INVESTIGATION OF THE STR HUMLIPOL IN AUSTRIAN CAUCASOID INDIVIDUALS: SEQUENCE DATA AND ALLELE FREQUENCIES

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INTRODUCTION

Short tandem repeat polymorphisms (STR's) are tracts of di- to hexa- or heptanucleotides, which are tandemly repeated. They are highly polymorphic in respect to the number of repeats and widely spread throughout the human genome (Tautz 1989; Edwards 1992).
Since the introduction of PCR amplification techniques these microsatellite markers have become useful tools for forensic casework including paternity testing, but they are also employed in genomic mapping linkage analysis and anthropology.
Application of sequenced allelic ladders as internal size standards is a very precise and reliable method of allele assignment in test samples, which allows allele designation according to the number of repeats.

In this work we present the sequence data of a ladder of the STR polymorphism in intron 6 of the lipoprotein lipase gene (Hammond et al. 1994; Zuliani and Hobbs 1990), designated HumLIPOL, and allele frequencies obtained in an Austrian Caucasian population sample using the AMPFLP-technique (N=550).

MATERIALS AND METHODS

Samples

Genomic DNA was extracted from peripheral blood of 550 healthy, unrelated Austrian individuals by the „salting out method" described by Miller et al. (1988).

Primers

5`-ATC TGA CCA AGG ATA GTG GGA TAT A-3` (forward primer, TTTA strand)
5`-CCT GGG TAA CTG AGC GAG ACT GTG TC-3` (reverse primer, TAAA strand)
(Zuliani and Hobbs 1990).

PCR

Amplifications of the population samples were performed with 20ng template, 0.6µM each primer, 2U polymerase (Dynazyme™, Finn Zymes Oy), 1x PCR buffer (50mM KCl, 10mM TrisCl pH=9.0 at 25°C, 0,1% Triton-X-100 and 1.5mM MgCl2) and 200µM of each dNTP, overlaid with 50µl paraffine oil, using a Hybaid Omnigene thermocycler.
A modified PCR protocol (based on Zuliani and Hobbs 1990) was used in all amplifications:
first denaturing 10 min 98°C, 1 cycle; 10 min 68°C, 1 cycle, adding the polymerase; followed by 1 min 94°C and 6 min 68°C, 10 cycles; 1 min 90°C and 6 min 68°C, 18 cycles; final annealing 10 min 68°C, 1 cycle.

Electrophoretical Methods

PAGE was carried out as described by Schwartz et. al. (1994) on 7% native polyacrylamide gels in 120mM Tris-Acetic Acid rehydration buffer and 200mM Tris-Tricine electrode buffer.
Subsequent silver staining was applied to visualize DNA (Bassam et al. 1991).
Typing was done by side by side comparison with the allelic ladder.

Allelic Ladder

Single bands of heterozygous population samples, corresponding to distinct alleles, were eluted from the gel. DNA was purified using Wizard PCR Preps, DNA Purification System (Promega, technical bulletin), diluted and reamplified. Equal concentrations of the amplification products were pooled to construct an allelic ladder.

Sequencing

Single stranded sequence determination of the different alleles took place on an automatic DNA-Sequencer (ALF™ Pharmacia LKB Technology AB) according to the protocol of the Pharmacia AutoRead™ Sequencing Kit (dye primers) on a 6% sequencing gel. For each allele both strands were sequenced.

Statistics

In a χ^2 test, performed to confirm the Hardy-Weinberg expectations, genotypes beyond expected values of 5 were grouped together.

RESULTS AND DISCUSSION

AMPFLP-typing of healthy, unrelated Caucasian individuals of Austria revealed 7 distinct alleles. One sample of each allele was used to construct the allelic ladder as decribed above.

Sequencing of 22 alleles (three alleles 7, four alleles 9, five alleles 10, three alleles each 11, 12, 13 and one allele 14) ,including the ladder alleles, showed a simple repeat structure, with 7 and 9 to 14 iterations of the [TTTA] repeat motif (table 1). Allele designation was in accordance with the repeat number following the recommendations of the International Society for Forensic Haemogenetics 1994.
In contrast to other STR loci (e.g. D21S11), which show a more complex structure and microheterogenities, side by side comparison of the sequencing pattern proved absolute length and sequence conformities of the 25 basepair 5` flanking regions and the 7 basepair 3`flanking regions.

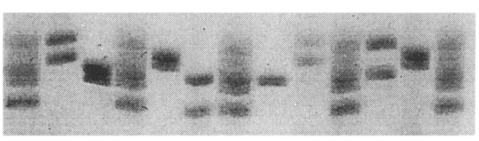
Allele sizes, which were determined by comparing the sequence pattern of the sense and antisense strand, range from 111 to 139 basepairs (Table 1). They were correlated with the sequence provided in Genbank (Accession No. X15736), which corresponds to our allele 11.

Table 1 Sequencing data of the HumLIPOL alleles

Allele designation	Repeat structure	Length (bp)	Number of sequenced alleles
7	$(TTTA)_7$	111	3
10	$(TTTA)_{10}$	123	5
11	$(TTTA)_{11}$	127	3
12	$(TTTA)_{12}$	131	3
13	$(TTTA)_{13}$	135	3
14	$(TTTA)_{14}$	139	1

The sequenced ladder was employed in testing 550 DNA samples (1100 chromosomes) of healthy, unrelated Austrian individuals on 7% native polyacrylamide gels (Fig. 1).
We found three common alleles 10, 11 and 12, two rare alleles 9 and 13 and two very rare alleles 7 and 14 (Table 2).
Observed heterozygosity is 72.9 %, not significantly deviating from the expected value of 70.8 %. Population data met the Hardy-Weinberg expectations (p=0.195).
Power of discrimination is 0.86, mean exclusion chance is 0.52 and discrimination index is 0.13.

Fig. 1 AMPFLP-typing for HUMLIPOL alleles employing the allelic ladder containing alleles 7 and 9 to 14



13 10 12 10 10 14 13 11
11 9 11 7 10 12 10 12

Table 2 Allele frequencies at the HumLIPOL STR locus in an Austrian Caucasoid population sample (N=550)

Allele Designation	Observed Alleles	Allele Frequency ±SE (%)[a]
7	3	0.3.± 0.2
9	22	5.3 ± 0.7
10	190	38.5 ± 1.5
11	143	27.8 ± 1.4
12	126	25.1 ± 1.3
13	17	2.6 ± 0.5
14	2	0.4 ± 0.2

$\chi^2 = 10.56$; 6 df; p = 0.195
Mean exclusion chance (Krueger et al. 1968) W = 0.52
Power of discrimination (Kloosterman et al. 1993) PD = 0.86
Discrimination index (Wong et al. 1987) DI = 0.13

[a] Standard error was calculated according to Edwards et al. (1992)

The application of the allelic ladder described in this paper allows a clear designation of alleles in unknown test samples in a precise and reliable way and there is no need for an additional size marker. The results of this study suggest that the HumLIPOL polymorphism could be suitable for forensics and other application fields as well.

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