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Analysis of the 19 Y-STR and 16 X-STR loci system in the Han population of Shandong province, China

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Genet. Mol. Res. 16 (1): gmr16019573
Received December 9, 2016
Accepted February 8, 2017
Published March 30, 2017
DOI http://dx.doi.org/10.4238/gmr16019573

ABSTRACT. The sex-linked short tandem repeats (STR), Y-STR and X-STR, are important for autosomal STRs in forensic paternity testing. We evaluated the forensic parameters of 19 Y-STRs and 16 X-STRs in the Han population of Shandong province, China. A Goldeneye 20Y kit (DYS391, DYS389I, DYS390, DYS389II, DYS348, DYS456, Y-GATA-H4, DYS447, DYS19, DYS392, DYS393, DYS388, DYS439, DYS635, DYS448, DYS460, DYS458, DYS437, DYS385 a/b) was used to analyze the forensic parameters of 534 unrelated males. A Goldeneye17X system (DXS6795, DXS9902, DXS8378, HPRTB, GATA165B12, DXS7132, DXS7424, DXS6807, DXS6803, GATA172D05, DXS6800, DXS10134, GATA31E08, DXS10159, DXS6789, DXS6810, amelogenin) was used to analyze 97 unrelated males and 214 females. In addition, we used the kits to examine 5 cases

with abnormal amelogenin test results, as well as a male child with agenosomia typed by autosomal STR. We found 203 Y-STR haplotypes with allele frequencies ranging from 0.0019 to 0.7959, and GD ranging from 0.3429 to 0.9667. Expect in DXS6803, the allele frequencies of the other 15 X-STR loci showed no differences between females and males. PD_F ranged from 0.5504 to 0.9638, while PD_M ranged from 0.3176 to 0.8377. With the exception of DXS6803 and DXS6810, the allele frequencies of other X-STR loci were in accordance with Hardy-Weinberg equilibrium in females. One amelogenin negative case was characterized as a deletion of Y-DYS458. This paper provided data regarding the genetic polymorphism of Y-STRs and X-STRs in the Han population, and demonstrated the importance of Y-STR and X-STR in forensic autosomal STR analysis.

Key words: Forensic science; Autosomal short tandem repeats; Y-STR; X-STR; Amelogenin dropouts; Agenosomia

INTRODUCTION

Autosomal short tandem repeats (STRs) are used extensively in forensic paternity testing (Hallenberg and Morling, 2001). Sometimes mutations in autosomal STR loci can be a problem in paternity testing. Therefore, autosomal STR loci have limitations in forensic analysis. Y chromosome short tandem repeat (Y-STR), inherited solely through the paternal line, offers special application values in paternity tests and individual recognition (Underhill et al., 1996; Kayser et al., 2000; Johnson et al., 2003). At present, Y-STR analysis is a useful tool in sexual assault investigations and paternity testing (Jobling et al., 1997; Moore et al., 2016). Y-STR profiles can be obtained from samples with a male:female DNA ratio of 1:1000 (Mayntz-Press and Ballantyne, 2007). X chromosome has a special mode of inheritance. The X-STRs in females are passed down to both sons and daughters, but males only pass down X-STRs to their daughters. X-STRs are important complements to autosomal STRs and Y-STRs in forensic casework (Szibor et al., 2003; Szibor, 2007). As a gender marker, the amelogenin locus is included in most forensic autosomal STR kits. In routine cases, the amelogenin locus can be used for sex determination. However, dropouts of amelogenin Y or X (Amel Y/X) can lead to incorrect sex determination (Borovko et al., 2015). In males, the sex-determining region Y (SRY) gene is responsible for testis development and initiation of male sex determination in the fetus (Vakili Azghandi et al., 2016). Mutation or deletion in SRY may lead to disorders of sexual differentiation (Coutin et al., 1996). Here we investigated the genetic polymorphism of 19 Y-STRs and 16 X-STRs in the Han population of Shandong province, China. At the same time, we used the Y-STR and X-STR systems to settle 5 special cases with amelogenin deviants and one agenosomia case typed by autosomal STR kits (Goldeneye 20A and Identifiler system).

