AMPFLP-TYPING FOR THE HUMCD4 STR POLYMORPHISM IN AN AUSTRIAN CAUCASOID POPULATION SAMPLE: SEQUENCE DATA AND ALLELE DISTRIBUTION

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INTRODUCTION

Short tandem repeat (STR) polymorphisms are microsatellite loci containing repeat motifs 2 to 7 nucleotides long. Several hundred STR marker systems have been described until now. They are widely spread throughout the human genome and most of them show high levels of heterozygosity. Since STR sequences may even be amplified from small amounts of highly degraded template DNA, they have become very useful in human identification e.g. for forensic casework and paternity testing.
Application of allelic ladders as standard size markers is a reliable and precise method of allele designation in test samples and has been described for several VNTR and STR loci before (e.g. Puers et al.1993).
We used this procedure to evaluate the HUMCD4 (AAAAG) pentanucleotide polymorphism in the CD4 gene (Edwards M.C. et al. 1991; Wall et al. 1993) at 12pter-p12 (GenBank M86525) in an Austrian Caucasoid population sample of 300 healthy, unrelated individuals.

MATERIAL AND METHODS

Samples

Genomic DNA was extracted from peripheral blood of 300 healthy, unrelated Austrian individuals by standard techniques.

Primers

5`TTG GAG TCG CAA GCT GAA CTA GC-3` (forward primer, CTTTT strand)
5`-GCC TGA GTG ACA GAG TGA GAA CC-3` (reverse primer, AAAAG strand)
(Edwards M.C. et al. 1991).

PCR

PCR amplifications of the population samples were performed in 50µl volume with 8ng template, 0.4µM of each primer, 1U polymerase (Dynazyme™, Finn Zymes Oy), 0.8x PCR buffer (1x: 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 upon Wall et al. 1993) was used for all amplifications: 10 min 98°C, without polymerase,1 cycle; then 10 min 58.5°C, 1 cycle adding the polymerase; followed by 1 min 94°C and 45 seconds 58.5°C for 10 cycles; then 1 min 90°C and 45 sec 58.5°C for 20 cycles; final annealing 58.5°C, 10 min, 1 cycle.

Electrophoretical Methods

Electrophoresis on 9% native polyacrylamide gels in 112mM Tris-Acetic Acid rehydration buffer and 200mM Tris-Tricine electrode buffer and subsequent silver staining was carried out as described by Schwartz at al. (1994). 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 build up 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 Pharmacia AutoRead™ Sequencing Kit.

For each allele the strand and anti-sense strand were sequenced. Preceding strand separation was performed with support of Streptavidin attached magnetic beads.

Statistics

In a χ^2 test alleles 6,7,8,10 and 11 were grouped.

RESULTS AND DISCUSSION

DNA samples of healthy, unrelated Austrian Caucasian individuals were amplified revealing 8 individual alleles, which were used to construct an allelic ladder by pooling amplification products of the purified allele DNAs.

Sequencing of both strands of 35 alleles including the ladder alleles (six alleles 4, six alleles 5, one allele 6 and one 7, two alleles 8, four alleles 8`, seven alleles 9, five alleles 10 and three alleles 11) showed a regular repeat structure with only one polymorphic repeat motif (table 1). The five shorter alleles 4-8 contain 4 to 8 iterations of the (AAAAG) repeat unit. The three longer alleles show 6 to 8 regular repeat units, then one repeat unit with an A to G transition changing AAAAG to AAAGG, and subsequently two copies of the core repeat, which led us to designate them 9,10 and 11 following the rule of the first four alleles and counting the AAAGG unit for a AAAAG. (Allele designations were done following the recommendations of the DNA commission of the International Society of Forensic Haemogenetics 1994).

Table 1 Repeat Structures and Frequencies of the HUMCD4 Alleles

Allele designation	Sequence structure	Length (bp)	Allele frequency ±SE[a] (%)
4	-$(AAAAG)_4$-	86	36.3 ± 2.0
5	-$(AAAAG)_5$-	91	31.6 ± 1.9
6	-$(AAAAG)_6$-	96	0.2 ± 0.2
7	-$(AAAAG)_7$-	101	0.2 ± 0.2
8	-$(AAAAG)_8$-	106	1.2 ± 0.4[b]
8`	-$(AAAAG)_5$ AAAGG $(AAAAG)_2$-	106	-
9	-$(AAAAG)_6$ AAAGG $(AAAAG)_2$-	111	27.5 ± 1.8
10	-$(AAAAG)_7$ AAAGG $(AAAAG)_2$-	116	2.5 ± 0.6
11	-$(AAAAG)_8$ AAAGG $(AAAAG)_2$-	121	0.5 ± 0.3

