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Detection of the deletion on Yp11.2 in a Chinese population

Wenjing Chen [a,1], Weiwei Wu [b,1], Jianding Cheng [a], Yinming Zhang [a], Yong Chen [a], Hongyu Sun [a,*]

[a] Department of Forensic Medicine, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou 510089, PR China
[b] Department of Criminal Investigation of Zhejiang Provincial Public Security Bureau, Hangzhou 310009, PR China

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ABSTRACT

Sex determination tests based on Amelogenin gene as part of commercial PCR multiplex reaction kits have been widely applied in forensic DNA analysis. Mutations that cause dropout of Y chromosomal Amelogenin gene (AMELY) could lead to errors in gender determination and mixture interpretation. To infer the mechanism and estimate the dropout frequency of AMELY and adjacent Y-STRs, we studied 3 samples with AMELY dropout combined with DYS458 and/or DYS456 and 37 samples with DYS456 dropout. DYS456, DYS458 and AMELY are located in the Yp11.2 region. The singleplex amplification system showed the null alleles could be caused by fragment deletion in Yp11.2 rather than a point mutation in the primer binding region. After detection of the 17 Y-STR and 77 STS markers, the deletion map showed different patterns. The DYS456-AMELY-DYS458 deletion pattern was the largest, breaking from 3.60 Mb to 8.29 Mb in the Y chromosome, and the overall frequency was 0.0077%. The AMELY-DYS458 deletion pattern was broke from 6.74 Mb to 9.17 Mb, with a 0.0155% frequency. The DYS456 negative pattern was concentrated in two main deletion regions, with a 0.8220% frequency. The frequency of all negative pattern was 0.0155%. All the AMELY-DYS458 and DYS456-AMELY-DYS458, and 92% of the DYS456 deletion patterns belonged to Hg O3, the rest belonged to Hg Q. The DYS456 deletion pattern was first reported in Chinese population. The current and previous findings suggest additional gender test for ambiguous sex determination may be required.

1. Introduction

Male-specific region of the human Y chromosome (MSY), also called non-recombining region of human Y chromosome (NRY), accounts for 95% of the Y chromosome which does not recombine with the X chromosome during meiosis [1–3]. Due to the unique characteristics of the Y chromosome, Y-chromosome specific short tandem repeat loci (Y-STR) and the Amelogenin genes (*AMELX* and *AMELY*) have been widely used in paternity testing, personal identification, prenatal testing, preimplantation genetic diagnosis, and deoxyribonucleic acid (DNA) databasing [3–10].

The Y chromosome bears a particularly large proportion of submicroscopic structural rearrangement, including deletions, insertions, duplications, large-scale copy number variants and inversions [3,7,9]. Abnormal phenomena have been identified during routine *AMELY* and/or Y-STR genotyping casework. *AMELY* dropouts will cause the false genotyping of male samples as female. Base variation in the primer binding region is one of the causes for the dropout of the *AMELX* and *AMELY*, but the *AMELY* dropout is predominantly due to fragment deletion on Yp11.2 region, and is often combined with the absence of adjacent Y-STR locus DYS458 [1,3,4,11–14]. In our study, one case showed the DYS456-*AMELY*-DYS458 deletion pattern and 37 cases showed the DYS456 deletion pattern. DYS456 is 2.4 Mb upper than the *AMELY* loci and DYS458 is 1.1 Mb lower than the *AMELY* loci on the Yp11.2 region [4,12], a possible hotspot of Y chromosome deletion [4,11,12]. Yp11.2 deletion mapping data has been reported in different populations [5,14–17]. Although cases with *AMELY* and Y-STR nulls have been reported in the Chinese population, no systematic analysis of the deletion region has been performed [3,18]. The present study described the four deletion patterns and determined the deletion map in the Yp11.2 region that causes the absence of *AMELY*, DYS458 and DYS456, some of which has been reported in a previously published article [14].

2. Materials and methods

2.1. Samples

Buccal swab samples 1 to 38, obtained from DNA database of 4501 unrelated Chinese Han males in Zhejiang provinces [19],

* Corresponding author at: No. 74 Zhongshan Road II, Guangzhou 510089, PR China. Tel.: +86 20 87330557; fax: +86 20 87334353.
E-mail addresses: sunhy@mail.sysu.edu.cn, sunhongyu2002@163.com (H. Sun).
[1] Wenjing Chen and Weiwei Wu contributed equally to the article.

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indicated null-alleles in DYS456 or DYS458 when genotyping using an AmpF/STR® Yfiler Kit (Applied Biosystems, USA), in which the samples 1 to 37 showed null alleles at the locus DYS456 and the sample 38 showed null alleles at the locus DYS458. Samples 39–42 were collected from 8414 unrelated phenotypically normal males in Guangdong province with informed consents, indicated null-allele for *AMELY* when genotyping using Powerplex® 16 system (Promega Corporation, Madison, USA). Genomic DNA from 42 blood stain samples was extracted by the Chelex-100 method.

2.2. Genotyping of AMELY nulls with 17 Y-STR markers

Four samples (39, 40, 41 and 42) with *AMELY* allele dropout were genotyped using the AmpF/STR® Yfiler system.

2.3. Genotyping with singleplex amplification systems for AMELY, DYS456 and DYS458

To verify the reason for allele dropout, tests using a singleplex amplification system were carried out using primer sequences from published data, binding to alternative sites (Table 1) [14,20–23].

