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SHORT COMMUNICATION

L. Casarino · A. Mannucci · C. P. Kimpton
S. Presciuttini · G. Bruni · M. G. Costa F. De Stefano

Forensic evaluation of HUMCD4: an Italian database

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Abstract The YTTTC pentanucleotide short tandem repeat polymorphism HumCD4 was studied in an Italian population sample. PCR products were compared to an allelic ladder by manual PAGE and silver staining. A total of 6 alleles ranging from 5 to 12 repeats were represented in the analysed sample, of which 3 alleles (10, 6 and 5 repeats) were predominant and displayed a combined frequency of 0.91. Successful amplification was obtained from different sources such as blood and urine stains, teeth and paraffin embedded tissues. Results were also determined in cases of severely degraded DNA. We consider that the HUMCD4 polymorphism may be a useful tool for individual identification, paternity testing, population studies and have also employed this locus to monitor engraftment of bone marrow transplantation.

Key words PCR · HUMCD4 · Identification · Paternity testing

Introduction

The HUMCD4 polymorphism was first described by Edwards et al. (1991) and further studies demonstrated the reliability of automated profiling of its alleles (Kimpton et al. 1993). Urquhart et al. (1994) sequenced a range of alleles and described the locus as a simple repeat STR although one allele displayed one repeat unit sequence CTTTC instead of the TTTTC consensus sequence. The low molecular weight and allelic variability of HUMCD4 indicate that it may be useful in forensic analysis (Kurosaky et al. 1993), paternity determination and genetic diversity studies. Moreover, for diagnostic purposes, the HUMCD4 marker may also have great usefulness in monitoring engraftment of allogenic bone marrow transplantation.

In order to establish the forensic utility of this locus an allelic ladder was created as recommended by ISFH (1994), allele genotype frequencies on a sample of 134 unrelated individuals were studied and DNA extracted from several different kinds of stain was typed.

L. Casarino · A. Mannucci (✉) · G. Bruni · M. G. Costa
F. De Stefano
Istituto di Medicina Legale, Università di Genova,
via De Toni 12, I-16132 Genova, Italy

C. P. Kimpton
Forensic Science Service, Priory House, Gooch St. North,
Birmingham B5 6QQ, UK

S. Presciuttini
Departamento Scienze Ambiente e Territorio, Università di Pisa,
via San Giuseppe 22, I-56100 Pisa, Italy

Materials and methods

DNA samples were obtained from 134 unrelated individuals from the Genova area. Blood and urine stains, teeth and paraffin embedded tissues from casework were used. In order to confirm mendelian inheritance of alleles, 102 meioses from paternity tests were studied and exclusions were confirmed by other DNA polymorphisms. Bone marrow transplantation engraftments from the Ematology Division of the Ospedale S. Martino, Genova, were kept under surveillance for 1 year.

Phenol/chlorophorm extraction was used for fresh blood samples. Paraffin embedded tissues and teeth were extracted according to the protocols described by Goelz et al. (1985) and Yamada et al. (1989). DNA from blood and urine stains was Chelex extracted. For amplification 10 ng DNA or 10 µl of Chelex extraction were used in a final volume of 50 µl, using 12.5 pmoles of the primers stated by Edwards et al. (1991) and PCR was carried out for 32 cycles, 94°C for 45 s, 60°C for 60 s and 72°C for 30 s, on a GeneAmp PCR System 9600 thermal cycler (Perkin Elmer). PCR amplification for automated profiling was performed with primer 1 (CTTTT strand), coupled via an aminohexyl linker to the fluorescent dye "FAM" (5-carboxy-fluorescein). The conditions were: 28 cycles at 94°C for 45 s, 60°C for 60 s, 72°C for 60 s.

Aliquots of 1–5 µl from each amplification reaction were loaded on a 8% polyacrylamide native gel, electrophoresed for 90 min at constant current (30 mA) and silver stained (Bassam et al. 1991). Determination of alleles was made by comparing bands to an allelic ladder. For automated detection, aliquots of 1–4 µl of each amplification were loaded onto standard 6% polyacrylamide denaturing sequencing gels and were run for 3.5 h at constant power (42 W) on an Applied Biosystems automated 373A DNA sequencer.