MATERIAL AND METHODS

Samples

In this study, 534 unrelated male individuals were typed using a Goldeneye 20Y kit. In

addition, 97 unrelated male and 214 female individuals were analyzed using a Goldeneye 17X kit. Among the 7500 civil parentage tests, 4 father/mother/son cases and a mother/daughter case showed abnormalities in their amelogenin test when typed by an autosomal STR system. We also found another mother/child case where the child's sex organs appeared abnormal. All samples were obtained from volunteers in the Han population of Shandong province, China, and informed consent were obtained from all subjects. This study was approved by the ethics committee of Jinan Central Hospital, which is affiliated to the Shandong University.

Reagents

The Goldeneye 20Y system included 19 Y-STR loci (DYS391, DYS389I, DYS390, DYS389II, DYS348, DYS456, Y-GATA-H4, DYS447, DYS19, DYS392, DYS393, DYS388, DYS439, DYS635, DYS448, DYS460, DYS458, DYS437, DYS385 a/b); the Goldeneye17X system included 16 X-STRs (DXS6795, DXS9902, DXS8378, HPRTB, GATA165B12, DXS7132, DXS7424, DXS6807, DXS6803, GATA172D05, DXS6800, DXS10134, GATA31E08, DXS10159, DXS6789, DXS6810) and an amelogenin locus (Peoplespot, Beijing, China; http://www.peoplespot.com.cn).

Polymerase chain reaction (PCR) and gene typing

Genomic DNA was extracted using Chelex-100 (Walsh et al., 1991). A Goldeneye 20Y system and a Goldeneye 17X system was used for PCR, according to manufacturer's instructions. Electrophoresis of amplified PCR products were carried out on the AB PRISM 3500 Genetic Analyzer. The data were analyzed using the Gene Mapper ID-X 1.3 software.

Data analysis

Y-STR allele and haplotype frequencies were calculated using the SPSS16.0 software; the genetic diversity (GD) of a single marker was calculated directly using the Nei's formula: $GD = n\ (1-\Sigma Pi^2)/(n-1)$ (Nei and Tajima, 1981; Coble et al., 2013). The allele number and haplotype frequencies of X-STRs were estimated using the SPSS16.0 software. Forensic parameters were computed based on allelic frequencies: power of discrimination in females (PD_F) and power of discrimination in males (PD_M) (Desmarais et al., 1998). Differences in allelic frequencies between female and male were assessed by the Chi-square test, and $P < 0.05$ was considered to be statistically significant. Hardy-Weinberg equilibrium analysis on female samples was performed with the Arlequin v3.1 software (Excoffier et al., 2007). Heterozygosity (Het) and polymorphism information content (PIC) were evaluated using the Powerstats v1.2 software (Shin et al., 2005).

RESULTS

Forensic parameters of 19 Y-STRs in the Han population of Shandong province from China

In this study, 203 Y-STR haplotypes were found in 534 unrelated healthy male individuals using the Goldeneye 20Y kit. The allelic frequencies ranged between 0.0019 and

0.7959; GD ranged between 0.3429 (DYS391) and 0.9667 (DYS385 a/b) (Table 1). Both DYS391 and DYS438 showed GD < 0.5, and the other loci showed GD > 0.5.

Table 1. Forensic parameters of 19 Y-STR loci in Shandong Han population of China.

DYS391		DYS389I		Y-GATA-H4		DYS437		DYS393		DYS460		DYS438		DYS19	
A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq
6	0.0056	11	0.0019	10	0.0506	13	0.0037	11	0.0056	8	0.0056	8	0.0019	11	0.0019
9	0.0487	12	0.5225	11	0.3352	14	0.6030	12	0.5712	9	0.3464	9	0.0262	13	0.0618
10	0.7959	13	0.2903	12	0.5150	15	0.3670	13	0.2079	10	0.3876	10	0.6873	14	0.2378
11	0.1479	14	0.1835	13	0.0936	16	0.0262	14	0.1742	11	0.2378	11	0.2247	15	0.4513
12	0.0019	15	0.0019	14	0.0056			15	0.0356	12	0.0187	12	0.0468	16	0.1667
								16	0.0056	13	0.0037	13	0.0112	17	0.0749
												14	0.0019	18	0.0056
GD	0.3429	GD	0.6102	GD	0.6122	GD	0.5019	GD	0.6000	GD	0.6741	GD	0.4750	GD	0.7038

