ANALYSIS OF THE SHORT TANDEM REPEAT POLYMORPHISM TC 11 (HUMTH01): ALLELE FREQUENCIES AND FAMILY STUDIES.

P. Berschick, L. Henke, J. Henke

Inst. für Blutgruppenforschung, Otto-Hahn-Str. 39,
P.O. Box 13 04 68, D-40554 Duesseldorf, Germany

Introduction

Short tandem repeat (STR) polymorphisms provide a potential tool to forensic casework. In order to use the system TC 11 (HUMTH01: human tyrosine hydroxylase intron 1 on chromosome 11 (1)) in forensic DNA analysis, detailed knowledge of allele frequencies is most important. In paternity cases it is also essential to know the mutation rate. For this reason we analysed unrelated individuals and families from Northern Germany.

Material and Methods

DNA was extracted from whole blood according to standard procedures. PCR was carried out with the primers (1):

TC 11/1: 5'-GTG GGC TGA AAA GCT CCC GAT TAT-3'
TC 11/2: 5'-ATT CAA AGG GTA TCT GGG CTC TGG-3'

The amplification conditions were: 50 mM KCl, 10 mM Tris HCl pH 8.3, 0.1% Triton X-100, 0.2 mM each dNTP, 0.5 μM each primer, 1 U Taq polymerase, 5-10 ng DNA. The reaction volume was 25 μl. The thermal cycler (Biometra TRIO-Thermoblock) was set to 80^{o}C for 5 min for hot start conditions and the reaction was started by adding primer TC 11/2 and Taq polymerase to the heated reaction mix (including DNA, TC 11/1 and $MgCl_2$). The reaction conditions were as followed:

94^{o}C - 1 min, 64^{o}C - 1 min, 72^{o}C - 2 min, for 30 cycles

8 μl of the amplification product were loaded on a 1 mm thick, 20 cm long polyacrylamidegel (6%T, 5%C) with bisacrylamide as crosslinker and seperated in 1x TBE for 3 h at constant 30 W (about 380-400V) until xylencyanol dye reached the anodal end of the gel. After electrophoresis the separated products were stained with ethidiumbromide (1 μg/ml) and visualized by means of UV light. The alleles were typed by comparing the products with an alleleic ladder which was constructed from alleles observed during this study.

DNA samples were collected from 268 unrelated individuals, 105 three member families and two 7 member families from Northern Germany.

Results and Discussion

We could find five different TC 11 alleles. These were the alleles 6, 7, 8, 9 and 10. Table 1 shows the allele frequencies of altogether 536 analysed alleles. The allele with the highest frequency is allele 10 with about 35%. The allelic distribution is comparable to those found by Edwards et al.(2). Allele 8 has the lowest frequency with 10%. The heterozygosity index is 75.7%. With the nondenaturating gel used in our experiments we were not able to separate alleles 9.3 and 10. For this reason we typed both alleles as allele 10.

Tab. 1: Allele frequency of TC 11 (HUMTH01) in the German population. Alleles 9.3 and 10 could not be separated and are therefore typed as 10.

REPEAT	%
5	-
6	21.8
7	16.0
8	10.3
9	17.2
9.3 + 10	34.7
11	-

The distribution of observable phenotypes in a population sample of 268 unrelated individuals are shown in Fig.1 and Tab.2. The heterozygosity index is 75.7% The distribution of the observed phenotypes is not different from the expected distribution according to Hardy-Weinberg equilibrium (X^2-test, d.f.: 10, $0.95 < P < 0.98$). As expected it could be stated that the alleles associated randomly with each other at this locus and there is no detectable population heterogeneity.

Up to now we analysed 246 meioses in the family studies and we could not find any de novo mutation. For calculating mutation rates we need to analyse more families.

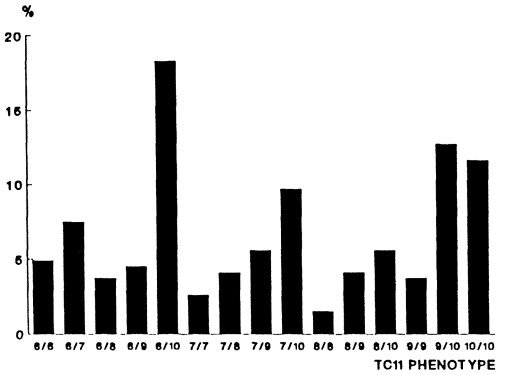

Fig. 1: Phenotype frequencies of TC 11 (HUMTH01) of 268 unrelated individuals of Northern Germany. Phenotypes are plotted against frequency in %.

Tab. 2: Phenotype frequencies of TC 11 (HUMTH01) of 268 unrelated individuals of Northern Germany. Heterozygosityindex is 76.7%. Distribution fits the Hardy-Weinberg equilibrium (χ^2-test, df: 10, $0.95 < P < 0.98$).

Phenotype	observed		expected
	number	%	%
6/6	13	4,9	4.8
6/7	20	7.5	7.0
6/8	10	3.7	4.5
6/9	12	4.5	7.5
6/10	49	18.3	15.2
7/7	7	2.6	2.6
7/8	11	4.1	3.3
7/9	15	5.6	5.5
7/10	26	9.7	11.1
8/8	4	1.5	1.0
8/9	11	4.1	3.5
8/10	15	5.6	7.1
9/9	10	3.7	2.9
9/10	34	12.7	11.9
10/10	31	11.6	12.0

In conclusion separation of TC 11-PCR products on nondenaturating polyacrylamide gels followed by ethidium bromide staining allows to resolve and detect the alleles of TC 11. Alleles 9.3 and 10 differ only by 1 basepair and could only be separated by denaturating sequencing gels.

Literature

1. Edwards, A., Civitello, A., Hammond, H.A., Caskey, T. 1991. DNA typing and genetic mapping with trimeric and tetrameric tandem repeats. Am. J. Hum. Genet. 49: 746-756.

2. Edwards, A., Hammond, H.A., Jin, L., Caskey, T., Chakraborty, R. 1992. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 12: 241-253.