Fragment sizes were determined automatically using Genescan 672 software (ABD), employing the Local Southern method. The

single components of the allelic ladder were compared to sequenced alleles.

Statistical Analysis. Hardy-Weinberg equilibrium was tested by the chi-squared method. Smith's test (Smith 1986) was used to cover expectations even smaller than 0.5. Gene diversity and standard error were computed as $1-\Sigma pi^2$ and $\{2[\Sigma pi^3-(\Sigma pi^2)^2]/N\}$ respectively, pi being the frequency of allele i and N the total number of sampled individuals (Nei 1987). The fixation index F (measuring deviation from panmixia), was computed as $1-Het/(1-\Sigma pi^2)N$ where Het is the total number of heterozygous genotypes.

Results

A total of six different alleles were observed from 134 blood samples of which three (5, 6 and 10 repeats) were abundant and uniformly represented and their gene frequencies were 0.35, 0.28 and 0.27, respectively. The remaining alleles were rare with frequencies below 0.05. This distribution is similar to that reported for other Caucasian populations (Kimpton et al. 1993).

A reamplifiable allelic ladder was developed from individuals with the desired genotypes. Automated profiling of the ladder and corunning with previously sequenced alleles (Urquhart et al. 1994) confirmed the ladder fragments to be 2 bp shorter than that reported by Edwards et al. (1991) (Table 1). According to data described elsewhere (Urquhart et al. 1994), alleles were designated by the number of repeat units present (ISFH Recommendations 1994).

Hardy-Weinberg equilibrium was satisfied by the chi-squared test when considering all the possible genotypes (21 classes, 15 d.f.; $\chi^2 = 15.22$, P = 43.6%). Even when Smith's test (Smith 1986) was employed we found good agreement (z = 0.13, P = 44.8%). However, in considering genotype distribution (Table 1) it emerged that homozygotes were generally more frequent than expected. In fact, the homozygosity test was significant for an excess of homozygotes (Total observed 49, Expected 37.6, $\chi^2 = 4.76$, d.f. = 1, P = 0.03). With these values, the fixation index F was 11.8%, indicating a dramatic deviation from random union of gametes. Gene diversity was 0.719 ± 0.010.

Family studies confirmed mendelian inheritance of alleles. No mutations were observed in 102 meioses and cases of paternity exclusions were confirmed by other DNA polymorphisms (Fig. 1).

Analysis of biological remains from casework (blood, urine, teeth and paraffin embedded tissues) showed clear results and alleles were easily detectable even when severely degraded DNA was recovered.

In addition more than 20 cases of bone marrow engraftment were succesfully surveyed by this locus for one

Table 1 HumCD4 in an Italian population A) Allelic ladder bands with molecular weight, number of repeats and frequency. B) Genotype frequencies with expected values

A)

Ladder band	True size*	N° of repeats**	Frequency
1	86 (88)	5	0.3507
2	91 (93)	6	0.2836
3	106 (108)	9	0.0410
4	111 (113)	10	0.2724
5	116 (118)	11	0.0373
6	121 (123)	12	0.0149

* N° in parenthesis denotes allele sizes in Edwards et al. (1991)
** Repeat Unit = YTTTC, where Y is either C or T

B)

Genotype N° of repeats	bp	Observed	Expected
12/12	121/121	0	0.0
12/11	121/116	0	0.1
12/10	121/111	1	1.1
12/ 9	121/106	0	0.2
12/ 6	121/ 91	3	1.1
12/ 5	121/ 86	0	1.4
11/11	116/116	0	0.2
11/10	116/111	3	2.7
11/ 9	116/106	0	0.4
11/ 6	116/ 91	3	2.8
11/ 5	116/ 86	4	3.5
10/10	111/111	16	9.9
10/ 9	111/106	2	3.0
10/ 6	111/ 91	14	20.7
10/ 5	111/ 86	21	25.6
9/ 9	106/106	0	0.2
9/ 6	106/ 91	5	3.1
9/ 5	106/ 86	4	3.9
6/ 6	91/ 91	13	10.8
6/ 5	91/ 86	25	26.7
5/ 5	86/ 86	20	16.5
Total		134	134.0

