TECHNICAL NOTE

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HumCD4—Validation of a STR System for Forensic Purposes in an Austrian Caucasian Population Sample

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ABSTRACT: The short tandem repeat system HumCD4 was amplified by the polymerase chain reaction (PCR) on blood samples from 304 unrelated Austrian Caucasians and analyzed by horizontal, non-denaturing polyacrylamide electrophoresis. The mean exclusion chance was 0.417, the discriminating power 0.850 and the heterozygosity rate 0.628. The observed phenotype distribution is in Hardy-Weinberg equilibrium. In 100 families (200 meioses) no mutations were found. Sufficient amplification could be achieved with as little as 80 pg of high molecular weight cell-line DNA. In a degradation experiment DNA extracted from bloodstains stored for up to 28 days in a moist chamber and DNA boiled for up to 18 min could be amplified. A duplex PCR with TH01 is proposed.

KEYWORDS: forensic science, DNA typing, short tandem repeat, HumCD4, Austria, population study, polymerase chain reaction, HumTH01

DNA typing based on amplification of short tandem repeat loci (STRs) is a promising means to overcome major problems encountered in forensic practice such as typing of minute amounts of DNA, highly degraded DNA, or mixtures of DNA from different individuals (1).

Recently a large number of short tandem repeat systems have been made available for forensic purposes such as stain analysis and paternity testing (2). Although most of them have a distinctly lower heterozygosity than restriction fragment length polymorphisms (RFLPs) and some amplifiable fragment length polymorphisms (AmpFLPs), this disadvantage is consequently reduced by the increasing number of systems available and the introduction of multiplex PCR (3). Whereas many studies have been published on some STRs in different populations (e.g., TH01 and HumVWA (4,5)), HumCD4 (6) is one of those not having been sufficiently evaluated by now. Therefore the present study was performed to check the forensic usefulness HumCD4 in an Austrian population sample.

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Materials and Methods

Blood samples from 304 unrelated Austrians were extracted using the SuperQuickGene kit (AGTC, Denver, CO). For the sensitivity studies cell line DNA (K 562, Promega, USA) was serially diluted down to 10 pg/μL. For the DNA quality studies DNA was degraded by boiling for 6 to 24 min and examined for high molecular weight DNA by agar electrophoresis followed by ethidium bromide staining. Additionally blood stains on white cotton were kept in a moist chamber at room temperature for 1 to 40 days, and afterwards stored at −80°C until extracted by the Chelex 100 method (7). A cotton fabric saturated with blood was used to test the size of a blood stain necessary for amplification following Chelex extraction.

PCR protocol and primers: Primer 1: TTGGAG TCGCAA GCTGAA CTA GC; Primer 2: GCCTGA GTGACA GAGTGA GAA CC; 1U Taq polymerase, 200 μM each dNTP, 2 μL 10 × PCR buffer, 1 μM each primer, 25 μL final reaction volume. Cycling conditions: 30 cycles of 94°-1 min, 62°-1 min and 72°, 1.5 min in a Trio-thermoblock (Biometra, Göttingen, FRG) (Protocol for the 11th collaborative exercise of the German DNA profiling group (GEDNAP); (personal communication, B. Brinkmann, Münster).

Duplex PCR—A duplex PCR of CD4 and TH01 was tested. The assay contained 0.6 μM each CD4 primer and 0.6 μM each TH01 primer (8) and 1.5 units Taq polymerase at the same dNTP and buffer concentrations. The same cycling conditions were used.

Electrophoresis—Three types of native, horizontal polyacrylamide gels (9) were tested: First, a 8%T, 3%C gel (Tris-sulfate as buffer, 6-cm separation distance, 0.145-mm thickness, 30 min running time) (referred to as "short gel") was used for typing the CD4 locus in both the monoplex and the duplex reaction. Second, "long gels" (19-cm separation distance, 4-h running time) as described (4) were used for typing the TH01 locus coamplified in the duplex reaction. Third, a slight modification of this "long gel" (7% T instead of 6% T) was used for typing the CD4 locus. A sequenced allelic ladder comprising of the alleles 5 through 13 was kindly provided by Professor B. Brinkmann, Münster, FRG.

Statistics—The mean exclusion chance (MEC) was calculated according to Krüger et al. (10), the discriminating power was

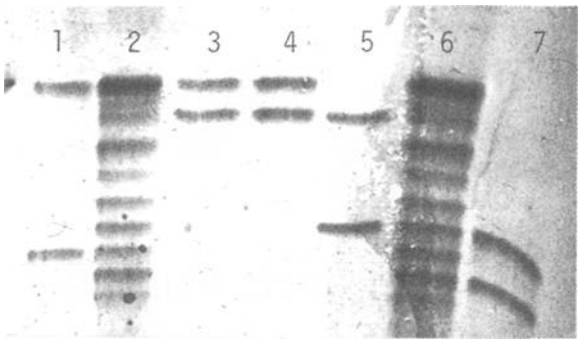

FIG. 1—*"Long" non-denaturing electropherogram of the CD4 locus. Lane 1: 5,11; lane 2 and 6: reamplifiable sequenced allelic ladder comprising of the alleles 5 through 13; lane 3: 5,6; lane 4: 5,6 lane 5: 6,10; lane 7: 10,12.*

calculated as 1-Σ(expected phenotype frequencies)^2 (11). For checking the Hardy-Weinberg expectations, a Chi-square test (phenotypes with less than five observations were pooled (12)), a comparison of the total number of distinct homozygous and heterozygous genotypes (13), and a permutation version of the exact test (14) were performed. The frequency profile comparison between different populations was carried out using a test for genetic heterogeneity (RxC contingency test; G. Carmody, Ottawa, Canada).

