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SHORT COMMUNICATION

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Allele detection and population study in Japanese using two STR loci (CYP19 and HUMTH01)

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Abstract Allele fragments at two polymorphic short tandem repeat (STR) loci, CYP19 and HUMTH01, were simultaneously amplified in a sample of 200 unrelated Japanese individuals living in the Kanto area, including Tokyo. After electrophoresis in PAG, the new alleles 7, 10, 11, 12, and 13 were detected at the CYP19 locus in Japanese. HUMTH01 allele frequencies in Japanese differed greatly from those reported for Caucasians and Asians living in the US. The polymorphism information content (PIC) in Japanese was calculated as 0.46 for CYP19 and 0.66 for HUMTH01. The power of discrimination (PD) was 0.7 for CYP19 and 0.86 for HUMTH01, and the combined PD was calculated as 0.96. No significant deviations from Hardy-Weinberg equilibrium could be observed for these systems.

Key words Short tandem repeats · HUMTH01 · CYP19 · Simultaneous PCR · Japanese

Introduction

Many short tandem repeat systems (STRs) are widely used in the field of forensic medicine [2, 4, 8, 9]. CYP19 is a small (140–172 bp) STR locus (repeat unit: 4 bp; core sequence: TTTA) located on chromosome 15q21.1 [6, 10]. HUMTH01 is a high polymorphic and widely used locus (size: 179–201 bp; repeat unit: 4 bp; core sequence: AATG) located on chromosome 11p15.5 [2]. In this study we investigated the genotype and allele frequencies in Japanese, using simultaneous amplification for the CYP19 and HUMT01 loci, and calculated the power of discrimination [12] and polymorphism information content [1].

Materials and methods

DNA was extracted from the blood of 200 healthy unrelated Japanese living in the Kanto area, including Tokyo, by phenol/chloroform extraction.

The PCR reaction mixtures (total volume of 50 µl) contained 10 ng of template DNA, 10 × Perkin Elmer buffer (2.5 m*M* $MgCl_2$, 10% DMSO, 200 µ*M* dNTP), 2.5 units of Taq DNA polymerase (Perkin Elmer), and 5 pmol each primer for CYP19 (forward primer: 5′-CTTCTTTTTTGTCTATGAATGTGCCT-3′, our design; reverse primer [10]) and HUMTH01 [2], respectively.

Amplifcation conditions were: 94° C 1 min for 1 cycle, 94° C 1 min, 59° C 1 min, 72° C 1.5 min for 10 cycles, 92° C 1 min, 59° C 1 min, 72° C 1.5 min for 20 cycles and 72° C 10 min for 1 cycle (Techne, Dri-Block, UK). The amplified fragments were separated on 6% polyacrylamide denaturing gels containing 7 *M* urea (20 × 40 cm, 1 mm thick), and visualized by silver staining (Silver Stain Kit, Wako, Japan).

Results and discussion

In the preliminary study, we used the primers proposed by Polymeropoulous et al. [10] to detect the CYP19 locus and as a results we discovered new alleles with 7, 10, 11, 12, and 13 repeats. We designed a new sense primer because the fragments 10–13 overlapped with the HUMTH01 locus fragments. The repeat numbers of these alleles were determined by the Taq-Dye Terminator Cycle sequencing method [7].

In CYP19, alleles 7 and 11 accounted for approximately 91% of the total. In HUMTH01, the frequencies of alleles 6, 7, and 9 accounted for approximately 90%. The distributions of the observed genotypes and allele frequen-

Table 1 Allele and genotype frequencies of the CYP19 system in the Japanese population ($n = 200$)

Genotype	Observed	Frequency
7– 7	67	0.335
7– 8	1	0.005
7–10	1	0.005
7–11	82	0.410
7–12	17	0.085
11–11	18	0.090
11–12	11	0.055
11–13	1	0.005
12–12	2	0.010
Power of discrimination (PD)		0.701
Heterozygosity (HT)		56.5%
Polymorphism information content (PIC)		0.46

