TECHNICAL NOTE

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Swiss Caucasian Population Data for 13 STR Loci Using AmpFISTR Profiler Plus and Cofiler PCR Amplification Kits*

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ABSTRACT: Allele and genotype frequencies for the 13 core STR loci (D3S1358, VWA, FGA, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, THO1, TPOX, CSF1PO, and D16S539) were determined in a Swiss Caucasian population sample ($n = 206$) using two commercially available multiplex PCR kits (AmpFISTR Profiler Plus and AmpFISTR Cofiler) and subsequent electrophoresis on an ABI PRISM CE 310 Genetic Analyzer instrument. All loci meet Hardy-Weinberg expectations. In addition, there is little evidence for association of alleles among the 13 loci. The allelic frequency data can be used in forensic analyses and paternity tests to estimate the frequency of a multiple STR locus DNA profile in the Swiss population.

KEYWORDS: forensic science, Switzerland, Caucasian, population genetics, short tandem repeats, Hardy-Weinberg equilibrium, polymerase chain reaction, DNA typing, DS51358, VWA, FGA, D8S1179, D21511, D18S51, D5S818, D13S317, D7S820, THO1, TPOX, CSF1PO, D16S539

Short tandem repeat (STR) loci are becoming routine markers for the forensic identification of biological materials and for paternity testing (1,2). Forensic laboratories in the United States recently established the 13 STR loci that will comprise the Combined DNA Index System (CODIS) database. Several commercial kits are available to enable multiplex amplification of these 13 core STR. For example, the AmpFISTR Profiler Plus kit (Perkin-Elmer/ABD, Foster City, CA) is designed for coamplification of nine STRs and the Amelogenin locus in a single PCR; and the AmpFISTR Cofiler kit (Perkin-Elmer/ABD, Foster City, CA) enables coamplification of the four remaining STR loci, as well as two loci (D3S1358, D7S820) also found in AmpFISTR Profiler Plus kit.

This paper presents allele frequency data in the Swiss population for these 13 STR loci. The data demonstrate that these loci are useful for providing estimates of the frequency of a DNA profile in identity testing cases.

Material and Methods

Sample Preparation

Blood samples from 206 unrelated Swiss Caucasian donors were provided from the Swiss Red Cross (Basel, Switzerland) and from disputed cases of paternity from our laboratory. Two hundred μL blood samples were placed onto cotton cloth, air dried, and a portion (2 mm by 2 mm) was used for extraction. The DNA was extracted using the organic phenol-chloroform-isoamyl alcohol method (3). All DNA extracts were quantitated using the Quantiblot® Human DNA Quantitation Kit (Perkin Elmer, Norwalk, CT) (4).

STR Typing and Data Analysis

The coamplification of D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, and D7S820 loci was performed using the AmpFISTR Profiler Plus kit (Perkin-Elmer/ABD, Foster City, CA). Reactions for PCR were prepared according to the manufacturer's recommendations except that the PCR volume was 12.5 μL using 2 ng of template DNA and AmpliTaq Gold DNA polymerase (Perkin-Elmer). PCR conditions were: 95°C for 11 min, followed by 28 cycles at 94°C for 1 min, 59°C for 1 min, and 72°C for 1 min. A final extension was conducted at 60°C for 45 min. A Perkin-Elmer GeneAmp PCR System 9600 thermal cycler was used for the PCR. Coamplification of D3S1358, D16S539, Amelogenin, THO1, TPOX, CSF1PO, and D7S820 loci was performed using the AmpFISTR Cofiler kit (Perkin-Elmer/ABD, Foster City, CA) according to the manufacturer's recommendations except that the PCR volume was 12.5 μL. 2 ng of template DNA and AmpliTaq Gold DNA polymerase were used for the PCR. The PCR conditions were the same as shown above.

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* Names of commercial manufacturers are provided for identification only, and inclusion does not imply endorsement by the authors.