Results

Sixty individuals were typed using both the "long" (Fig. 1) and the "short" gels (Fig. 2). All samples were typed for the same alleles in both types of gels. The remaining individuals were typed only on the short gels. A total of 14 genotypes (Table 1) corresponding to 7 alleles were observed (Table 2). The mean exclusion chance (MEC) was 0.417, discriminating power DP was 0.850, the heterozygosity rate 0.628%. No deviations from Hardy Weinberg expectations were found in the tests applied (Table 1). Highly significant differences between the Austrian and a Sub-Saharan African, an Asian, and a native American population (15) were found (Table 2).

Family Studies—CD4 was also tested in 100 families (200 meioses) which had been validated by 22 bloodgroup and enzyme polymorphisms and the STRs HumVWA and HumTH01 (W > 99.75). The segregations followed Mendelian inheritance and no mutations were found.

Sensitivity Studies—K562 cell line DNA was diluted stepwise and amplified. Standard PCR as described yielded typeable results down to approximately 80 pg of K562 cell line DNA. When 32 instead of 30 cycles were used, the threshold was not lowered. In another experiment, cotton threads saturated with blood of varying size were extracted and amplified. Correct typing was possible with threads of 0.5-mm length.

Both experiments gave the same results for both the singleplex amplifications of CD4 ("singleplexing") and the duplex amplifications of CD4 and TH01 ("duplexing") (Fig. 3).

DNA Quality Studies—Correct typing was accomplished for blood stains stored for up to 28 days in a moist chamber. The extractions were repeated twice with consistent results. After boiling the DNA, amplification could be achieved after up to 18 min of boiling, although no high molecular weight DNA was detectable. Both experiments gave the same results for both single- and duplexing.

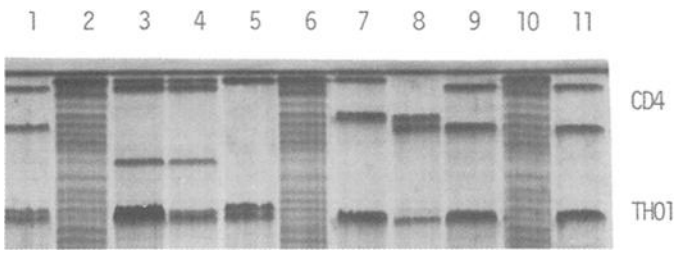

FIG. 2—*Electropherogram of a "short gel" of the duplex reaction: Lanes 1 and 5: allelic ladder comprising of the alleles 5 through 13; lane 2: 5,6; lane 3: 5,6; lane 4: 5,5; lane 6: 5,9; lane 7: 9,10. The lower bands are the fragments of the TH01 locus. Lanes 2 and 3 show an extra band migrating between both loci. This extra band was constantly found in the 5,6 genotype and did not interfere with the typing process.*

TABLE 1—*Genotypes for CD4 in Austria found in this study.*

Genotype	Observed (O)	Expected (E)	(O-E)2/E
5,5	39	32.89	1.135
5,6	61	72.37	1.786
5,9	4	1.64*	
5,10	50	52.96	0.166
5,11	6	5.59	0.030
5,12	1	1.32*	
6,6	50	39.80	2.613
6,10	54	58.26	0.311
6,11	5	6.15	0.220
7,10	1	0.27*	
9,11	1	0.14*	
10,10	24	21.32	0.338
10,11	5	4.50	0.060
10,12	3	1.05*	
Others	0	5.74*	
*Pooled obs.	10	10.16	0.006
Total	304	304	6.65

*Observations pooled for the Chi-square test:
Chi-square: 6.65; df: 6; $0.3 < p < 0.4$.
Exact-test: $p = 0.052$.
Expected number of distinct homozygous genotypes: 3.25 ± 0.88.
Observed number of distinct homozygous genotypes: 3.
Expected number of distinct homozygous genotypes: 11.64 ± 2.76.
Observed number of distinct homozygous genotypes: 11.

