

Controversial cases of human gender identification by amelogenin test

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ABSTRACT

Gender identification is essential on forensic science to provide the initial information for criminal investigation. However, gender identification by amplification of amelogenin gene sometimes showed controversial cases, in which male samples was falsely identified as females. We reported the anomalous amelogenin results of 8 samples in Taiwan. The frequency was about 0.00625 % for males and 0.015 % for females, and lower than those of the other populations reported. To confirm amelogenin results of the 8 controversial samples, DNA was further used in SRY and Y-STR typing. All samples but one showed with Y chromosome. From the results, it was highly suggested that for the controversial cases of human gender identification with amelogenin tests, amplification of SRY gene or/and Y-STR markers will be adopted to confirm the gender.

Keywords: gender identification, amelogenin gene, SRY gene, Y-STR, forensic science

Introduction

Sex typing is essential in medical diagnosis of sex-linked disease and forensic science. Gender for criminal evidence of offender is usually as the initial information for investigation. For individualization, identification of gender is performed in addition to the STR markers recently. The most popular gene included in the commercial individualization kits for human gender identification is amelogenin gene. However, it was reported that two males were typed as females in 1998 by PCR amplification of amelogenin [1], and later other laboratories also observed the anomalous amelogenin results. Allele dropout of X amelogenin was observed in males, and proved to result from the polymorphism of the primer binding sites [2]. Nevertheless, structural variation

is particularly prevalent on the Y chromosome. Higher frequency of deletion for the Y amelogenin region in Indian population group was observed [3, 4] and deletion of the fragment Y(p)11.2 was determined [5]. The male samples would be identified as females in such cases. For Austrian study, the samples which lacked the amplicon of Y amelogenin in males were also identified [6, 7]. These samples were confirmed to be from males by amplification of Y-STR and SRY (sex-determining region of Y chromosome) gene. The phylogenetic context, origins and implications for male amelogenin dropouts were reported [8]. We reported the controversial cases, not only for males but also for females, of human gender identification by amelogenin test in Taiwan.

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Materials and methods

DNA sources

There were 8 DNA samples with the anomalous amelogenin results collected from approximate 100,000 tested individuals, for 80,000 males and 20,000 females. These DNA samples were from 5 males (named from M1 to M5) and 3 females (named from F1 to F3) identified

by phenotypes and other background information. The anomalous amelogenin results tested with AmpFISTR Identifier PCR Amplification Kit (Applied Biosystems) were showed in Table 1.

Table 1 Results of gender identification by amelogenin gene, SRY gene and Y-STR.

Sample	Phenotype ^a	Amelogenin	SRY ^b	Y-STR ^b	Chromosome detected ^c
M1	Male	Y	+	+	Y
M2	Male	X	-	-	X
M3	Male	Y	+	+	Y
M4	Male	X	+	+	XY
M5	Male	X	+	+	XY
F1	Female	XY	+	+	XY
F2	Female	XY	+	+	XY
F3	Female	XY	+	+	XY

^aPhenotypes were identified by according to the records of identification cards.

^bThe symbols of “+” and “-” represented the positive and negative results respectively.

^cChromosome detected showed the result of combing amelogenin, SRY and Y-STR tests.

Gender identification with SRY gene and Y-STR

DNA samples were amplified with Quantifiler Y Human Male DNA Quantification Kit (Applied Biosystems) and AmpFISTR Yfiler PCR Amplification Kit (Applied Biosystems) to test the SRY gene and Y-STR respectively. They were performed following the manufacturer's protocols.

Results and discussion

The anomalous amelogenin results of the 8 controversial samples were showed in Table 1. For the 5

male samples, from M1 to M5, two of them (M1 and M3) showed only Y amelogenin amplicon; and the other three (M2, M4 and M5) showed only X amelogenin amplicon. For the 3 female samples, from F1 to F3, all showed both X and Y amelogenin amplicon. The frequency of anomalous cases was about 0.00625 % (5/80,000) for males, and 0.015 % (3/20,000) for females in this study. For Indian, Malay and Austrian populations, they were 3.2-3.6 %, 0.6-0.88 % and 0.02 % for males respectively [4, 6]. The frequency was higher in Indians than other populations, and the possibility was reported that partial Y deletion formed a relatively old and stable haplotype

in the Indian ethnic group [4]. To confirm amelogenin results of the 8 controversial samples, furthermore, the DNA was used in other sex tests, such as SRY and Y-STR typing.

To test SRY gene, DNA was amplified with the commercial kit for Y DNA quantification by SRY gene (Quantifiler Y Human Male DNA Quantification Kit) due to its high sensitivity of real-time PCR amplification and fluorescence detection. The results were showed in Table 1. All the samples but M2 showed SRY gene. This gene was only on the Y chromosome. The results indicated that although no Y amelogenin amplicon for M4 and M5, Y chromosome may exist in both samples.

To test Y-STR, DNA was amplified with the AmpFISTR Yfiler PCR Amplification Kit to test 17 Y-STR markers. The results were showed in Table 1. All the samples but M2 showed at least 8 Y-STR markers. The results strongly suggested that no Y chromosome for M2.

The result for combining the tests of amelogenin, SRY and Y-STR was showed in Table 1. It indicated that only X-chromosome and no Y-chromosome existed in M2. For M1 and M3, only Y-chromosome detected may result from the polymorphism of the primer binding sites on X amelogenin gene [2]. For M4 and M5, no Y amelogenin was amplified but SRY and Y-STR were detected, it may result from deletion of the Y amelogenin region. The three females (F1 to F3), all showed with X and Y chromosomes by amelogenin test, and the presence of Y-chromosome was confirmed by SRY and Y-STR tests. The results also showed the consistence of SRY and Y-STR.

From the results, it was highly suggested that for the controversial cases of human gender identification with amelogenin tests, amplification of SRY gene or/and Y-STR markers will be adopted to confirm the gender.

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