

Screening of Y-chromosome STR Loci in Taiwanese Populations

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Received: March 3, 2002/ Received in revised from: March 19, 2002/ Accepted: April 7, 2003

ABSTRACT

This study examines the diversity of nine Y-chromosome STR in nine aboriginal tribes of Taiwanese. The STR loci were DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, DYS385 and DYS388. The populations studied in this survey included Ami, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tsou and Tao tribes. A total of 113 haplotypes for all nine Y-chromosome STR loci were found in 173 unrelated healthy males. The diversity of the nine Y-chromosome STR loci was more variable in Chinese Han population than in nine aboriginal tribes. The minimum diversity for the populations increased significantly with nine Y-STR markers compared to eight markers.

Keywords: Y-chromosome STR, haplotypes, dw_{\min} value

Introduction

The use of Y-chromosome short tandem repeat (STR) loci have become increasingly common in forensic science, due in part to the loci being recombination-free during meiosis and paternally inheritance. The analysis of Y-chromosome STRs in paternity testing of potential paternal relationships is very important especially in cases where a potential father is not available [1]. In sexual assault cases, Y-chromosome STRs were used to identify the male offender [2]. M. Prinz et al. reported that the male DNA was detectable even in the ratio of 1:2000 (male:female) by Y-chromosome STR analysis [3]. Their applications also included human evolution [4], genealogical [5] and population studies [6]. This paper examines the population diversity of the aborigi-

nal populations in Taiwan.

There are two major populations living in Taiwan, one was Han population and the other was indigenous population [7]. Han population included plain people in Taiwan and the immigrating Chinese population. The indigenous population included eight tribes (Ami, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat and Tsou) living in the mountains and the only other tribe (Tao tribe) living in Orchid island. The analysis of Y-chromosome STRs for the Han population in Taiwan has been performed [7], however the analysis of Y-chromosome STRs for the aboriginal population has not been the subject of such a study. We report here the haplotype diversity of Y-chromosome STRs in the nine aboriginal tribes (Ami, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tsou and Tao).

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Materials and methods

Sample sources

The blood samples from 173 unrelated healthy males were randomly collected with informed consent by Kaohsiung Medical University and the Central Police University in Taiwan. Samples were collected from 9 aboriginal tribes including 13 Bununs, 13 Atayals, 36 Amis, 9 Puyumas, 16 Paiwans, 29 Taos, 22 Tsous, 10 Rukais, and 25 Saisiats. The parents of each collected sample were from the corresponding tribe respectively. DNA was extracted with salt-chloroform method [8] and quantified with the QuantiBlot kit (Roche Molecular Systems, Alameda, Calif.).

PCR amplifications and electrophoresis of PCR products

PCR amplifications were performed in one quadruplex, one triplex and two singleplex reactions. The loci analyzed in the quadruplex reactions were DYS19, DYS389I/II and DYS390, in the triplex reaction for DYS391, DYS392 and DYS393, and in the two singleplex reactions for DYS385 and DYS388 respectively. The primers adopted in this study were designed according to the report of Kayser et al. [9]. One of each primer pair was labeled with fluorescent dye FAM, HEX or TET. The primer sequences and respective labeled dyes are shown in Table 1. PCR amplifications were performed in a reaction mixture of 25 μ l, which contained 15-20 ng of isolated genomic DNA, 0.06-0.3 μ M each of primers, reaction buffer (10 mM Tris-HCl, pH 8.3, 2.5 mM MgCl₂, 50 mM KCl, 0.01% (w/v) gelatin), 200 μ M dNTP, and 1 unit of AmpliTaq Gold™ DNA polymerase (Applied Biosystems). The PCR amplifications were performed according to the program of 95 °C for 10 minutes and followed by 35 or 40 cycles of 94 °C for 1 minute, 55 °C for 1 minute and 72 °C for 1-2 minutes in a thermal cycler (GeneAmp PCR System 480 Applied Biosystems). PCR products were separated and detected by 4% denatured polyacrylamide gel and ROX500 (Applied Biosystems) was used as the internal standard on a PRISM 377 DNA sequencer (Applied Biosystems).

Statistical analysis

The minimum diversity within the population (dw_{\min}) was calculated following the equation [10]:

$$dw_{\min} = 1 - \sum_{i=1}^h (X_i / N_x)^2$$

X_i represented the sample number of i^{th} haplotype, and h represented the total number of haplotypes in all samples (N_x).

