PCR TYPING OF ALU ELEMENTS - MOLECULAR GENETICS AND FORENSIC APPLICATION

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Introduction

Alu repeats belong to the family of short interspersed elements (SINEs) and are among the most abundant repetitive DNA sequences in the mammalian genome. They represent mobile genetic elements ancestrally derived from the 7SL RNA gene and have presumably spread within the genome by retroposition (reviewed in [1]). A particular group of Alu repeats appears to be human-specific (HS subfamily) and has expanded only recently within the human genome as indicated by distinct dimorphisms at various loci due to the presence or absence of an Alu repeat. In recent extensive studies, the frequency distributions of Alu insertions at selected loci in various human racial groups and populations were determined. Significant differences in frequency distribution between these populations were observed which could be used to determine the evolutionary origin as well as the phylogenetic relationship between racial groups [2,3]. These genetic markers should therefore be useful to obtain clues on the race of an unknown stain donor in forensic casework. In the present study, we have determined the frequency and segregation behaviour of Alu insertions at six loci in the German population.

Material and Methods

In the population study, DNA samples from 49 unrelated individuals as well as 23 children from South-Western Germany were tested. All individuals were obtained from consecutive routine paternity cases, whereby family relationships had been been determined using conventional blood group as well as DNA VNTR systems.

Table 1: Primer sequences for amplification of Alu insertions

Repeat	*Chromosomal location*	*5'-primer (5'->3')*	*3'-primer (5'->3')*
D1	3	TGCTGATGCCCAGGGTTAGTAAA	TTTCTGCTATGCTCTTCCCTCTC
ACE	17	CTGGAGACCACTCCCATCCTTTCT	GATGTCGCCATCACATTCGTCAGAT
TPA25	8	GTAAGAGTTCCGTAACAGGACAGCT	CCCCACCCTAGGAGAACTTCTCTTT
APO	11	AAGTGCTGTAGGCCATTTAGATTAG	AGTCTTCGATGACAGCGTATACAGA
FXIIIB	1	TCAACTCCATGAGATTTTCAGAAGT	CTGGAAAAAATGTATTCAGGTGAGT
PV92	16	AACTGGGAAAATTTGAAGAGAAAGT	TGAGTTCTCAACTCCTGTGTGTTAG

The six Alu repeat loci D1, ACE, TPA25, APO, FXIIIB, and PV92 were typed using the polmerase chain reaction (PCR) with locus-specific primers flanking the Alu insertion site. The PCR primer sequences as well as the chromosomal locations of the six systems are given in Tab. 1 [3]. Standard PCR amplification was carried out using 100 ng template DNA, 0.5 µM of each primer, 200 µM dNTP's, 1.5 mM $MgCl_2$, and 2U of Taq polymerase (Gibco Life Technologies GmbH) in a 1x PCR buffer (provided by the

Table 2: Observed and expected genotypes of six Alu loci in the German population (n=49)

		Observed		*Expected*		*p*
Locus	*Genotype*	*(n)*	*(%)*	*(n)*	*(%)*	*(exact test)*
D1	+ +	7	14.3	6.6	13.5	
	+ –	22	44.9	22.8	46.5	0.64
	– –	20	40.8	19.6	40.0	
ACE	+ +	13	26.5	13.8	28.2	
	+ –	26	53.1	24.4	49.8	0.66
	– –	10	20.4	10.8	22.0	
TPA25	+ +	6	12.2	5.9	12.0	
	+ –	22	44.9	22.2	45.3	0.87
	– –	21	42.9	20.9	42.6	
APO	+ +	46	93.9	46.0	94.0	
	+ –	3	6.1	2.9	5.9	0.52
	– –	0	0.0	0.1	0.1	
FXIIIB	+ +	14	28.6	11.3	23.0	
	+ –	19	38.8	24.5	49.9	0.12
	– –	16	32.7	13.3	27.1	
PV92	+ +	1	2.0	1	2.0	
	+ –	12	24.5	12	24.5	0.78
	– –	36	73.5	36	73.5	

+ + homozygous with insertion, + – heterozygous, – – homozygous without insertion

The observed and expected genotype frequencies as well as the p values of the exact test are shown in Tab. 2. No significant deviations from Hardy-Weinberg equilibrium were found. Also, no mutation or aberrant segregation behaviour was observed in the family study comparing the genotypes of parents and offspring in 46 meioses. Except for the APO locus, heterozygosity is between 24 and 53 % in the German population.