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TABLE 1—*Observed allele frequencies in a Swiss population sample (206 individuals).*

Allele	D3S1358	VWA	FGA	D8S1179	D21S11	D18S51	D5S818	D13S317	D7S820	D16S539	TH01	TPOX	CSF1PO
5	—	—	—	—	—	—	—	—	—	—	0.005	—	—
6	—	—	—	—	—	—	—	—	—	—	0.243	0.002	—
7	—	—	—	—	—	—	0.002	—	0.034	—	0.172	—	—
8	—	—	—	0.015	—	—	0.002	0.129	0.168	0.029	0.104	0.549	0.007
9	—	—	—	0.007	—	—	0.046	0.078	0.189	0.131	0.143	0.078	0.015
9.3	—	—	—	—	—	—	—	—	—	—	0.316	—	—
10	—	—	—	0.095	—	0.007	0.071	0.046	0.235	0.068	0.017	0.053	0.284
11	—	—	—	0.095	—	0.015	0.328	0.330	0.199	0.313	—	0.296	0.296
12	—	—	—	0.133	—	0.155	0.364	0.294	0.141	0.286	—	0.022	0.291
13	0.002	—	—	0.269	—	0.148	0.170	0.087	0.029	0.153	—	—	0.095
14	0.097	0.119	—	0.209	—	0.139	0.015	0.036	0.005	0.020	—	—	0.012
15	0.221	0.109	—	0.136	—	0.153	0.002	—	—	—	—	—	—
16	0.284	0.223	—	0.036	—	0.143	—	—	—	—	—	—	—
17	0.202	0.286	—	0.005	—	0.097	—	—	—	—	—	—	—
18	0.175	0.170	0.022	—	—	0.066	—	—	—	—	—	—	—
19	0.019	0.080	0.075	—	—	0.044	—	—	—	—	—	—	—
20	—	0.010	0.155	—	—	0.017	—	—	—	—	—	—	—
21	—	0.003	0.189	—	—	0.007	—	—	—	—	—	—	—
21.2	—	—	0.005	—	—	—	—	—	—	—	—	—	—
22	—	—	0.146	—	—	0.007	—	—	—	—	—	—	—
22.2	—	—	0.005	—	—	—	—	—	—	—	—	—	—
23	—	—	0.138	—	—	—	—	—	—	—	—	—	—
23.2	—	—	0.003	—	—	—	—	—	—	—	—	—	—
24	—	—	0.138	—	—	0.002	—	—	—	—	—	—	—
25	—	—	0.083	—	—	—	—	—	—	—	—	—	—
25.2	—	—	0.002	—	—	—	—	—	—	—	—	—	—
26	—	—	0.032	—	0.002	—	—	—	—	—	—	—	—
26.2	—	—	—	—	0.005	—	—	—	—	—	—	—	—
27	—	—	0.007	—	0.032	—	—	—	—	—	—	—	—
28	—	—	—	—	0.131	—	—	—	—	—	—	—	—
28.2	—	—	—	—	0.002	—	—	—	—	—	—	—	—
29	—	—	—	—	0.209	—	—	—	—	—	—	—	—
30	—	—	—	—	0.269	—	—	—	—	—	—	—	—
30.2	—	—	—	—	0.044	—	—	—	—	—	—	—	—
31	—	—	—	—	0.068	—	—	—	—	—	—	—	—
31	—	—	—	—	0.068	—	—	—	—	—	—	—	—
31.2	—	—	—	—	0.090	—	—	—	—	—	—	—	—
32	—	—	—	—	0.019	—	—	—	—	—	—	—	—
32.2	—	—	—	—	0.100	—	—	—	—	—	—	—	—
33.2	—	—	—	—	0.024	—	—	—	—	—	—	—	—
34.2	—	—	—	—	0.005	—	—	—	—	—	—	—	—
Ob. Ho.*	0.223	0.209	0.107	0.189	0.131	0.146	0.320	0.277	0.209	0.223	0.233	0.393	0.301
Ex. Ho†	0.208	0.191	0.131	0.170	0.158	0.123	0.274	0.227	0.179	0.225	0.218	0.396	0.261
HWE-T.‡	0.600	0.527	0.296	0.460	0.292	0.328	0.139	0.089	0.262	0.965	0.601	0.923	0.188
Ex. Test§	0.408	0.944	0.283	0.100	0.96	0.064	0.388	0.283	0.570	0.068	0.658	0.292	0.602

*Observed Homozygosity; †Expected Homozygosity (unbiased); ‡ HWE-Homozygosity Test; § Exact Test.

