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Analysis of the Alu insertion polymorphism in Taiwanese Han population

Hsing-Mei Hsieh,[1] Ph.D.; Chih-Wen Su,[2] M.S.; Li-Chin Tsai,[1] M.S.; Nu-En Huang,[2] M.A.; Rocky Tai-Ping Shih,[2] M.D.; Adrian Linacre,[3] Ph.D.; James Chun-I Lee,[1,*] Ph.D.

[1] Department of Forensic Science, Central Police University, 56 Shu Jen Road, Taoyuan 333, Taiwan ROC.

[2] Criminal Investigation Bureau, National Police Administration, Taiwan ROC.

[3] Forensic Science Unit, Department of Pure & Applied Chemistry, University of Strathclyde, Glasgow G1 1XW, UK.

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ABSTRACT

We report on a comprehensive study of six Alu insertions within the Taiwanese Han population. Specific primer pairs were designed to amplify and detect the presence (insertion) or absence of human-specific Alu fragments at the loci PLAT, ACE , APO, B65, HS3.23 and HS4.32 in 178 individuals of the Taiwanese Han population and 44 family pairs. The frequencies of Alu insertion were found to be 0.7135 (ACE), 0.5197 (PLAT), 0.9551 (HS3.23), 0.4186 (HS4.32), 0.9354 (APO) and 0.4326 (B65). The discrimination powers ranged from 0.6203 for B65 to 0.1606 for HS3.23 and the combined discrimination power was 0.985. The power of exclusion (PE) for trio case in parentage testing of these six loci ranged from 0.0410 to 0.1873. The joint power of exclusion was approximately 0.5908. The results indicated that the polymorphism of Alu insertion could assist the individual identification and paternity determination in Taiwanese Han population.

Keywords: Alu insertion, Discrimination power, Individual identification, Paternity determination

Introduction

The Alu insertion is via an RNA-mediated transposition process and is about 300 bp in length. Alu inserts are abundant in the human genome of about 500,000 copies comprising approximately 5% of the human genome [1,2]. Alu sequences have been proved to be useful genetic markers in anthropological and evolutionary studies [3-9] although these loci have limited use in forensic studies [10]. The population study on Taiwanese has been carried out with the mitochondrial DNA, Y-STR markers and autosomal STRs [11-13]. To date no previous examination of the distribution of Alu inserts in the Han Taiwanese population has been undertaken.

In this study primer pairs were designed to amplify six Alu insertion loci including PLAT on chromosome 8, ACE on chromosome 11, APO on chromosome 22, B65 on chromosome 11, HS3.23 on chromosome 7 and HS4.32 on chromosome 12. A total of 178 unrelated individuals of the Taiwanese Han population were studied along with 44 families of parent and child groups from the same population. The data of genotypes were established, and the probabilities of individual identification and parentage testing were evaluated.

* Corresponding author, e-mail:jimlee@sun4.cpu.edu.tw

Materials and methods

Population samples

Blood samples were collected from 178 unrelated individuals and 44 father (mother)/child combinations of 44 families in the Taiwanese Han population. The Taiwanese Han population in this study is an admixtures of individuals who migrated from the Han area of mainland China from approximately 400 years ago and from all 35 mainland provincial areas of China in 1949. DNA was extracted by the QIAamp Tissue/Blood kit (Qiagene) and quantified with ultra-violet detection by spectrophotometer.

PCR amplification and cycle sequencing

The loci of Alu insertions analyzed in the study included PLAT, ACE, APO, B65, HS3.23 and HS4.32. The primer sequences and annealing temperatures for

each PCR amplification are shown in Table 1. PCR amplifications were performed in a volume of 50 µl, containing 40 ng of genomic DNA, 1X reaction buffer (25 mM Tris-HCl, pH 8.5, 10 mM $MgCl_2$, 2.5 mM KCl, 1 mM β-mercaptoethanol), 0.2 mM of each dNTP; 2 units of Taq DNA polymerase (BerTaq) and 100 ng of each primer. The PCR amplifications were carried out in a GeneAmp 9600 (applied Biosystems) using the program of denaturing at 94˚C for 1 min, annealing at the temperatures shown in Table 1 for 2 min and extension at 72˚C for 2 min, for 30 cycles.

