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Allelic Ladder Characterization of the Short Tandem Repeat Polymorphism in Intron 6 of the Lipoprotein Lipase Gene and Its Application in an Austrian Caucasian Population Study

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ABSTRACT: The short tandem repeat (STR) polymorphism HumLPL $(TTTA)_n$, which is located in intron 6 of the lipoprotein lipase gene, was investigated by AMPFLP (amplification fragment length polymorphism)-technique using an allelic ladder consisting of amplified alleles of this locus as a standard size marker.

The allelic ladder was prepared by pooling equal concentrations of six separate alleles, which were identified by their different electrophoretic mobility in native polyacrylamide gel, eluted and subsequently amplified. Sequence analysis of the ladder alleles and allele 7, which is not included in the ladder, showed a regular repeat structure with 7 and 9 to 14 repetitions of the core repeat.

The allelic ladder was employed in the analysis of the genotypes of 550 unrelated Caucasoids of Austria. No new alleles were found.

The population investigated showed no deviation from Hardy-Weinberg equilibrium ($P = 0.195$).

KEYWORDS: forensic science, DNA, short tandem repeats, allelic ladder, population genetics, human identification, HUMLPL

Short tandem repeats (STRs) are microsatellite DNA sequences of di- to hexa- or heptanucleotides that are tandemly repeated (1,2,3). They are highly polymorphic in respect to the number of repeats and widely spread throughout human genome (4). Since the introduction of PCR-amplification techniques, they have become a useful tool in human identification for forensic and anthropological purposes (5–8), paternity testing (9,10) and genetic mapping (11, 12). As a fact of their small overall size they have many advantages over the previously used much longer repeat motifs, called VNTRs or minisatellites, because STRs require only a small amount of template and may produce results even if the DNA is highly degraded (13). The distinct alleles of a sample can be typed by electrophoresis of PCR-amplification fragments on native or denaturing polyacrylamide gels. Bands are visualized by ethidium bromide- or silver staining (14). Application of allelic ladders as internal size standards simplifies the designation of alleles in test samples and is a very precise and reliable method.

This approach has been described for example, for the VNTR loci D1S80 (15,16), Col2A1 (17) and for the STR loci HPRTB (3), HumTH01 (18), HumF13A01 (19), HumVWA, HumD21S11 and HumFES/FPS (20).

In this paper, we present the characteristics of a ladder of the STR polymorphism in intron 6 of the lipoprotein lipase gene (21, 22) and the allele frequencies obtained in an Austrian Caucasian population sample using the AMPFLP-technique.

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Material and Methods

Genomic DNA was extracted from peripheral blood of healthy, unrelated Austrian blood donors by the "salting out method" of Miller et al. (23). DNA concentration was adjusted to 8 ng/μL by optical densitometry.

The oligonucleotide primers used were 5′-ATCTGACCAAG-GATAGTGGGATATA-3′ (forward primer, TTTA strand) and 5′-CCTGGGTAACTGAGCGAGACTGTGTC-3′ (reverse primer, TAAA strand) (21). PCR amplifications of the population samples were performed in 50 μL volume with 20 ng template, 0.6 μM of each primer, 2U polymerase (Dynazyme™, Finn Zymes Oy), 1× PCR buffer (50 mM KCl, 10 mM TrisCl pH = 9.0 at 25°C, 0,1% Triton-X-100 and 1.5 mM MgCl2) and 200 μM of each dNTP using a Hybaid Omnigene thermocycler. A modified PCR protocol (based upon Zuliani and Hobbs 1990 (21)) was used in all amplifications: 98°C, 10 min without polymerase, 1 cycle; 10 min at 68°C adding the polymerase, 1 cycle; followed by 1 min, 94°C and 6 min, 68°C, 10 cycles; then 1 min, 90°C and 6 min, 68°C, 18 cycles; final annealing 10 min, 68°C, 1 cycle.

PAGE was carried out in horizontal mode as described by Schwartz et al. 1994 (24) on 7% gels in 120 mM Tris-Acetat rehydration buffer and 200 mM Tris-Tricine electrode buffer. (Initial 1200V, 12 mA, 30W for 20 minutes, then 1200V, 20 mA, 30W until the bromophenol blue marker front had reached the anodal end of the gel.) Subsequent silver staining was applied to visualize DNA (14).

Single bands of heterozygous population samples, corresponding to distinct alleles, were eluted from the gel in 100 μL elution buffer, described for the "crush and soak method" by Sambrook et al. (25). DNA was purified using Wizard PCR Preps, DNA Purification System (Promega, technical bulletin), deluted $1:10^3$ and reamplified. Equal concentrations of the amplification products were pooled to construct an allelic ladder.

