TECHNICAL NOTE

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Frequencies of D8S384 Alleles and Genotypes in European, African-American, Chinese, and Japanese Populations

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ABSTRACT: D8S384 is a tetranucleotide tandem repeat locus. In order to evaluate the forensic validation of D8S384, the genotype distributions and allele frequencies in ten populations from three main ethnic groups were investigated, including Germans, Slovakians, African Americans, Japanese, and Chinese (Jilin, Guangzhou, Nanning, Hailaer, Dali, and Chengdu). A total of 1011 unrelated individuals, 41 pedigrees, 30 disputed paternity trios and three personal identification cases were analyzed for D8S384 by Amp-FLP technique. Many kinds of tissues, body fluids, secreta and stains have been tested. The alleles were determined by comparison with a human allele ladder. The results showed that D8S384 typing was both precise and reliable. There were eight alleles in these populations. The genotype distributions conformed to Hardy-Weinberg equilibrium predictions. No mutation events were observed. With a maximum likelihood method, the mutation rate was indirectly estimated as 2.14×10^{-5}. The heterozygosity was 0.704 ± 0.014 at D8S384 locus. All these results suggest that D8S384 locus is a useful marker for forensic identification and paternity analysis.

KEYWORDS: forensic science, DNA typing, population genetics, short tandem repeat, D8S384, mutation rate, Caucasian, African-American, Japanese, Chinese

The fragments of short tandem repeats (STR) are relatively short and hence amplifications of them are suitable for highly degraded DNA (1). Moreover, STR loci are abundantly throughout the human genome. Most of them are highly polymorphic. Therefore, STR loci were considered as an important genetic marker system in forensic science. However, single STR locus has few alleles compared with VNTR and its mutation rate is usually higher than mean mutation rate of the human genome (2). Exclusionary results could not be present for only one STR marker in paternity cases. Therefore, amount of STR loci must be used in forensic work after necessary validation experiment. We presented a tetranucleotide repeat marker D8S384 (Genbank Accession number L18535). It was initially isolated as simple sequence repeat containing sequence tagged site (STS) from the human genome and named as Human chromosome 8 STS UT1205 by the Utah marker development group (3). We investigated its distribution feature in three main ethnic groups and evaluated its validation in forensic work.

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Material and Methods

Population Samples—Blood samples were obtained from 105 Germans (Bremen), 111 Slovakians (Bratislava), 119 Japanese (Yamanashi), and 592 Chinese (Table 1), which came from unrelated donors of blood banks. Ethnic origin was determined by appearance and self-declaration. The 84 bloodstains from African Americans were taken from bodies delivered to the office of the Chief Medical Examiner of New York City at the time of death. Ethnic origin was determined by skin color and country of birth.

Family Samples—A total of 133 blood samples was collected from 41 Chinese pedigrees in Chengdu.

Casework Samples—A total of 91 blood samples came from 30 disputed paternity cases. Seventeen samples were derived from one violent death case and two sexual assault cases, which include five blood stains, nine cigarette ends, two semen stains, and one mixed stain collected from victims, accused individuals and the scenes of crimes.

DNA Extraction, Amplification, and Typing—DNA was extracted using the Chelex method (4). Quantification of DNA was undertaken using a primate-specific alpha satellite probe assay (5). PCR amplification was carried out using the primers 5-TTTCTCAGTATTCTACACAGG-3 and 5-GTTCCTG TCTTC TTCTAGAG-3 according to the Utah marker development group (3), which were synthesized by GIBCO BRL Custom Primers, HK (Life Technologies Inc, USA). Each PCR reaction contained 2-40 ng human genomic DNA, 1 × Taq buffer (Life Technologies Inc, USA), 1.5 mM $MgCl_2$ (Life Technologies Inc, USA), 200 μM each nucleotide (Phamacia, Sweden), 1.5 U Taq polymerase (Life Technologies Inc, USA), 0.25 μM each primer in a total of 37.5 mL. A

TABLE 1—*Geographic distribution of ten populations and the number of samples.*

Nation	Area	Longitude	Latitude	Ethnic	Nationality	Number of Samples
China	Jilin	125°E	44°N	Mongoloid	Han	102
China	Guangzhou	113°E	23°N	Mongoloid	Han	99
China	Nanning	108°E	23°N	Mongoloid	Zhuang	105
China	Hailaer	120°E	49°N	Mongoloid	Mongolian	95
China	Dali	100°E	26°N	Mongoloid	Bai	96
China	Chengdu	104°E	31°N	Mongoloid	Han	95
Japan	Yamanashi	38°E	35°N	Mongoloid		119
Germany	Bremen	9°E	53°N	Caucasoid		105
Slovak	Bratislava	17°E	48°N	Caucasoid		111
U.S.A.	New York	74°W	41°N	African American		84

