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Swiss population data and forensic efficiency values on 3 tetrameric short tandem repeat loci-HUMTH01, TPOX, and CSF1PO – derived using a STR multiplex system

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Abstract Allele and genotype frequencies for 3 tetrameric short tandem repeat loci were determined in a Swiss population sample (n = 100) using the GenePrint STR Multiplex System, electrophoresis of the PCR products in DNA sequencing gels and subsequent detection of allelic fragments by silver staining. The loci are HUMTH01, TPOX, and CSF1PO. The observed heterozygosities are 83.0%, 60.0%, and 72.0%, respectively. The discrimination power determined for the individual loci is 0.914, 0.780, and 0.860, respectively, and the combined discrimination power for the triplex is 0.997. All loci meet Hardy-Weinberg expectations and after Bonferroni correction there was no evidence that the population sample deviates from expectations of independence. Moreover, independence of alleles at these STR loci with other PCR-based loci derived from the same Swiss population sample, previously reported, were considered. These loci were DQA1, LDLR, GYPA, HBGG, D7S8, GC and D1S80. Again, after Bonferroni correction there was no evidence that the population sample deviates from expectations of independence among alleles at the 10 different PCR-based loci. Thus, the allelic frequency data can be used in human identity testing to estimate the frequency of a multiple PCR-based DNA profile in the Swiss population.

Key words DNA-STR's · HUMTH01 · TPOX · CSF1PO-Polymarker · Population genetics · Switzerland · Hardy-Weinberg expectations · Linkage equilibrium · PCR

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Zusammenfassung In einer Schweizer Bevölkerungs-Stichprobe (n = 100) wurden mittels der GenePrint STR Multiplex System und nach Auftrennung auf Sequenziergelen und Silberfärbung die Allel- und Genotyp-Frequenzen der drei Short-Tandem-Repeat Loci HUMTH01, TPOX und CSF1P0 bestimmt. Die beobachteten Heterozygotieraten sind 83.0%, 60.0% bzw. 72.0%. Die Diskriminationsindices der einzelnen Loci betragen 0.914, 0.780, bzw. 0.860, der kombinierte Diskriminationsindex 0.997. Alle Loci erfüllen die Hardy-Weinberg Kriterien und nach Bonferroni-Korrektur finden sich keine Hinweise für eine Koppelung von Allelen der drei Loci. Darüber hinaus wurde die Unabhängigkeitsregel zwischen diesen drei Loci und anderen Loci aus der gleichen Schweizer Bevölkerungs-Stichprobe, deren Daten bereits publiziert wurden, überprüft. Diese Loci sind DQA1, LDLR, GYPA, HBGG, D7S8, GC and D1S80. Es ergaben sich nach Bonferroni-Korrektur wiederum keine Hinweise auf eine Koppelung von Allelen der insgesamt zehn auf PCR basierenden Loci. Die ermittelten Allelfrequenzen können somit zur Abschätzung der Häufigkeit eines aus diesen Loci zusammengesetzten DNA Profils in der Schweizer Bevölkerung herangezogen werden.

Schlüsselwörter DNA-STR's · HUMTH01 · TPOX · CSF1PO-Polymarker · Populationsgenetik · Schweiz · Hardy-Weinberg Gleichgewicht · Kopplungsanalyse · PCR

Introduction

Short tandem repeat (STR) loci are a subgroup of the highly polymorphic variable number of tandem repeat (VNTR) loci [1, 2] that are very useful for the analysis of forensic DNA evidence [3–6]. Puers et al. [7, 8] described an analytical system comprised of 3 STR loci, HUMTH01 [1, 2, 9], TPOX [10], and CSF1PO [11], which can be amplified simultaneously by PCR. The multiplex STR products can be typed by electrophoresis in denatured polyacrylamide gels and silver stained. Since the sizes of the 3 STR loci in

the multiplex analysis do not overlap, unequivocal typing of the 3 loci is possible. Currently there are little data on allele frequencies and genotype distributions in various populations for the STR loci HUMTH01, TPOX, and CSF1PO [12]. This paper presents allele/genotype frequency data in a Swiss population sample for these 3 tetrameric STR loci generated using a STR Multiplex System. Moreover, these loci were tested for departures from independence expectations with other previously reported PCR-based loci from the same Swiss population sample – HLA DQA1, LDLR, GYPA, HBGG, D7S8, GC, and D1S80 [13] to evaluate if the allelic frequency data at the 10 different PCR-based loci can be used to estimate the frequency of a multiple PCR-based DNA profile.