DYS392		DYS439		DYS390		DYS456		DYS388		DYS389II		DYS635		DYS448	
A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq‡
10	0.0094	8	0.0019	16	0.0019	12	0.0056	10	0.1798	24	0.0037	16	0.0019	17	0.0112
11	0.1386	10	0.0524	19	0.0019	13	0.0262	11	0.0019	26	0.0112	18	0.0019	18	0.1592
12	0.1685	11	0.3970	21	0.0037	14	0.1910	12	0.6685	27	0.0749	19	0.1161	18.2	0.0019
13	0.3577	12	0.3764	22	0.0487	15	0.5318	13	0.1292	28	0.2940	20	0.2303	19	0.3670
14	0.2678	13	0.1386	23	0.4194	16	0.1873	14	0.0094	29	0.3464	21	0.3689	19.2	0.0019
15	0.0543	14	0.0318	24	0.3240	17	0.0468	15	0.0019	30	0.1910	22	0.1835	20	0.3258
16	0.0037	15	0.0019	25	0.1891	18	0.0094	16	0.0056	31	0.0637	23	0.0655	21	0.1105
				26	0.0112	25	0.0019	17	0.0037	32	0.0112	24	0.0299	22	0.0187
										33	0.0037	25	0.0019	23	0.0037
GD	0.7511	GD	0.6790	GD	0.6821	GD	0.6438	GD	0.5049	GD	0.7485	GD	0.7600	GD	0.7225

DYS447		DYS458		DYS385a/b											
A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq	A	Freq
18	0.0019	12	0.0019	9/17	0.0019	11/15	0.0037	12/17	0.0730	13/17	0.0243	14/20	0.0056	16/16	0.0056
19	0.0019	14	0.0112	10/11	0.0037	11/16	0.0112	12/18	0.0562	13/18	0.0693	14/21	0.0019	16/17	0.0037
20	0.0019	15	0.1030	10/14	0.0019	11/17	0.0337	12/19	0.0581	13/19	0.0581	14/22	0.0037	16/18	0.0037
21	0.0131	15.1	0.0019	10/16	0.0019	11/18	0.0318	12/20	0.0300	13/20	0.0300	14/23	0.0019	16/19	0.0037
22	0.0318	16	0.2247	10/17	0.0019	11/19	0.0225	12/21	0.0075	13/21	0.0056	15/16	0.0056	16/20	0.0037
23	0.2322	17	0.3034	10/18	0.0019	11/20	0.0056	12/22	0.0037	13/22	0.0019	15/17	0.0056	16/21	0.0037
24	0.2603	18	0.1948	10/19	0.0056	11/21	0.0037	12/23	0.0019	14/14	0.0037	15/18	0.0112	17/22	0.0019
25	0.1873	19	0.1067	10/20	0.0019	12/12	0.0337	12/26	0.0019	14/15	0.0019	15/19	0.0056	19/19	0.0019
26	0.1161	19.2	0.0019	11/11	0.0075	12/13	0.0300	13/13	0.0281	14/16	0.0019	15/20	0.0019		
27	0.1086	20	0.0337	11/12	0.0206	12/14	0.0094	13/14	0.0206	14/17	0.0206	15/21	0.0150		
28	0.0281	21	0.0150	11/13	0.0150	12/15	0.0112	13/15	0.0056	14/18	0.0300	15/22	0.0094		
29	0.0150	22	0.0019	11/14	0.0131	12/16	0.0449	13/16	0.0243	14/19	0.0243	15/24	0.0037		
30	0.0019														
GD	0.8173	GD	0.7975											GD	0.9667

A: allele; Freq: frequencies; GD: genetic diversity.

