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SHORT COMMUNICATION

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D20S161 data for three ethnic populations and forensic validation

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Abstract In order to evaluate the forensic applicability of the STR locus D20S161 and construct a preliminary database, the genotype distributions and allele frequencies in five populations from three main ethnic groups were investigated, including Germans, Slovakians, African Americans, Japanese and Chinese. A total of 512 samples from unrelated individuals and 85 confirmed father/mother/child triplets were analyzed by PCR and allele determination was carried out by comparison with a sequenced human allelic ladder. The results showed that D20S161 typing was both precise and reliable. A total of 7 alleles was found in these populations and no evidence of deviation from Hardy-Weinberg equilibrium was observed. Pairwise comparisons between populations showed that there were significant differences in the distributions of the allele frequencies among the three main ethnic groups. No mutation events were observed from the confirmed father/mother/child triplets. With a maximum likelihood method, the mutation rate was indirectly estimated as 2.5×10^{-5}. These results suggest that D20S161 is a useful marker for forensic casework and paternity analysis.

Key words Short tandem repeats · D20S161 · Polymerase chain reaction · Genetic polymorphism · Mutation rate · Forensic haemogenetics

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Introduction

Analysis of short tandem repeat (STR) loci has been shown to be both robust and reproducible for use in forensic science. However, there is a need to develop more STR markers, because the discriminating power of each STR locus is limited. When a new STR marker is chosen, some considerations are required. Firstly, dinucleotide or trinucleotide repeat markers should not be considered because of stutter bands for the former and the potential risk of dynamic mutation for the latter. Secondly, the electrophoretical behavior of the PCR products has been demonstrated to give problems in typing some STRs (Gill et al. 1994; Kimpton et al. 1995; Pestoni et al. 1995). Thirdly, the distribution of allele frequencies in different ethnic groups should be investigated to construct an appropriate database (Brinkmann et al. 1996). Fourthly, the mutation rate of STR markers should be estimated. According to those considerations, we describe a tetranucleotide repeat marker D20S161(GenBank Accession number L16405), which was initially isolated as a simple sequence repeat containing sequence tagged site (STS) from the human genome and named as Human chromosome 20 STS UT1674 by the Utah marker development group (1995).

Materials and methods

Population samples

Blood samples were obtained from 100 Germans (Bremen), 173 Chinese (Chengdu), 99 Japanese (Yamanashi) and 90 Slovakians (Bratislava), which were unrelated donors from blood banks. Ethnic origin was determined by self declaration. The 50 bloodstains from African Americans were taken from bodies delivered to the Office of the Chief Medical Examiner of New York City at time of death. Ethnic origin was determined by skin color and country of birth.

Family samples

A total of 255 blood samples was collected from 85 confirmed father/mother/child triplets of Chinese living in Chengdu.

Experimental details

DNA was extracted using the Chelex method (Singer-Sam et al. 1989). PCR amplification was carried out using the primers 5-CCCCTTCAACTTGTCAGC-3 and 5-TCCTTCCAACTGGTATCTTG-3 according to the Utah marker development group (1995). Each PCR reaction contained 2–40 ng human genomic DNA, 1 × Taq buffer, 1.5 mM $MgCl_2$, 200 μM each nucleotide, 1.5 U Taq polymerase (Promega), 0.25 μM each primer in a total volume of 37.5 μl. A total of 28 cycles was carried out in a thermocycler (Biometra) with denaturation for 1 min at 94 °C, annealing for 1 min at 58 °C and extension for 2 min at 72 °C. The PCR products were analyzed using nondenaturing polyacrylamide gel electrophoresis with a discontinuous buffer system (Allen et al. 1989; Hou et al. 1994a; Hou and Walter 1996) and the gels were silver stained.

Nomenclature

Allele determination was carried out by comparison with a sequenced human allele ladder according to the recommendations of the International Society of Forensic Haemogenetics (DNA Commission of the ISFH 1994; Bär et al. 1997). The allele classification for the D20S161 locus was based on the number of repeat motifs.

Statistical calculations

A modified χ^2-test (Hou et al. 1994b) was used to verify whether the genotype distribution conformed to Hardy-Weinberg equilibrium predictions. The expected heterozygosity was calculated according to the equation $h = 2n(1-\Sigma Xi^2)/(2n-1)$ (Nei 1978). The mutation rate at the D20S161 locus was estimated directly from family studies and indirectly using a maximum likelihood method (Chakraborty and Neel 1989). The power of discrimination and the chance of exclusion were calculated as described by Fisher (1951) and Ohno et al. (1982), respectively.

Results and discussion

Typing for D20S161

Figure 1 displays a representative result of typing for D20S161 with nondenaturing polyacrylamide gel electrophoresis. This STR locus amplified authentically and provided easily interpretable results. The amplified fragment size ranged from 156 bp to 187 bp, while the number of core motifs varied from 14 to 22. There was a good parallel relationship between the amplified fragment size and the number of repeat motifs when a nondenaturing polyacrylamide gel electrophoresis was employed to analyze the PCR products. Also, the phenotypes at this locus can be determined easily with the denaturing gel electrophoresis described by the Utah marker development group, which initially sequenced this locus (1995). Therefore, typing for D20S161 is to be both precise and reliable.