Materials and methods

Whole blood was obtained in EDTA vacutainer tubes by venipuncture from 100 unrelated Swiss Caucasians by the Swiss Red Cross (Basel, CH). The DNA was extracted as described previously [14] and the quantity of DNA was estimated by UV-absorbance. For the multiplex analysis of HUMTH01, TPOX, and CSF1PO the same samples were used as had been typed for the loci HLA DQA1, LDLR, GYPA, HBGG, D7S8, GC, and D1S80.

Multiplex PCR analysis

The multiplex analysis of HUMTH01, TPOX, and CSF1PO was performed as described by Huang et al. [12] and the manufacturer's recommendations [15] using the GenePrint™ STR Multiplex System (Promega Corporation, Madison, WI, USA). Briefly, the PCR was carried out in 50 µl reaction volumes each containing 2 ng genomic DNA in a Perkin Elmer 480 thermal cycler. PCR products (5 µl) were mixed with 2.5 µl 3 × STR loading dye and 2.5 µl of this mix was loaded onto a denaturing polyacrylamide gel (4% T, 5% C, 31 cm long and 0.4 mm thick) containing 7 *M* urea and 0.5 × Tris-Borate-EDTA buffer. Electrophoresis was carried out on an SA 32 Electrophoresis Apparatus (BRL, Gaithersburg, MD, USA). The conditions for electrophoresis were set a constant power of 40 W and carried out at ambient temperature. Electrophoresis was stopped when the xylene cyanol dye migrated 6 cm from the anode (approximately 1 h 15 min).

Allele designations were determined by comparison of the sample fragments with those of the allelic ladders supplied in the kit. Allele designations were made according to recommendations of the DNA commission of the International Society for Forensic Haemogenetics [16].

Statistical analysis

The frequency of each allele for each STR 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. [2]. The expected number of distinct homozygous and heterozygous genotype classes and their standard error (SE) was calculated according to the method described by Chakraborty et al. [17, 18]. Possible divergence from Hardy-Weinberg expectations (HWE) was determined by calculating the unbiased estimate of the expected homozygote/heterozygote frequencies [17, 19, 20], the likelihood ratio test [2, 18, 21], and the exact test [22]. An interclass correlation criterion [23] for two-locus association was used for detecting disequilibrium between the STR loci. Independence across all loci (HLA, DQA1, LDLR, GYPA, HBGG, D7S8, GC, HUMTH01, TPOX, CSFPO, and D1S80) was determined by examining whether or not an observed variance of the number of heterozygous loci in the population sample is outside is confidence interval under the presumption of independence [24]. The discrimination power was calculated according to the method of Jones [25].

Results and discussion

All 100 samples were typed successfully for the 3 STR loci. The distributions of observed allelic frequencies for HUMTH01, TPOX, and CSF1PO are shown in Tables 1–3. All alleles differed in size by one repeat unit (i.e., 4

Table 1 HUMTH01 allele frequencies in a sample of 100 unrelated Swiss Caucasians

Allele	Frequency
5	0.010
6	0.250
7	0.185
8	0.110
9	0.155
9.3	0.280
10	0.010
11	0.000

Observed homozygosity = 0.170
Expected homozygosity (unbiased) = 0.208
HWE – Homozygosity Test ($P = 0.355$), Likelihood Ratio Test ($P = 0.402$), and Exact Test ($P = 0.565$)

