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SHORT COMMUNICATION

M. Gené · E. Huguet · P. Moreno · C. Sánchez
A. Carracedo · J. Corbella

# Population study of the STRs HUMTH01 (including a new variant) and HUMVWA31A in Catalonia (northeast Spain)

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**Abstract** Allele and genotype frequencies for 2 short tandem repeat loci were determined in a population sample from Catalonia (Spain) using the polymerase chain reaction. After denaturing PAG electrophoresis, seven common and one variant (13.3) alleles were identified for HUMTH01 in a sample of 234 unrelated individuals, and seven alleles were found for HUMVWA31A in 162 individuals. No deviation from Hardy-Weinberg equilibrium was found. The observed heterozygosities are 76.92 and 79.62 respectively. The discrimination power determined for the individual loci is 0.928 and 0.937 respectively.

**Key words** STR · HUMTH01 · HUMVWA31A · Population genetics · Paternity testing

## Introduction

Tetrameric short tandem repeats (STRs) represent a rich source of highly polymorphic markers in the human genome that may be studied with the polymerase chain reaction (PCR). In order to obtain data from a western Mediterranean population, the allele frequencies and genotype distribution of HUMTH01 and HUMVWA31A polymorphisms were studied in a sample population from Catalonia (northeast Spain).

M. Gené (✉) · E. Huguet · C. Sánchez · J. Corbella
Forensic Genetics Laboratory, Department of Legal Medicine, Faculty of Medicine, University of Barcelona, Avda. Joan XXIII s/n, E-08028 Barcelona, Spain

P. Moreno
Department of Animal Biology, Section of Anthropology, Faculty of Biology, University of Barcelona, Avda. Diagonal 645, E-08028 Barcelona, Spain

A. Carracedo
Institute of Legal Medicine, Faculty of Medicine, E-15705 Santiago de Compostela, Galicia, Spain

## Materials and methods

DNA was extracted from healthy unrelated individuals living in Catalonia, using the phenol-chloroform-isoamyl alcohol method [1]. Amplification of HUMTH01 and HUMVWA31A was achieved using the primers described by Edwards et al. [2] and Kimpton et al. [3] respectively. The reaction assay and the amplification conditions were carried out as described by Wiegand et al. [4] and Möller et al. [5]. Singleplex PCR amplifications were performed in a Perkin Elmer PE1 Thermocycler, together with negative and positive control samples.

Separation was carried out on 6% (w/v acrylamide/bisacrylamide) polyacrylamide denaturing high-performance DNA sequencing gels (Ready Mix Gel ALF grade, Pharmacia). All fresh PCR products were typed twice. The electrophoresis was carried out on the Automated Laser Fluorescent (ALF) DNA Sequencer (Pharmacia) at 1450 V, 38 mA, 45 W and 50° C with laser power at 3 mW for 220 min.

Amplified DNA was mixed with internal fluorescent labelled standard sizers. External lane ladders were also used for adjustment. A cocktail of samples containing all observed alleles was used as an allelic ladder.

A standard $\chi^2$ goodness-of-fit statistic was calculated to assess conformity to Hardy-Weinberg expectations. Where expected genotype frequencies were less than 5, these were pooled collectively according to the system outlined by Dickinson-Gibbons [6]. The Catalonia data were compared with Spanish and other European sample populations using an RxC contingency table $\chi^2$ test for homogeneity.

## Results and discussion

The distributions of observed genotypes and allele frequencies for HUMTH01 and HUMVWA31A in a population from Catalonia are shown in Tables 1 and 2, respectively. The HUMTH01 analysis revealed 20 genotypes, representing products of the seven common alleles and one variant allele. Alleles 6 and 9.3 showed the highest frequencies. The variant allele designated 13.3 was located above the allelic ladder, with a size of 215 bp. The 13.3 allele was isolated in agarose low melting gel electrophoresis, purified and used as a template to sequence by the direct sequencing cycle method. The observed structure of the tandem repeat is: $(CATT)_3(CAT)_1(CATT)_8(CGTT)_1(CATT)_1$ (Fig. 1).