Forensic parameters of 16 X-STRs in the Han population of Shandong province, China

The 16 X-STR allelic frequencies of 97 males and 214 females are shown in Table 2. With the exception of DXS6803 (P = 0.002), the allelic frequencies of other 15 X-STRs (DXS6795, DXS9902, DXS8378, HPRTB, GATA165B12, DXS7132, DXS7424, DXS6807, GATA172D05, DXS6800, DXS10134, GATA31E08, DXS10159, DXS6789, DXS6810) were comparable between females and males in all loci examined. PD_F ranged from 0.5504 to 0.9638; PD_M ranged from 0.3176 to 0.8377. In the female samples, the Het of DXS6800 was < 0.5, and the PIC of the 6 loci were < 0.5; all the other loci had high polymorphism (PIC > 0.5, Het > 0.5) (Table 3). In the female samples, allele frequencies at all loci were in accordance with Hardy-Weinberg equilibrium, with the exception of DXS6803 and DXS6810.

Table 2. Allele frequencies of 16 X-STR loci in Shandong Han population of China.

GATA165B12			DXS6810			DXS9902			DXS6795			DXS7132			GATA172D05		
A	F	M	A	F	M	A	F	M	A	F	M	A	F	M	A	F	M
9	0.2383	0.2474	14	0.0047	0.0103	9	0.0210	0.0103	9	0.0491	0.0309	11	0.0093	-	6	0.0421	0.0309
10	0.5187	0.5567	17	0.1869	0.2268	10	0.4136	0.4124	10	0.1752	0.1546	12	0.0771	0.0928	7	0.007	0.0103
11	0.1939	0.1649	18	0.4626	0.4330	11	0.3715	0.3918	11	0.2593	0.3196	13	0.2126	0.2268	8	0.1355	0.1237
12	0.0444	0.0309	19	0.3364	0.2990	12	0.1729	0.1443	12	0.0257	0.0412	14	0.3294	0.3711	9	0.1519	0.0928
13	0.0047	-	20	0.0093	0.0309	13	0.0187	0.0412	13	0.4486	0.4433	15	0.2827	0.2474	10	0.3902	0.4124
						14	0.0023	-	14	0.0397	0.0103	16	0.0748	0.0515	11	0.2243	0.2577
									15	0.0023	-	17	0.0140	0.0103	12	0.0491	0.0722
DXS8378			HPRTB			DXS6800			DXS6807			GATA31E08			DXS7424		
A	F	M	A	F	M	A	F	M	A	F	M	A	F	M	A	F	M
8	0.0023	-	10	0.0023	-	11	0.0023	-	9	0.0023	-	6	0.0023	-	9	0.0047	-
9	0.0140	0.0103	11	0.0631	0.0515	16	0.7967	0.8144	11	0.4463	0.3608	7	0.0514	0.0515	11	0.0093	-
10	0.5491	0.5670	12	0.2991	0.2680	17	0.0023	-	12	0.0117	0.0103	8	0.0467	0.0412	12	0.0023	0.0103
11	0.2640	0.2887	13	0.3949	0.3711	18.1	0.0023	-	13	0.0374	0.0103	9	0.1846	0.1753	13	0.0701	0.0722
12	0.1472	0.1031	14	0.1776	0.2268	19	0.1051	0.1237	14	0.3201	0.4124	10	0.2290	0.2165	14	0.1355	0.1340
13	0.0187	0.0309	15	0.0467	0.0619	20	0.0023	-	15	0.1612	0.1753	11	0.3598	0.3918	15	0.2944	0.3196
14	0.0047	-	16	0.0117	0.0206	21	0.0047	-	16	0.0164	0.0206	12	0.1168	0.1134	16	0.3621	0.4227
			17	0.0047	-	22	0.0841	0.0619	17	0.0047	0.0103	13	0.0093	-	17	0.1005	0.0206
												14	-	0.0103	18	0.0187	0.0206
															19	0.0023	-
DXS10159			DXS6789			DXS6803			DXS10134								
A	F	M	A	F	M	A	F	M	A	F	M	A	F	M			
18.2	0.0023	-	13	-	0.0103	9.3	-	0.0103	29	0.0023	-	37.3	0.0327	0.0412			
22	0.0140	-	14	0.0070	0.0103	10	0.0140	-	30	0.0047	-	38	0.1098	0.1031			
22.2	0.0023	-	15	0.1425	0.1959	10.3	-	0.0206	31	0.0070	-	38.1	0.0023	-			
23	0.0818	0.0722	16	0.2897	0.1959	11	0.1355	0.1340	32	0.0187	0.0206	38.3	0.0117	-			
24	0.3131	0.1959	17	0.0327	0.0309	11.3	0.0981	0.0515	32.2	0.0047	-	39	0.0514	0.0515			
25	0.2383	0.3196	19	0.0280	0.0412	12	0.1332	0.1546	33	0.0537	0.0309	40	0.0187	-			
26	0.1612	0.1856	20	0.2407	0.2577	12.3	0.4299	0.5155	34	0.0841	0.1134	40.3	0.0023	0.0103			
27	0.1192	0.1753	21	0.1659	0.1546	13	0.0794	0.0103	35	0.1822	0.2062	41.3	-	0.0103			
28	0.0607	0.0515	22	0.0771	0.1031	13.3	0.0701	0.0722	36	0.2220	0.2577	42.3	0.0070	-			
29	0.0070	-	23	0.0117	-	14	0.0164	-	37	0.1846	0.1546						
			24	0.0047	-	14.3	0.0140	0.0309									
						15	0.0093	-									