PCR products for cycle sequencing were also conducted using a GeneAmp 9600 (Applied Biosystems) with the following conditions: 25 cycles of 95˚C for 45 sec, 50˚C for 15 sec and then 72˚C for 4 min. Sequencing was performed using the 5' end or 3' end primer and the BigDye™ Terminator Kit (ABI PRISM™ BigDye™ Terminator Cycle Sequencing Ready Reaction Kit). The cycle sequencing products were separated by 5% denatured Long Ranger™ gel (FMC BioProducts, Rockland, Maine, USA) and detected using a PE Applied Biosystems 373A DNA sequencer.

Statistical methods

The paternity index for each locus was calculated as X/Y, assuming that X was the probability that the putative father could be the biological father of the child, and that Y was the probability that a random man could be the father of the child. The combined discrimination power was calculated as $1-\prod_i Pm_i$, Pmi stands for the probability of match of each locus. The power of exclusion was the probability that a falsely-accused putative father will be excluded as the biological father of a particular child, and it can be calculated for a trio when the genotypes of the alleged father, the mother and the child were known. The general equation used for this situation was given below:

$PE_{trio} = \sum_{i=1}^{n} P_i(1-P_i^2) + \sum_{i=1}^{n}\sum_{j=i+1}^{n} (P_iP_j)^2(3P_i+3P_j-4)$, Pi was the frequency of the ith allele and n is the number of alleles at the locus [17].The equation for the joint power of exclusion for several independent loci was given below:

$PE_{joint} = 1-\prod(1-PE_{locus})$.

Results and discussion

The primer pairs were used to amplify the six Alu insertion loci of PLAT, ACE, APO, B65, HS3.23 and

Table 1 The analyzed loci, primer sequences and annealing temperatures for each PCR amplification.

Locus	5' end primer (5' → 3')	3'end primer (5'→ 3')	Annealing temperature	Reference
APO	AAGTGCTGTAGGCCATTTAGATTAG	AGTCTTCGATGACAGCGTATACAGA	50˚C	[14]
ACE	CTGGAGACCACTCCCATCCTTTCT	GATGTGGCCATCACATTCGTCAGAT	58˚C	[14]
B65	ATATCCTAAAAGGGACACCA	AAAATTTATGGCATGCGTAT	59˚C	[15]
PLAT	GTGAAAAGCAAGGTCTACCAG	GACACCGAGTTCATCTTGAC	60˚C	[16]
HS3.23	GGTGAAGTTTCCAACGCTGT	CCCTCCTCTCCCTTTAGCAG	60˚C	[3]
HS4.32	GTTTATTGGGCTAACCTGGG	TGACCTGCTAACTTGTACTTTAACC	60˚C	[3]

Table 2 Genotype frequencies of Alu loci in this study.

	ACE	PLAT	HS3.23	HS4.32	APO	B65
Ppp	0.2591	0.0729	0.8320	0.0307	0.7656	0.0350
Ppq	0.1672	0.2492	0.0074	0.2369	0.0146	0.2410
Pqq	0.0067	0.0532	0.0000	0.1143	0.0000	0.1037
Pm	0.4330	0.3754	0.8394	0.3819	0.7802	0.3797
Pd	0.5670	0.6246	0.1606	0.6181	0.2198	0.6203

Note P_{pp}, P_{pq}, and P_{qq} represent the probability of homozygote with Alu insertion, heterozygote and homozygote without Alu insertion, respectively. Pm stands for probability of match, and Pd for probability of discrimination.

