TECHNICAL NOTE

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Analysis of the HUMTH01 Allele Frequencies in the Spanish Population

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ABSTRACT: Genetic marker typing based on DNA amplification by the polymerase chain reaction (PCR) increasingly is being employed in forensic casework and for paternity testing. The allele frequencies were determined using PCR for 120 unrelated Spanish Caucasians for the locus HUMTH0H1. Six alleles were observed, with frequencies ranging from 0.013 (allele 11) to 0.254 (allele 10). The observed heterozygosity was 75.8%, and the power of discrimination is 0.92. The genotype distribution meets Hardy-Weinberg expectations.

KEYWORDS: pathology and biology, PCR, STR, DNA, HUMTH01, forensic genetics

Human tetrameric repeats are a source of genetic markers that can be useful for identity testing purposes. These short tandem repeat (STR) loci can exhibit a high degree of variability. Edwards et al. [*1,2*] have reported on a number of potential candidate STR loci that are amenable to polymerase chain reaction (PCR) analysis [*3*]. One of these STR loci, called HUMTH01, is reasonably polymorphic and has been reported by Brinkmann et al. [*4*] to be rather robust for forensic analyses. HUMTH01 is a tetranucleotide repeat (AATG) located in chromosome 11 within intron 1 of the tyrosine hydroxylase gene [*1,2*].

For HUMTH01 to be useful for identity testing some population data are desirable. Therefore, this short technical note describes allele and genotype data for HUMTH01 alleles from a Spanish sample population and compares these findings with that observed in another Caucasian (American) sample population [*1,2*].

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Materials and Methods

Whole venous blood samples were drawn in EDTA-vacutainer tubes from 120 unrelated Spanish caucasians living in Andalucia (southern Spain). The DNA was isolated from the blood by organic extraction, as described by Brinkmann et al. [*4*] and quantified by slot-blot analysis using the probe D17Z1, following the procedure of Waye et al. [*5*].

The primers were 5′ -GTGGGCTGAAAAGCTCCCGATTAT-3′ and 5′ -ATTCAAAGG-GTATCTGGGCTCTGG-3′ [*1,2*]. The components of the PCR were 1 ng of genomic DNA, 1 μL of each dNTP (10 mM stocks), 5 μL of 10X PCR buffer (Perkin-Elmer), 1 μL of each primer (from 12.5 μM stock solutions), and 2.5 units of Taq polymerase. The final volume was brought to 50 μL with sterile doubled distilled H_2O.

The PCR was carried out in a Perkin-Elmer 9600 Thermal Cycler. The cycles were 95°C for 45 s for denaturing, primer annealing at 60°C for 30 s, and primer extension at 72°C for 30 s. The total number of cycles was 27.

After PCR the amplified alleles were separated in a discontinuous horizontal ultrathin-layer polyacrylamide gel (7% T, 5% C). The cross-linker was piperazine di-acrylamide, as described by Budowle et al. [*6*]. The gel buffer was 60 mM tris-formate, pH 9.0. The electrode buffer was 0.28M tris-borate pH 9.0, containing a final concentration of 0.01% of bromophenol blue to serve as dye marker for the moving boundary [*6*]. Paper blotting strips (1 cm × 13.5 cm) (BRL, Gaithersburg, MD) were soaked in the electrode buffer and excess buffer was removed by lightly blotting with paper towels. The paper strips (two layers thick) were placed on the anodal and cathodal ends of the polyacrylamide gel approximately 20 cm apart from each other.

Five μL of amplified product were applied to fiber glass application tabs (2.5 × 5mm). The tabs were placed on the gel surface one cm from the cathode. An EC apparatus (EC Corporation, Saint Petersburg, FL,) was used for electrophoresis. The electrophoresis power supply was set at 600 V, 20 mA, and maximum watts. The temperature of the electrophoretic cooling platen was maintained at 15°C. The electrophoretic process was stopped when the moving boundary migrated to the anodal plug.

The alleles were visualized using a silver staining procedure, according to Budowle et al. [*6*].

Results and Discussion

Since allele are codominant, and all genotypes are distinguishable, frequencies were estimated by the traditional count method. The allele frequencies and genotype distribution are shown in Fig. 1 and Tables 1 and 2.

The alleles were designated 6-11, based on the number of tandem repeats per allele reported by Edwards et al. [*1,2*] and by comparison with a control cell line D.N.A. (K562). There were six alleles detected in 120 unrelated Spanish Caucasians, with frequencies ranging from 0.013 (allele 11) to 0.254 (allele 10).

Recently, a variant called 9.3, which is a relatively common HUMTHO1 allele in Caucasians according to Hochmeister et al. [*7*], has been reported. The 9.3 allele is 1 base pair smaller in size than the 10 allele, and by using horizontal 20 cm long acrylamide gels, allele 9.3 could not be distinguished from allele 10. Thus, the 9.3 and 10 alleles were pooled and designated as a 10.

