Investigation of the STR locus HUMTH01 using PCR in Caucasian samples from England and Galicia, NW Spain

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INTRODUCTION

Short tandem repeat (STR) loci consist of variable numbers of tandemly repeated units (VNTRs) which are typically dimeric, trimeric, tetrameric or pentameric in length. The loci are widespread throughout the genome and normally display a discrete number of alleles. Unlike other types of fragment length polymorphism, they can often be successfully amplified from degraded, low molecular weight DNA, since loci are shorter. With such characteristics STR loci would appear to be ideal candidates for use in forensic analyses.

HUMTH01 is an STR locus, found within intron 1 of the tyrosine hydrolase gene located at chromosome 11p15.5 - p15. It consists of variable numbers, from 5 to 11, of AATG repeat units, giving 7 alleles ranging in size from 187 - 211 base pairs using the listed primers.

A survey of unrelated Caucasians, from the UK and from Galicia (NW Spain), was performed and the populations compared. The genotyping technique used on the UK samples employed agarose electrophoresis, whilst the samples from Galicia were tested using polyacrylamide electrophoresis. The genetic stability of HUMTH01 and its applicability as a polymorphic marker in paternity analysis was investigated by constructing false family trios.

MATERIALS AND METHODS

Blood samples from 200 UK and 210 Galician Caucasians were used. Some of the samples were from paternity cases. Mother-child pairs from these cases were used to construct a total of 80 false family trios (30 from the UK and 50 from Galicia).

DNA was extracted directly from whole blood or bloodstains using a chelating resin (Sigma Chemical Co) following the procedure developed by Singer-Sam et al. (Amplifications, 3, 11, 1989) with extracts being used in the PCR reaction mix. Amplification was performed using the 24mer primers described by Edwards et al. (Am J Hum Genet, 49, 746-756, 1991) and synthesized by the phosphoramidite method in a 380A DNA synthesizer and purified through OPC columns (Applied Biosystems). The primer sequences were:

5' GTG/GGC/TGA/AAA/GCT/CCC/GAT/TAT 3' (AATG strand)
5' ATT/CAA/AGG/GTA/TCT/GGG/CTC/TGG 3' (TTAC strand)

A 30 cycle PCR reaction was used: denaturation at 94°C, 45 sec; annealing at 60°C, 30 sec; elongation at 72°C, 30 sec. In a total reaction volume of 25µl, 10µl of DNA extract was amplified with 0.25µMx2 primers and 200µMx4 nucleotides with 1 unit of Taq1 DNA polymerase. Reaction buffer (10x) consisted of 100mM tris-HCl pH8.3, 500mM KCl, 15mM MgCl and 0.01% gelatin. The quality of amplification was assessed on an agarose minigel.

Agarose gels (8cm x 10.5cm) were prepared using 4.5% MetaPhor (FMC Bioproducts) in 40ml 1xTAE, poured at 70°C. The gel and 1xTAE tank buffer were cooled to 4°C before the run and 20µl samples loaded into 1mmx5mm wells with 5x loading buffer.

Gels were run at 80V for 5 hours. Tank buffer (at 4°C) was replaced three times during run. Ethidium bromide (0.005%) concentration, was added to buffers.

Polyacrylamide gels, cross-linked with bisacrylamide and polymerized with TEMED/ammonium persulphate, were made to a concentration of C=10, T=3.3 using 33mM tris-sulphate pH9.0 gel buffer. 17cm wide x 22cm long x 0.5mm gels, with 5mm x 1.5mm x 0.25mm wells, 2cm from end, were cast on glass-supported Gelbond (FMC Bioproducts). 1cm thick electrodes were prepared from 2% agarose in 0.52M tris-borate buffer, pH8.5. 4µl samples were pipetted into the wells and run at 15°C at a constant 450V, 25mA for 2½ hr after a 45 min pre-run. Gels were silver stained using a standard protocol (Allen R et al., BioTechniques, 7, 736-744, 1989).

A seven allele ladder, supplied as part of a European DNA Profiling (EDNAP) collaborative exercise, was successfully amplified, but problems were encountered in obtaining clearly defined bands for all seven alleles. Therefore, for routine genotyping, a 6.8.10 allele ladder, constructed by combining authenticated heterozygote and homozygote controls, was run at regular intervals across the gel.

RESULTS

Typical electrophoretic results are shown in Figs 1 and 2. Genotype frequencies are listed in Table 1. Fig 3 shows the allele frequencies. Fifty samples, tested in duplicate using both electrophoretic techniques, showed no discrepancies in genotype assignment. There was no evidence of departure from Hardy-Weinberg equilibrium or allele dropout. The discrimination power of the HUMTH01 locus was estimated as 90.3% (UK) and 91.8% (Galicia).

50% of the UK false family trios and 56% of the Galician false family trios, showed an exclusion of paternity. Expected exclusion rates were 53.7% and 57.0% respectively. The 80 meioses observed in the mother-child pairs showed normal segregation with no examples of mutation or non-Mendelian inheritance.