AmpFISTR Profiler Plus and AmpFISTR Cofiler amplification products were subjected to electrophoresis on an ABI PRISM 310 Genetic Analyzer instrument. After amplification, 1 μL of PCR product and 1 μL of GeneScan™-500 (Rox) Internal Lane Size Standard (GeneScan™-350 (Rox) was used for the Cofiler PCR products) were added to 24 μL of deionized formamide, denaturated at 95°C for 3 min and immediately placed on ice. The PCR products were injected 5 s and electrophoresis was performed at 15 kV in Performance Optimized Polymer 4 (POP4™; 1 mL syringe). Data were collected using ABI PRISM 310 Collection software application, with the module GS POP4 F (virtual filter set F). Genotyper software (Perkin-Elmer) was used for the automated genotyping of the samples.

Statistical Analysis

Statistical evaluations were performed using a software package kindly provided by Ranijt Chakraborty (University of Texas School of Biomedical Sciences, Houston, Texas). The frequency of each allele for each locus was calculated from the numbers of each observed genotype in the sample set (i.e., the gene count method). Unbiased estimates of expected heterozygosity were computed as described (2). Possible divergence from Hardy-Weinberg expectations (HWE) was tested by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies (5–7) and the exact test (8), based on 2000 shuffling experiments. An interclass correlation criterion (9) for two-locus associations was used for detecting disequilibrium between the STR loci. The power of discrimination (PD) and the probability of exclusion (PE) were also calculated for each locus. A 2 × N contingency table exact test was used to generate a G-statistic (2000 shuffling experiments) (10,11) to test for homogeneity between sample populations.

Results and Discussion

The observed allele frequencies in the Swiss population sample for the 13 core STR loci are shown in Table 1. All loci are highly polymorphic. The observed heterozygosities for the STR loci range from 60.7% for the TPOX locus to 89.3% for the FGA locus. The discrimination power and probability of exclusion for the STR loci are displayed in Table 2. Based on it's high discrimination power and probability of exclusion, the combination of these 13 STRs makes these loci a powerful choice for databasing purposes. The overlap of two STR loci (D3S1358 and D7S829) and the amelogenin locus also provides the laboratory with a quality control check against sample mixing.

TABLE 2—*Power of discrimination (PD) and probability of exclusion (PE) for STR loci in a sample population from Switzerland.*

Locus	PD (Obs)*	PD (Exp)†	PE
1 D3S1358	0.92105759	0.92304652	0.58371923
2 vWA	0.93543218	0.93566361	0.61939022
3 FGA	0.96196626	0.96747463	0.72998605
4 D8S1179	0.94456536	0.94882844	0.66057803
5 D21S11	0.95442549	0.95654622	0.68779644
6 D18S51	0.96649072	0.97106873	0.74572836
7 D5S818	0.88406070	0.87750004	0.48665452
8 D13S317	0.91290414	0.91491018	0.56752886
9 D7S820	0.94085211	0.94174642	0.63730041
10 D16S539	0.90734282	0.91462616	0.56508198
11 THO1	0.91747573	0.91810642	0.57169107
12 TPOX	0.77335281	0.78154240	0.34853480
13 CSF1PO	0.88735979	0.88361852	0.49438247
	>.99999999	>.99999999	0.99999430

* PD was calculated using observed genotype frequencies.
† PD was calculated using expected genotype frequencies.

