THE POLYMORPHISM OF NEW Y-STR A10 AND C4 LOCI IN CHINESE

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Abstract: Y-STR loci A10 and C4 were investigated by duplex PCR amplification with fluorescent FAM labeled primers. 6 alleles were identified for A10 locus and 8alleles in Chinese males and 8 for C4 in 100 unrelated Chinese. 24 haplotypes were observed in 100 unrelated Chinese males. The gene diversity was 0.6505 at A10 locus and was 0.7659 at C4 locus. Sequencing of alleles proved that both loci A 10 and C4 consisted of simple GATA consensus structure without any variation of inter-structure of alleles.

Key words: short tandem repeat A10 and C4 multiplex amplification gene frequency gene diversity DNA sequencing

Introduction

Y-STR markers on Y chromosome have been found to be very useful in individual identification of males and paternity testing. Now new 7 Y-STR loci (A 7.1, A7.2, A10, C4, H4, A4 and A8) have been reported in 1999[1]. Five of them (A 7.1, A7.2, A10, C4, H4) is specific for male, DNA from female sample could not be amplified. All of them showed a high level of Y chromosomal heterogeneity within and between populations.

The aim of this study was to evaluate the genetic variation of A10 and C4 loci in Japanese male, their consensus structure and construct a sequenced allelic ladder.

Methods

1 Preparation of DNA

Genomic DNA was extracted from blood samples of 100 unrelated Chinese males lived in southern area with classical phenol-chloroform method [].

2. Duplex amplification of PCR

Primer sequences

A10 Primer1：5*-CCT GCC ATC TCT ATT TAT CTT GCA TAT A-3*

A10 Primer2：5*-ATA AAT GGA GAT AGT GGG TGG ATT-3*

C4 Primer1：5*-AGT GTC TCA CTT CAA GCA CCA AGC AC-3*

C4 Primer2：5*-GCA GCA AAA TTC ACA GTT GGA AAA ATG T-3*

The primers were labeled with fluorescence 6-FAM at their 3'-end.The amplification was performed in a volume of 25μl. The mixture contained 20ng genomic DNA, 15pmol each primer, 0.16mmol/L dNTPs, 8mmol/L Tris-HCl (pH8.3), 40mmol/LKCl, 1.5mmol/L $MgCl_2$, 800ng/ml BSA and 2 U of gold Taq DNA polymerase (Perkin-Elmer, Norwalk. CT. USA).

Cycling parameter for duplex amplification in GeneAmp PCR system 2400 themocycle, Perkin-Elmer) is as followings:

95^0C 10 min, 1 cycle, then

94 ^{0}C 15 sec, 58^{0}C 20Sec, 72 ^{0}C 15Sec, 5 cycles,
94^{0}C 15 sec, 56^{0}C 20sec, 72 ^{0}C 15 sec ,30 cycles.

Electrophoresis

A 0.4μl of PCR products were mixed with 0.8μl of 2X loading buffer and 0.4μl of internal lane standard Genescan-500 TAMURA (Perkin-Elmer, Norwalk, CT, USA) to make the loading mixture. The mixture was denatured at 100 ^{0}C for 3 min, followed by rapidly put into ice water. A μl of the denatured mixture was separated on 1 4%denatured polyacrylamide gel on Automated Sequencer 377(Perkin-Elmer, Norwalk, CT, USA), 1x TBE served as electrode buffer. The allele size was auto-calculated with GeneScan analysis ver2.0.2.

Sequencing

The alleles were sequenced by Dye terminator/direct sequencing with –21M 13 primer.

Allelic ladders and nomenclature

The designation of alleles followed by the recommendations of the International Society of Forensic Haemogenetics (DNA Commission of the ISFH 1997) based on the number of variable repeats.

Allelic ladders for each locus were made sequenced alleles and were run on every gel to ensure correct allele typing.

Statistical evaluation

Gene or haplotype diversity calculated according to the formula $D=1-[(n/n-1)(\Sigma f_i^2)]$, N: The number of the samples, f_i:the frequency of the I allele or haplotype.

