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Polymorphism of two new Y-STR loci in a Chinese population

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Abstract

Human chromosome Y-specific short tandem repeat (Y-specific STR) markers have useful properties for forensic applications. However, there is a need to develop more Y-specific STR markers because the discriminating power of each STR locus is limited. In the present study, we describe our results on two new Y-specific STR markers, which were initially reported by White et al. [Genomics 57 (1999) 433] as Y-GATA-C4 and Y-GATA-A10. The distributions of alleles and haplotypes for both Y-specific STR loci were studied in a Chinese Han population sample. Allele determination was carried out by comparison with sequenced allelic ladders which were made in-house. Following the recommendations of the International Society of Forensic Genetics, the allele classification for both Y-specific STR loci was based on the number of repeat motifs. The results show that the haplotype diversity, the power of discrimination and the exclusion probability in the Chinese population for both Y-specific STR loci were 0.9090. The implication from this study is that Y-GATA-C4 and Y-GATA-A10 are useful Y-specific STR markers for forensic DNA typing in Chinese populations.

Keywords: Y-specific short tandem repeat; Haplotypes; Y-GATA-C4; Y-GATA-A10

1. Introduction

Human chromosome Y specific short tandem repeat (Y-specific STR) markers have useful properties for forensic analysis. A panel consisting of seven Y-specific STR markers has been recommended for forensic applications [1]. However, there is a need to develop more Y-specific STR markers because the discriminating power of each

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STR locus is limited. Two sets of Y-specific STR markers were recently published [2,3]. In both cases, the number of individuals and ethnic groups that were tested was limited and further characterization of the new markers is necessary. Two of the new Y-specific STR markers, Y-GATA-C4 and Y-GATA-A10 [2], were used in this study. One example of each allele at two Y-specific STR loci was sequenced. In order to reveal the amount of genetic polymorphism shown by these new Y-specific markers in Chinese, we also investigated the allele and haplotype frequencies in a Chinese Han population sample.

2. Materials and methods

2.1. Sample

EDTA-blood specimens were collected from 104 unrelated male Han volunteers, who donated blood for blood banks in Chengdu, Sichuan province, China. Ethnic origin was determined by self-declaration. DNA was extracted using the Chelex method.

2.2. Typing for Y-specific STR

PCR amplification for the Y-GATA-A10 locus was carried out using the primers according to White et al. [2]. The primers for Y-GATA-C4 locus were modified in order to reduce the amplified fragment size. The new primers for Y-GATA-C4 locus were redesigned by us as following.

P1:5′ -gtggaaccagcccaaatatc-3′
P2:5′ -aatgctctcttggcttctcact-3′

All loci were amplified in singleplex polymerase chain reactions. Each PCR reaction contained 2–20 ng of DNA, 1 × Taq buffer, 1.5 mM $MgCl_2$, 200 μM each nucleotide (Pharmacia Biotech, Sweden), 1.5 U Taq polymerase (Life Technologies, USA), 0.25 μM each primer in a total volume of 37.5 μl. In the PCR protocol, the DNA was initially denatured at 94 °C for 2 min. This was followed with 94 °C for 30 s, 54 °C for 40 s and 72 °C for 30 s. A total of 30 cycles were carried out in a GeneAmp PCR System 9600 (Perkin-Elmer, USA). The PCR products of two Y-specific STR loci were analyzed using a horizontal non-denaturing polyacrylamide gel electrophoresis with a discontinuous buffer system [4].

2.3. Sequence analysis

PCR products were eluted from the gels and purified before sequencing. An example of each allele was sequenced on an ABI 377 automated sequencer using a Dye Terminator Cycle Sequencing kit (PE Applied Biosystems, USA). The alleles were also cloned using the pGEM®-T Easy Vector System I (Promega, USA) according to the manufacturer's

instruction. The DNA clones were sequenced with the ABI 377 automated sequencer to verify the allele sequences.

2.4. Nomenclature

The allele classification for each Y-specific STR locus was based on the number of repeat motifs according to the recommendations of the International Society of Forensic Genetics (ISFG) [1].

