ALLELE FREQUENCIES OF THREE STRs IN AN ITALIAN POPULATION SAMPLE

E. D'ALOJA, M. DOBOSZ, M. PESCARMONA, A.MOSCETTI, L. GRIMALDI, and V.L.PASCALI

Immunohematology Laboratory, Department of Forensic Medicine, Catholic University, largo F. Vito 1, 00168 Roma, Italy

Several short tandem repeat (STR) (1) polymorphisms have been recently described which are potentially useful in procedures of criminal identification and in paternity tests. STRs consist of short DNA stretches arranged in 2-6 base pair long tandem repeats. They are abundant in the human genome and generally are highly polymorphic. Because of their small size (generally 300 bp) STRs are more likely to be investigated from degraded DNA samples. Tetranucleotide-repeat microsatellites are of primary interest, because they are more easily genotyped.
Preliminar to their use in forensics, an archive of data to adopt as reference should be made available. In conformity with this guideline, we promoted a study aimed at obtaining the allele distribution of some recently described STRs. We here report frequencies of HUMTH01 (11p15.5-p15) (2), MIT-MH26 (D7S460) (3), HUMACTBP2 (chr 5 or 6) (4) in our reference population (Central and Southern Italy).

MATERIALS AND METHODS

Genomic DNA was extracted from whole blood samples, obtained by venipuncture from at least 100 unrelated Italian donors. Standard PCR protocols were adopted to amplify HUMTH01, MITMH26 and HUMACTBP2 loci, with some modifications apported whenever fluorescent primers use was required. Amplified products were separated in polyacrylamide gels (native or denaturing) followed either by silver staining or by analysis with an automated apparatus relying on argon fluorescence (A.L.F., Pharmacia).
Both the non-denaturing gels and the automatic apparatus (6% T and 7 M urea) were able to simultaneously separate the allelic patterns of the three loci. However, with the native horizontal polyacrilamide gels, different acrilamide sieves had to be used, according to the size of alleles (8% T for HumACTBP2, 7.5% T for HumTH01 and MIT-MH26, 5% C common to all the systems). For HumACTBP2, an accurate typing was achieved only by automated laser fluorescence.

RESULTS AND DISCUSSION

In Table 1, the distribution of observed and expected HUMTH01 and MIT-MH26 genotypes is shown. Fragments were identified and

classified with the help of home made admixtures of different individuals. Individual alleles apparently originated from iteration (6 to 11 repeat for HUMTH01, 1 to 7 repeats for MIT-MH26) of a four base pair-long repeat unit. Interestingly, in MIT-MH26 distribution some alleles were missing in the virtually complete ladder of sizes (fragments with 2 and 5 tandem repeats). HUMTH01 distribution showed a complete scale of alleles (from 6 to 11 repeat units), but an additional allele with incomplete repeat unit (three base pair unit instead of the expected four) was present in a significant pool of individuals (the so called 10-1 allele). A high average heterozygosity was evident in both systems (HUMTH01:.75; MIT- MH26:.72). Unlike the two systems just dealt, HUMACTBP2 was a more complex polymorphism to study. It encompassed a larger DNA region in which the occurrence of incomplete repeat units had its natural counterpart in a wider number of alleles. Additionally, alleles with apparently the same size were supposed to underlie internal sequence differences by slightly irregular migration of the relevant bands. Owing to these peculiarity, a correct definition of the entire HUMACTBP2 pattern of alleles was only - and partially - achieved by the use of the automated sequencer. Also, setting a current allelic ladder containing the most frequent alleles was a proportionally difficult task. Due to these difficulties, a tentative distribution of observed and expected genotypes is achieved by PAGE and silver staining procedures. An A.L.F. typing of all genotypes so far detected is presently in progress. The average heterozigosity of the system amounted to .92 and no mutation were observed in family segregation. We presently believe that HUMACTBP2 system will be useful in a fewer forensic instances than those envisioned for other STRs.

REFERENCES

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Table 1
Distribution of observed and expected HUMTH01 and MIT-MH26 phenotypes.

MITMH26

Type	Observed	Expected	Chi square
1-1	1	0.82	0.04
3-3	7	5.40	0.47
4-4	18	16.71	0.10
6-6	12	9.60	0.60
7-7	1	1.45	0.14
1-3	5	4.20	0.15
1-4	6	7.39	0.26
1-6	4	5.60	0.46
1-7	4	2.18	1.52
3-4	16	19.00	0.47
3-6	16	14.40	0.18
3-7	3	5.60	1.21
4-6	23	25.33	0.21
4-7	14	9.85	1.75
6-7	5	7.47	0.81
sum	135		

HUMTH01

Type	Observed	Expected	Chi square
6-6	10	9.76	0.01
7-7	5	3.66	0.49
8-8	1	2.56	0.95
9-9	14	10.00	1.60
10-10	15	8.57	4.81
11-11	0	0.01	0.01
6-7	7	11.95	2.05
6-8	11	10.00	0.10
6-9	22	19.76	0.25
6-10	19	18.29	0.03
6-11	1	0.49	0.54
7-8	8	6.13	0.57
7-9	14	12.10	0.30
7-10	10	11.20	0.13
7-11	0	0.30	0.30
8-9	10	10.13	0.00
8-10	9	9.38	0.02
8-11	1	0.25	2.25
9-10	7	18.52	7.17
9-11	0	0.49	0.49
10-11	0	0.46	0.46
sum	164		