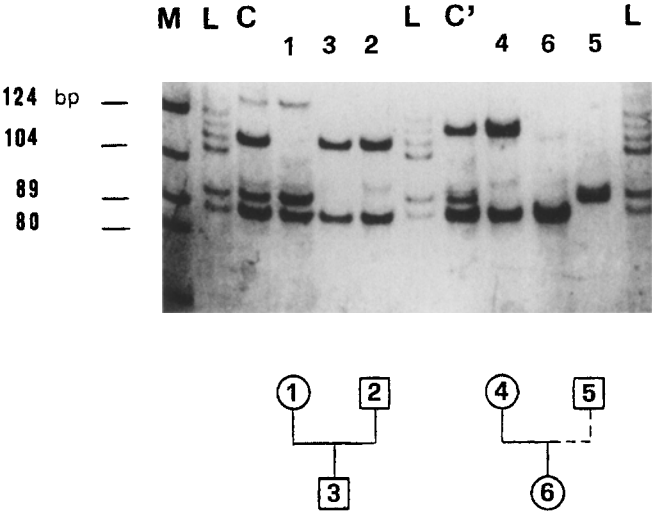

Fig. 1 8% native PAGE of PCR products from paternity tests. The ideograms show the parental trees, family 1 samples 1–3; family 2, samples 4–6. M, molecular weight marker pBR322/HaeIII digested (Marker V, Boehringer). L: allelic ladder. C: Co-migration of all the samples of each family. The exclusion in family 2 was confirmed by other markers

year. In all the cases results were confirmed by other STR markers.

Discussion

A short pentanucleotide tandem repeat located within the first intron of the human CD4 gene was studied. In the Italian population sample tested, six alleles were identified with a gene diversity of 0.72. This value was a little higher than that already reported in Caucasians by Kimpton et al. (1993). The pattern of gene variation was also very similar to that shown for Caucasians (Kimpton et al. 1993), given that allele 1 of this series is identical to the 5 repeats (86 bp) allele described by Kimpton (unpublished data).

Although the excess of homozygous types reached the significance level of 0.03, no particular genotype was predominantly responsible for this effect, the surplus being distributed over all 3 high-frequency alleles (5, 6 and 10). It is possible that the surplus is due to Wahlund's effect, or population admixture. It should be noted that the data were drawn from an area subjected in the past to a high level of immigration from other Italian regions – e.g. Sardinia – whose gene frequencies for other loci are generally different (Piazza et al. 1989a, b; Walter et al. 1989), although similar frequency distributions for four STR allele were observed in three ethnic groups in which 16 different populations were categorised (Gill and Evett 1995). Another less likely explanation is that our relatively small Italian population sample itself was not representative of the true population of the area sampled. An excess of homozygosity has been observed for another STR locus (HumF13A1) in samples from a British population, but disappeared when the size of the data was increased (Evett et al. in press). Nevertheless a similar pattern of CD4 alleles frequencies was described in Caucasians (Kimpton et al. 1993) but without excess homozygosity. The data from Evett et al. (in press) and these data suggest a larger Italian study may be useful. It is our aim to evaluate more individuals for this locus but if these results are confirmed in larger population samples, they will constitute an interesting subject for micro-evolutionary analyses.

The six alleles observed in this study were mixed to make an allelic ladder and compared with previously sequenced alleles by an automatic sequencer. This allelic ladder is now available for an easy manual classification of alleles, following the ISFH recommendations (1994).

Analysis of biological remains from casework allowed clear definition of alleles even on severely degraded DNA. In analysis of maltreated stains, no evidence of allelic drop out was found and reconfirmation of homozygosity was obtained after renewed DNA extraction and reamplification from fresh blood samples. Moreover results in casework from degraded DNA were consistent for heterozygosity even when the distance between alleles was larger than one repeat. Furthermore, the HUMCD4 marker allowed engraftment testing of allogenic bone marrow transplantations and in all cases results with other markers were consistent.

In conclusion, the results obtained show that the HUMCD4 locus may be considered a useful tool in forensic analysis for identification and in paternity determination. Moreover, the demonstrated variability permits population studies and monitoring of bone marrow transplantation.

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