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ORIGINAL ARTICLE

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D1S80 allele frequencies in a Chinese population

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Abstract Allele frequencies for the VNTR locus D1S80 were determined in a Chinese population sample using the polymerase chain reaction and subsequent analysis of the amplified products by polyacrylamide gel electrophoresis and silver staining. A total of 18 nominal D1S80 alleles were observed in 105 unrelated Chinese. The data demonstrate that D1S80 is highly polymorphic in Chinese with a heterozygosity of 90.5%. The D1S80 frequency distribution meets Hardy-Weinberg expectations. This D1S80 data can be used in forensic analyses and paternity tests to estimate the frequency of a DNA profile in a Chinese population.

Key words Chinese · Population Data · VNTR Hardy-Weinberg Expectations · PCR · D1S80

Zusammenfassung Allelfrequenzen für den VNTR Lokus D1S80 wurden in einer Chinesischen Populationsstichprobe mittels Polymerase-Kettenreaktion und anschließender Analyse der Amplifikationsprodukte durch Polyacrylamid-Gelelektrophorese und Silberfärbung bestimmt. Insgesamt 18 nominelle D1S80 Allele wurden bei 105 unverwandten Chinesen beobachtet. Die Daten zeigen, daß D1S80 in Chinesen hochpolymorph mit einer Heterozygosität von 90.5% ist. Die D1S80 Frequenzverteilungen sind im Hardy-Weinberg-Gleichgewicht. Diese D1S80 Daten können für forensische Analysen und Vaterschaftstests zur Abschätzung eines DNA-Profils in einer Chinesischen Population herangezogen werden.

Schlüsselwörter Chinesen · Populationsdaten · VNTR Hardy-Weinberg-Gleichgewicht · PCR · D1S80

Introduction

The most forensically characterized amplified fragment length polymorphism (AMP-FLP) marker is the polymorphism at the locus D1S80 (Budowle et al. 1991; Kasai et al. 1990; Kloosterman et al. 1993). However, there are scant population data on D1S80 for populations from the Orient (Kasai et al. 1990; Budowle et al. 1994). This paper presents the allele frequency data for the D1S80 VNTR locus in a Chinese population sample.

Materials and methods

Sample Preparation: whole blood was obtained in EDTA vacutainer tubes by venipuncture from 105 unrelated Chinese individuals collected by the Criminal Investigation Bureau DNA Laboratory in Taipei, Taiwan. The DNA was extracted by the phenol-chloroform method (Maniatis et al. 1982). The quantity of extracted DNA was estimated using the slot-blot procedure described by Waye et al. (1989) and/or by UV absorbance.

D1S80 Typing: DNA was amplified by using the D1S80 primers described by Kasai et al. (1990) and PCR according to the protocol of Baechtel et al. (1993). The amplified products were typed by horizontal ultrathin layer polyacrylamide gel (20 cm long) electrophoresis on an ICE apparatus (EC Corporation, Clearwater, FL) and subsequent silver staining according to previously described procedures (Budowle et al. 1991; Baechtel et al. 1993). Additionally, some samples were typed in vertical polyacrylamide gels using a variation of the horizontal electrophoretic procedure as described by Budowle et al. (1994). The allelic ladder, described by Baechtel et al. (1993), was used as a reference for typing the alleles in each sample.

Statistical Analysis: The frequency of each allele for D1S80 was calculated from the numbers of each genotype in the sample set (i.e., the gene count method). Unbiased estimates of expected

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heterozygosity were computed as described by Edwards et al. (1992). The expected numbers of distinct homozygous and heterozygous genotypes and their standard error (SE) were calculated; possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies, by the log likelihood ratio test criterion and the exact test (see Budowle et al. 1994; Edwards et al. 1992).

A 2×C contingency table exact test (Roff and Bentzen 1989) was used to test for homogeneity (1000 shuffling experiments) between D1S80 Oriental population samples.