Discrimination capacity (DC)= the number of halpotype/ the number of the sample analyzed.

Results and discussion

1. The frequency distribution of alleles

In 100 Chinese male lived in southern area, 6 (allele 11-16) different alleles at A10 locus and 8(allele 10-17,18) at C4 locus were found. The gene diversity for A10 and C4 is 0.6505 and 0.7659, respectively. Table 1 and 2 showed the allele frequency and gene diversity. There was no significant difference to other reports[1].

2. Sequence of the structure of alleles

20 alleles of the A10 locus and 19 alleles of c4 locus were sequenced. The results showed both of the loci were constructed of simple $(TATG)_n$ repeat structure, without further any variation of inter-structure. However, the size of the allele at A10 locus was found more 2 bp than that of P.Scott white[1], while consisted with data in Genebank supplied by P.Scott white. Figure 1 showed the census sequence of the locus A10 and C4.At locus C4, there were 2 TATC repeat units and 3 TATG repeats without any changes. The size of alleles at locus C4 is less than that of P.Scott White[1], while consisted with data in Genebank supplied by P.Scott White.

3. The polymorphism of the Haplotype of A10 and C4 locus

There were 24 haplotype of A10 and C4 to be found, 11 of which appeared only in one time. Table 3 showed the frequencies of the haplotypes. The Discrimination capacity(DC) and haplotype diversity (HD) was 0.24 and 0.8787,respectively. Combined with other DYS (19, 389II, 390, 391, and 392, 393), there

were more haplotypes to be observed and the discrimination capacity increased. The increasing size of available database and the characterization of further highly informative. Y-STRs should increase the power of exclusion of Y chromosome haplotypes and the usefulness of these markers in crime investigation.

Like mtDNA, the non-recombining region of the Y chromosome is of special interest since it is haploid and does not recombine at meiosis. The Y chromosome markers in this region are transmitted together as haplotype from fathers to sons, thus establishing patrilineages. Therefore these markers are powerful tools for exclusion, particularly helpful in analyzing mixed DNA samples, as well as in deficiency cases in paternity testing where the alleged father is not available but other partitioned relatives are.

References

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Table1 The polymorphism of Chinese males at A10 locus

Size(bp)	allele(7)	n=100	frequency
154	10		
158	11	1	0.01
162	12	48	0.48
166	13	31	0.31
170	14	12	0.12
174	15	7	0.07
178	16	1	0.01

Gene diversity(GD)=0.6505

Table 2 the polymorphism of Chinese males at C4 locus

Size(bp)	allele(7)	n=100	frequency
246	10	1	0.01

250	11	11	0.11
254	12	31	0.31
258	13	30	0.30
262	14	17	0.17
266	15	6	0.06
270	16	3	0.03
274	17		
278	18	1	0.01
Gene diversity (GD)=0.7659			

Table 3. Informations of haplotype(A10-C4) in Chinese

A10-C4	Chinese	
haplotype	n(24)	frequency
11-14	1	0.01
12-10	1	0.01
12-11	7	0.07
12-12	13	0.13
12-13	17	0.17
12-14	6	0.06
12-15	4	0.04
13-11	2	0.02
13-12	11	0.11
13-13	9	0.09
13-14	6	0.06
13-15	1	0.01
13-16	1	0.01
13-18	1	0.01
14-11	1	0.01
14-12	3	0.03
14-13	4	0.04

14-14	3	0.03
14-15	1	0.01
15-11	1	0.01
15-12	4	0.04
15-14	1	0.01
15-16	1	0.01
16-16	1	0.01
total	100	1.000
Discrimination capacity (DC) = 0.24		
haplotype diversity (HD) = 0.8707		

Consensus structure at A10 locus:

5‘-P1(28bp)-46bp-(TATC)n-16bp-P2(24bp)-3’

Consensus structure at C4 locus:

5‘-P1(26bp)-68bp-(TATC)3(TATG)2(TATC)2(TATG)2(TATC)n-48bp-P2(28bp)-3’

Fig 1.Sequence structure of A10 locus and C4 locus