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Case report

Deletion mapping of the regions with AMELY from two Chinese males

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ABSTRACT

The amelogenin (AMEL) is widely used in many multiplex PCR kits for gender determination. However, the null of amelogenin Y (AMELY) can result in the incorrect genotyping of male samples as females. In this study, we report the deletion of AMELY in two cases with a deletion frequency of 0.019% (2/10526) in our laboratory. The deletion region with AMELY was mapped by using other twelve loci, which shows the class I deletion pattern. Further, the Y chromosome short tandem repeat (Y-STR) typing shows that these two cases share the same haplotype with other two cases from previous reports. The haplogroup of the two cases was predicted as O3 haplogroup with a 100% probability. Altogether, this study will provide evidence to further demonstrate the deletion of AMELY in Chinese population.

1. Introduction

The Y chromosome short tandem repeats (Y-STRs) are valuable genetic markers in forensic casework. However, the null of Y-STRs, such as DYS393, DYS19 and so on, has been widely reported in various populations [1,2]. Also, the protein-coding genes on Y chromosome, such as amelogenin (AMEL), azoospermia factor b (AZFb) and so on, were observed to be deleted in different regions and/or different ethnic populations [3,4]. As a single copy gene, AMEL is located on Xp22.1-Xp22.3 of X chromosome (AMELX) and Yp11.2 of Y chromosome (AMELY), respectively. It seems more important to investigate the cases with the null of AMELY since AMEL is widely used in many multiplex PCR kits for gender determination as a result of the 6 bp deletion in the amplification product of AMELX [5]. Generally, the male's AMELX is detected while AMELY is undetectable in AMELY-null cases, which will result in the incorrect genotyping of male samples as females. According to the previous studies, the null of AMELY can be caused by the abnormality of Y chromosomes [6,7], the mutation in primer binding sites [8] and/or the deletion encompassing AMELY on Yp11.2 [3,8–17]. In cases with abnormal Y chromosomes, Y-STRs on the q arms of Y chromosomes were not detected. In cases with the mutation in primer binding sites, the locus of AMELY could be detected by designing new primer sets and all Y-STRs detected in commercial kits could be well observed. In contrast, in cases with the deletion of region on Yp11.2, primers will not work on the amplification of AMELY. In these cases, some loci close to AMELY, such as DYS456 and DYS458, were not observed while other loci in the commercial kits could be detected by using testing kits [8,13–17].

Since the deletion of AMELY among populations are increasingly reported and might have a population-specific pattern, it is important to characterize the region with the deletion of AMELY [12]. During the routine paternity testing in our laboratory we observed two AMELY-null cases. In this study, we characterized the deletion region by the detection of sequence-tagged-sites (STSs) [18]. Also, we discuss the deletion type of AMELY based on the data from previous reports in China.

2. Material and methods

2.1. Samples and exaction

The first case is a baby boy (S1) with his alleged father and another is a 53-year-old male (S2) with his alleged daughter. DNA was extracted from the bloodstains on the filter paper by using the Chelex-100 and proteinase K protocol [19].

2.2. AMEL and STR typing

AMEL and autosomal STR profiles were obtained by using the Identifiler® PCR Amplification Kit (Applied Biosystems, USA), PowerPlex®21 System (Promega, USA) and Expressmarker 22 STR loci PCR Amplification Kit (AGCU, China). Meanwhile, Y-STR profiles were obtained by using the Y18 STR loci PCR Amplification Kit (AGCU, China). Allele designation was determined according

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to allelic ladders provided in the kits by using the GeneMapper ID software v3.2.

2.3. Amplification and analysis of STSs

The primer sequences for SRY and the 7 selected STSs (sY1240, sY1241, sY1242, sY57, sY1079, sY59 and sY1250) were obtained from UniSTS database (http://www.ncbi.nlm.nih.gov/unists/). The FAM™-labelled primers for the 8 loci were used for PCR amplification with the cycling conditions: 94 °C, 5 min followed by 32 cycles of 94 °C, 1 min; 60 °C, 1 min, 72 °C, 1 min and an extension of 72 °C, 10 min by using the GeneAmp PCR System 9700 (Applied Biosystems, USA) with positive and negative controls. Amplified PCR products were separated by capillary electrophoresis in ABI PRISM 3130xL Genetic Analyzer (Applied Biosystems, USA).

2.4. Analysis of deletion region, haplogroup and population frequency

The deletion maps were constructed using AMELY, SRY, 4 loci in Y-STR kit (DYS393, DYS456, DYS458 and DYS19) and 7 STSs. The position information of the Y-STRs was obtained from the studies of Hanson and Ballantyne [20], while the position information of SRY, AMEL, and STSs was obtained from the website of UCSC (http://genome.ucsc.edu/cgi-bin/hgTracks). The deletion patterns were classified according to the studies from Jobling et al. [18]. The Y chromosome haplogroups of the two cases in this study and cases in Ma et al.'s study were predicted by the 27 Haplogroup Program of Y Haplogroup Prediction from Y-STR Values (http://www.hprg.com/hapest5/hapest5b/hapest5.htm) based on the results of Y-STR genotyping. The population frequency was estimated according to the count of the unrelated males in our daily paternity testing and compared with the frequencies reported in previous studies.