Results and discussion

Alleles were confirmed by DNA sequencing and named according to the number of repeat units presented by sequencing results. There were 6 (DYS19), 4 (DYS389I), 6 (DYS389II), 5 (DYS390), 4 (DYS391), 5 (DYS392), 5 (DYS393), 27 (DYS385) and 3 (DYS388) alleles in 173 aboriginal males. The results are displayed in Table 2. There were two allele types for DYS385 locus in some haplotypes due to the duplicated tandem repeat structure on this locus. The most common allele of DYS19 was allele 16 in Puyuma, but allele 15 in the other tribes. The most common allele of DYS389I was allele 10 in Atayal and Bunun, but allele 9 in the other tribes. The most common allele of DYS389II was allele 24 in Saisiat, allele 25 in Ami, Paiwan, Rukai and Tao, allele 26 in Atayal, Bunun, and Tsou, and allele 25, 26, 28 in Puyuma. The most common allele of DYS391, DYS392, DYS393 and DYS388 was allele 10, 14, 13 and 12 respectively in all nine tribes. The most popular allele of DYS385 was allele 12-13 in Puyuma and Rukai, allele 13-13 in Ami, Atayal, Tsou, and Tao, allele 13-14 in Paiwan and Saisiat, allele 14-14 in Bunun. The frequency of the most popular allele was 1.000 in Atayal (DYS390 and DYS391), Bunun (DYS393 and DYS388), and Rukai (DYS389I, DYS391, DYS392, DYS393 and DYS388), but the representative may be restricted to the sample size of each tribe.

Totally there were 113 haplotypes of nine Y-chromosome STR loci in 173 unrelated healthy males (Table 2). The most popular haplotype was no. 13 (15/9/24/23/10/14/13/13-13/12) presented in Rukai, Saisiat and Tao tribes. There were 13 samples with this haplotype at a frequency of 7.51 %. Eighty-eight samples produced a haplotype detected only once, representing a frequency of 0.58%. The sample sizes of each tribe are too small to specify the unique haplotype in each aboriginal tribe of Taiwanese and to evaluate the similarity between them.

In a previous study of 183 unrelated individuals of Taiwanese Han population a total of 162 haplotypes were produced [7]. In the Han population the most popular haplotype was the same as that in this study, but the frequency was only 2.73 % [7]. The results indicated that

the nine Y-chromosome STR loci were more variable in Han population than in nine aboriginal tribes. It is possible that a greater number of intra-marriages occur within the aboriginal populations than the larger Han population.

The minimum diversity values (dw_{\min}) of nine tribes (this study), Han population [7], Japanese and Germans [11] in eight loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS388) are shown in Table 3. The haplotype numbers were 138, 107, 194 and 110 in 183 individuals of Han population, 173 individuals of nine tribes, 200 individuals of Japanese and 111 individuals of Germans respectively. The haplotypes were quite variable among and with in populations. The dw_{\min} value of nine tribes in this study was 0.9720, and lower than that of other populations. The dw_{\min} value of nine loci was 0.9822, higher than that of eight loci (0.9720). Some new Y-chromosome STR markers, such as DYS434, DYS437, DYS438, DYS439, DYS441, DYS442, DYS443, DYS444, DYS445 and GATA A10, have been analyzed in Portuguese or Japanese populations and the polymorphism was observed [12, 13]. Nineteen Y-chromosome STR markers have been amplified to evaluate their usefulness in forensic casework [14]. The dw_{\min} value will increase if more Y-chromosome STR loci were analyzed. Therefore, it could be valuable to analyze more Y-chromosome STR loci in Taiwan.

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Table 1 The primer sequences and fluorescent dyes used to amplify the nine Y-STR loci.

Y-STR locus	Primer sequences (fluorescent dye)
DYS19	5'-CTACTGAGTTTCTGTTATAGT-3' (HEX)
	5'-ATGGCATGTAGTGAGGACA-3'
DYS389 I / II	5'-CCA ACTCTCATCTGTATTATCTAT-3' (HEX)
	5'-TCTTATCTCCACCCACCAGA-3'
DYS390	5'-TATATTTTACACATTTTTGGGCC-3' (FAM)
	5'-GACAGTAAAATGAACACATTGC-3'
DYS391	5'-CTATTCATTCAATCATAACCCA-3' (FAM)
	5'-GATTCTTTGTGGTGGGTCTG-3'
DYS392	5'-TCATTAATCTAGCTTTTAAAAACAA-3' (TET)
	5'-AGACCCAGTTGATGCAATGT-3'
DYS393	5'-GTGGTCTTCTACTTGTGTCAATAC-3' (FAM)
	5'-AACTCAAGTCCAAAAAATGAGG-3'
DYS385	5'-AGCATGGGTGACAGAGCTA-3' (FAM)
	5'-TGGGATGCTAGGTAAAGCTG-3'
DYS388	5'-GTGAGTTAGCCGTTTAGCGA-3' (HEX)
	5'-CAGATCGCAACCACTGCG-3'

Table 2 Total of 113 haplotypes of nine Y-chromosome STR loci in 173 unrelated individuals.

