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Brief Communication

Species specificities among primates probed with commercially available fluorescence-based multiplex PCR typing kits

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ABSTRACT

To assess species specificities among primates of signals from short tandem repeat (STR) loci included in two commercially available kits, mainly the AmpFlSTR Identifiler kit and additionally the GenePrint PowerPlex 16 system, we analyzed 69 DNA samples from 22 nonhuman primate species representing apes, Old World Monkeys (OWMs), New World Monkeys (NWMs), and prosimians. Each prosimian species and the NWM cotton-top tamarin apparently lacked all STR loci probed. Only one peak, the amelogenin-X peak, was evident in samples from all other NWMs, except the owl monkey. In contrast, several loci, including the amelogenin-X peak, was evident in samples from each OWM species. Notably, for each ape sample, the amelogenin peaks were concordant with morphological gender of the individual. Among the primates, especially in apes, the numbers of alleles for STR loci were increasing according to their phylogenetic order: prosimians < NWMs < OWNs < apes, and so among apes: agile gibbons < white handed gibbons < orangutans < gorillas/common chimpanzees/bonobos. The species specificities among primates for a few commercially released multiplex STR kits examined in this study would contribute to forensic examinations.

1. Introduction

Short tandem repeat (STR) analyses are most commonly used for two purposes in forensic sciences: (1) personal identification and (2) paternity tests. For those purposes, commercial kits for fluorescence-based multiplex PCR amplification and typing of STR loci, including the CODIS 13 core STRs, are available. The AmpFlSTR Identifiler kit (ID) and the GenePrint PowerPlex 16 system (PP16) each produce data for 16 of the following 18 loci: CSF1PO, D2S1338, D3S1358, D5S818, D7S820, D8S1179, D13S317, D16S539, D18S51, D19S433, D21S11, FGA, Penta D, Penta E, TH01, TPOX, vWA, and Amelogenin. There may be substantial homology at STR loci among primate species, including humans [1,2]; however, manuals for these commercially available kits only minimally address nonhuman studies of the target STRs. For example, amplification peaks are evident for most loci with samples from higher primates based on the PowerPlex manual [3]. Similarly, data from the AmpFlSTR manuals indicate significant sequence homologies between nonhuman primate DNA and human DNA based on sequences of amplified products from ape DNA samples (gorilla, chimpanzee, and orangutan) [4–7]. However, none of the manuals provides a concrete, comprehensive description of the differences between nonhuman primates and humans. In other previous studies, a relatively small number of loci or species were investigated with regard to STR variability and specificity [8–16].

In the present study, we used two commercially available kits, ID and PP16, to investigate 17 STR loci and amelogenin in DNA samples from 69 individuals, representing 22 species of nonhuman primates and including apes, Old World Monkeys (OWM), New World Monkeys (NWM), and prosimians. We have documented the patterns of amplification peaks generated from nonhuman primate DNA samples with these commercially available kits and some supplementary genotyping methods. Moreover we have compared the differences of allele patterns between human and nonhuman primate species or among nonhuman primate species to make it possible to determine primate genus and species and to distinguish individuals, and to apply the results to forensic fields.

2. Materials and methods

2.1. Primate species and individuals included in the analysis

The numbers, group, and species of the nonhuman primates analyzed were as follows: 19 apes (3 pygmy chimpanzees (bonobos); *Pan paniscus*, 3 common chimpanzees (common chimps);

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Pan troglodytes, 7 gorillas; *Gorilla gorilla*, 2 orangutans (orans); *Pongo pygmaeus*, 2 white handed gibbons; *Hylobates lar*, and 2 agile gibbons; *Hylobates agilis*), 21 OWMs (3 hamadryas baboons; *Papio hamadryas*, 2 pig-tailed macaques; *Macaca nemestrina*, 3 rhesus macaques; *Macaca mulatta*, 3 japanese macaques; *Macaca fuscata*, 4 crab-eating macaques; *Macaca fasciculas*, 2 stump-tailed macaques; *Macaca arctoides*, a patas monkey; *Erythrocebes patas*, and 3 african green monkeys; *Cercopithecus aethiops*), 13 NWMs (2 squirrel monkeys; *Saimiri sciureus*, 4 capuchin; *Cebus apella*, a black-handed spider monkey; *Ateles geoffroyi*, a long-haired spider monkey; *Ateles belzebuth*, 2 owl monkeys; *Aotus trivirgatus* and 3 cotton-top tamarins; *Saguinus oedipus*), and 6 prosimians (2 greater galagos; *Otolemur garnettii* and 4 ring-tailed lemurs; *Lemur catta*). Data for each of these individuals are listed in Table 1.

2.2. DNA extraction and quantification

Conventional organic extraction was used to purify DNA from blood samples or muscle tissue, and DyNA Quant 200 (Hoefer Pharmacia Biotech, CA) was used to fluorometrically measure the concentration of DNA in each purified sample.