3. Results and discussion

Two cases from paternity testing claimed to be males and all the autosomal STRs were well genotyped (Fig. 1A). However, the AMELY failed to be detected by using Identifiler® kit, PowerPlex®21 System, and Expressmarker 22 kit (Fig. 1A). Since the three different commercial identification kits have different primer sets for AMEL, the null of AMELY in these two cases should not be due to the mutation in primer binding sites. The Y18 kit was used to detect other STR loci on Y chromosome to characterize the null of AMELY. Results from genotyping of Y-STRs showed the additional deletion of DYS458 in the two cases (Fig. 1B), which suggests that the null of AMELY is due to the deletion of Yp11.2 region. Also, the abnormality of Y chromosomes could be excluded due to the presentence of other 14 Y-STRs on the q arms of Y chromosomes.

To approximately map the deletion region on Y chromosome, the primer sets for the additional 8 loci were used. In fact, the presence of SRY in two cases shows that the biological samples were from males (Fig. 2). Furthermore, sY1240, sY1241, sY59 and sY1250 were detected in electrophoresis, while sY1242, sY57 and sY1079 were undetectable in the two cases (Fig. 2). Based on the obtained data, the deletion map was constructed, which could be classified as class I deletion pattern according to the reports of Jobling et al. [18] (Fig. 3).

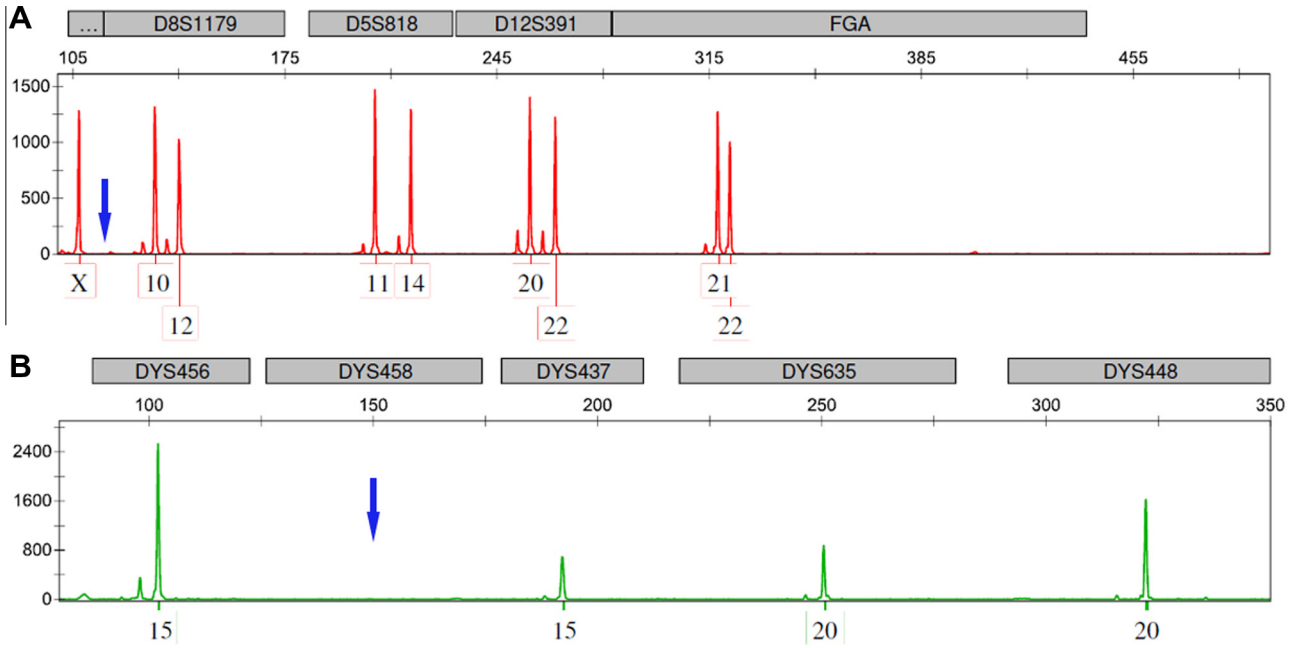

Fig. 1. The electrophoretograms of genotyping from S2 by using Expressmarker 22 kit (A) and Y18 kit (B). The deletion of alleles was shown by the arrow.

