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Amelogenin Y negative males: Multiple origins

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Abstract. Examination of the approximate size of a deletion in the short arm of the Y-chromosome, incorporating the amelogenin locus, and associated Y chromosome STR haplotypes, in five samples, indicates that the phenotype amelogenin-Y negative has multiple origins.

Keywords: Amelogenin; Y-chromosome; Sex typing; STR; Forensic

1. Introduction

Amelogenin testing is a part of many genetic profiling multiplex systems used by forensic scientists to determine if the sample being tested is of male or female origin [1–4]. There have been a small number of reports on the absence of amelogenin-Y sequence in some males [5–12]. The accumulated data suggest that they are more likely due to a deletion rather than sequence variation at the primer binding site(s). We have investigated five samples, each of which is known to have come from a male that provided an 'X only' result for amelogenin when tested using Powerplex 1.2 (Promega) or AmpF*l*STR Profiler Plus (Applied Biosystems). It is of interest to know if the phenomenon giving rise to amelogenin-Y negative males is the same or different among the five samples. The samples were typed for eight sites located on the short arm of the Y chromosome, on either side of the amelogenin locus, to determine approximate deletion sizes. They were also typed for 11 Y-STRs to generate haplotypes for phylogenetic analysis.

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2. Materials and methods

The following Y-chromosome markers were used to determine the approximate deletion size: G66289 (position 6699 kbp), 6718K (6718), 6832K (6832), G66294 (6840), DYS266 (6849), sY1320 (7022), G66276 (7430), DYS288 (7700) [13]. They were typed using standard electrophoresis procedures after amplification using primers and conditions (with extra cycles for some) described at [13]. Table 1 identifies the Y chromosome markers used to generate the STR haplotypes. Standard nomenclature, amplification and typing protocols were used for the STRs [14,15].

3. Results

After scoring the five amelogenin-Y negative samples for the presence or absence of eight sequences lying 5′ and 3′ to amelogenin, two different sized deletions were observed. The breakpoint of all amelogenin-Y deletions occurred between 59 and 78 kbp upstream of the amelogenin locus and extended to a maximum of ~653 kbp (samples 2 and 4) or to between 653 and 923 kbp downstream of the amelogenin locus (samples 1, 3 and 5). Deletions were either 304–731 kbp or 712–1001 kbp in size.

Y-STR haplotype analysis indicated that each of the five samples had a different haplotype, with between 2 and 11 mutation steps separating any two samples (Table 1). Sample 1 is the most distinct, being eight mutation steps from the most similar sample (sample 2).

4. Discussion

Though males 1, 3 and 5 cannot be distinguished using these eight deletion size markers, it does not mean that they share identical deletions. The Y-haplotype data indicates that male 1 is very different from males 3 and 5 and it is likely that the deletion has a different origin. Males 2 and 4 appear to have the same deletion, as the apparent size using these markers is the same, and they differ only by one repeat unit at 2 of the 11 haplotype loci tested.

These data imply that there are multiple origins of amelogenin-Y negative males. More investigation is required to detect the frequency of amelogenin-Y negative males and if they are more prevalent in males of particular ethnic origins. Consideration should be

Table 1
Y-chromosome STR typing in five amelogenin-Y negative males

Marker	Sample 1	Sample 2	Sample 3	Sample 4	Sample 5
DYS19	13	15	15	15	16
DYS389I	13	12	12	12	12
DYS390	24	24	23	24	24
DYS391	11	11	10	11	10
DYS392	13	11	11	11	11
DYS393	13	13	12	12	12
DYS434	9	9	9	9	NT
DYS435	11	11	11	11	NT
DYS437	9	9	9	9	NT
DYS438	12	9	9	9	9
DYS439	12	12	12	11	NT

given to using an additional gender-typing marker in those cases where gender identification is relevant to an investigation.

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