A: allele; F: female; M: male.

Table 3. Forensic parameters of 16 X-STR loci in Shandong Han population of China.

Loci	Het	P value	PD_f	PDm	PIC	Loci	Het	P value	PD_f	PD_m	PIC
DXS6795	0.6961	0.2154	0.8613	0.6747	0.5129	DXS6803	0.7575	0.0003	0.9173	0.6830	0.6400
DXS9902	0.6603	0.8431	0.8184	0.6538	0.4298	GATA172D05	0.7518	0.9066	0.9034	0.7333	0.6306
DXS8378	0.6066	0.6860	0.7867	0.5835	0.2970	DXS6800	0.3470	0.8360	0.5504	0.3176	0.4964
HPRTB	0.7168	0.2100	0.8729	0.7321	0.5564	DXS10134	0.8567	0.5259	0.9638	0.8377	0.8157
GATA165B12	0.6346	0.2626	0.8099	0.6007	0.4090	GATA31E08	0.7655	0.7226	0.9109	0.7516	0.6555
DXS7132	0.7545	0.9804	0.8998	0.7382	0.6341	DXS10159	0.7944	0.5402	0.9292	0.7865	0.7098
DXS7424	0.7483	0.3835	0.8985	0.6951	0.6217	DXS6789	0.8023	0.2343	0.9334	0.8194	0.7242
DXS6807	0.6706	0.6794	0.8338	0.6683	0.4535	DXS6810	0.6377	0.0075	0.7974	0.6706	0.3754

Het: Heterozygosity with female samples; P value: P value using Hardy-Weinberg equilibrium with female samples; PD_f: power of discrimination with female samples; PD_m: power of discrimination in males; PIC: polymorphism information content with female samples.

Amelogenin test abnormalities by autosomal STR kits

In a father/mother/son case typed by autosomal STR, both the father and the son had amelogenin Y dropouts and deficiency of amelogenin Y when analyzed by Goldeneye 17X. However, both only showed deficiency of DYS458 when Goldeneye 20Y kit was used. Thus, both father and son had amelogenin Y-DYS458 deletion, as the son's mutation was inherited from his father. In 2 father/mother/son cases, 2 boys had dropout of amelogenin Y. In another father/mother/son case, the son had deficiency of amelogenin X. Including the amelogenin

locus, the gene types of the 3 boys were normal based on analysis using the Goldeneye 20Y and 17X kits, and their mothers and fathers were of normal gene type. They only had deficiency of amelogenin X or Y according to Goldeneye 20A and the Identifiler system. In a mother/daughter case, both individuals exhibited addition of amelogenin Y (XY) according to autosomal STR kits; amelogenin type was also found to be XY according to the Goldeneye 17 X-STR kit. However, no gene type was determined using the Goldeneye 20Y kit.

