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Allele Frequency and Its Forensic Application of STR Y27 in Korean Males

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The allele frequency and mutation rate in a Short Tandem Repeat locus, Y27 were studied in 247 unrelated Korean males using polymerase chain reaction followed by high-resolution polyacrylamide gel electrophoresis,a procedure called the amplification fragment length polymorphism technique. Six alleles were noted ranging from 190 bp to 210 bp. They existed as discrete bands with 4 bp discrepancy. Among which DY3(198 bp), DY4(202 bp) were common with the frequencies of 0.408, 0.356 respectively. Other alleles, DY1(190 bp, frequency 0.020), DY2(194 bp, frequency 0.121), DY5(206 bp, frequency 0.089), DY6(210 bp, freuency 0.004) were relatively uncommon. In a 78 subject father-son study with parenthood confirmed through other genetic studies, no case of mutation was noted. As the allele number was not as large as 6 and two alleles were dominant, the discrimination power in routine individual identification was thought to be low. But in selective cases such as father-son determination or sex determination, this locus could be a valuable genetic marker and we thought these results to be common for the Korean population. These results were also compared with that of other race.

Key Words : *Short Tandem Repeat, Y27, Allele frequency, Mutation rate, Korean*

INTRODUCTION

Repetitive DNA sequences are an abundant source of polymorphism, and these are distributed over the whole genome(Nakamura et al., 1987). They are usually not expresed, and can be typed by a simple procedure called amplification fragment length polymorphism (AMP-FLP), in which the ploymerase chain reaction (PCR) products are separated by high-resolution polyacrylamide gel electrophoresis(PAGE) (Budowle et al., 1991). With its increased sensitivity and specificity, this method over several repetitive sequences(Kasai et al., 1990, Ludwig et al., 1989) has replaced the Southern-Hybridization method and serologic test in the field of individual identification and has become a popular test.

Contrary to autosome, the Y chromosome manifests a low degree of polymorphism(Jakubiezka et al., 1989, Malaspina et al., 1990) and only a limited number of reports have been presented. Arneman et al.(1986) identified a tetranucleotide(GATA)n repetative sequence in a Y chromosomal cosmid library and mapped the sequence to the short arm. Roewer and Epplen(1992) designed an appropriate PCR primer set and studied the polymorphism of this locus using AMP-FLP. They found four alleles, and the allele size ranged from 186 bp to 198 bp. Before any genetic polymorphism in a locus can be used for individual identification, population data including allele distribution and mutation rate

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must be known. But these vary according to race, and native population data is necessary in a given population. The current report describes the availability of the short tandem repeat(STR) Y27 locus in a Korean population and the population data such as allele frequency and mutation rate. Also a comparison with other populations was done.

MATERIALS AND METHODS

Ninety placentae and 157 blood samples were collected in Seoul National University(SNU) Hospital and Department of Forensic Medicine in SNU Medical College in Seoul. The sampling was random and only the unrelated objects in paternal lineage were collected. Seventy-eight father-son pairs were subjected to family study. Total DNA was extracted from placental tissue and blood as described elsewhere(Sambrook et al., 1987). About 10 ng of DNA was subjected to amplification using two primers, Y-27H39.1 (5'-CTACTGAGTT TCTGTTATAGT-3') and Y-27H39.2 (5'-ATGGCATGTAGT GAGGACA-3') in 20 ul reaction volume with the condition of 67 mM Tris-HCl pH 8.3, 1.5 mM magnesium chloride, 16.6 mM ammonium sulfate, 10 mM 2-mercaptoethanol, 170 ug/ml bovine serum albumin, 10% dimethyl sulfoxide, 2.5 mM dNTP mixture, 0.5 U Taq polymerase, 2 uM of each primer. The temperature profile for 32 cycles of amplification was 94℃ for 10 sec, 57℃ for 15 sec, 62℃ for 30 sec with GeneAmp PCR System 9600(Perkin-Elmer Corporation, Foster City, CA, USA). The products were separated on 9.4% polyacrylamide gel, and the result was interpretated after silver staining.