There were 17 out of a possible 21 genotypes observed in this Spanish sample population (Table 2). The power of discrimination (PD), according to Fisher's formula [*8*], was 0.92. The observed heterozygosity was 75.8%, which is not significantly different from its expectations, 80.1%, under the Hardy-Weinberg assumption ($P = 0.245$).

The genotype distribution (Table 2) observed in this sample of the Spanish gene pool met Hardy-Weinberg expectations, as judged by the three currently employed test criteria

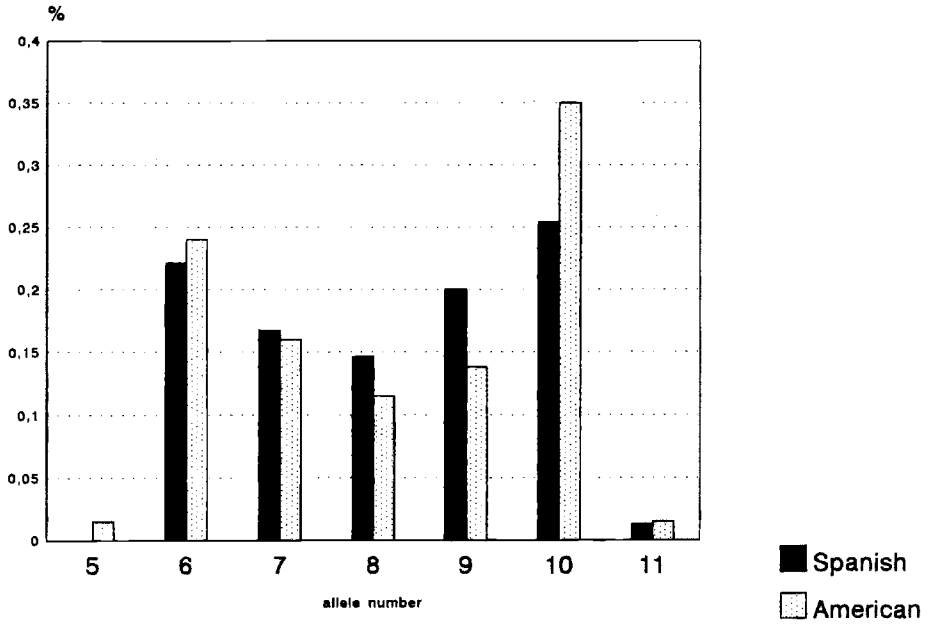

FIG. 1—*Comparison of HUMTH01 allele frequencies in Spanish and American (USA) caucasians* [2].

that can be used for analyzing hypervariable loci. First, the test based on total number of heterozygotes and homozygotes gives a chi-square value of 1.35 with 1 df (based on the unbiased estimate of the heterozygosity [*9,10*] at this locus in the sample, 80.1%), which is not significant ($P = 0.245$). Second, comparison of the frequencies of each specific genotype with their expectations under the Hardy-Weinberg assumptions yields a log likelihood ratio test criterion ($-2\ln L$) of 15.75, which is also not statistically significant ($P = 0.411$, determined empirically as well as using the large sample chi-square test with 15 df [*11,12*]. Third, Guo and Thompson's [*13*] exact test, with 2.000 replications of permutations of alleles, yields an empirical probability of 0.379. These data demonstrate that there is no detectable deviation from Hardy-Weinberg expectations for HUMTH01 in the Spanish sample population.

After running a number ($n = 8$) of common control samples to ensure a similar allele designation, the Spanish data were compared with an American Caucasian sample population [*1,2*] using a R × C contingency table χ^2 test for homogeneity (the program was kindly

TABLE 1—*HUMTH01 allele frequencies in a sample of 120 unrelated Spanish caucasians.*

Allele	Frequency ± s.e.
6	22.08 ± 2.68
7	16.67 ± 2.41
8	14.58 ± 2.28
9	20.00 ± 2.58
10	25.42 ± 2.81
11	1.25 ± 0.08

TABLE 2—*HUMTH01 genotypes in a sample of 120 unrelated Spanish caucasians.*

Genotype	Observed	Expected
6,6	5	5.86
6,7	11	8.85
6,8	7	7.74
6,9	11	10.61
6,10	12	13.47
6,11	2	0.69
7,7	5	3.35
7,8	3	5.85
7,9	6	8.02
7,10	9	10.18
7,11	1	0.52
8,8	6	2.56
8,9	7	7.01
8,10	6	8.90
9,9	4	4.80
9,10	16	12.19
10,10	9	7.74
all other genotypes	0	1.66
Total	120	120.00

provided by G. Carmody, Carleton University, Ottawa, Canada). The allele frequency distributions were similar ($P = 0.163$) (Fig. 1).

In conclusion, HUMTH01 appears to be an informative genetic marker for identity testing purposes in the Spanish population, as well. Since as little as 1 ng of human genomic DNA was required for PCR, it would appear that sensitivity of detection would not be a serious concern for typing this locus. Furthermore, Brinkmann, et al. [*9*] recently reported successful typing of HUMTH01 from DNA derived from urine. This suggests that HUMTH01 could be useful for genetic characterization of forensic biospecimens. We currently are investigating further the forensic utility of HUMTH01.

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