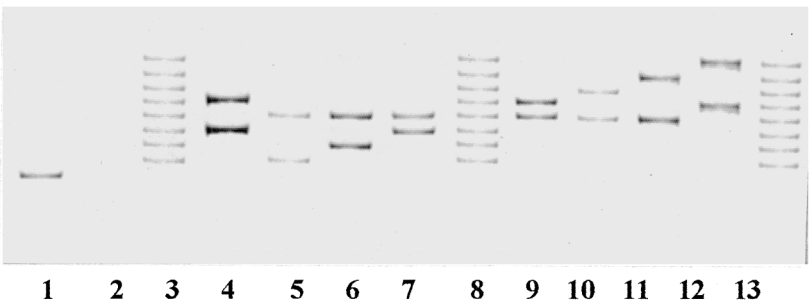

FIG. 1—*D8S384 typing in nondenaturing gel. D8S384 human allele ladder composed of alleles with 5, 6, 7, 8, 9, 10, 11, 12 repeats. Anode on the bottom. Lane 1: molecular weight ladder (200 bp); Lane 2: negative control; Lane 3: human allele ladder; Lane 4: positive control (7–9); Lane 5: 5–8; Lane 6: 6–8; Lane 7: 7–8; Lane 8: human allele ladder; Lane 9: 8–9; Lane 10: 8–10; Lane 11: 8–11; Lane 12: 9–12; Lane 13: human allele ladder.*

total of 30 cycles was carried out in a GeneAmp PCR System 9600 (PERKIN ELMER, USA) with denaturation for 1 min at 94°C, annealing for 1 min at 60°C and extension for 2 min at 72°C. The PCR products were separated by nondenaturing polyacrylamide gel electrophoresis and visualized by silver staining (6).

Nomenclature—The size of unknown alleles was determined by comparison with a human allele ladder according to the recommendations of the International Society of Forensic Haemogenetics (7,8). The human allele ladder was constructed by mixing products of PCR with different genotype. Classification of allele for D8S384 locus was based on the number of repeat motifs.

Statistical Calculations—A modified χ^2-test (9) was used to verify whether genotype distribution conformed to Hardy-Weinberg equilibrium predictions. The expected heterozygosity was calculated according to the equation $h = 2n(1 - \sum Xi^2)/(2n - 1)$ (10). The mutation rate at the D8S384 locus was estimated directly from family studies and indirectly with a maximum likelihood method (11). The power of discrimination and the chance of exclusion were calculated as described by Fisher (12) and Ohno et al. (13), respectively. The Statistical Program for Social Science (SPSS) was used for χ^2-test. A computer program written by Hou was used for other calculations. It is available from the corresponding author.

Results and Discussion

Typing for D8S384—A total of 8 alleles were observed in the three ethnic groups. The allele fragment length ranged from 204 to 232 bp. Allele designations were determined by the size of repeat motif. The ranges included alleles with 5, 6, 7, 8, 9, 10, 11, and 12 repeats, respectively. Figure 1 displays the electrophoretic pattern of the alleles in nondenaturing gels. The result shows that alleles are easily observed at D8S384 locus. All samples from both blood and blood stains were testable in D8S384 locus in this study. The D8S384 is robust and amplifies with fidelity. With the allele ladder, this STR locus provided easily interpretable results.

Population Genetics—The genotypes of 1011 individuals in ten populations from three ethnic groups were determined in order to study the intra- and inter-population variation of D8S384. Allele 5, 6, 11, and 12 were pooled because of their low frequency. The result of analysis shows that the genotype distributions have no significant deviation from Hardy-Weinberg equilibrium (Table 2). There were significant different distributions among three main ethnic groups according to results of χ^2-test. However, population subheterogeneity within each ethnic group was not clear compared with distributions among three ethnic groups. Meanwhile, the differences of distribution were more significant between national groups than within them. The same trend could be observed between and within nationality groups. The results are consistent with other studies (14,15). Allele frequencies exhibited overall unimodal distribution. Allele with 7, 8, and 9 repeat motifs were the common among all ethnic groups with allele 8 being the most common.

Mutation Rate—A total of 41 pedigrees were investigated. The result shows that D8S384 locus conforms to the Mendelian laws of the inheritance while delivering from generation to generation. No mutation events were found. An indirect estimate of the mutation rate gives value 2.14×10^{-5}. It is reasonably low in STR system compared with other published trinucleotide and tetranucleotide repeat loci ($2.36 \times 10^{-5} - 1.86 \times 10^{-4}$) (16,17). For instance, the mutation rate of HUMTH01 is 3.5×10^{-5}, HUMF13A01 is 6.60×10^{-5}, HUMARA 1.59×10^{-5}, HUM-