For the determination of exact allele size and confirmation of the repetition unit, sequencing was performed for two alleles, Y2 and Y3. First, the agarose gel containing the target bands were excised under UV light, and DNA was eluted from the gel using Qiagen kit(QIAGEN Inc, Chatsworth, CA, USA). After phosphorylation with 1 mM ATP, the DNA was used as a sequencing template. Using pUC 19 as a vector, transformation was done with E. coli XL1-Blue, and several positive colonies were selected. Plasmid DNA was prepared from the broth using QIA prep spin plasmid kit(QIAGEN Inc, Chatsworth, CA, USA). Sequencing reaction was performed using a DyeDeoxy Terminator cycle sequencing kit and a ABI automatic sequencer(Applied Biosystem, Foster City, CA, USA) following the manufacturer's recommendation.

Table 1. Y27 allele frequencies in Koreans comparing with that of Brazilians. Data of Brazilians were from Santos et al.(1993). The number means the number of cases found among 247 Koreans. The data of Brazilians showing the similar frequencies, not the same size, with that of Koreans were arranged in same column.

Allele	DY1	DY2	DY3	DY4	DY5	DY6
Number	5	30	101	88	22	1
Frequency	0.020	0.121	0.408	0.356	0.089	0.004
Brazilian		0.19	0.49	0.24	0.07	0.01

RESULTS

After amplification, each allele could be detected as a discrete band with silver staining(Fig. 1). A total of six alleles were found. These alleles were separated with the repetition unit, 4 bp, and could be designated according to repetition number without remark of the exact size. The smallest allele DY1 was 196 bp and the largest allele DY6 was 210 bp. All the alleles found were shown in Table 1 with the frequency. Each allele could be determined by comparing it with the previously known standard allelic ladder with ease. DY3 and DY4 were rather commonly met with the frequencies 0.408 and 0.356 respectively. Other alleles were less frequent.

In 78 father-son pairs, all the alleles of the offsprings could be traced to their fathers. No case of mutation was noted.

The nucleotide sequences of DY2 and DY3 were as Fig. 2. The exact size and GATA repetition units were confirmed. The allele DY2 has 16 repetitions of the unit [GATA] and allele DY3 has 17 repetitions of the unit [GATA]. A focus of repetition unit change from GATA to GGTA was noted, and the sites were same in both DY2 and DY3.

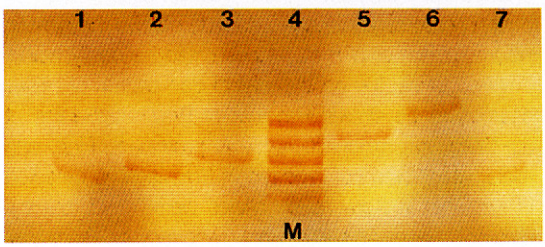

Fig. 1. Silver stained polyacrylamide gel after PCR amplification of Y27 in DNA from 6 unrelated males. Each allele could be well differentiated and could be designated compared with the size marker, lane 4. Allele number of each lane was as follows, lane 1: DY1, lane 2: DY2, lane 3: Y3, lane 5: DY4, lane 6: DY5, lane 7: DY1. The numbers above designate the lane number, and the five bands in marker(M) lane indicate each allele, DY1 to DY5 from below.

