TECHNICAL NOTE

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The Analysis of Three Short Tandem Repeat (STR) Loci in the Slovene Population by Multiplex PCR

REFERENCE: Drobnič K, Budowle B. The analysis of three short tandem repeat (STR) loci in the Slovene population by multiplex PCR. J Forensic Sci 2000;45(4):893–895.

ABSTRACT: Allele frequencies for three tetrameric short tandem repeat (STR) loci D3S1358, HUMVWA, and HUMFGA were determined in a Slovene Caucasian population sample. DNA samples from a total of 221 Slovenes were amplified by multiplex PCR using the commercial kit AmpFISTR Blue (Perkin-Elmer). Separation and detection of the amplified STR fragments were carried out using a 377 automated genetic analyzer (Applied Biosystem Division/Perkin Elmer). Seven alleles at the D3S1358 locus, 8 alleles at the HUMVWA31A locus, and 13 alleles at the HUMFGA locus were observed. A deviation from Hardy-Weinberg equilibrium was observed, only at the HUMVWA31A locus ($p = 0.045$, exact test). The departure at this locus was not significant after Bonferroni correction. There were no detectable departures between pairwise comparisons of the loci. The combined power of discrimination for all three loci is 0.9998, and the power of exclusion is 0.9526. The observed allele frequencies for the loci D3S1358, HUMVWA31A, and HUMFGA are similar to those in European and U.S. Caucasian populations.

KEYWORDS: forensic science, DNA typing, short tandem repeats, polymerase chain reaction, D3S1358, vWA, FGA, Slovenia, population genetics, Hardy-Weinberg equilibrium

Microsatellites, or short tandem repeat (STR) loci, are polymorphic regions found throughout the human genome, are based on 2 to 7 base pair repeat motifs, and are amenable to amplification by PCR (1–3). The polymorphism at a STR locus is the result of variation in the number of tandemly repeated units. The tetranucleotide repeat STR loci have been studied and widely used for forensic purposes (4–6). Detection of amplified STR products can be achieved by the use of fluorescence detection technology using an automated instrument, such as the ABI PRISM 377 DNA genetic analyzer (7). Amplification of three loci D3S1358 (8), HUMVWA31A (9,10), and HUMFGA (11) can be achieved using a commercially available kit. In order to apply these three STR loci to DNA profiling in forensic identification testing cases in Slovenia, allele frequencies in a Slovene sample population were determined.

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Received 20 Apr. 1999; and in revised form 3 Sept. 1999; accepted 7 Sept. 1999.

Materials and Methods

Sample Preparation

Samples from 221 unrelated individuals residing in Slovenia were collected and processed as described previously (12). The quantity of recovered DNA was determined by slot blot hybridization using the Quantiblot kit (Perkin-Elmer, Foster City, CA).

PCR Amplification and Typing

PCR multiplex amplification was performed using the AmpFISTR Blue kit (Perkin Elmer, Foster City, CA) according to the manufacturer's recommendations. One to 2 ng of template DNA were used in each PCR. The amplifications were carried out in a Perkin Elmer, GeneAmp 9600 thermal cycler. Electrophoresis was carried out in an ABI PRISM 377 automatic genetic analyzer (Perkin Elmer, Foster City, CA) in accordance with the manufacturer's instructions. Allele designation was achieved by comparison with an allelic ladder corresponding to each STR system.

Statistical Analysis

The frequency of each allele for each locus was calculated from the numbers of each genotype in the sample set (i.e., the gene count method). Unbiased estimates of expected heterozygosity were computed as described by Edwards et al. (5). Possible divergence from Hardy-Weinberg equilibrium (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (13–15) and the exact test (16), based on 2000 shuffling experiments. An interclass correlation criterion (17) for two-locus associations was used for detecting disequilibrium between the loci.

The power of discrimination (PD) was calculated from the genotype data on the basis of an equation derived by Fisher ($PD = 1 - \sum P_j^2$, where P_j is the frequency of each genotype) (18). These analyses were facilitated using a software package kindly provided by Dr. R. Chakraborty (Houston, TX).

Results and Discussion

The allelic frequencies observed for each of the three STR loci in the Slovene population sample are shown in Table 1. Seven alleles and 21 genotypes were detected for locus D3S1358 and allele frequencies ranged from 0.045 (allele 13) to 0.2670 (alleles 15). The most frequent alleles were 15, 16, and 17. There were eight alleles and 27 genotypes observed at the HUMVWA locus, with allele frequencies ranging from 0.0091 (allele 13) to 0.3100 (allele 17). The most common alleles were 16, 17, and 18. The HUMFGA locus had a greater number of detected alleles and genotypes compared with the D3S1358 and HUMVWA loci. Thirteen different alleles and 49 genotypes were observed. The observed heterozygosity for the loci D3S1358, HUMVWA, and HUMFGA was 80.5, 72.9, and 86.0%, respectively (Table 2).

All loci except HUMVWA ($p = 0.045$) meet HWE (Table 2). After employing the Bonferroni correction (19) for the number of loci analyzed (i.e., three loci per database), this borderline significant departure was no longer significant. The PD and PE for the three STR loci are displayed in Table 2. The most informative locus is HUMFGA (PD = 0.9644). The overall power of discrimination (PD) was 0.9998.

An interclass correlation test analysis did not detect any departures from independence in the three pairwise comparisons of the three STR loci. The data demonstrate that the expectation of gametic phase equilibrium holds for these STR loci.

The allele frequencies at the three STR loci in the Slovene population generally are similar to those reported for other Caucasian populations (20–30). Thus, a three locus profile frequency comprised of D3S1358, HUMVWA, and HUMFGA would be similar for any reported Caucasian population groups.

TABLE 1—*Allele frequencies for the three STR loci in the Slovene population (n = 221).*

Allele	D3S1358	HUMVWA	HUMFGA
13	0.0045	0.0091	…
14	0.0973	0.1177	…
15	0.2670	0.0928	…
16	0.2647	0.1833	…
17	0.1901	0.3100	…
18	0.1652	0.2195	0.0181
19	0.0113	0.0543	0.0634
20	…	0.0136	0.1403
20.2	…	…	0.0091
21	…	…	0.1561
22	…	…	0.2172
22.2	…	…	0.0091
23	…	…	0.1290
23.2	…	…	0.0091
24	…	…	0.1471
25	…	…	0.0792
26	…	…	0.0204
27	…	…	0.0023

TABLE 2—*Statistical values of interest for the Hardy-Weinberg equilibrium.*

	D3S1358	HUMVWA	HUMFGA
Observed homozygosity	19.5%	27.1%	14.0%
Expected homozygosity	21.3%	20.2%	13.9%
Homozygosity test (*p*-value)	0.513	0.010	0.950
Exact test (*p*-value)	0.994	0.045	0.904
Power of discrimination (PD)	0.9202	0.9301	0.9644
Power of exclusion (PE)	0.5765	0.6044	0.7172

Conclusions

A Slovene population database has been established for three STR loci: D3S1358, HUMVWA, and HUMFGA. The genotype data at the three STR loci meet HWE. Thus, the allele frequencies can be used to estimate the frequency of a multiple locus DNA profile in the Slovene population. The data demonstrate that the power of discrimination for all three loci together is highly informative for characterization of forensic stains or person analysis in Slovenia. Two of the loci (HUMVWA and HUMFGA) are part of the established common loci determined by the European Network of Forensic Science Institutes (ENFSI), and all three STR loci are part of the core STR loci of CODIS.

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