TECHNICAL NOTE

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Northeast Italy Population Data Using Multiplex PCR (HUMCD4, HUMTH01, HUMTPOX, and HUMCSF1P0) Loci

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ABSTRACT: Allele and genotype frequencies for four short tandem repeat (STR) loci (HUMCD4, HUMTH01, HUMTPOX, and HUMCSF1P0) were determined in 100 unrelated individuals from Veneto (Northeast Italy). After a multiplex polymerase chain reaction (PCR)-amplification, semi-automatic DNA profiling was performed using an A.L.F.express DNA Sequencer. Conditions were optimized for the PCR co-amplification of these four STR loci and the quadruplex PCR was performed on various forensic DNA samples such as whole blood, blood-stains, teeth, and saliva from Caucasians living in the Northeast Italy. The distribution of the genotype frequencies showed no significant deviation from Hardy-Weinberg expectations in the sampled population. The combined Power of Discrimination (PD) of the quadruplex was 0.9999.

KEYWORDS: forensic science, DNA typing, population genetics, HumCD4, HumTH01, HumTPOX, HumCSF1P0, Veneto, Italy

DNA profiling using short tandem repeat (STR) loci has become widely used for human identification and paternity testing in forensic sciences (1,2). STRs are highly polymorphic markers that consist of a tandemly repeat sequence of 2–7 base pairs (bp). By multiplex PCR amplification of several STR loci, it was possible to decrease the quantity of DNA required for analysis than if each locus were analyzed separately. This is very useful in forensic casework (3,4).

In this work, we have applied this multiplex amplification strategy to four STR loci: HumCD4, HumTH01, HumTPOX, and HumCSF1P0 located on chromosomes 12, 11, 2, and 5, respectively. None of these STR loci have overlapping allele size ranges. This multiplex has a high power of discrimination.

The amplified products were separated on a denaturing polyacrylamide gel by a monochrome Automated Laser Fluorescent (A.L.F.express, Pharmacia Biotech, Uppsala, Sweden) DNA sequencer and identified with locus specific allelic ladders (5,6). A population sample of 100 unrelated individuals living in Veneto (a Northeast region of Italy) was analyzed, and then we calculated its genetic-populational parameters (allelic frequencies, heterozygosity, fit to the Hardy-Weinberg equilibrium, PD and PIC).

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Materials and Methods

Sample Preparation

The samples were obtained from 100 unrelated individuals from the region of Veneto (Northeast Italy). DNA was extracted from whole blood samples, collected into EDTA vacutainer tubes, by the "GenomicPrep blood DNA isolation kit" (Pharmacia Biotech, Uppsala, Sweden), from teeth as previously described by Smith et al. (7), and modified by using only a phenol-chloroform extraction; while DNA was extracted from blood stains and saliva using the Chelex extraction procedure described by Walsh et al. (8). DNA was quantified by spectrophotometry.

Multiplex Amplification

Co-amplification by PCR of the four STR loci was performed using 1 to 10 ng of human genomic DNA, 5 μL of 10X PCR buffer II (500 mM KCl, 100 mM Tris-HCl pH 8.3), 1.5 mM $MgCl_2$, 0.2 mM of dNTP, 1.25 units of AmpliTaq DNA polymerase (Perkin Elmer Applied Biosystems, Foster City, CA), 0.25 μM of each HumCD4 primer (9) and 0.3 μM of each HumTH01 (10), HumTPOX (11), and HumCSF1P0 (12) primer (Pharmacia Biotech, Uppsala, Sweden), diluited to a final volume of 50 μL with double distilled sterile water.

The quadruplex PCR amplification conditions were 95°C for 2 min, followed by 30 cycles at 95°C for 30 s, 67°C for 30 s, 72°C for 30 s, and a final extension step at 72°C for 10 min, in a GeneAmp® PCR System 2400 thermal cycler (Perkin Elmer Applied Biosystems, Foster City, CA).

STR Typing

The forward primer STR locus was labeled at the 5′ end with fluorescent dye marker Cy5 (an activated di-carboxyl derivate of a cyanine compound) from Pharmacia Biotech (Uppsala, Sweden). The fluorescent labeled PCR products were resolved by electrophoresis through a 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gel (Ready Mix Gel ALF grade, Pharmacia-Biotech, Uppsala, Sweden)

containing 1X TBE buffer and 7 M urea, and detected by laser scanning on the A.L.F.express (Pharmacia-Biotech, Uppsala, Sweden) DNA Sequencer at 1500 V, 36 mA and 45°C for 240 min. The PCR products were mixed with internal fluorescently labeled size standard (50 bp, Pharmacia-Biotech, Uppsala, Sweden) to compensate for any mobility shift between the lanes (13). The allelic ladders for the four STR loci were loaded as an external marker in some lanes on each gel for the identification of the amplified alleles with fragment software V1.2. The four ladders (consisting of a mix of sequenced amplified products) were constructed from blood samples previously typed using the Promega ladders (Madison, WI).