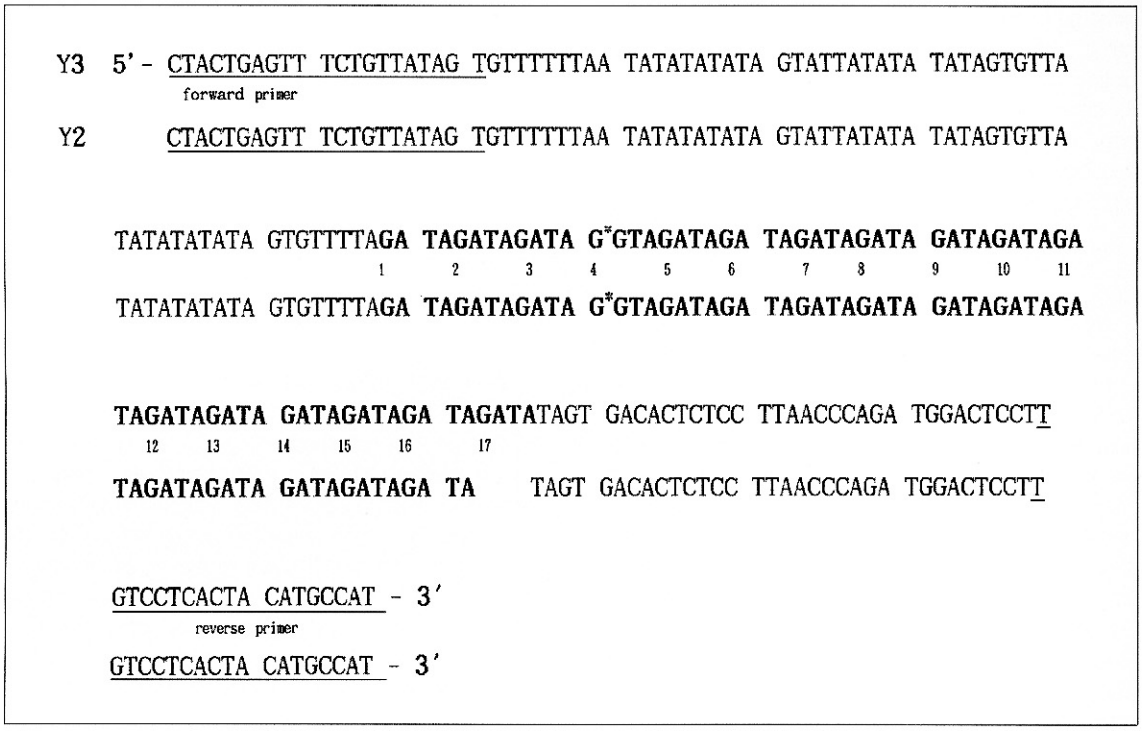

Fig. 2. Nucleotide sequence of two alleles, DY3 and DY2. Primer sequences are underlined in each end and the repetitive portion are in bold letter with repetition number. * denotes the site of sequence variation within repetition unit.

DISCUSSION

AMP-FLP analysis is known to be a powerful tool in individual identification for VNTR of STR loci, which reveals a high level of polymorphism. It was shown that the method could be operative in Y27 locus, and even one repetition difference, 4 bp could be differentiated on a high percentage of polyacrylamide gel. So the genetic polymorphism of STR Y27 locus could be easily typed.

At a glance, the general distribution pattern of alleles seemed to be similar with that of Brazilians(Santos et al., 1993), but racial difference is prominent. First, one allele, which was not reported in Brazilians, is detected in Koreans. In Koreans six alleles were found, but the smallest allele DY1 was not reported in Brazilians. Roewer and Epplen(1992) found only four alleles, although the object number was not designated. As the discrimination power of a genetic locus correlates with the number of allele, the usefullness of the Y27 was higher in Koreans than in Brazilians. Second, the most frequent allele in Koreans, DY3 was 198 bp, but in Brazilians it was 194 bp. As the whole sequence data and the number of the [GATA] repetition unit are not known in Brazilians, we don't know what make these racial differences between Koreans and Brazilians. The different repetition number and the different flanking sequence are two possible explanations for those differences, but more data are needed for confirmation. But the above finding confirmed that native population data is necessary in a given population for practical usage, and this would be the first report in Koreans.

In practical casework, discrimination power of a genetic locus determines its usefulness as an individual identification marker. The probability of paternity exclusion in Y27 locus is $P(Y) = 1 - \Sigma(Pi)^2$ (Chakraborty 1985), and this approaches 0.67. This is not so little compared to other popular genetic markers. As this locus was in the Y chromosome, the recombiantion rate is quite low. Furthermore as it exists in haploid, its allele frequency distribution does not need the precondition of Hardy-Weinberg equilibrium for statistical analysis. These points make the practical application of the

Y27 locus more convenient. The polymorphism in Y chromosome seems to be low, but the probability of paternity exclusion matches with that of autosome. So STR Y27 could be a valuable genetic marker especially when determining the paternal lineage or in genealogical comparison(Oakey and Smith 1990). Rape is another situation suitable for this study. The victim as a female reveals no amplification, and there is no worry of confusion between the victim and assailant. Recently two other $[GATA]_n$ repetitive loci on chromosome 12 (4804LR and 4815LR, Roewer and Epplen 1992) was recognized. As these loci have similar repetitive structure and the products of three loci differ in size, these loci can be co-amplified in a relatively simple PCR condition. Such multiplex PCR increases the usefulness of the amplification and reduces the amount of DNA needed for reaction.

As the Y27 resides in the sex chromosome, it could be used in sex typing. In females a distinct band was not found on amplification. As the amplification product is relatively small, this locus could be typed in archaelogical material. Positive band upon amplification suggests that the sample is from a male. But the possibility of a false negative case needs reliable control sample. Also genetic locus which are located on both X and Y chromosome, such as amelogenin gene, would be helpful in sex typing.

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