TABLE 3—*Two locus inter-class correlation test for STR loci in a sample population from Switzerland.*

Loci	p-value	Loci	p-value
1 D3S1358/2 vWA	0.737	4 D8S1179/11 THO1	0.196
1 D3S1358/3 FGA	0.109	4 D8S1179/12 TPOX	0.138
1 D3S1358/4 D8S1179	0.942	4 D8S1179/13 CSF1PO	0.491
1 D3S1358/5 D21S11	0.712	5 D21S11/6 D18S51	0.783
1 D3S1358/6 D18S51	0.183	5 D21S11/7 D5S818	0.032*
1 D3S1358/7 D5S818	0.737	5 D21S11/8 D13S317	0.599
1 D3S1358/8 D13S317	0.612	5 D21S11/9 D7S820	0.405
1 D3S1358/9 D7S820	0.414	5 D21S11/10 D16S539	0.132
1 D3S1358/10 D16S539	0.970	5 D21S11/11 THO1	0.634
1 D3S1358/11 THO1	0.009***	5 D21S11/12 TPOX	0.497
1 D3S1358/12 TPOX	0.198	5 D21S11/13 CSF1PO	0.365
1 D3S1358/13 CSF1PO	0.135	6 D18S51/7 D5S818	0.751
2 vWA/3 FGA	0.197	6 D18S51/8 D13S317	0.097
2 vWA/4 D8S1179	0.369	6 D18S51/9 D7S820	0.991
2 vWA/5 D21S11	0.324	6 D18S51/10 D16S539	0.761
2 vWA/6 D18S51	0.825	6 D18S51/11 THO1	0.865
2 vWA/7 D5S818	0.858	6 D18S51/12 TPOX	0.566
2 vWA/8 D13S317	0.113	6 D18S51/13 CSF1PO	0.282
2 vWA/9 D7S820	0.543	7 D5S818/8 D13S317	0.475
2 vWA/10 D16S539	0.648	7 D5S818/9 D7S820	0.395
2 vWA/11 THO1	0.924	7 D5S818/10 D16S539	0.131
2 vWA/12 TPOX	0.978	7 D5S818/11 THO1	0.213
2 vWA/13 CSF1PO	0.783	7 D5S818/12 TPOX	0.470
3 FGA/4 D8S1179	0.825	7 D5S818/13 CSF1PO	0.891
3 FGA/5 D21S11	0.464	8 D13S317/9 D7S820	0.162
3 FGA/6 D18S51	0.364	8 D13S317/10 D16S539	0.366
3 FGA/7 D5S818	0.772	8 D13S317/11 THO1	0.956
3 FGA/8 D13S317	0.052	8 D13S317/12 TPOX	0.703
3 FGA/9 D7S820	0.756	8 D13S317/13 CSF1PO	0.259
3 FGA/10 D16S539	0.196	9 D7S820/10 D16S539	0.042*
3 FGA/11 THO1	0.179	9 D7S820/11 THO1	0.902
3 FGA/12 TPOX	0.136	9 D7S820/12 TPOX	0.050
3 FGA/13 CSF1PO	0.438	9 D7S820/13 CSF1PO	0.659
4 D8S1179/5 D21S11	0.801	10 D16S539/11 THO1	0.774
4 D8S1179/6 D18S51	0.232	10 D16S539/12 TPOX	0.392
4 D8S1179/7 D5S818	0.936	10 D16S539/13 CSF1PO	0.968
4 D8S1179/8 D13S317	0.178	11 THO1/12 TPOX	0.896
4 D8S1179/9 D7S820	0.169	11 THO1/13 CSF1PO	0.772
4 D8S1179/10 D16S539	0.466	12 TPOX/13 CSF1PO	0.013*

* Department from linkage equilibrium.

An inter-class correlation test analysis detected four departures from independence out of 78 pair-wise comparisons of the 13 STR loci (Table 3). This number of pair-wise departures is not substantially more than would be expected. The Swiss population allele frequency data for these 13 PCR based loci do not differ substantialy from other Caucasian data for the same loci (an Italian database (12) and a Maine Caucasian population DNA database using 12 STR loci (13), data not shown). Eleven out of 13 loci compared with the Italian database and ten out of 12 loci compared with the U.S. Caucasian database were statistically similiar. Thus, overall there is little difference between these population samples.

In conclusion, a Swiss population database has been established for the 13 core STR loci for CODIS (D3S1358, VWA, FGA, Amelogenin, D8S1179, D21S11, D18S51, D5S818, D13S317, D7S820, THO1, TPOX, CSF1PO, and D16S539). The data support other studies and demonstrates that valid estimates of a multiple STR locus profile frequency can be derived for identity testing purposes using the product rule under the assumption of independence.

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