χ^2=8.18; 6df; p=0.23
Heterozygosity observed = 0.73
Heterozygosity expected = 0.69

Mean exclusion chance (Krueger et al.1968) :W= 0.44
Power of discrimination (Kloosterman et al. 1993)=0.85
Discrimination index (Wong et al. 1987)=0.13

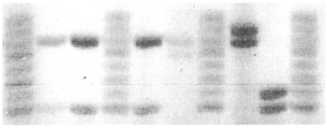
[a] SE indicates standard error calculated according to Edwards et al. 1992
[b] Common frequency of the electrophoretically indistinguishable alleles 8 and 8´

In addition we encountered a variant form of allel 8, which doesn`t show 8 continous copies of the repeat motif, but contains the A to G transition. This variant allele 8` appeared with a frequency of 4 out of 6 sequenced alleles. It was not included in the allelic ladder because it was indistinguishable from allele 8 in population sample testing on native polyacrylamid gels. Side by side comparison of the sequences revealed absolute length and sequence conformities of the 5`and 3` flanking regions of all sequenced alleles. No other microheterogenities were found. Allele sizes ranging from 86 to 121 basepairs were correlated with the sequence provided in GenBank (Accession No.M86525), which corresponds to our allel 9.

The sequenced ladder was employed to characterize 600 chromosomes from unrelated Austrian Caucasoids by AMPFLP-typing on native polyacrylamide gels (fig. 1). A total of eight distinguishable alleles appearing with frequencies between 0.2% to 36.3% were observed within our population sample. There are three common alleles

4, 5 and 9, two rare alleles 8 (and 8`) and 10 and three very rare alleles 6, 7 and 11. No deviations from Hardy-Weinberg eqilibrium could be observed (table 1).

Fig.1 AMPFLP-typing of Austrian Caucasoid population samples employing the sequenced allelic ladder for the HUMCD4 STR locus containing alleles 4 to 11.



9 9 9 9 10 5
4 4 4 8 9 4

The data obtained in this study suggest that the HUMCD4 pentanucleotide polymorphism is a potential marker for personal identification applications. The assignment of alleles using a well characterized allelic ladder is a simple, precise and reliable method independent of the used electrophoresis systems. Further advances should be made to obtain such ladders for all practicable STR loci, especially for those applied in forensic cases and paternity testing.

REFERENCES

Bassam BJ, Caetano Anolles G, Gresshoff PM (1991) Fast and sensitive silver staining of DNA in polyacrylamide gels. Anal Biochem 196:80-83

DNA comission of the ISFH (1994) DNA recommendations- 1994 report concerning further recommendations of the DNA comission of the ISFH regarding PCR-based polymorphisms in STR (short tandem repeat) systems. Int J Leg Med 107:159-160

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

Edwards MC, Clemens PR, Tristan M, Pizzuti A, Gibbs RA (1991) Pentanucleotide repeat length polymorphism at the human CD4 locus. Nucleic Acids Res 19:4791

Kloosterman AD, Budowle B, Daselaar P (1993) PCR-amplification and detection of the human D1S80 VNTR locus. Int J Leg Med 105:257-264

Krueger J, Fuhrmann W, Lichte KH, Steffens C (1968) Zur Verwendung des Polymorphismus der sauren Erythrozytenphosphatase bei der Vaterschaftsbegutachtung. Deutsch Z Gerichtl Med 64:127-146

Puers C, Hammond HA, Jin L, Caskey CT, Schumm JW (1993) Identification of repeat sequence heterogeneity at the polymorphic short tandem repeat locus HUMTH01[AATG]n and reassignment of alleles in population analysis by using a locus-specific allelic ladder. Am J Hum Genet 53:953-958

Schwartz DWM, Jungl EM, Krenek OR, Mayr WR (1994) Simple and rapid typing for VNTR polymorphisms using high resolution electrophoresis of PCR products on rehydratable polyacrylamide gels. Advances in Forensic Haemogenetics 5:170-172, Springer, Berlin, FRG

Wall WJ, Williamson R, Petrou M, Papaioannou D, Parkin BH (1993) Variation of short tandem repeats within and between populations. Hum Mol Genet 2:1023-1029

Wong Z, Wilson V, Patel I, Povey S, Jeffreys AJ (1987) Characterization of a panel of highly variable minisatellites cloned from human DNA. Ann Hum Genet 51:269-288

Zuliani G, Hobbs HH (1990) Tetranucleotide repeat polymorphism in the LPL gene. Nucleic Acids Res 18:4958

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