Statistical Analysis

The frequencies of each allele for each polymorphic locus were calculated from the numbers of each genotype in the population sample by the traditional counting method. Evaluation of the Hardy-Weinberg equilibrium (HWE) was based on comparison of observed and expected genotype frequencies (genotype with less than five observations pooled) using the Chi-square test (χ^2). Polymorphism Information Content (PIC) values were obtained as described by Botstein et al. (14), while the power of discrimination PD (the probability that two individuals chosen at random from a given population have different phenotypes) for each locus was calculated using Fisher's equation (15). The allelic diversity (h) of the four loci and the standard error were determined as described by Nei and Roychoudhury (16).

Results and Discussion

The loci studied were chosen because they proved to coamplify reliably and to be highly polymorphic. The location of these loci on four different chromosomes eliminates the possibility of physical genetic linkage, and furthermore, their small size (generally less than 350 bp) enables analysis of many degraded DNA samples.

The quadruplex PCR performed on blood samples permitted an efficient co-amplification of all four loci as shown on Fig. 1 and allowed the simultaneous development of the data bases for HumCD4, HumTH01, HumTPOX, and HumCSF1P0. The multiplex PCR reaction conditions described (see Materials and Methods) allowed us to obtain equilibrated amplifications at the different loci within the quadruplex and all STR electrophoregrams were free of artifactual bands.

The allele frequencies for the four STR loci in the Northeast Italy population sample are displayed in Table 1.

HumCD4 locus—We found a total of 6 alleles and 12 genotypes out of 21 possible genotypes; alleles 7 and 8 were not observed, while the most frequent alleles were 5, 6, and 10. The Chi-square test performed on our population sample did not show any significant deviation from Hardy-Weinberg Equilibrium ($\chi^2 = 23{,}47$; d.f. $= 15$; $P = 0{,}07$). The observed heterozygosity, expected heterozygosity, allelic diversity, power of discrimination, and polymorphism information content for this locus are listed in Table 2.

HumTH01 Locus—Six alleles and 16 genotypes have been identified for this locus. Allele 5 was not detected, while the most frequent allele was 9.3. The observed heterozygosity, expected heterozygosity, allelic diversity, power of discrimination, and polymorphism information content for the HumTH01 locus are listed in Table 2. For this system the population sample analyzed meets HWE expectations ($\chi^2 = 23{,}01$; d.f. $= 15$; $P = 0{,}08$).