Results and discussion

The distribution of allele frequencies for Chinese is shown in Table 1. There were 18 different nominal alleles observed in our population sample of 105 people. Because of the potential for observing anodal and cathodal off-ladder variants by horizontal polyacrylamide gel electrophoresis, nominal alleles were binned proximal to a fragment in the D1S80 reference ladder. There was one allele out of a total of 210 chromosomes which migrated approximately equidistant between alleles 26 and 27 of the reference ladder by horizontal polyacrylamide gel electrophoresis (Table 1). By vertical polyacrylamide gel electrophoresis this middle variant migrated to the same position as allele 27 and therefore was classified as allele 27. All other alleles migrated proximal to one of the steps of the allelic ladder. It has been suggested by Budowle et al. (1994) and demonstrated by Reynolds (Roche Molecular Systems; Alameda, CA, personal communication) that anodal and cathodal D1S80 variants observed after horizontal polyacrylamide gel electrophoresis may be the result of sequence variation among similar sized alleles. Conformational effects on the DNA fragments due to sequence polymorphisms are less likely to manifest themselves during vertical gel electrophoresis which provides a warmer environment than when carrying out electrophoresis with horizontal gels (Budowle et al. 1994).

The observed heterozygosity for D1S80 in the Chinese sample population is 90.5%. The level of heterozygosity was equivalent to that (i.e., 90.7%) reported by Budowle et al. (1994) for an Oriental database. Thus, Orientals exhibit the highest heterozygosity levels for D1S80 compared with other sample populations (Budowle et al. 1994). A test for independence for the alleles within a locus, based on the number of distinct heterozygote and homozygote genotypes, was performed. There was no deviation from expected values. Four different homozygote and 41 different heterozygote genotypes were observed, while the expected number of homozygote and heterozygote classes was 4.7 ± 1.2 and 40.1 ± 4.1, respectively. Additionally, the distribution of D1S80 genotypes does not deviate from HWE based on the homozygosity test, log likelihood ratio test criterion, and the exact test (Table 1).

Presently, there are scant data on D1S80 allele frequency distributions in Oriental populations. Kasai et al. (1990) reported D1S80 data, but their data set was a composite of 67 unrelated Caucasian and Japanese individuals. Therefore, it was not possible to obtain the D1S80 population data for only Japanese. Budowle et al. (1994) reported D1S80 data for Orientals, however, these data were not defined by country of origin (predominately Japanese and Chinese). The D1S80 population described in the current study is comprised solely of Chinese. The Chinese D1S80 data were compared with Oriental data described by Budowle et al. (1994). While Chinese D1S80 population data are different compared with Caucasians and African Americans, there were no significant differences in allele frequencies between the two Oriental population groups using the exact test ($P = 0.245$).

In conclusion, a Chinese population database has been established for the VNTR locus D1S80. The distribution of the genotype frequencies meet HWE. The data demonstrate that estimates of genotype frequencies can be obtained for identity testing purposes using the product rule under the assumption of independence.

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Table 1 D1S80 allele frequencies in a sample of 105 unrelated Chinese

Allele	Frequency
14	0.005
15	0.000
16	0.010
17	0.000
18	0.129
19	0.014
20	0.000
21	0.038
22	0.014
23	0.010
24	0.276
25	0.052
26	0.005
27	0.062
28	0.067
29	0.024
30	0.148
31	0.114
32	0.014
33	0.000
34	0.000
35	0.000
36	0.010
37	0.000
38	0.000
39	0.000
40	0.000
41	0.000
> 41 [a]	0.010

[a] All alleles migrating slower than the largest allele in the ladder (i.e., allele # 41) are placed in the > 41 allele class.
[b] Observed Homozygosity = 0.095
[c] Expected Homozygosity (unbiased) = 0.138
[d] HWE – Homozygosity Test ($P = 0.207$), Likelihood Ratio Test ($P = 0.277$), Exact Test ($P = 0.380$)

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