TABLE 2—*Allele frequencies for CD4 found in this study (n:604 chromosomes), and for a Sub-Saharan African (n:806), an Asian (n:600), and a native American population (n:704) (15).*

Alleles	Austrians	Sub-Saharan Africans	Asians	Native Americans
4	0	0.002	0	0
5	0.330	0.233	0.604	0.553
6	0.362	0.065	0.048	0.019
7	0.002	0.039	0.003	0
8	0	0.081	0.003	0.001
9	0.008	0.029	0.004	0.001
10	0.265	0.172	0.310	0.421
11	0.028	0.210	0.023	0.004
12	0.007	0.114	0.002	0.001
13	0	0.029	0	0
14	0	0.022	0	0
15	0	0.005	0	0

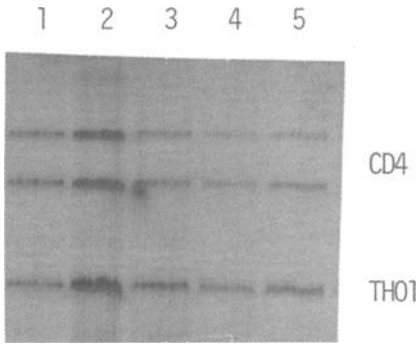

FIG. 3—*"Short gel" of the duplex reaction of the simulated blood stains: Lane 1: 1 cm of blood saturated cotton thread; lane 2: 5 mm; lane 3: 2 mm; lane 4: 1.5 mm; lane 5: 1 mm; lane 6: 0.5 mm.*

Forensic Case Work—Up to now the STR system CD4 has been used in our laboratory in 12 criminal cases. Altogether 70 traces (35 bloodstains, 9 hair roots, 1 handkerchief with nasal secretion, 5 semen stains, and debris from 20 fingernails) that had previously been typed successfully with HumTH01 and HumVWA were amplified. Amplification was successful in all cases and the results matched those of the persons who were suspected.

Duplexing—CD4 was coamplified with HumTH01. The sensitivity and the resistence against degradation was the same as that for singleplex reactions. The typing of the Duplex reactions required two electrophoretic steps as none of the gels tested allowed sufficient resolution of both loci. "Short gels" were used for typing the CD4 locus, "long gels" were used for typing the TH01 locus.

Discussion

Unlike most STRs routinely used in a forensic laboratory, CD4 is a pentameric STR and the amplified fragments are among the shortest ones (allelic range: 85–120 bp). Thus the electrophoretic resolution of this STR is higher than that of many other STRs. In our hands it was possible to type this STR in a native gel with only 6-cm separation distance and 30-min running time ("short gels"), whereas other STRs have to be typed on "long gels" (4). Although the alleles migrate close to each other in these short gels (comparable to those of the locus ACTBP2 in native gels (4)), they can be easily typed, as proven by the fact that the samples run on both long and short gels were assigned the same genotypes in both types of gels. When using these short gels a stain can be easily extracted, amplified, and typed within the same day.

Furthermore, the short fragment length should make this locus resistent against denaturing of the template. In this study blood stains stored for up to 28 days in a moist chamber could be typed whereas the same samples could not be typed after 25 days using the STR FES/FPS (fragment length 222–250 bp) in a prior study (16). Parameters like MEC and DP, however, are considerably smaller than those for other STRs (17,18). This disadvantage is most limiting for paternity testing, because some of its advantages (e.g., high sensitivity or resistence against degradation of DNA) are of minor importance in this field.

Studies on Sub-Saharan African populations, however, (15) revealed a considerably higher polymorphicity of the locus CD4 (Table 2). Therefore this locus might be more interesting for US laboratories. On the other hand, Asian and native American populations are considerably less variable. Thus the racial differences in the distribution of the allelic frequencies might allow assessing the race of an unknown stain donor as recently proposed for other STRs (19) (e.g., in criminal cases in which the race of the perpetrator is not known).

Coamplification with another STR system, as demonstrated for HumTH01 in this study, increases the power of an assay considerably. Using Austrian population data for TH01 (12), the combined MEC of these systems is 81.00% and the combined DP is 98.5%.

The duplex reaction is as sensitive as a singleplex reaction. Although theoretically the considerably shorter alleles of the CD4 locus should be more resistant to degradation, the experiments with the humidified samples gave the same stability for both loci amplified in the duplex reaction.

The typing of the TH01 alleles is not possible in the short gels. Therefore a second electrophoresis in a long gel is required. This does not imply an increased consumption of the amplificate because in our laboratory, a "short gel" is used to check for the success of any amplification for a long time and material consuming gel is run.

Typing of both loci on one native gel is possible by using a slight modification of the long gels (T = 7% instead of T = 6%) (data not shown). However, because the discrimination between the alleles 9.3 and 10 of the TH01 locus is deteriorated, this strategy was not successful. Testing several longer STRs (FES/FPS (20), D21S11 (21), and ACTBP2 (22)) as third partner in a triplex system was not successful, as the sensitivity of the assay was greatly reduced or extra bands led to false typing.

Conclusion

CD4 proved to be satisfying concerning sensitivity and sufficiently resistent to degradation of DNA. The high electrophoretic resolution of this locus which allows a running time of only 30 min is most unique. Although MEC and DP were relatively low, a coamplification with TH01 overcomes this limitation without consuming more template or time. The STR system CD4, therefore, seems to be useful for analyzing biological traces for forensic purposes.

Acknowledgments

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