Haplotype no. (population)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 388	Sample size
1 (Ts)	13	9	24	23	9	14	13	13 18	10	1
2 (Pa)	13	9	25	22	10	13	12	11	12	2
3 (Am, Sa, Ts)	14	9	24	25	11	14	12	13 19	10	3
4 (Pa)	14	9	24	23	9	14	13	13 15	12	1
5 (Ru)	14	9	25	23	10	14	13	13 14	12	2
6 (Sa)	14	9	25	24	11	15	12	13 17	10	1
7 (Sa)	14	9	25	24	11	14	12	13 17	10	1
8 (Pu)	14	10	26	23	11	13	14	12 13	12	1
9 (Pa)	14	10	27	24	10	14	12	13 18	10	1
10 (Sa)	14	11	27	24	10	14	14	16 21	12	2
11 (Pu)	15	8	23	23	10	14	13	12 13	12	1
12 (Sa)	15	9	23	23	10	14	13	13	12	1
13 (Ru, Sa, Ta)	15	9	24	23	10	14	13	13	12	13
14 (Pa)	15	9	24	23	9	14	13	13	12	2
15 (Sa)	15	9	24	23	10	13	13	13 14	12	5
16 (Sa)	15	9	24	23	10	14	13	13 14	12	3
17 (Sa)	15	9	24	23	10	13	13	13 15	12	1
18 (Sa, Ta)	15	9	24	23	10	13	13	13	12	3
19 (Sa)	15	9	24	23	10	14	13	13 15	12	1
20 (Am)	15	9	24	24	10	14	13	13 14	12	1
21 (Ts)	15	9	24	24	10	12	12	13 16	12	1
22 (Ta)	15	9	25	21	9	14	14	12 14	13	1
23 (Ru)	15	9	25	22	10	14	14	13 15	12	1
24 (Am)	15	9	25	23	10	15	13	13	12	2
25 (Am)	15	9	25	23	10	14	13	13 14	13	1
26 (Ru, Ts, Ta)	15	9	25	23	10	14	13	12 13	12	9
27 (Pa)	15	9	25	23	9	14	13	13	12	1
28 (Pa)	15	9	25	23	10	14	13	13	12	1
29 (Pu)	15	9	25	23	10	14	11	12	12	1
30 (Pu)	15	9	25	23	10	14	11	12 14	12	1
31 (Ts)	15	9	25	23	9	14	13	12 13	12	1
32 (Ts)	15	9	25	23	10	14	12	15 18	10	1
33 (Ta)	15	9	25	23	10	12	13	13 14	12	2
34 (Am)	15	9	25	24	11	13	14	13 15	13	1
35 (Am)	15	9	25	24	10	13	14	14 19	12	1
36 (Am)	15	9	25	24	10	13	14	13 15	13	1
37 (Am)	15	9	25	24	10	13	14	13 16	13	1

Table 2 Total of 113 haplotypes of nine Y-chromosome STR loci in 173 unrelated individuals. (continued)

Haplotype no. (population)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 388	Sample size
38 (Am)	15	9	25	24	10	13	14	14 18	12	1
39 (Am)	15	9	25	24	11	13	14	13 16	13	2
40 (Ru)	15	9	25	24	10	14	13	13 15	12	2
41 (Pa)	15	9	25	24	12	13	14	13 16	13	1
42 (Pa)	15	9	25	24	10	13	12	12	12	1
43 (Pa)	15	9	25	24	10	14	13	13 14	12	1
44 (Pa)	15	9	25	24	10	14	13	13 15	12	1
45 (Ta)	15	9	25	24	10	14	13	12 13	12	3
46 (Ta)	15	9	25	24	11	13	13	13 16	13	1
47 (Am)	15	9	25	25	10	13	14	13 14	13	1
48 (Bu)	15	9	26	23	10	14	13	13 14	12	1
49 (Sa)	15	9	26	23	10	14	13	13	12	1
50 (Ts)	15	9	26	23	10	14	12	13	12	1
51 (Ta)	15	9	26	23	10	12	12	11 12	12	1
52 (Ts)	15	9	27	23	10	14	13	13	13	1
53 (Am)	15	9	27	25	10	14	15	16	12	1
54 (Ta)	15	9	28	23	10	13	13	10 17	12	1
55 (Am)	15	10	25	24	11	16	13	12 13	12	1
56 (Am)	15	10	25	24	11	13	13	11 16	13	1
57 (At)	15	10	25	24	10	13	12	13 20	10	1
58 (Bu)	15	10	26	22	10	14	13	14	12	1
59 (Bu)	15	10	26	22	10	14	13	14 15	12	1
60 (Bu)	15	10	26	22	11	14	13	14	12	1
61 (At)	15	10	26	23	10	14	13	13 14	12	4
62 (At, Bu)	15	10	26	23	10	14	13	13	12	4
63 (Am)	15	10	26	24	10	13	14	13 16	13	1
64 (Ts)	15	10	26	24	10	13	12	13 21	12	1
65 (Bu)	15	10	26	23	11	14	13	14	12	1
66 (At)	15	10	27	23	10	14	13	13	12	2
67 (Sa)	15	10	28	23	10	13	12	12	12	1
68 (Ta)	15	10	28	24	10	13	12	12 19	12	1
69 (Am)	15	10	28	25	10	15	14	14 19	12	1
70 (Am)	15	10	28	23	10	14	15	13 17	12	1
71 (At)	15	11	26	23	10	14	13	13 14	12	1
72 (At)	15	11	27	25	10	14	13	13 11	12	1
73 (Ts)	15	11	27	23	10	13	14	13 14	12	1
74 (Sa)	15	11	28	23	10	13	13	10 18	12	1