Single stranded sequence determination of the different alleles (amplified using the previously described protocol with 0.4 μM forward primer and 0.2 μM 5′ biotin labelled reverse primer) took place on an automatic DNA-Sequencer (ALF™ Pharmacia LKB

TABLE 1—*Sequences of the HumLPL alleles.*

Allele	Sequence	Length (bp)
7	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_7$ TTTTTGA-Y	111
9	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_9$ TTTTTGA-Y	119
10	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_{10}$ TTTTTGA-Y	123
11	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_{11}$ TTTTTGA-Y	127
12	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_{12}$ TTTTTGA-Y	131
13	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_{13}$ TTTTTGA-Y	135
14	X-GAAGAAAAAACATTCCAAGAATTAT $(TTTA)_{14}$ TTTTTGA-Y	139
X: ATCTGACCAAGGATAGTGGGATATA (forward primer)		
Y: GACACAGTCTCGCTCAGTTACCCAGG (reverse primer target sequence)		

Technology AB) according to the protocol of Pharmacia Auto-Read™ Sequencing Kit (which employs T7-polymerase in a chain termination reaction) on a 6% sequencing gel in 0.6× TBE at 32 mA and 34W for 6 hours. The preceding strand separation was done with support of Streptavidin-attached magnetic beads (Dynabeads®, Dynal®) following the included protocol (26)

Results and Discussion

AMPFLP-typing of 550 healthy unrelated Caucasian individuals of Austria revealed seven different alleles, of which six were pooled to construct an allelic ladder. Sequencing of 22 alleles from 13 heterozygous individuals (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. Allele designation was in accordance with the repeat number following the recommendations of the International Society for Forensic Haemogenetics (27). 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. No microheterogeneities were observed. 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.

Using the described PCR conditions reproducible results could be obtained from 20 ng template DNA down to a minimum threshold of 400 pg. No "slippage problems" have been observed.

The sequenced ladder, containing alleles 9 to 14, was employed in testing 550 DNA samples (1100 chromosomes) of unrelated Austrian Caucasoids on 7% native polyacrylamide gels (Fig. 1). As preferential amplification of the very rare allele 7 caused imbalance of allele concentrations in reamplification of the allelic ladder, it was not considered. Although we used native electrophoresis conditions, bands in Fig. 1 seem to be "doublets." This phenomenon might be due to the polymerization state of the gel, because it appeared to increase with extensive degasing periods of the gel solution.

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%, power of discrimination (PD) is 0.86. Population data met the Hardy-Weinberg expectations ($P = 0.195$).

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 HumLPL polymorphism could be a suitable marker for personal identification applications in the Austrian population.

FIG. 1—*Typing of LPL alleles on a 7% non-denaturing polyacrylamide gel. Lanes 1, 4, 7, 10 and 13 contain the allelic ladder with alleles 9 to 14. Lane 2: 10, 12; Lane 3: 11, 12; Lane 5, 6: 10, 12; Lane 8, 11: 11, 11; Lane 9: 11, 12 and Lane 12: 9, 10.*

TABLE 2—*Allele frequencies at the HumLPL STR Locus in an Austrian Caucasoid population sample* (n = *550).*

Allele designation	Observed alleles	Allele frequency ±SE (%)*
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
	6 df $\chi^2 = 10.56$ $P = 0.195$	

*Standard error was calculated according to Edwards et al. 1992 (3).

References

(1) Udalova IA, Nedospasov SA, Webb GC, Chaplin DD, Turetskaya RL. Highly informative typing of the human TNF locus using six adjacent polymorphic markers. Genomics 1993;16(1):180–86.
(2) Tautz D, Trick M, Dover GA. Cryptic simplicity in DNA is a major