2.5. Analysis of haplotypes in Chinese Han population

The Chinese Han haplotypes were established using the allelic typing results. Allele determination was carried out by comparison with the sequenced allelic ladders, which were made in-house and contained all the alleles found in this study. The gene diversity or haplotype diversity was calculated according to the equation [5]

$$h = n(1 - \Sigma x^2)/(n - 1)$$

where n is the number of individuals, h is the gene diversity or haplotype diversity and x is the allele frequency or haplotype frequency in the given population sample). Standard errors were calculated according to the equation [5]

$$\mathrm{SE} = \{2\{\Sigma x^3 - (\Sigma x^2)^2\}/n\}^{1/2}$$

3. Results

3.1. Sequencing data

Tables 1 and 2 show the DNA sequences of the alleles at the two loci as observed in our population sample.

Table 1
DNA sequences of alleles at Y-GATA-A10 Y-specific STR locus

Consensus structure	P1(28bp)cttatccatttatttattcatccatctctttctttctctccatcca(tatc)11taatctatcatctatcP2(24bp)
Allele (bp)	Sequence
11 (158)	P1(28bp)...46bp...(tatc)11...16bp...P2 (24bp)
12 (162)	P1(28bp)...46bp...(tatc)12...16bp...P2 (24bp)
13 (166)	P1(28bp)...46bp...(tatc)13...16bp...P2 (24bp)
14 (170)	P1(28bp)...46bp...(tatc)14...16bp...P2 (24bp)
15 (174)	P1(28bp)...46bp...(tatc)15...16bp...P2 (24bp)

Table 2
DNA sequences of alleles at Y-GATA-C4 Y-specific STR locus

Consensus structure	P1(22bp) ttgcatagaatctc(tatc)3(tatg)2(tatc)2(tatg)2(tatc)9acatttttctttatccatcattgattgatg P2(20bp)
Allele (bp)	Sequence
9 (157)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)9...29bp...P2(20bp)
10 (161)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)10...29bp...P2(20bp)
11 (165)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)11...29bp...P2(20bp)
12 (169)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)12...29bp...P2(20bp)
13 (173)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)13...29bp...P2(20bp)
14 (177)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)14...29bp...P2(20bp)
15 (181)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)15...29bp...P2(20bp)
16 (185)	P1(22bp)...14bp...(tatc)3(tatg)2(tatc)2(tatg)2(tatc)16...29bp...P2(20bp)

3.2. Population data

The distribution of haplotypes in the Chinese Han population is shown in Table 3. A total of 23 different haplotypes were observed in 104 males. The haplotype diversity for two Y-specific STR loci in the Chinese Han population was calculated to be 0.9090 and the standard error was calculated to be 0.0097.

Table 3
Y-specific STR haplotypes in a Chinese Han population

Haplotype	Y-GATA-C4	Y-GATA-A10	N	Frequency
1	9	15	1	0.0096
2	10	12	2	0.0192
3	10	13	7	0.0673
4	10	14	6	0.0577
5	10	15	1	0.0096
6	11	11	1	0.0096
7	11	12	5	0.0481
8	11	13	14	0.1347
9	11	14	8	0.0770
10	12	12	22	0.2116
11	12	13	12	0.1155
12	12	14	6	0.0577
13	13	11	2	0.0192
14	13	12	3	0.0288
15	13	13	2	0.0192
16	13	14	2	0.0192
17	14	12	2	0.0192
18	14	13	1	0.0096
19	14	14	1	0.0096
20	15	12	2	0.0192
21	15	13	2	0.0192
22	15	14	1	0.0096
23	16	14	1	0.0096
Total			104	1

4. Discussion

For Y chromosome specific markers, the allele distribution for each marker is less important than the haplotype diversity for the whole array of loci. The haplotype diversity value of the Y-chromosome corresponds to the value of the power of discrimination (PD) and the chance of exclusion (CE) for unrelated males. Since we used a random population sample to estimate the haplotype diversity for the total, the equation, $h = n(1 - \Sigma x^2)/(n - 1)$ for an unbiased estimate of the haplotype diversity was employed according to Nei's [5] reccomendation. The haplotype diversity for two Y-specific STR loci in the Chinese Han population was calculated to be 90.9%. This means that the power of discrimination (PD) and the chance of exclusion (CE) for unrelated males are also 90.9%. This implies that the new markers will be valuable additions to the current test panel and will improve the exclusion probabilities in forensic and kinship cases. Further characterizations of these loci should include sequencing data for more ethnic groups, mutation rates and an evaluation of the performance of these STR's for different types of forensic evidence.

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