAM* (R2), his wife (W.R2) and daughter (D.R2). Red bands, which are common to all lanes are the molecular weight markers. Other colour bands represent various STR alleles. Note the inheritance of 50% of STR alleles from father to daughter

(Fig. 1 A) and we propose to call them "*d*eleted-*a*melogenin *m*ales" (*DAMs*). Of the five *DAMs*, one was from a parentage case (R2), three were random males (M60, B16 and K96) belonging to different caste populations and one was an infertile male (A151). However, *SRY* was present in all the five *DAMs* (Fig. 1 B). In Southern hybridisation using the 50f2 probe, all the five *DAMs* showed deletion of the A and B loci (Fig. 1 C), which encompass about 1 Mb in the p-arm of the Y chromosome, ruling out the possibility of a point mutation in the priming site on the Y chromosome. In addition to deletion of the A and B loci, one infertile male (A151) also showed a deletion in the q-arm of the Y chromosome (C and E loci) corresponding to the AZFb and AZFc regions, which are implicated in male infertility. STR profiling and genotyping of one *DAM*, along with his wife and child, revealed a paternal contribution of 50% of the STR alleles (Fig. 1 D), confirming that despite a large deletion on the p-arm of the Y chromosome, the individual is a fertile male. The six Y chromosome STR loci studied on the *DAMs* revealed four haplotypes (Table 1) and two individuals who shared the same haplotype belonged to the same religious group (Muslim). However, they came from different regions of India and were part of a different study group. Y-haplotype analysis suggests that there are four different paternal lineages carrying this deletion.

In many forensic cases, sex identification is crucial and it is absolutely essential in rape cases where there is a possibility of contamination of DNA from both the victim and the culprit. Selection of markers is, therefore, very important in such cases to unambiguously identify the male and female DNA in a single reaction. Santos et al. (1998) have reported deletions of *AMEL Y* in two Sri lankan males. Steinlechner et al. (2002) have also demonstrated the lack of Y-specific amelogenin in 6 out of 29,432 (0.02%) Austrian males. For the first time we report the amelogenin deletion in the Indian population. Although the AmpF*l* STR Profiler Plus kit has been used worldwide and has been found suitable for forensic identification and parentage testing (Han et al. 2000; Pawlowski and Maciejewska 2000; Steinlechner et al. 2002), we suggest that users do not rely on amelogenin for sex testing.

Even though the frequency of the amelogenin deletion is low (1.85%), considering the fact that the future of an individual is based on the reliability of this test, one should not make conclusions about the gender simply by using the amelogenin alone. Sex identification of unborn children for prenatal diagnosis of diseases specifically affecting the male child, particularly in families with histories of such diseases, can be disastrous in the light of the present study. Any false sex assignment may lead to litigation. We, therefore, suggest the inclusion of additional Y chromosome markers such as *SRY*, STR, STS and/or other Y chromosome markers in the existing multiplex STR kits for gender identification. Among the routinely used Y-STRs, DYS389 is more appropriate, because this marker detects

Table 1 Y chromosome STR-based haplotypes of five *d*eleted-*a*melogenin *m*ales (*DAM*s)

Samples	Y chromosome STR alleles					
DAMs	DYS19	DYS389I	DYS389II	DYS390	DYS391	DYS393
R2	14	10	27	22	10	14
M60	14	10	27	22	10	14
B16	16	11	27	24	11	12
K96	17	10	27	25	10	12
A151	14	10	27	21	11	14

two loci (Thangaraj et al. 1999), ranging from 239–263 bp and 353–385 bp, respectively. We also suggest the inclusion of a fifth dye to the Y chromosome specific marker(s) for easy detection and to avoid confusion.

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