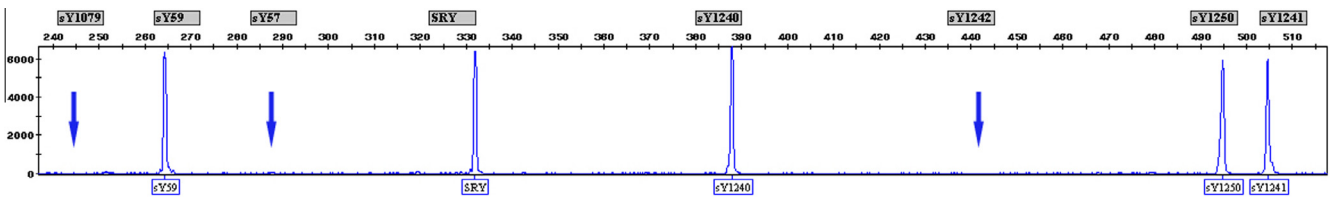

Fig. 2. The electrophoretogram of other 8 loci from S2. The null of alleles was shown by the arrow.

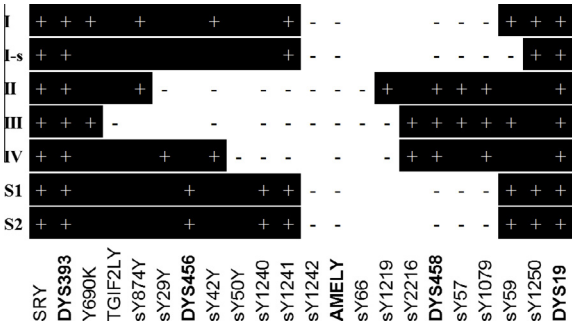

Fig. 3. The deletion map based on the data in this study and comparison with the classification described by Jobling et al. [18].

Interestingly, two cases (S1 and S2) in this study and another two cases (38 and S14) from the previous studies share the same haplotype [8,17] (Table S1). The genotyping of autosomal STRs did not show a relationship between two males in this study. Since the alleged father was excluded as the biological father of the child, we did not know the true birth place of the boy baby. Another three cases come from Hubei province, Zhejiang province, and Guangdong province, respectively, in China. It seems that these three cases have no relationship to each other. Since the four cases share the same haplotype, they might have the same male ancestor.

In the kit used in this study, there are four loci close to AMELY in the Yp11.2 region of Y chromosome including DYS393, DYS456, DYS458 and DYS19. Compared with DYS456 at 4.31 Mbp and DYS458 at 7.91 Mbp of Y chromosome, DYS393 at 3.17 Mbp and DYS19 at 10.11 Mbp are far away from AMELY. Although Ma et al. recently reported one case with the AMELY-DYS458-DYS19 deletion [8], it is rarely reported about the deletion of AMELY together with DYS393 or DYS19 [1,2]. Therefore, AMELY-null cases can be speculated into two major deletion patterns including DYS456-AMELY deletion pattern corresponding to the class II and III deletion patterns of Jobling et al. [18] and AMELY-DYS458 deletion pattern corresponding to the class I and I-s patterns (Fig. 3). Nowadays, PowerPlex® Y23 system as a new Y-STR kit contains two loci located on Yp11.2 region including DYS576 at 7.09 Mbp and DYS481 at 8.47 Mbp, which will allow us to categorize deletion patterns in more detail.

The haplogroup of the two cases in our study was predicted as O3 haplogroup with a 100% probability. Based on previous reports and our results, we further investigate the relationship between deletion patterns and haplogroup in Chinese population. The O3 haplogroup is the most common haplogroup in China with a 0.74 frequency in Han Chinese [21]. The AMELY-DYS458 deletion pattern was observed in 7 cases of O3 haplogroup and 5 cases of J2 haplogroup, while no case with DYS456-AMELY deletion pattern was observed in both O3 and J2 haplogroups. The observation was consistent with the data in Jobling et al.'s study [18].

The frequency of AMELY-null male cases was estimated as 0.019% (2/10526) by counting the unrelated male samples in our laboratory. The frequency was consistent with previous reports that the frequency of AMELY-DYS458 deletion pattern was 0.010% (8/79304) in Ma et al.'s study and was 0.016% (2/12915) in Chen et al.'s study in Chinese population [8,17]. Although the frequency of J2 haplogroup is relatively low in Chinese population, the high frequency of the AMELY deletion in J2 haplogroup has been shown. In contrast, the AMELY deletion has a low frequency in O3 haplogroup. Based on reports in the literature to date, the total frequency of the AMELY deletion in Chinese population is relatively lower than that in other populations [3,12,14,16].

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.legalmed.2014.05.002.

