Int J Legal Med (1998) 111 : 107–109

SHORT COMMUNICATION

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A study on the short tandem repeat systems HumCD4, HumTH01 and HumFIBRA in population samples from Yemen and Egypt

Received: 20 May 1997 / Received in revised form: 1 September 1997

Abstract The short tandem repeat systems (STRs) HumCD4 (CD4), HumTH01 (TH01) and HumFIBRA (FGA) were amplified by the polymerase chain reaction (PCR) on blood samples from 100 unrelated Yemenians and 100 unrelated Egyptians. PCR products were separated on native horizontal discontinuous gel electrophoresis followed by silver staining. The distribution of observed phenotypes did not deviate from Hardy-Weinberg equilibrium. While significant differences between both Arab populations and an European population from Austria were found at all loci, differences between the Egyptian and the Yemenian samples were found only for CD4. In a number of verified Austrian families (TH01: 426 meioses, CD4: 275 meioses, FGA: 144 meioses) no mutations were found. The observation of a TH01 allele consisting of 4 repeats was confirmed by sequencing. Moreover we report the structure of a TH01 allele 6.3 observed in a Hungarian Caucasian population.

Key words HumCD4 · HumTH01 · HumFIBRA · Egypt · Yemen · Population genetics · STR

Introduction

The forensic usefulness of short tandem repeat systems (STRs) such as HumCD4 (CD4) [1], HumTH01 (TH01) [2] and HumFIBRA (FGA) [3] has been demonstrated [4]. For these purposes, however, it is desirable to obtain population data on these polymorphisms in various populations. As no data from Arab populations are available for the STRs CD4 and FGA and only a Moroccan database is available for TH01 [5], these systems were tested in both a Yemenian and an Egyptian population sample. Furthermore, family studies were performed to estimate the frequency of mutations in these STRs in confirmed Austrian families.

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Materials and methods

Sample preparation

Whole blood was obtained by venipuncture from 100 unrelated Yemenians and 100 unrelated Egyptians. Bloodstains were prepared on sterilized cotton cloth and subsequently air dried. The DNA was extracted by a slightly modified alkaline lysis protocol [6] where bloodstains measuring 2×2 mm were lysed in 10 µl 0.2 N NaOH without initial incubation in distilled water and 90 µl 0.04 M Tris buffer pH 7.5 was used to normalize the pH of the extract. Previously extracted DNA samples from paternity cases in Austria (Graz area) were used for the family studies. In all cases the probability of paternity was in excess of 99.75% using 18 conventional serological systems and a varying number of STRs.

PCR typing

Aliquots of 2.5 µl of the extracts were used for amplification without prior quantification. The systems CD4 and TH01 were coamplified in a duplex reaction containing 0.6 µM each CD4 primer [1] and 0.6 µM each TH01 primer [2], $1 \times$ PCR buffer (Finnzymes, Espoo, SF), 2 mM $MgCl_2$, 200 µM each dNTP and 1U of Dynazyme DNA polymerase (Finnzymes, Espoo, SF) diluted to a total volume of 25 µl with double distilled water. Cycling conditions were 30 cycles of 94°–1 min, 62°–1 min and 72°–1.5 min in a Trio-thermoblock (Biometra, Göttingen, FRG).

FGA was amplified in a singleplex reaction containing 1 µM each primer (Primer 1: GCC CCA TAG GTT TTG AAC TCA; Primer 2: TGA TTT GTC TGT AAT TGC CAG), $1 \times$ PCR buffer, 200 µM each nucleotide, 0.5 U of Dynazyme DNA polymerase diluted to a total volume of 25 µl with double distilled water. (Protocol for the 13th collaborative exercise of the German DNA profiling group (GEDNAP XIII); personal communication B. Brinkmann, Münster). Electrophoretic separation and typing were performed using native horizontal polyacrylamide gels as previously

described [7, 8]. Sequenced allelic ladders for all STRs were kindly provided by B. Brinkmann, Münster.

Sequencing

For sequencing, the rare TH01 alleles were eluted from the gels using the QIAEXII kit (Qiagen, FRG) and reamplified with 0.1 μM biotinylated forward primer and 0.1 μM unlabeled reverse primer. Strand separation and single strand sequencing (solid phase) on an A.L.F. automatic sequencer (Pharmacia, Sweden) were conducted as previously described [9].

Statistical analysis

The mean exclusion chance (ME) was calculated according to Krüger et al. [10] and the discriminating power (DP) was calculated as 1-Σ (expected phenotype frequencies)^2 [11]. For checking the Hardy-Weinberg expectations, χ^2-tests were performed (phenotypes with less than 5 observations were pooled). Comparisons of the allele frequencies between different populations were performed using two-way contingency tables. The computer program was kindly provided by G. Carmody, Ottawa.

Results and discussion

The distribution of the observed allele frequencies for the three STRs tested are compared to a European population sample from Austria in Table 1. The Austrian database includes already published data [8, 12, 13] and samples recently typed for the family studies. No deviations from Hardy Weinberg expectations were found for either of the STRs ($P > 0.05$). The forensically relevant parameters calculated for the three loci are given in Table 2. These parameters were comparable between the European and the Arab databases for TH01 and FGA, while those for CD4 were significantly higher in the Arabs. This finding is consistent with the reported direct correlation between the polymorphicity of this locus and the geographic distance of the studied population to Sub-Saharan Africa [14].

Highly significant differences between the European population sample from Austria and the two Arab populations were found for each of the three STRs (Table 3). However, evidence of population heterogeneity between both Arab population samples, one from Africa, one from the Arab peninsula, was found only for CD4. Moreover, in previous studies no significant differences between different Arab populations were found for HLA-DQA1 and D1S80 [15, 16] and for other STRs using the same DNA samples [1]. This finding further supports the suggested suitability of CD4 [8] for assessing the probable race of a stain donor [18, 19].