Table 2 Total of 113 haplotypes of nine Y-chromosome STR loci in 173 unrelated individuals. (continued)

Haplotype no. (population)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 388	Sample size
75 (Pa)	16	9	24	23	10	14	13	13 14	12	1
76 (Am)	16	9	25	23	10	15	13	13	12	1
77 (Am)	16	9	25	23	10	14	13	13 14	13	1
78 (Am)	16	9	25	23	11	13	13	13 15	12	1
79 (Am)	16	9	25	23	10	13	13	11 16	13	1
80 (Am)	16	9	25	23	10	14	12	12 14	12	1
81 (At, Pa)	16	9	25	23	10	14	13	13 14	12	3
82 (Ru)	16	9	25	23	10	14	13	12 13	12	2
83 (Pa)	16	9	25	23	10	14	13	13 15	12	1
84 (Pu)	16	9	25	23	10	14	13	12	12	1
85 (Am)	16	9	25	24	10	14	13	13	12	5
86 (Ta)	16	9	25	24	10	13	13	12 17	13	1
87 (Sa)	16	9	25	25	10	13	12	13 20	12	1
88 (Bu)	16	9	26	23	10	14	13	13 14	12	1
89 (Pu)	16	9	26	23	10	14	14	13 14	12	1
90 (Pu)	16	9	26	23	10	13	13	13 17	13	1
91 (Sa)	16	9	26	23	10	14	12	12 14	12	1
92 (Ta)	16	9	26	23	10	14	13	12 13	12	1
93 (Am)	16	9	26	24	10	14	13	13	12	1
94 (Am)	16	9	26	24	10	14	13	12 13	12	1
95 (Bu)	16	10	26	22	10	13	13	14 15	12	1
96 (Bu, Ts)	16	10	26	22	11	14	13	14	12	3
97 (Ts)	16	10	26	23	9	14	13	11 12	12	1
98 (Bu)	16	10	27	22	10	14	13	14	12	1
99 (Ta)	16	10	27	24	10	13	15	14 19	12	1
100 (Pu)	16	11	28	23	10	14	13	12 13	12	2
101 (Am)	17	9	25	23	10	15	13	13	12	1
102 (Am)	17	9	25	23	10	14	13	13 14	12	1
103 (Am)	17	9	25	23	10	14	13	12 14	12	2
104 (Ru)	17	9	25	23	10	14	13	12 13	12	1
105 (Am)	17	9	25	24	10	13	13	13 16	13	1
106 (Ts)	17	9	25	25	10	13	12	12 19	12	1
107 (Bu)	17	10	26	22	11	14	13	14	12	1
108 (Bu)	17	10	26	22	10	12	13	14	12	1
109 (Ts)	17	10	26	23	10	14	13	13	13	1
110 (Ts)	17	10	26	23	10	14	13	13 14	12	1
111 (Ts)	17	11	27	23	10	14	12	13	13	1

Table 2 Total of 113 haplotypes of nine Y-chromosome STR loci in 173 unrelated individuals. (continued)

Haplotype no. (population)	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 388	Sample size
112 (Ts)	18	9	25	23	10	14	13	13	12	1
113 (Ts)	18	10	26	23	10	14	13	13	12	3

The allele was named according to the number of repeat unit. Am, At, Bu, Pa, Pu, Ru, Sa, Ta and Ts represented Ami, Atayal, Bunun, Paiwan, Puyuma, Rukai, Saisiat, Tao and Tsou respectively.

Table 3 The dw_{\min} values of nine tribes, Han population, Japanese and Germans.

	Han	Nine tribes	Japanese	Germans
Sample size	183	173	200	111
Haplotype no.	138	107	194	110
dw_{\min}	0.9893	0.9720	0.9947	0.9908