source of genetic variation. Nature 1986;322(6080):652–56.
(3) Edwards A, Hammond HA, Jin L, Caskey CT, Chakraborty R. Genetic variation at five trimeric and tetrameric tandem repeat loci in four human population groups. Genomics 1992;12:241–53.
(4) Tautz D. Hypervariability of simple sequences as a general source for polymorphic DNA markers. Nucl Acids Res 1989;17(16):6463–71.
(5) Sajantila A, Budowle B, Strom M, Johnsson V, Lukka M, et al. PCR amplification of alleles at the DIS80 locus: comparison of a Finnish and a North American Caucasian population sample, and forensic casework evaluation. Am J Hum Genet 1992;50(4):816–25.
(6) Devlin B, Risch N. Ethnic differentiation at VNTR loci, with special reference to forensic applications. Am J Hum Gen 1992; 51(3): 534–48.
(7) Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, et al. Identification of the remains of the Romanov family by DNA analysis. Nature Gen 1994;6(2):130–35.
(8) Kurosaki K, Matsushita T, Ueda S. Individual DNA identification from ancient human remains. Am J Hum Gen 1993;53:638–43.
(9) Morling N, Hansen HE. Paternity testing with VNTR DNA systems. I. Matching criteria and population frequencies of the VNTR systems D2S44, D5S43, D7S21, D7S22, and D12S11 in Danes. Int J Leg Med 1993;105(4):189–96.
(10) Hansen HE, Morling N. Paternity testing with VNTR DNA systems. II. Evaluation of 271 cases of disputed paternity with the VNTR systems D2S44, D5S43, D7S21, D7S22, and D12S11. Int J Leg Med 1993;105(4):197–202.
(11) Dawson E, Shaikh S, Weber JL, Wang Z, Weissenbach J, et al. A continuous linkage map of 22 short tandem repeat polymorphisms on human chromosome 12. Genomics 1993;17(1):245–48.
(12) Kumar Singh R, Humphries P. Isolation and genetic mapping of four microsatellite repeats from chromosome 3p21 using 40 CEPH pedigrees. Genomics 1993;18:3717–19.
(13) Jeffreys AJ, Wilson V, Neumann R, Keyte J. Amplification of human minisatellites by the polymerase chain reaction: towards DNA fingerprinting of single cells. Nucl Acids Res 1988;16(23):10953–71.
(14) Bassam BJ, Caetano Anolles G, Gresshoff PM. Fast and sensitive silver staining of DNA in polyacrylamide gels. Analyt Biochem 1991;196(1):80–83.
(15) Budowle B, Chakraborty R, Giusti AM, Eisenberg AJ, Allen RC. Analysis of the VNTR locus D1S80 by the PCR followed by high-resolution PAGE. Am J Hum Gen 1991;48(1):137–44.
(16) Skowasch K, Wiegand P, Brinkmann B. pMCT 118 (D1S80): a new allelic ladder and an improved electrophoretic separation lead to the demonstration of 28 alleles. Int J Leg Med 1992;105(3):165–68.
(17) Rand S, Puers C, Skowasch K, Wiegand P, Budowle B, et al. Population genetics and forensic efficiency data of 4 AMPFLP's. Int J Leg Med 1992;104:329–33.
(18) Puers C, Hammond HA, Jin L, Caskey CT, Schumm JW. 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 Gen 1993;53(4):953–58.
(19) Puers C, Hammond HA, Caskey CT, Lins AM, Sprecher CJ, et al. Allelic ladder characterization of the short tandem repeat polymorphism located in the 5′ flanking region to the human coagulation factor XIII A subunit gene. Genomics 1994;23(1):260–64.
(20) Moeller A, Meyer E, Brinkmann B. Different types of structural variation in STRs: HumFES/FPS, HumVWA and HumD21S11. Int J Leg Med 1994;106(6):319–23.
(21) Zuliani G, Hobbs HH. Tetranucleotide repeat polymorphism in the LPL gene. Nucl Acids Res 1990;18(16):4958.
(22) Wall WJ, Williamson R, Petrou M, Papaioannou D, Parkin BH. Variation of short tandem repeats within and between populations. Hum Molec Gen 1993;2(7):1023–29.
(23) Miller SA, Dykes DD, Polesky HF. A simple salting out procedure for extracting DNA from human nucleated cells. Nucl Acids Res 1988;16(3):1215.
(24) Schwartz DWM, Jungl EM, Krenek OR, Mayr WR. Simple and rapid typing for VNTR polymorphisms using high resolution electrophoresis of PCR products on rehydratable polyacrylamide gels. Advances in Forensic Haemogenetics 1994;5:170–72.
(25) Sambrook J, Fritsch EF, Maniatis T, editors Molecular cloning: a laboratory manual. 2nd ed. Isolation of DNA fragments from polyacrylamide gels. Cold Spring Harbor Laboratory Press, 1989; 6.46–6.48.
(26) Hultman T, Stahl S, Hornes E, Uhlen M. Direct solid phase sequencing of genomic and plasmid DNA using magnetic beads as solid support. Nucl Acids Res 1989;17(13):4937–46.
(27) DNA recommendations-1992 report concerning recommendations of the DNA Commission of the International Society for Forensic Haemogenetics relating to the use of PCR-based polymorphisms. Int J Leg Med 1992;105(1):63–64.

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