Allele frequencies: 7: 0.5875; 8: 0.0025; 10: 0.0025; 11: 0.325; 12: 0.08; 13: 0.0025. $\chi^2 = 3.67$; df = 8; $0.75 < P < 0.9$

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Table 2 Allele and genotype frequencies of the TH01 system in the Japanese population (n= 200)

Genotype	Observed	Frequency
5– 9	1	0.005
5– 9.3	1	0.005
6– 6	15	0.075
6– 7	24	0.120
6– 8	8	0.040
6– 9	37	0.185
6– 9.3	4	0.020
7– 7	12	0.060
7– 8	3	0.015
7– 9	48	0.240
7– 9.3	2	0.010
8– 9	12	0.060
8– 9.3	1	0.005
9– 9	27	0.135
9– 9.3	4	0.020
9–10	1	0.005
Power of discrimination (PD)		0.860
Heterozygosity (HT)		73.0%
Polymorphism information content (PIC)		0.66

Allele frequencies: 5: 0.005; 6: 0.2575; 7: 0.2525; 8: 0.06; 9: 0.3925; 9.3: 0.03; 10: 0.0025. $\chi^2 = 16.4$; df = 15; $0.25 < P < 0.5$

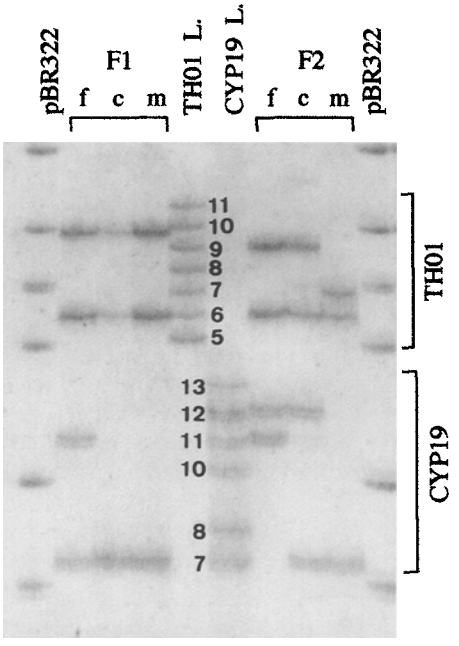

Fig. 1 Amplification patterns in two Japanese families (F1, F2) for HUMTH01 and CYP19 loci. PAGE after silver staining. Allele size and repeat numbers were determined using the allelic ladder. f = father, c = child, m = mother, L = allelic ladder. F1 (f: 6/9.3 (TH01), 7/11 (CYP19); c: 6/9.3, 7/7; m: 6/9.3, 7/7). F2 (f: 6/9, 11/12; c: 6/9, 7/12; m: 6/7, 7/7)

cies for the two STR loci in the Japanese population sample are shown in Tables 1 and 2, respectively. The Hardy-Weinberg equilibrium test was conducted for CYP19 and HUMTH01 using the individual alleles [5] and the χ^2 values showed no significant deviations between the expected and observed numbers (Tables 1 and 2).

The mode of inheritance of the alleles at both CYP19 and HUMTH01 was investigated in 10 Japanese families with a total of 20 children. It was found that the mode of inheritance of the alleles was codominant (Fig. 1).

The allele frequencies of CYP19 locus were unevenly distributed with a resulting PIC value [1] slightly higher than that of the red blood cell system MNSs (0.456) in Japanese [3]. The most frequenlty expressed alleles in Japanese at the HUMTH01 locus were 9, 7, and 6 in descending order which was significantly different ($\chi^2 = 31.7$, df = 5, $P < 0.005$) from results previously reported for Asians living in the United States [11]. Moreover, a comparison with reports on Caucasians [11] showed a large significant difference ($\chi^2 = 82.0$, df = 5, $P < 0.005$), the frequencies of alleles 9, 9.3, and 7 being markedly different (Table 2). The cumulative power of discrimination (PD) [12] for the CYP19 and HUMTH01 loci was calculated as 0.96, which was slightly better than that of 3 different red blood cell systems in the Japanese subjects [3], ABO, MNSs and P1 (0.955).

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