Polymerase chain reaction (PCR) amplification was carried out with 12.5 μL 2 × GoTaq® Green Master Mix (Promega, USA), 1 μL of each of the primers (10 μmol/L) and 5–7 ng DNA template. Nuclease-free water was added to a total volume of 25 μL. The thermo cycling conditions were: 95 °C for 2 min, 32 cycles at 94 °C for 1 min, 60 °C for 1 min, and 72 °C for 1 min, with a final extension at 72 °C for 15 min. PCR products were separated by non-denaturing polyacrylamide gel electrophoresis (T = 6%, C = 3.3%) and subsequently visualized with silver staining.

2.4. Y chromosome haplogroup prediction

Based on Y-STR genotyping, the haplogroup (Hg) of Y chromosome was predicted using the Whit Athey's Haplogroup Predictor 27-haplogroup program (http://www.hprg.com/hapest5/hapest5b/hapest5.htm, as updated on 10/12/2012), selecting "Equal Priors" in the Area Selection menu.

2.5. Deletion mapping using STS markers in Yp11.2 region

Seventy-seven sequence-tagged site (STS) markers, spaced at approximately 20–600 kb, were selected to detect the deletion region. These STS markers were selected from DYS393 (located in 3.13 Mb segment of the Y chromosome) to DYS19 (located in 9.52 Mb segment of the Y chromosome), containing the DYS456, *AMELY* and DYS458 null alleles (Table 2).

Table 1
Primer pairs for *AMELY*, DYS456 and DYS458.

Markers	Primer sequence (5′–3′)	Product size (Repeats)
AMEL	F-ACCTCATCCTGGGCACCCTGG R-AGGCTTGAGGCCAACCATCAG	*AMELX*: 212 bp *AMELY*: 218 bp
DYS456	1: F-GGACCTTGTGATAATGTAAGATA R-CCCATCAACTCAGCCCAAAAC	141–161 bp (13–18)
	2: F-ACTCGGACTGGCTCATCTTG R-CCCATCAACTCAGCCCAAAAC	189–209 bp (13–18)
	3: F-TCAGCCTGCAGATGGTCT R-TTTTGAACTCTTGGCCTCAA	318–338 bp (13–18)
DYS458	1: F-GCAACAGGAATGAAACTCCAAT R-GTTCTGGCATTACAAGCATGAG	132–160 bp (13–20)
	2: F-GGGTGGTGGAGGTTACTGTG R-CTAGAGGTTCCTGCCACCAC	308–336 bp (13–20)
	3: F-GGTGGTGGAGGTTACTGTGA R-TTCCTGACCTTGTGATCCAG	221–249 bp (13–20)

STS primers and PCR conditions were obtained from the National Institute of Health website (http://www.ncbi.nlm.nih.gov, as accessed on 15/06/2012). Special care was taken to ensure the STS markers' effectiveness. Only the markers with a single copy, or the PCR products that were wide enough to be separated from the homologous X chromosome, were used to show the deletion map of the Yp11.2 region.

3. Results

3.1. Deletion patterns

After using the alternative single primer sets, all samples continued to show the same results as before, which suggests a fragment deletion of the Y chromosome rather than a base mutation within the annealing region of the primers. This is consistent with previous studies [1,4,11,12,14,24–28].

According to Y-STR and STS genotyping, four patterns of deletion were detected. These were the DYS456 deletion pattern for samples 1–37, the *AMELY*-DYS458 deletion pattern for samples 38 and 39, and the DYS456-*AMELY*-DYS458 deletion pattern for sample 40. Samples 41 and 42 were negative for all 17 Y-STR markers and *AMELY* (Fig. 1), therefore no further investigation was performed.

3.2. Hg prediction based on Y-STRs haplotypes

Twenty-five different Haplotypes were identified in 40 samples (1–40). Two Hg, O3 and Q, were predicted based on Y-STRs haplotypes (Table 3). Samples 7, 12 and 36 of the DYS456 deletion pattern belonged to Hg Q. All other samples belonged to Hg O3.

3.3. Deletion map of DYS456 deletion pattern

Samples 1–37 showed the DYS456 deletion pattern and exhibited similar fragment deletion around the DYS456.

As shown in Fig. 1, the deletion regions were found on two main non-consecutive sites. The upper breakpoint was varied from upper marker sY3020 (No. 2 at 3.22 Mb) to lower marker DXYS112 (No. 9 at 3.90 Mb). The most notable breakpoint was sY3024 (No. 7 at 3.76 Mb), where seventeen (46%) samples were breaking from this marker, and thirty-five (95%) samples were negative at this marker. Twenty-six (70%) samples began to show positive results after DXYS6 (No. 10 at 4.05 Mb). The first main deletion region was ranged from 3.76 Mb to 4.05 Mb at the Y chromosome, where twenty-nine (78%) samples showed negative results.

After thirty-two (86%) samples indicated positive results in sY221 (No. 11 at 4.08 Mb), all the samples indicated no results from sY3040 or sY3041 (No. 13 or No. 14 at 4.13 Mb), where the second main deletion region began from. And this deletion was extended to sY3026 (No. 27 at 4.87 Mb). Some positive parts appeared between the second deletion region. The most notable one was eighteen (49%) samples showed positive in sY3103 (No. 16 at 4.20 Mb), and majority (61%, 11/18) of them were prolonged to sY3104 (No. 17 at 4.21 Mb). Ignoring the interspersed positive results, the second main deletion region was from 4.13 Mb to 4.87 Mb, where thirty-five (95%) samples showed negative results.

A negative result was considered more important than a positive result, as negative results could indicate either a true deletion or a variation at the markers. The longest breakpoint boundary surrounding DYS456 extended from 3.22 Mb to 4.89 Mb (sample 8), while the shortest breakpoint extended from 3.76 Mb to 4.23 Mb (sample 17). Ignoring the sporadic positive results, the lengths of the deletion region extended from 0.47 Mb to 1.67 Mb (Fig. 1).