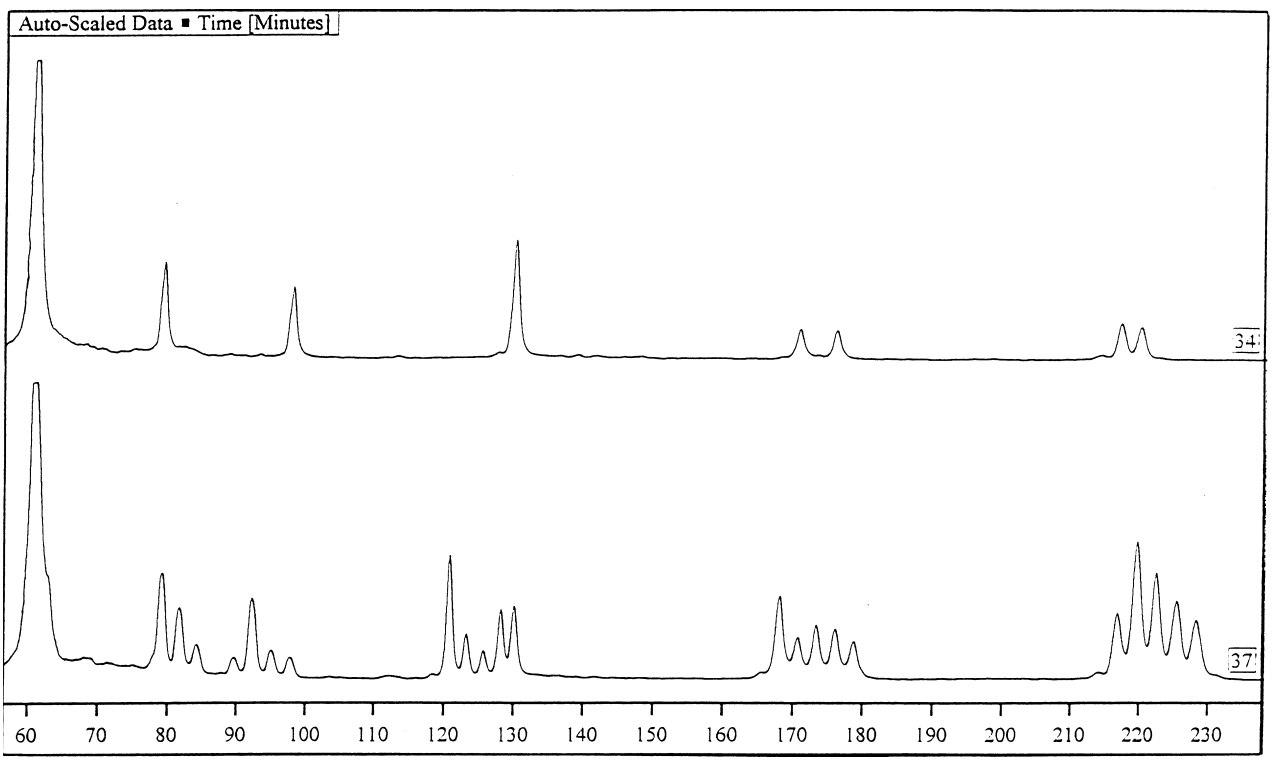

FIG. 1—*Electropherogram of the quadruplex PCR products (HUMCD4, HUMTH01, HUMTPOX, and HUMCSF1P0) on a 6% denaturing polyacrylamide gel containing 1X TBE buffer and 7 M urea on the A.L.F.Express DNA sequencer. The blood sample of one random individual is analyzed in lane 34. The size of amplified alleles are determined against the locus specific allelic ladders in lane 37. The allelic ladders include alleles 5, 6, 7, 9, 10, 11, and 12 for HUMCD4; alleles 6, 7, 8, 9, and 9.3 for HUMTH01; alleles 8, 9, 10, 11, and 12 for HUMTPOX and alleles 9, 10, 11, 12, and 13 for HUMCSF1P0.*

TABLE 1—*Distribution of allele frequencies count of HumCD4, HumTH01, HumTPOX, and HumCSF1P0 loci in 100 unrelated Northeast Italy individuals.*

Locus	Allele	Allele Frequency
HumCD4		
	5	0.335
	6	0.305
	7	…
	8	…
	9	0.005
	10	0.310
	11	0.035
	12	0.010
HumTH01		
	5	…
	6	0.245
	7	0.135
	8	0.105
	9	0.160
	9.3	0.333
	10	0.025
HumTPOX		
	8	0.455
	9	0.140
	10	0.085
	11	0.275
	12	0.045
HumCSF1P0		
	9	0.040
	10	0.280
	11	0.275
	12	0.350
	13	0.055

TABLE 2—*Statistical parameters for CD4, TH01, TPOX, and CSF1P0 in Northeast Italy population sample.*

	CD4*	TH01*	TPOX*	CSF1P0*
H. obs.	0.63	0.81	0.72	0.71
H. exp.	0.69	0.77	0.69	0.72
h (s.e.)	0.83 ± 0.037	0.92 ± 0.027	0.86 ± 0.034	0.89 ± 0.031
PD	0.85	0.91	0.85	0.87
PIC	0.67	0.76	0.68	0.69

* H. obs. = observed heterozygosity; H. exp. = expected heterozygosity; h (s.e.) = allelic diversity and standard error; PD = discrimination power; PIC = polymorphism information content.

HumTPOX Locus—We observed 5 different alleles (8–12) giving 12 genotypes. The most frequent allele was 8 (Table 1). The Northeast Italy TPOX phenotypes distribution is in Hardy-Winberg Equilibrium (χ^2 = 10,65; d.f. = 10; P = 0,38) and the observed heterozygosity, expected heterozygosity, allelic diversity, power of discrimination, and polymorphism information content were calculated (Table 2).

HumCSF1P0 Locus—A total of 5 alleles (9–13) and 12 genotypes were detected for HumCSF1P0. The most frequent alleles were 10, 11, and 12. The statistical analysis of the Northeast Italy population sample clearly showed that CSF1P0 meets HWE expectations (χ^2 = 7,89; d.f. = 10; P = 0,64). Table 2 indicates the observed heterozygosity, expected heterozygosity, allelic diversity, power of discrimination, and polymorphism information content of the locus.

For this quadruplex STR system, the combined power of discrimination (PD) was 0.9999. The Northeast Italian population allele frequency data for these four STR loci do not differ substantially from other Caucasian data for the same loci (data not shown). The quadruplex PCR amplification of the four loci is easy to perform and gives a good amplification signal with small amounts of DNA template when using a monochrome automated laser fluorescence sequencer.

In conclusion, the efficiency, precision, and high degree of discrimination of this quadruplex STR system suggests its possible application in numerous routine forensic analyses for human identification and paternity testing in forensic sciences.

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