One case report of male sex organ malformation

In a mother/child case, the mother regarded the child as a girl. However, autosomal STR results showed X/Y on the amelogenin locus, while results of the Y-STR and X-STR analyses showed that the child was male. Results of chromosome nuclear type analysis of peripheral blood showed the genotype (46, XY). Physical examination results reported that the child was anatomically female.

DISCUSSION

As forensic genetic markers, highly polymorphic Y-STR systems are useful in forensic science cases (Kayser et al., 2000). X-STRs are also important for autosomal STRs and Y-STRs (Szibor et al., 2003; Szibor, 2007). The haplotype GD of Y chromosome is determined based on the discrimination power and power of exclusion (Bosch et al., 2000; Carracedo et al., 2001). We found that the 19 Y-STRs and the 16 X-STRs were highly polymorphic in the Han population of Shandong province, China. In the 16 X-STRs system, the allelic frequencies of DXS6803 in females differed from that of males; DXS6803 and DXS6810 were not in accordance with Hardy-Weinberg equilibrium in female samples. The significant deviation from the expected values for some loci may be due to the limited number of samples (Huang and Yang, 2004).

When using only amelogenin as a sex marker in autosomal STR kits, dropouts in amelogenin Y or X may be troublesome in forensic caseworks during sex affirmation (Ma et al., 2012; Davis et al., 2012; Ou et al., 2012). X-STRs and Y-STRs are important complements to autosomal STR analysis in some forensic cases (Yang et al., 2016). In this study, we found 5 cases with amelogenin test abnormalities using the autosomal STR kits (Goldeneye 20A and Identifiler system). In one father/son case, both had deletions of amelogenin Y-DYS458. Previous studies showed that the deletion frequency of the amelogenin Y-DYS458 was 0.0155% (Chen et al., 2014). Two cases had amelogenin Y dropouts, and one individual showed deficiency of amelogenin X, but were all found to have normal amelogenin when analyzed using the Goldeneye 20Y and 17X kits. An amelogenin mutation or STR primer design can result in deletion of amelogenin Y or X (Maciejewska and Pawłowski, 2009). At present, there are several autosomal STR kits which include amelogenin and other X- or Y-STR loci to overcome shortcomings such as deletion of amelogenin Y or X. One such example is the Goldeneye 25A kit, which contains amelogenin as well as the Y-STR DYS391 (Peoplespot, Beijing, China; http://www.peoplespot.com.cn).

Several studies revealed that specific STR loci on the X chromosome can exchange genetic material with the Y chromosome. While very few individuals show such phenomenon, it may lead to unusual mode of inheritance or gene types (Dupuy et al., 2000; Von Wurmb-Schwark et al., 2007). Sometimes there are homologous regions among X and Y chromosome in

human (Iwase M, et al., 2010). In this study, both mother and daughter had Y-type amelogenin. The mother's Y-type amelogenin may have come from an X chromosome interchange with the Y chromosome from her parents, and this interchange may be inherited by her daughter. We found one child with male sex organ malformation, his sex was verified by X-, Y-STRs and chromosome nuclear type analysis. The gender determining gene, sex-determining region Y (SRY), is located on Yp11.3. Deletion of SRY can cause a male to develop as a female (Sinclair et al., 1990; Harley et al., 1992; Harley et al., 2003). We did not detect SRY in this case, but the results declared that the boy was an agenosomia male.

CONCLUSION

Our results indicated that 19 Y-STRs and 16 X-STRs are highly polymorphic in the Han population of Shandong province, China, and that these data can be used in forensic paternity testing. At the same time, we used the two kits to investigate 5 abnormal amelogenin cases and an agenosomia case. The results confirmed that X-STR and Y-STR kits are a necessary complement to autosomal STR kits in forensic cases.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

Research supported by the National Natural Science Foundation of China (grants #81625013, #81330073, and #81302620) and the Ministry of Science and Technology of China (grant #2016YFC0800703).

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