By comparing Alu insertion frequencies from different racial groups, significant differences were observed by comparing the results from the present study with frequencies of the African-American population [3] for the loci APO and FXIIIB (see Fig. 2). The combined exclusion chances for all six loci are 61.2 % in paternity tsting and 98.8 % in identification cases for Germans and 66.8 % and 99.4 % for African-Americans, respectively. Thus the Alu insertion polymorphisms provide another useful tool in forensic DNA analysis with the potential to obtain information on the possible race of an unknown stain donor.

References

1. Deininger PL, Batzer MA (1993) Evolution of retroposons. Evolutionary Biol 27:157-196
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4. Guo SW, Thompson EA (1992) Performing the exact test of Hardy-Weinberg proportion for multiple alleles. Biometrics 48:361-372

polymerase manufacturer). Routinely, 32 cycles of a three-step PCR were performed in a TC-1 thermocycler (Perkin-Elmer GmbH) using the following protocol: denaturation for 1' at 94°C, annealing for 1' at various temperatures (55°C for APO and PV92, 56°C for FXIIIB, 58°C for ACE and TPA25, 61-58°C touchdown PCR for D1), extension for 1' at 72°C with a final extension of 7' at 72°C. Ten µl of each PCR reaction were separated by electrophoresis in a 1.2 % agarose gel. A size difference of approx. 300 bp indicated the presence or absence of the Alu insertion at a given locus. The observed homozygous and heterozygous genotypes were counted and possible deviations from the Hardy-Weinberg equilibrium was analysed using an exact test [4]. Exact test and exclusion chances were calculated using the paternity analysis software PATER (C. Brenner, Berkeley, CA).

Results and Discussion

The typical DNA fragment patterns of heterozygous genotypes (from individuals with and without Alu insertions) from all six loci are depicted in Fig. 1. The PCR fragments are easy to discriminate and vary in size between 100 bp for the alleles without insertion (APO and TPA25) and approx. 800 bp for the allele with insertion (FXIIIB). In a series of dilution experiments to test the sensitivity, amplification of the FXIIIB-specific fragments and detection by ethidium bromide staining could be achieved with 2-5 ng of template DNA (not shown).

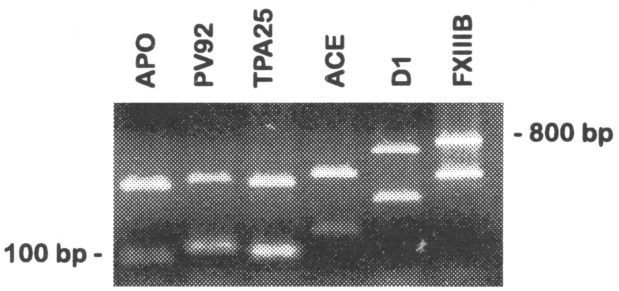

Fig. 1: Heterozygous genotypes of the six Alu insertion loci analysed. The fragments were visualized after separation in a 1.2% agarose gel by ethidium bromide staining.

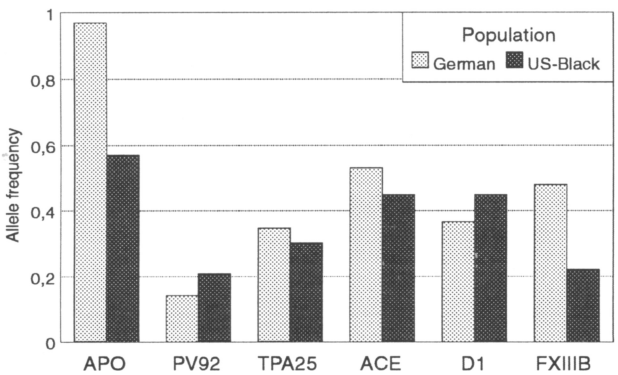

Fig. 2: Comparison of Alu insertion allele frequencies in the German (n=49) and the US-African-American populations (n=46, from ref. [3]).