Table 2 TPOX allele frequencies in a sample of 100 unrelated Swiss Caucasians

Allele	Frequency
8	0.575
9	0.060
10	0.065
11	0.265
12	0.035

Observed homozygosity = 0.400
Expected homozygosity (unbiased) = 0.407
HWE – Homozygosity Test ($P = 0.888$) Likelihood Ratio Test ($P = 0.976$), and Exact Test ($P = 0.968$)

Table 3 CSF1PO allele frequencies in a sample of 100 unrelated Swiss Caucasians

Allele	Frequency
7	0.000
8	0.000
9	0.025
10	0.245
11	0.275
12	0.375
13	0.065
14	0.005
15	0.010

Observed homozygosity = 0.280
Expected homozygosity (unbiased) = 0.278
HWE – Homozygosity Test ($P = 0.958$), Likelihood Ratio Test ($P = 0.327$), and Exact Test ($P = 0.289$)

Table 4 Observed and expected heterozygous and homozygous classes for HUMTH01, TPOX, and CSF1PO in 100 unrelated Swiss Caucasians (SE = standard error)

	HUMTH01	TPOX	CSF1PO
Heterozygotes Observed	13	9	11
Heterozygotes Expected ± SE	13.2 ± 1.5	8.0 ± 1.0	10.9 ± 1.5
Homozygotes Observed	4	2	4
Homozygotes Expected ± SE	4.6 ± 0.6	2.8 ± 0.7	3.4 ± 0.5

Table 5 Two loci interclass correlation test for the 3 tetrameric short tandem repeat loci and other, previously reported [13] PCR-based loci in 100 unrelated Swiss Caucasians

Loci	Two-sided probability	Loci	Two-sided probability
HUMTH01/TPOX	0.057	GYPA/TPOX	0.831
HUMTH01/CSF1PO	0.287	HBGG/TPOX	0.702
TPOX/CSF1PO	0.157	D7S8/TPOX	0.972
DQA1/HUMTH01	0.898	GC/TPOX	0.525
LDLR/HUMTH01	0.696	D1S80/TPOX	0.299
GYPA/HUMTH01	0.422	DQA1/CSF1PO	0.378
HBGG/HUMTH01	0.843	LDLR/CSF1PO	0.790
D7S8/HUMTH01	0.187	GYPA/CSF1PO	0.355
GC/HUMTH01	0.037	HBGG/CSF1PO	0.974
D1S80/HUMTH01	0.849	D7S8/CSF1PO	0.334
DQA1/TPOX	0.465	GC/CSF1PO	0.691
LDLR/TPOX	0.357	D1S80/CSF1PO	0.381

base pairs) for all loci, except for the HUMTH01 allele 9.3. The 9.3 allele is one base pair smaller in size than the 10 allele [7].

The observed heterozygosities for HUMTH01, TPOX, and CSF1PO are 83.0%, 60%, and 72.0%, respectively (Tables 1–3). The discrimination power determined for the individual loci is 0.914, 0.780, and 0.860, respectively, and the combined discrimination power for the triplex is 0.997. A test for independence of the alleles within a locus based on the number of distinct heterozygote and homozygote genoytype classes was performed (Table 4). There were no deviations from expected values for the 3 STR loci. Furthermore, the 3 loci did not deviate from HWE based on the homozygosity test, likelihood ratio test, and the exact test (Tables 1–3). An inter-class correlation test analysis demonstrated that there is little evidence for correlation between the alleles at any of the pair-wise comparisons of loci (Table 5). After Bonferroni correction [26] there was no evidence that the population sample deviates from expectations of independence. One could argue that a population sample size of 100 is insufficient for testing for departures from HWE and linkage equilibrium expectations. However, the tests used in this study have sufficient power to detect departures from expectations that would affect estimates of the likelihood of occurrence of a PCR-based DNA profile for forensic identity testing. Thus the data in this report can be used for human identity testing. In conclusion, a Swiss population database has been established for HUMTH01, TPOX, and CSF1PO and it has been shown that the allelic frequency data at 10 different PCR-based loci can be used to estimate the frequency of a multiple PCR-based DNA profile in the Swiss population.

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