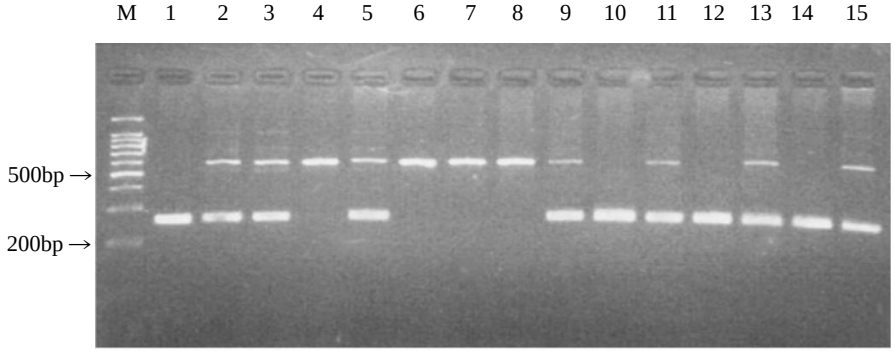

Fig. 1 Electrophoresis of PCR products of homozygote with Alu insertion (lanes 4, 6, 7 and 8), heterozygote (lanes 2, 3, 5, 9, 11, 13 and 15) and homozygote without Alu insertion (lanes 1, 10, 12 and14) on PLAT locus from 15 samples. M represents the size marker of 100 bp ladder.

Table 3 The power of exclusion and joint power of exclusion for the six loci in parentage testing.

	ACE	PLAT	HS3.23	HS4.32	APO	B65
PE	0.1626	0.1873	0.0410	0.1841	0.0568	0.1852
joint PE = 0.5908						

HS4.32 from the 178 unrelated samples and 44 family groups in Taiwanese Han population. Successful amplification was achieved for all the samples. Figure 1 was one of the examples for Alu tests in this study. The lengths of PCR products, including the priming sites and flanking DNA, ranged from approximately 400 bp to 600 bp. The fragments were about 300 bp longer than the PCR products without an Alu insertion. The presence of an Alu insert was confirmed by cycle sequencing for all the PCR products.

The frequencies of Alu insertion from the 178 samples were 0.7135 for ACE, 0.5197 for PLAT, 0.9551 for HS3.23, 0.4186 for HS4.32, 0.9354 for APO and 0.4326 for B65. The frequencies of APO and B65 were similar to a previous report of the African population [8] where APO had a frequency of 0.93 and B65 was at a frequency of 0.54. Our results showed that ACE had a higher frequency compared to that of the African population (0.7135 compared to 0.50) [8]. Tests for the goodness of fit of observed genotype proportions to Hardy-Weinberg equilibrium proportions revealed that all loci except one (B65) fit Hardy-Weinberg expectations. The standard deviations were 0.023963 (ACE), 0.026479 (PLAT), 0.010980 (HS3.23), 0.026145 (HS4.32), 0.013028 (APO), and 0.026257 (B65). The discrimination powers (Pd) of these six loci were 0.5670 (ACE), 0.6246 (PLAT), 0.1606 (HS3.23), 0.6181 (HS4.32), 0.2198 (APO) and 0.6203 (B65) as shown in Table 2. The combined discrimination power was 0.985. It is clear that in this study some loci are more informative (ACE, PLAT, HS4.32 and B65) than others (HS3.23 and APO). It is most likely that the wide variation corresponds to the time point that the Alu insertion in the genome occurred for a longer time.

The Alu inserts in four Taiwanese aboriginal tribes (Ami, Atayal, Bunun and Paiwan) have previously been described [18]. The genetic distances between the Han population, which is the largest population in Taiwan, and these aboriginal populations were calculated, using the formula according to Nei [19]. Using these data a neighbor-joining tree was constructed (data not shown), however, this result is not consistent with the result of microsatellite DNA [20]. Sample size of the aboriginal populations may be the major reason for the difference.

The Alu insertion polymorphisms were analyzed in 44 family combinations for these six loci. The average paternity index (PI) and probability of paternity (PP) were 6.706388 and 87.02% respectively. Due to the nature of the loci, the PP for one family group ranged from 98.52% to 28.25%. The variations were caused by the differences of the power of exclusion (PE) in Alu loci. The power of exclusion for trio case in parentage testing of these six loci showed in Table 3. The joint power of exclusion was approximately 0.5908.

The six Alu loci analysed in this study were dimorphic, however, another locus HSC3N1 may be monomorphic, without a non-insertion allele in the 178 samples tested (data not shown).

The combined discrimination power of these six loci was 0.985. There is potential to increase this discrimination power by the addition of more loci as it is estimated that there may be as many as 400 Alu insertion polymorphisms in the human genome [21]. Several additional Alu insertion polymorphisms are currently being characterized. It is therefore expected that the applications of Alu insertion polymorphism will assist the individual identification and parentage testing that performed by current STR technology.

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