At the TH01 locus one allele migrating faster than the shortest allele in the ladder (5) was observed in this study. Moreover we want to report another rare TH01 allele migrating between 6 and 7 which was recently observed by one of the authors in a Hungarian Caucasian population sample. Sequencing confirmed that the TH01 allele found in the Arabs consisted of 4 AATG repeat units (Table 4). To our knowledge, no such short allele has yet been reported for humans, while they are common in primates [20]. These nonhuman alleles, however, show several base substitutions in the flanking regions, whereas the flanking regions of our allele coincided perfectly with published human consensus sequences [20]. The rare TH01 allele found in the Hungarians consisted of 6 AATG repeats and a single ATG triplet and was thus denominated 6.3 (Table 4). While 9.3 is the the most common allele in Caucasian populations [21], other .3 variants are extremely rare. Other investigators [5], however, have reported 8.3 and 10.3 variants, while to our knowledge no 6.3 allele has yet been observed.

In Austrian families which had been validated by conventional systems and other STRs (W > 99.75%) no mutations were found for the TH01 (426 meioses), CD4 (275 meioses) and FGA loci (144 meioses). Thus the as-

Table 1 Allelic frequencies for the STRs CD4, TH01, and FGA in Arab population samples from Egypt and Yemen and an European population sample from Austria. The most common alleles are in bold print (*n*: number of individuals in the database)

Allele	Egyptians	Yemenians	Austrians
CD4	*n*: 100	*n*: 100	*n*: 321
5	0.315	0.300	0.331
6	0.290	0.280	0.361
7	0.005	0.010	0.002
8	0.025	0.025	0
9	0.050	0	0.008
10	0.235	0.270	0.264
11	0.070	0.105	0.028
12	0.005	0.010	0.007
13	0.005	0	0
FGA	*n*: 100	*n*: 100	*n*: 525
17	0	0.005	0.001
18	0.015	0.025	0.010
19	0.075	0.020	0.063
20	0.095	0.075	0.147
21	0.140	0.135	0.170
21.2	0.010	0.005	0.001
22	0.160	0.200	0.207
22.2	0.005	0.005	0.008
23	0.165	0.150	0.131
23.2	0	0	0.001
24	0.185	0.225	0.145
25	0.075	0.085	0.083
25.2	0.005	0	0
26	0.040	0.055	0.025
27	0.010	0.010	0.007
28	0.010	0.005	0.001
29	0.005	0	0
> 29	0.005	0	0
TH01	*n*: 100	*n*: 100	*n*: 457
4	0.005	0	0
5	0	0	0.003
6	0.185	0.290	0.214
7	0.225	0.185	0.162
8	0.100	0.125	0.114
9	0.295	0.235	0.182
9.3	0.165	0.160	0.314
10	0.025	0.005	0.010

Table 2 Forensically relevant parameters for the three STRs in the three populations studied (ME: Mean exclusion chance; DP: Discriminating power; H obs: observed heterozygosity)

		CD4	TH01	FGA
H. obs	Egyptians	0.65	0.77	0.83
	Yemenians	0.69	0.78	0.83
	Austrians	0.63	0.82	0.87
DP	Egyptians	0.905	0.922	0.964
	Yemenians	0.899	0.922	0.956
	Austrians	0.847	0.92	0.962
MEC	Egyptians	0.528	0.587	0.733
	Yemenians	0.511	0.575	0.704
	Austrians	0.409	0.574	0.711

Table 3 R × C comparisons of the Egyptian (Egy.), the Yemenian (Yem.), and the Austrian (Austr.) population samples

Populations		CD4	*P*		TH01	*P*		FGA	*P*
Egy.-Yem.	χ^2	13.66	0.072	χ^2	10.79	0.083	χ^2	15.85	0.561
	G	17.93	0.026	G	11.46	0.094	G	18.22	0.623
Austr.-Yem.	χ^2	42.18	$< 10^{-3}$	χ^2	21.8	0.002	χ^2	35.01	0.004
	G	38.85	$< 10^{-3}$	G	24.06	$< 10^{-3}$	G	35.47	$< 10^{-3}$
Austr.-Egy.	χ^2	46.01	$< 10^{-3}$	χ^2	34.72	$< 10^{-3}$	χ^2	42.05	0.002
	G	40.73	$< 10^{-3}$	G	34.61	$< 10^{-3}$	G	33.45	0.011

Table 4 Sequences of the repeat regions of the rare TH01 alleles found in this study and other previously reported .3 variants

Allele designation	Sequence	PCR fragment length bp	Reference
4	5′-$(AATG)_4$-3′	175	This study
6.3	5′-$(AATG)_3ATG(AATG)_3$-3′	186	This study
8.3	5′-$(AATG)_5ATG(AATG)_3$-3′	194	[5]
9.3	5′-$(AATG)_6ATG(AATG)_3$-3′	198	[5, 21]
10.3	5′-$(AATG)_6ATG(AATG)_4$-3′	202	[5]

sumed rare occurrence of mutations in STRs (approx. 0.1%-1%[5, 22]) cannot be disproved by our data.

In conclusion, the three STRs TH01, CD4, and FGA proved to be valuable tools for forensic purposes in Arabs. Especially CD4 was found to have a higher polymorphism in Arabs than in Europeans.

Acknowledgements This work was partially supported by grant EM0075 to N. Al-Hammadi from the World Health Organization and by grant 6523/1 from the Jubilee fund of the Austrian National Bank to M. Klintschar.

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