HumTH01 ALLELE FREQUENCIES IN ITALY - REPORT OF THE GEFI COLLABORATIVE STUDY

E. d'Aloja* and R. Domenici✦ editors

(*) Immunohematology laboratory, Istituto Medicina Legale, Università Cattolica, Largo F.Vito,1, 00168 Roma, Italy; (✦) Istituto Medicina Legale, Università di Pisa

INTRODUCTION

In order to achieve an harmonisation among protocols for STRs typing and create a national database, since 1991 the GEFI (Gruppo Ematologi Forensi Italiani) promotes (Pascali 1994, Presciuttini 1994) every two years an inter-laboratory exercise to identify the polymorphic loci more suitable for exchange and comparison of forensic data.

In 1993 two markers of different classes were chosen (namely, D1S80 as a Variable Number of Tandem Repeats, VNTR, and HumTH01 as a Short Tandem Repeats, STRs) according with the aim to investigate the reproducibility of several typing procedure.

Participants were asked to use the typing methodology currently employed in their labs in forensic casework and to identify two bloodstains at both loci investigated.

A comparison of the different methods and the allele frequencies resulted for HumTH01 locus (11p15,5-p15) (Edwards 1991) are here reported.

MATERIALS AND METHODS

Twenty-two laboratories were invited to this experiment covering almost all the area of Italian Peninsula, with the only exclusion of Sardinia.

Each participating laboratory was asked to analyse at least 50 unrelated individuals, resident in the region, and was supplied with two "unknown" samples, an HumTH01 allelic ladder (containing all the alleles from 6 to 11 but 9.3) and the relevant primers (according to Edwards).

Laboratories were set free to use whichever methods for amplification, electrophoresis and detection they had chosen for routine analysis.

However a basic protocol of amplification (Edwards with minor modifications) and separation (PAGE 8% T and 5% C) was suggested by the group responsible for the allelic ladder supply.

Allele designation was achieved by side to side comparison with the allelic laddder supplied.

All groups involved in paternity analysis were aldo asked to give information about the mutation rate at this locus.

RESULTS

Results are shown in table n.1 and in Fig.1 as the following allele frequencies were ascertained.

The allelic designation given by the participating labs was not always uniform due to the fact that 5 out 15 laboratories pooled togheter the 9.3 and 10 allele frequencies. In the present table only results concerning 1305 individuals were considered, representing the population sample typed for all the eigth major alleles.

Observed genotypes

Gen	Obs	Gen	Obs	Gen	Obs	Gen	Obs
6 - 6	63	**6 - 7**	103	**7 - 8**	55	**8 - 10**	5
7 - 7	29	**6 - 8**	89	**7 - 9**	70	**9 - 9.3**	120
8 - 8	17	**6 - 9**	149	**7 - 9.3**	122	**9 - 10**	9
9 - 9	46	**6 - 9.3**	148	**7 - 10**	11	**9.3 -10**	6
9.3-9.3	87	**6 - 10**	13	**8 - 9**	60	**9.3 - 11**	1
10 -10	1	**6 - 11**	1	**8 - 9.3**	98	**5 - 9.3**	2

Allele frequencies

Allele	Frequency	Allele	Frequency
5	**0.001**	**9**	**0.192**
6	**0.242**	**9.3**	**0.257**
7	**0.160**	**10**	**0.018**
8	**0.131**	**11**	**0.001**

COMMENTS

Results were returned by fifteen laboratories for a total number of 1611 unrelated individuals and over 200 meiosis.

A number of different electrophoresis and detection systems were utilised by the participants. The majority of laboratories employed native polyacrilamide gel as electrophoretic system and silver staining as detection (86%); denaturing polyacrilamide gels using either an automated laser fluorescent apparatus (7%) or radioactive detection (7%) were also employed.

Eight alleles, ranging from allele five (two observations) to the one containing eleven repeats (two observations), were detected although not all the laboratory showed the same ability to clearly identify allele 9.3 and 10, mainly due to the choice of the native separation method employed.

All GEFI laboratories participating in this exercise successfully typed the DNA from the two 'unknown' stains at the HumTH01 locus. This was achieved despite variation in the amplification, electrophoresis and detection systems utilised by individual laboratories. The results demonstrate that this simple STR locus is ideal for standardisation in the forensic community, where different laboratories have varying resources.

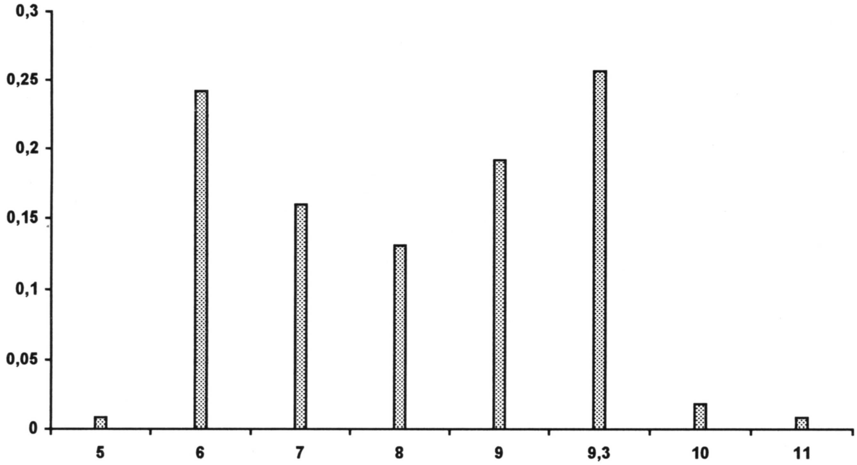
REFERENCES

Edwards A, Civitello A, Hammond HA, Caskey CT (1991). DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am. J. Hum. Genet. 49:746-756.

Pascali VL, Domenici R (1994) Garda1 (D2S44 SBA) - Harmonization of protocols and a collaborative database. In: W. Bar, A. Fiori, U. Rossi (eds.) Advances in Forensic Haemogenetics 5. Springer-Verlag, Berlin Heidelberg New York pg 545.

Presciuttini S, De Stefano F (1994) An Italian collaborative study on the HLA-DQA1 locus (GEFI's "Garda 1" project). In: W. Bar, A. Fiori, U. Rossi (eds.) Advances in Forensic Haemogenetics 5. Springer-Verlag, Berlin Heidelberg New York pg 438.

Fig. 1: HumTH01 Italian Sample Population - Allele frequencies



List of participating laboratories

Istituto di Medicina Legale, Università di **Ancona**; Istituto di Medicina Legale, Università di **Bologna**; Istituto di Medicina Legale, Università di **Brescia**; Istituto di Medicina Legale, Università di **Catania**; Istituto di Medicina Legale, Università di **Genova**; Istituto di Medicina Legale, Università di **Modena**; Istituto di Medicina Legale, Università di **Padova**; Istituto di Medicina Legale, Università di **Parma**; Istituto di Medicina Legale, Università di **Pavia**; Istituto di Medicina Legale, Università di **Pisa**; Istituto di Medicina Legale, Università di **Roma UCSC**; Istituto di Medicina Legale, Università di **Roma "La Sapienza"**; Istituto di Medicina Legale, Università di **Perugia** (sezione distaccata di **Terni**); Istituto di Medicina Legale, Università di **Trieste**; Istituto di Medicina Legale, Università di **Verona**.