TABLE 2—*Allele frequencies of D8S384 in ten populations.*

	Chinese									African	
	Jilin	Guangzhou	Nanning	Hailaer	Dali	Chengdu	Japanese	Germans	Slovakians	Americans	Total
						Frequencies of allels					
5	0.010	0.005	0.000	0.000	0.005	0.016	0.000	0.000	0.000	0.000	0.003
6	0.029	0.020	0.005	0.047	0.000	0.005	0.008	0.024	0.032	0.030	0.020
7	0.113	0.162	0.214	0.158	0.151	0.153	0.164	0.276	0.266	0.220	0.188
8	0.510	0.419	0.410	0.284	0.474	0.437	0.374	0.433	0.369	0.589	0.426
9	0.235	0.293	0.281	0.269	0.292	0.310	0.328	0.210	0.288	0.137	0.267
10	0.088	0.086	0.071	0.174	0.677	0.074	0.092	0.052	0.041	0.018	0.077
11	0.014	0.015	0.019	0.047	0.010	0.005	0.034	0.005	0.004	0.000	0.016
12	0.000	0.000	0.000	0.021	0.000	0.000	0.000	0.000	0.000	0.006	0.003
						Test for Hardy-Weinberg equilibrium					
$\chi 2$	8.098	12.207	11.194	20.393	14.118	8.511	10.397	15.196	7.003	8.864	
df	12	10	6	17	8	9	9	8	7	5	
P	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	>0.05	

TABLE 3—*Forensic value of D8S384 in ten populations*.

	Chinese									African	
	Jilin	Guangzhou	Nanning	Hailaer	Dali	Chengdu	Japanese	Germans	Slovakians	Americans	Total
OH	0.676	0.727	0.733	0.726	0.563	0.726	0.756	0.714	0.606	0.595	0.691
EH	0.666	0.708	0.705	0.791	0.666	0.687	0.719	0.692	0.710	0.588	0.704
SE	0.047	0.046	0.045	0.042	0.048	0.048	0.041	0.045	0.043	0.054	0.014
DP	0.844	0.864	0.859	0.923	0.831	0.846	0.871	0.849	0.859	0.778	0.864
CE	0.432	0.462	0.450	0.584	0.408	0.432	0.473	0.434	0.448	0.344	0.461

OH: Observed heterozygosity. EH: Expected heterozygosity. SE: Standard error.
DP: Discrimination power. CE: Chance of exclusion.

FESFPS 4.30 $\times$ 10^{-5}, HUMCD4 4.81 $\times$ 10^{-5}, D6S366 6.13 $\times$ 10^{-5} (16,17). The mutation rate indicates that the D8S384 STR locus is inherited with fidelity from generation to generation and would be an informative genetic marker for paternity analysis along with other genetic markers. In addition, result of typing of difference tissues from the same individual shows that no somatic mutation was detected. So the conclusion can be reached that all kinds of tissue, body fluid and secreta can be used as sample for typing of D8S384.

Forensic Applications—According to our population data, the forensic validation of D8S384 was evaluated (Table 3). This locus exhibits a moderate heterozygosity, chance of exclusion, and discrimination power. All these calculations indicate that D8S384 is a useful genetic marker in forensic work, especially in Mongolians and Caucasians. A total of 30 disputed paternity cases was analyzed with D8S384 locus as well as other genetic markers, such as ABO, MN, PGM1, GC, HLA, D3S1545, D20S161, HUMTH01, HUMVWA31, D19S400, DXY156, and HUMFES. As a genetic marker, D8S384 shows a satisfied result with high successful rate in both exclusion and inclusion cases. In 11 of 30 cases the disputed paternity was excluded by at least two of the STR markers. In the middle of them, the paternity was excluded by D8S384 locus in six cases. In the other 19 cases, the paternity was not excluded by all markers, including D8S384. All samples can be tested in D8S384 locus in the forensic identification cases, including blood stains, cigarette ends, semen stains, and blood/semen mixed stains. Figure 2 displays a result of D8S384 typing in a violent death case. It accords with results from other markers.

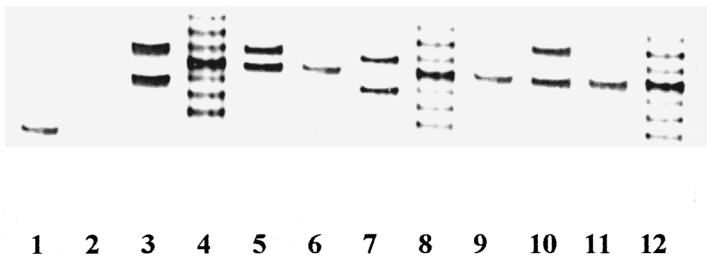

FIG. 2—*D8S384 locus was used in a violent death case. Anode on the bottom. Lane 1: molecular weight ladder (200 bp); Lane 2: negative control; Lane 3: positive control (7–9); Lane 4: human allele ladder; Lane 5: victim blood stain (8–9); Lane 6: cigarette end in the criminal scene (8–8); Lane 7: suspected man 1 (7–9); Lane 8: human allele ladder; Lane 9: suspected man 2 (8–8); Lane 10: suspected man 3 (8–10); Lane 11: suspected man 4 (8–8); Lane 12: human allele ladder.*

Conclusion

D8S384 amplifies with fidelity. Varieties of tissues, body fluid, and secret fluid are testable in this locus. Distributions of D8S384 genotype are in conformance with the prediction of Hardy-Weinberg equilibrium in the three main ethnic groups. The mutation rate seems to be reasonably low at D8S384. Moreover, the expected heterozygosity, the discrimination power, and the chance of exclusion of D8S384 were reasonably high. Combined with the results, D8S384 may be an effective investigative genetic marker for forensic use.

Acknowledgments

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