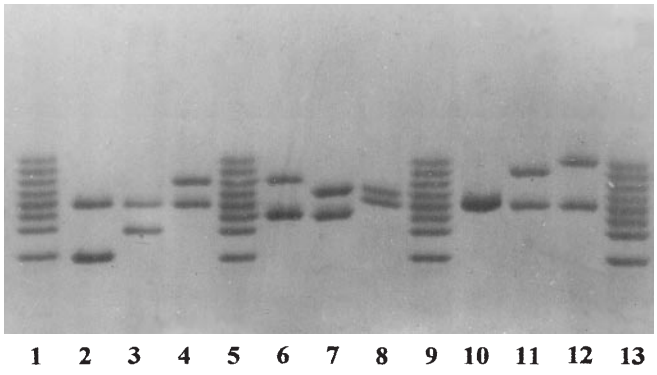

Fig. 1 Typing for D20S161 with nondenaturing polyacrylamide gel electrophoresis Lanes 1, 5, 9, 13: Allele ladder including alleles 14, 16, 17, 18, 19, 20, 21, 22 Lane 2:14–18, lane 3:16–18, lane 4:18–20, lane 6:17–20, lane 7:17–19, lane 8:18–19, lane 10:18, lane 11:18–21, lane 12:18–22. The sequence of allele 19 as an example is given as follows: cccctttcaacttgtcagc tttaatgataaagaaatacgggtgtagaaaagttaagtgatttgtccaccatgaca (taga)3(tagg)4(taga)12tggaataaatgattataggg caagataccagttggaagga

Population genetics

A total of seven alleles was observed in these population samples. No evidence of deviation from Hardy-Weinberg equilibrium in these populations was observed by the modified χ^2-test (Hou et al. 1994b). Pairwise comparisons between populations show that the distribution of allele frequencies is significantly different among the main ethnic groups. In Asian populations most of the possible 28 genotypes were observed, while only 16 out of the possible 28 genotypes appeared in European populations. The African American population from New York revealed another pattern. The allele 14 was frequently observed in this population but not in Asian and European populations (Table 1). This means that there is a population substructure at D20S161 in humans.

Mutation rate

Before applying this marker in paternity analysis, it is important to evaluate the mutation rate. A total of 85 confirmed father/mother/child triplets was analyzed but no mutation events were observed. Although the number of

Table 1 Allele frequencies of D20S161 in five populations

D20S161	Chinese	Japanese	Germans	Slovakians	African Americans
14	0	0	0	0	0.1100
16	0.0491	0.0253	0.0200	0	0
17	0.2254	0.1313	0.1750	0.1778	0.1400
18	0.3237	0.4293	0.5100	0.4778	0.2300
19	0.1532	0.1313	0.2050	0.2222	0.2600
20	0.1301	0.1515	0.0600	0.0889	0.1800
21	0.0983	0.0909	0.0250	0.0278	0.0600
22	0.0202	0.0404	0.0050	0.0055	0.0200
Sample size	173	99	100	90	50
HWE					
χ^2	21.3225	13.0692	6.9474	9.9177	17.4808
df	19	14	6	7	13
P	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$	$P > 0.05$

HWE: Test for Hardy-Weinberg equilibrium

Table 2 Forensic values for D20S161 based on data from five populations

D20S161	Chinese	Japanese	Germans	Slovakians	African Americans
H(obs)	0.7514	0.7677	0.7300	0.6778	0.8800
H(exp)	0.7938	0.7515	0.6659	0.6858	0.8196
SE	0.0308	0.0434	0.0472	0.0489	0.0544
CE	0.5973	0.5488	0.4287	0.4431	0.6257
DP	0.9276	0.9079	0.8427	0.8534	0.9378

H: heterozygosity; obs = observed; exp = expected
SE: standard error
CE: chance of exclusion
DP: discrimination power

father/mother/child triplets studied is too low to draw definite conclusions, the mutation rate for D20S161 seems to be reasonably low. Using data from these populations, the mutation rate for D20S161 was indirectly estimated with the maximum likelihood method (Chakraborty and Neel 1989) as 2.5×10^{-5}.

Forensic value of D20S161

Table 2 displays the forensic value of D20S161 based on data from five populations. Compared with other STR markers, including CYP19, FABP and TPOX loci, the D20S161 revealed higher values both for the power of discrimination and the chance of exclusion in three main ethnic groups, especially in Asian and African American populations. As shown in this study, all of 50 postmortem bloodstains from New York were analyzed for D20S161. Also, D20S161 typing was used in 34 cases in our laboratory in China. Both gave positive results of D20S161 typing. Therefore, D20S161 typing revealed a high success rate for the analysis of difficult bloodstains.

In conclusion, D20S161 typing is both precise and reliable. The distribution of allele frequencies in different ethnic groups has been investigated and the population data are available. The mutation rate at D20S161 locus seems to be reasonably low. A rather high success rate of D20S161 typing for bloodstains from casework has been observed. Therefore, this study implicates that D20S161 is a useful marker for forensic casework and paternity analysis.

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