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Forensic evaluation and haplotypes of 19 Y-chromosomal STR loci in Koreans

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Abstract

In this study, 19 Y-specific STR loci (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, DYS385, DYS388, DYS434, DYS435, DYS436, DYS437, DYS438, DYS439, DYS446, DYS449, and DYS464) were analyzed in 301 unrelated Korean males by three multiplex PCR systems. The haplotype diversity using the classical set of Y-STRs (DYS19, DYS389I/II, DYS390, DYS391, DYS392, DYS393, and DYS385; multiplex I) was 0.9963. For the same individuals, the haplotype diversity value using the new set of highly informative Y-STRs (DYS385, DYS446, DYS449, and DYS464; multiplex III) was 0.9989, while that using the combined set of Y-STRs by adding DYS388 to the previously studied DYS434, DYS435, DYS436, DYS437, DYS438, and DYS439 (multiplex II) was 0.9509. A total of 297 different haplotypes were identified using the 19 Y-STR markers, of which 293 were unique and 4 were found twice. The overall haplotype diversity was 0.9999.

The evaluation of the information of selected markers by combination of each marker with the minimal haplotype showed that DYS434, DYS435, DYS436, DYS437, and DYS438 do not significantly contribute to increment of haplotype diversity. However, respective conjunction of DYS464, DYS449, and DYS446 with the minimal haplotype considerably increased the haplotype diversity. Especially, DYS464 is expected to be the most useful marker that can be included in the expanded minimal haplotype. These results including the haplotype data at 19 Y-STR loci in the present study would provide useful information in forensic practice in a Korean population.

Keywords: 19 Y-STRs; Minimal haplotype loci; DYS388; DYS434; DYS435; DYS436; DYS437; DYS438; DYS439; DYS446; DYS449; DYS464; Gene diversity; Haplotype; Koreans

1. Introduction

Short-tandem repeat (STR) markers on the Y chromosome have been very useful, not only because of their application to forensic studies, but also because of their use in studies of human migration and evolution [1–4]. Since the human Y-STR markers are haploidly inherited without recombination [5], the frequencies of individual markers cannot combine into one probability statement using the product rule [6]. The allele combination of a series of individual Y-STR loci has thus been considered a haplotype.

Y-STR Haplotype Reference database has been established for the Europeans, Americans and Asians (http://www.ystr.org, http://www.ystr.org/usa, and http://www.ystr.

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org/asia) [7–9], and the set of Y-STR database which is defined as the minimal haplotype consists of the loci DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393 and DYS385. The combination of these Y-STRs can distinguish approximately 76.1–95.5% of male individuals in various populations [7–12].

Despite the utility of minimal haplotype, additional Y-STR loci are required in order to improve the potential to distinguish different paternal lineages. Also, empirical studies of a number of Y-STR loci are needed to provide an efficient marker set with sufficient power of discrimination. Meanwhile, Redd et al. [13] have analyzed 14 novel loci and reported DYS446, DYS449, and DYS464 of 14 loci were highly diverse in Asian YCC cell lines.

In this study, we evaluated the usefulness of these three new Y-STRs (DYS446, DYS449, and DYS464) as well as DYS388 and the previously studied DYS434, DYS435, DYS436, DYS437, DYS438, and DYS439 [14] in conjunction with the minimal haplotype loci. We determined gene and haplotype diversities and assessed contributions of respective Y-STRs to the increase of haplotype diversity. Haplotype distributions of the 19-YSTRs were also examined. Finally, allelic associations across haplotype loci were discussed, since haplotype diversity is negatively dependent on the degree of gametic association [15,16].

2. Materials and methods

2.1. DNA samples

A sample of 301 unrelated Korean male buccal swabs already typed for the DYS434, DYS435, DYS436, DYS437, DYS438, and DYS439 [14] was selected and analyzed. Genomic DNA was extracted using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) and quantified fluorometrically with Picogreen® dsDNA Quantitation Reagent (Molecular Probes, Eugene, OR) using a TBS-380 Mini-Fluorometer (Turner Biosystems, Sunnyvale, CA). The 9948 male DNA (Promega, Madison, WI) was also analyzed to calibrate allelic ladders.

2.2. Multiplex PCR amplification conditions

A total of three multiplex PCR reactions for typing 19 Y-STRs were constructed. Primer sequences and concentrations are displayed in Table 1. Multiplex I included the core minimal haplotype loci (DYS19, DYS389I, DYS389II, DYS390, DYS391, DYS392, DYS393, and DYS385). PCR reaction was carried out in a 10.0 μl of reaction volume containing 1–2 ng DNA, 2.0 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA) and 1.6 μl of Gold ST*R 10X buffer (Promega, Madison, WI). Thermal cycling was conducted on a PTC-200 DNA engine (MJ Research, Waltham, MA) using the following conditions: 95 °C for 11 min; 30 cycles of 94 °C for 1 min, 55 °C for 1 min, and 72 °C for 1 min; and a final extension of 60 °C for 45 min.

Multiplex II included the seven STR loci (DYS388, DYS434, DYS435, DYS436, DYS437, DYS438, and DYS439), where DYS388 was added to already reported six STRs [14]. PCR reaction, primer sequences, and cycling conditions were the same as described by Lee et al. [14].

Multiplex III included the four STR loci (DYS385, DYS446, DYS449, and DYS464). PCR reaction was carried out in a 10.0 μl of reaction volume containing 1–2 ng DNA, 2.0 U AmpliTaq Gold DNA polymerase (Applied Biosystems, Foster City, CA) and 1.4 μl of Gold ST*R 10X buffer (Promega, Madison, WI). Thermal cycling conditions were as follows: 95 °C for 11 min; 30 cycles of 94 °C for 1 min, 59 °C for 1 min, 72 °C for 1 min; and a final extension of 60 °C for 45 min.

2.3. Genotyping of PCR products

The PCR products were mixed with GeneScan-500 (ROX) size standard (Applied Biosystems, Foster City, CA) and separated by capillary electrophoresis using an ABI PRISM 310 Genetic Analyzer (Applied Biosystems, Foster City, CA) and GeneScan software 3.1 (Applied Biosystems, Foster City, CA). The typing of PCR products at each 19 STR locus was performed by comparing them to an allelic ladder marker which was constructed after confirming the sequences. Allele designation was carried out using Genotyper 2.5 software (Applied Biosystems, Foster City, CA).

2.4. Construction of sequenced allelic ladders

Sequenced allelic ladders were constructed for 19 Y-STR loci by combining all observed alleles from each locus. Each allele, except DYS389I/II, DYS385, and DYS464, was purified using a QIAquick PCR Purification Kit (Qiagen, Hilden, Germany), and sequenced on an ABI 310 automated sequencer using a BigDye Terminator Cycle Sequencing v2.0 Ready Reaction kit (Applied Biosystems, Foster City, CA). The results were analyzed using Sequencing Analysis Software Version 3.4 and Sequence Navigator 1.01 (Applied Biosystems, Foster City, CA). For DYS389I/II, DYS385, and DYS464, each allele was cloned using pGEM®-T Easy Vector System I (Promega, Madison, WI) following the manufacturer's recommendations. Thereafter sequencing was performed as described above.

2.5. Statistical analysis

The relative frequencies of allele/haplotype occurrences and their gene/haplotype diversities were calculated using the Arlequin statistical analysis package Version 2.000 according to Nei [17]. A linkage disequilibrium exact test using a Markov chain method was also performed using the Arlequin software [18].