References

[1] Kim SH, Kim NY, Hong SB, Cho NS, Kim JJ, Han MS, et al. Genetic polymorphisms of 16 Y chromosomal STR loci in Korean population. Forensic Sci Int Genet 2008;2:e9–10.
[2] Balaresque P, Parkin EJ, Roewer L, Carvalho-Silva DR, Mitchell RJ, van Oorschot RA, et al. Genomic complexity of the Y-STR DYS19: inversions, deletions and founder lineages carrying duplications. Int J Legal Med 2009;123:15–23.
[3] Santos FR, Pandya A, Tyler-Smith C. Reliability of DNA-based sex tests. Nat Genet 1998;18:103.
[4] Lee BY, Kim SY, Park JY, Choi EY, Kim DJ, Kim JW, et al. Unusual maternal uniparental isodisomic x chromosome mosaicism with asymmetric y chromosomal rearrangement. Cytogenet Genome Res 2014;142:79–86.
[5] Sullivan KM, Mannucci A, Kimpton CP, Gill P. A rapid and quantitative DNA sex test: fluorescence-based PCR analysis of X-Y homologous gene amelogenin. Biotechniques 1993;15(636–8):40–1.
[6] Steinlechner M, Berger B, Niederstatter H, Parson W. Rare failures in the amelogenin sex test. Int J Legal Med 2002;116:117–20.
[7] Davis C, Illescas M, Tirado C, Lopez R, Budowle B, Cruz TD. A case of Amelogenin Y-null: a simple primer binding site mutation or unusual genetic anomaly? Leg Med (Tokyo) 2012;14:320–3.
[8] Ma Y, Kuang JZ, Zhang J, Wang GM, Wang YJ, Jin WM, et al. Y chromosome interstitial deletion induced Y-STR allele dropout in AMELY-negative individuals. Int J Legal Med 2012;126:713–24.
[9] Lattanzi W, Di Giacomo MC, Lenato GM, Chimienti G, Voglino G, Resta N, et al. A large interstitial deletion encompassing the amelogenin gene on the short arm of the Y chromosome. Hum Genet 2005;116:395–401.
[10] Mitchell RJ, Kreskas M, Baxter E, Buffalino L, Van Oorschot RA. An investigation of sequence deletions of amelogenin (AMELY), a Y-chromosome locus commonly used for gender determination. Ann Hum Biol 2006;33:227–40.
[11] Parkin EJ, Kraayenbrink T, Opgenort JR, van Driem GL, Tuladhar NM, de Knijff P, et al. Diversity of 26-locus Y-STR haplotypes in a Nepalese population sample: isolation and drift in the Himalayas. Forensic Sci Int 2007;166:176–81.
[12] Chang YM, Perumal R, Keat PY, Yong RY, Kuehn DL, Burgoyne L. A distinct Y-STR haplotype for Amelogenin negative males characterized by a large Y(p)11.2 (DYS458-MSY1-AMEL-Y) deletion. Forensic Sci Int 2007;166:115–20.
[13] Kumagai R, Sasaki Y, Tokuta T, Biwasaka H, Aoki Y. DNA analysis of family members with deletion in Yp11.2 region containing amelogenin locus. Leg Med (Tokyo) 2008;10:39–42.
[14] Takayama T, Takada N, Suzuki R, Nagaoka S, Watanabe Y, Kumagai R, et al. Determination of deleted regions from Yp11.2 of an amelogenin negative male. Leg Med (Tokyo) 2009;11(Suppl. 1):S578–80.
[15] Turrina S, Filippini G, Voglino G, De Leo D. Two additional reports of deletion on the short arm of the Y chromosome. Forensic Sci Int Genet 2011;5:242–6.
[16] Velarde-Felix JS, Salazar-Flores J, Martinez-Cortes G, Flores Garcia A, Munoz-Valle JF, Rios-Tostado JJ, et al. Deletion mapping and paternal origin of a Mexican AMELY negative male. Leg Med (Tokyo) 2011;13:262–4.
[17] Chen W, Wu W, Cheng J, Zhang Y, Chen Y, Sun H. Detection of the deletion on Yp11.2 in a Chinese population. Forensic Sci Int Genet 2014;8:73–9.
[18] Jobling MA, Lo IC, Turner DJ, Bowden GR, Lee AC, Xue Y, et al. Structural variation on the short arm of the human Y chromosome: recurrent multigene deletions encompassing Amelogenin Y. Hum Mol Genet 2007;16:307–16.
[19] Polski JM, Kimzey S, Percival RW, Grosso LE. Rapid and effective processing of blood specimens for diagnostic PCR using filter paper and Chelex-100. Mol Pathol 1998;51:215–7.
[20] Hanson EK, Ballantyne J. Comprehensive annotated STR physical map of the human Y chromosome: forensic implications. Leg Med (Tokyo) 2006;8:110–20.
[21] Wen B, Li H, Lu D, Song X, Zhang F, He Y, et al. Genetic evidence supports demic diffusion of Han culture. Nature 2004;431:302–5.