CONCLUSIONS

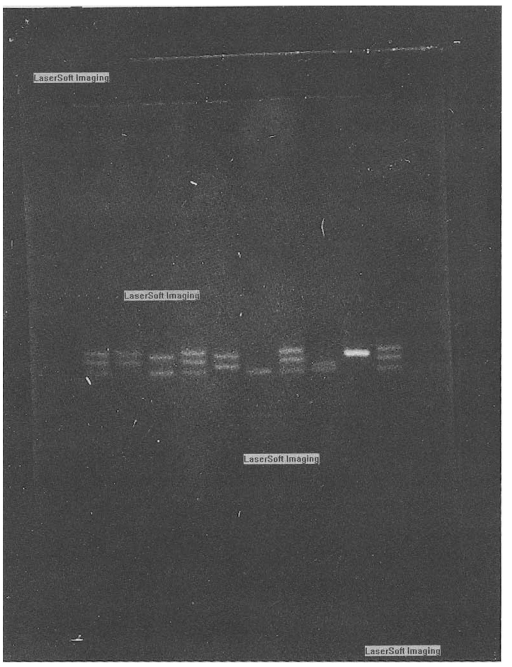
HUMTH01 is an informative polymorphism for which population statistics are readily established. The use of a 6-8-10 allelic ladder proved a simple and effective control for routine typing. Agarose electrophoresis, using a high-sieving gel such as MetaPhor, provides a simple and rapid technique for separating STRs and has the advantage of greater consistency between gels, when compared with polyacrylamide gels. Polyacrylamide electrophoresis, followed by silver staining, is, however, more sensitive than agarose at detecting low yield of product after amplification. Used in combination with other similar STR loci, HUMTH01 would provide a powerful extension to the analytical tools being used in forensic science today.

Genotype	UK observed	UK expected	Galicia observed	Galicia expected
10.10	25	24.15	21	20.74
9.10	17	19.81	23	24.20
8.10	13	11.81	18	16.97
7.10	27	23.28	25	22.31
6.10	32	35.44	24	26.71
9.9	6	4.06*	6	7.06
8.9	2	4.84*	10	9.90
7.9	7	9.55	13	13.02
6.9	18	14.53	19	15.58
8.8	1	1.44*	4	3.47*
7.8	6	5.69	6	9.13
6.8	11	8.67	12	10.93
7.7	5	5.61	6	6.00
6.7	17	17.08	14	14.37
6.6	12	13.01	8	8.60
5.9	1	0.14*	0	
5.7	0		1	0.17*
Other 5 types	0	0.86*	0	0.83*

Table 1: Population survey of UK (agarose) and Galician (polyacrylamide) Caucasians

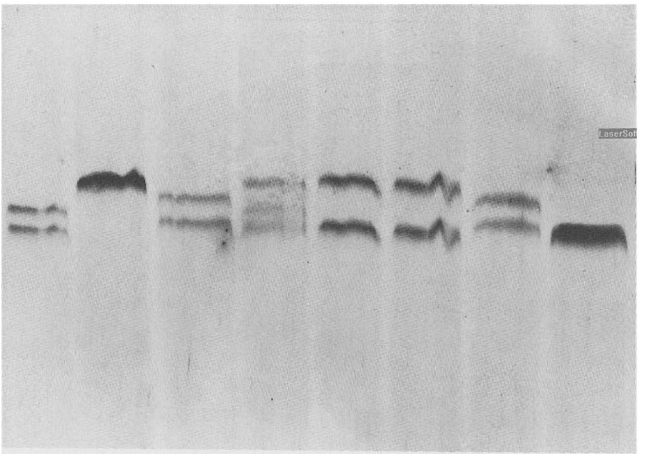
Chi-square 3.39, df=9, p>0.05 (UK); 3.93, df=7, p>0.05 (Galicia) (*classes pooled)

Heterozygosity = 0.755 (0.764 expected) (UK); 0.786 (0.785 expected) (Galicia)



Lane	1	6.8.10 ladder
	2	8.10
	3	6.9
	4	6.8.10 ladder
	5	7.9
	6	6.6
	7	6.8.10 ladder
	8	6.7
	9	9.9
	10	6.8.10 ladder

Fig1: HUMTH01 genotypes obtained with agarose electrophoresis



Lane	1	10.10
	2	7.9
	3	6.10
	4	6.10
	5	6.8.10 ladder
	6	7.9
	7	6.6
	8	8.10

Fig2: HUMTH01 genotypes obtained with polyacrylamide electrophoresis

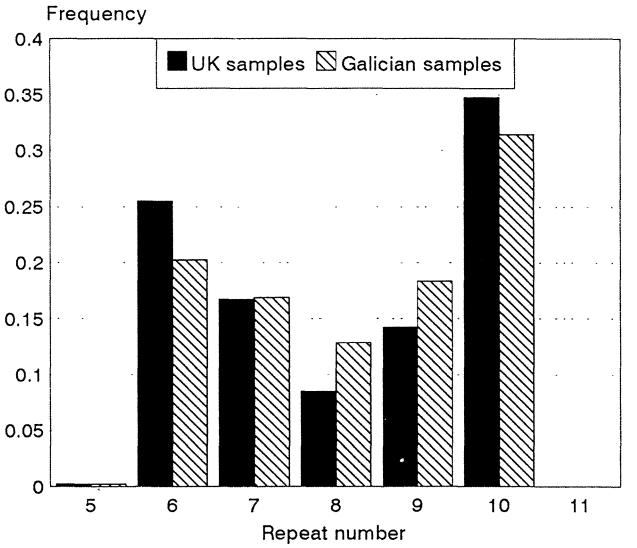


Fig 3: HUMTH01 allele frequencies