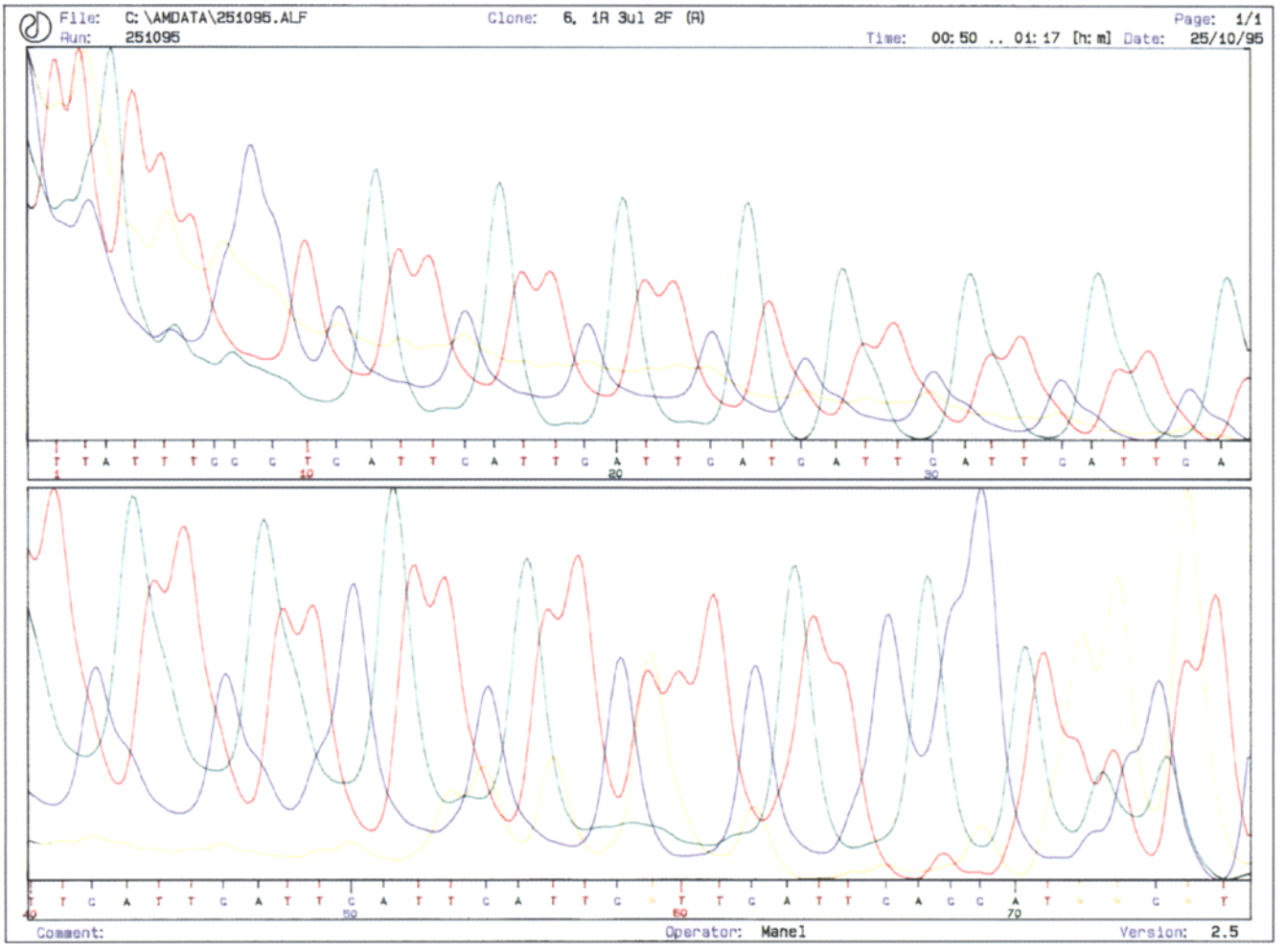


**Fig. 1** HUMTH01 new variant sequence: $(CATT)_3(CAT)_1(CATT)_8(CGTT)_1(CATT)_1$

**Table 1** Observed allele frequency and genotype values for HUMTH01 in 234 individuals from Catalonia

| Genotypes | Observed | Genotypes | Observed |
|---|---|---|---|
| 6– 6 | 15 | 8 – 8 | 8 |
| 6– 7 | 14 | 8 – 9 | 12 |
| 6– 8 | 13 | 8 – 9.3 | 15 |
| 6– 9 | 21 | 9 – 9 | 9 |
| 6– 9.3 | 31 | 9 – 9.3 | 21 |
| 6–10 | 4 | 9 –11 | 1 |
| 7– 7 | 4 | 9 –13.3 | 1 |
| 7– 8 | 13 | 9 –10 | 1 |
| 7– 9 | 18 | 9.3–9.3 | 17 |
| 7– 9.3 | 15 | 10 –10 | 1 |

| | |
|---|---|
| Index heterozygosity | = 76.9 |
| Power discrimination | = 0.93 |
| Mean exclusion chance | = 0.59 |
| Essen-Möller mean value | = 9.55 |

Allele frequencies (f) 6: 0.241; 7: 0.145; 8: 0.147; 9: 0.199; 9.3: 0.248; 10: 0.015; 11: 0.002, 13.3: 0.002

**Table 2** Observed allele frequency and genotype values for HUMVWA31A in 162 individuals from Catalonia

| Genotype | Observed | Genotype | Observed |
|---|---|---|---|
| 14–14 | 1 | 15–20 | 1 |
| 14–15 | 3 | 16–16 | 8 |
| 14–16 | 8 | 16–17 | 20 |
| 14–17 | 12 | 16–18 | 12 |
| 14–18 | 5 | 16–19 | 4 |
| 14–19 | 4 | 17–17 | 12 |
| 14–20 | 1 | 17–18 | 13 |
| 15–15 | 2 | 17–19 | 8 |
| 15–16 | 14 | 18–18 | 9 |
| 15–17 | 6 | 18–19 | 7 |
| 15–18 | 7 | 19–19 | 1 |

| | |
|---|---|
| Index heterozygosity | = 79.6 |
| Power discrimination | = 0.94 |
| Mean exclusion chance | = 0.62 |
| Essen-Möller mean value | = 9.52 |

Allele frequencies (f) 14: 0.108; 15: 0.120; 16: 0.228; 17: 0.256; 18: 0.191; 19: 0.089; 20: 0.006

The HUMVWA31A polymorphism showed 23 genotypes representing products of seven alleles, of which alleles 17 and 16 showed the highest frequency. The distribution of genotypes for HUMTH01 and HUMVWA31A, is in Hardy-Weinberg equilibrium (TH01: $\chi^2 = 7.352$, df = 10, $P = 0.675$; VWA: $\chi^2 = 10.267$, df = 9, $P = 0.329$).

The data from Catalonia for the HUMTH01 locus were compared with other European populations. No significant differences were observed with populations from Andalucia [7], Galicia [8], Italy [9] and Switzerland [10].

For HUMVWA31A, comparisons between Catalonia and other European populations show good agreement with those from France [11], Norway [12], northwest Germany [5] and the United Kingdom [13].

From these results it can be concluded that there is general uniformity for the HUMTH01 and HUMVWA31A systems in Caucasoid populations.

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