2.3. PCR amplification and typing

17 STR loci and the amelogenin loci were analyzed using two commercially released multiplex PCR amplification and typing kits, ID (AmpFlSTR Identifiler PCR amplification kit, Applied Biosystems, CA) and PP16 (GenePrint PowerPlex 16 system, Promega, WI). Additionally, three other multiplex PCR amplification kits, AmpFlSTR Profiler plus, COfiler, and SGM plus PCR amplification kits (Applied Biosystems, CA) and a D21S11 specific amplification primer set identical to that in ID were used for supplementary genotyping. The STR loci assessed were CSF1PO (CSF), D2S1338 (D2), D3S1358 (D3), D5S818 (D5), D7S820 (D7), D8S1179 (D8), D13S317 (D13), D16S539 (D16), D18S51 (D18), D19S433 (D19), D21S11 (D21), FGA, Penta D (Pe D), Penta E (Pe E), TH01 (TH), TPOX (TPO) and vWA. ID was used to analyze each nonhuman primate DNA sample; the D21 primer set and the other three Applied Biosystems kits were then used to confirm the assignment of each peak to the corresponding locus. In contrast, PP16 was used to analyze only the ape samples. For each analysis with ID or PP16, a 1-ng DNA sample was amplified according to the respective kit manual. A Genetic Analyzer 310 (Applied Biosystems, CA) was used according to the respective kit manual for electrophoresis of PCR products; GeneMapper ID 3.2 software (Applied Biosystems, CA) was then used to analyze each gel image.

3. Results and discussion

3.1. Species specificities among primates

Details of PCR products amplified from 69 primates representing 22 species using ID kits are summarized in Table 1. No amplified peaks were evident with any prosimian or cotton-top tamarin sample. Only one peak that corresponded to the amelogenin X region was evident with each DNA sample from each other NWM species, except for the owl monkeys; notably, these peaks were evident in samples from males and females. On the other hand, each owl monkey sample gave rise to one amelogenin X peak and one peak in the human D13 region. All of the OWM samples of both genders showed the amelogenin-X peak and the peaks in and/or near the ranges of the allelic ladders markers for TPO and/or CSF. Amelogenin-X peaks have been detected in some primate and nonprimate species previously [4–7,9,14,17]. For each ape sample, the amelogenin peaks were concordant with the morphological gender of the individual.

Among the primates, especially in apes, the numbers of peaks at each STR loci, some of which were genotyped as similar to human by the Gene Mapper ID software, were species specific according to their phylogenetic order: prosimians < NWMs < OWNs < apes, and so among apes: agile gibbons < white handed gibbons < orangutans < gorillas/common chimpanzees/bonobos. For some loci, the ranges of peak sizes might have differed between nonhuman primates and humans because of insertions and deletions; therefore, it could be difficult, using ID or PP16, to identify individual STR loci in primate samples based only on peak size. For example, peaks between D8 and D21 in chimps, gorillas, orans, and gibbons differed in size from the human peaks at those loci (Fig. 1). However, in this study, most STR loci in nonhuman primates could be identified without sequence analysis because peak size data generated with ID or PP16 could be compare with that generated with the other three STR kits, which used distinctly labeled fluorescent primers (data not shown). By comparing among data generated with six kits, we were able to distinguish each of the STR 16 loci with ID and PP16. As a result, the allele patterns (ON, OFF and OUT: see Table 1 footnote) of 16 loci with ID or PP16 would make it possible to distinguish between human and nonhuman primate clearly, and additionally to determine some primate genus and species, especially ape species. Some sequence data from BLAST searching also supported to determine which locus out-of-ladder peaks belonged to. For an example, the numbers of repeats at D5 locus is 11, 3, and 2 for human, bonobo and common chimp, respectively from NCBI database. These repeat numbers supported to be determined as "out of the shorter range of the allelic ladder" ("OUT*" in the footnote of Table 1).

Even though we happen to meet partial DNA profiles due to DNA degradation and/or minute template DNA and so on, some information to determine primate genera or species might be obtained from those allelic patterns. Therefore, such patterns would be useful in forensic fields.

3.2. Species specificities in apes

The results of genotyping for six ape species using ID and PP16 are shown in Tables 1 and 2, respectively. Representative electropherograms of ape data generated with ID are shown in Fig. 1a (bonobo), 1b (common chimp), 1c (gorilla), and 1d (oran).

For the bonobos, ID was used to genotype eight loci (D8, CSF, D3, TH, D13, D2, vWA and TPO), and PP16 was used for seven (D8, CSF, D3, TH, vWA, TPO and Pe E). However, peaks detected at four loci (D21, D16, D5 and FGA) with ID and at seven loci (D21, D7, D16, D18 D5, FGA and Pe D) with PP16 were designated off-ladder or out-of-ladder alleles. With ID, no peaks were observed at three loci (D7, D19 and D18), but only at one locus (D13) with PP16. PCR amplification with a D21-primer set made it clear that one or two peaks genotyped as D8 with ID and as D3 with PP16 were actually derived from D21. Results from the both kits seemed to have indicated that a peak observed in a region shorter than the D5 range was actually derived from D5. In one bonobo sample, one allele from the FGA locus was longer than the 42.2 allele; it is well-known that the range including the 42.2 allele is very specific to Africans among humans [7]. For each of the three bonobo samples, only one kind of allele was observed for three loci-D5, Pe E and Pe D.

For the common chimps, as for the bonobos, ID was used to genotype eight loci (D8, CSF, D3, TH, D13, D2, vWA and TPO), and PP16 was used for seven (D8, CSF, D13, TH, vWA, TPO and Pe E); notably, allele-peaks appeared at D13, instead of D3, in the common chimps. With common chimps, as with the bonobos, off-ladder or out-of-ladder alleles were evident at D21, D16, D5