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Short Communication

Identification of variant alleles at AmpF*l*STR SGM Plus STR loci in a sample population of Bangladesh

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DNA profiles obtained from 442 individuals using AmpF*l*STR® SGM Plus™ PCR amplification kit (Applied Biosystems, Foster City, CA, USA) were screened for variant alleles not included within allelic ladder provided by the manufacturer. A total of 5 distinct variants were identified at 3 of the 10 European core STR loci tested. This study identified one variant at D16S539 locus (allele 7), two variants at D21S11 locus (allele 29.3 and 34.1) and two other rare variants at D19S433 locus (allele 11.2 and 18). Of these 5 alleles, variant 29.3 and 34.1 found at D21S11 have been previously reported in Caucasians and African Americans. The rest three variants (allele 7 at D16S539, allele 11.2 and 18 at D19S433) have been separately reported in STRBase (http://www.cstl.nist.gov/biotech/strbase), but no published reports are available. All these alleles fell within the allelic range of the allelic ladder except allele 18 at D19S433 locus, which fell outside the range.

Key words: Allele, locus, variant, chelex, macro.

INTRODUCTION

Short tandem repeat (STR) analysis has become the gold standard for personal identification and paternity testing in most of the forensic laboratories in the world, since its introduction in mid 1990s (Butler, 2005; Fregeau and Fourney, 1993; Gill, 2002; Hammond et al., 1994; Lygo et al., 1994). The current method involves amplification of multiple STR loci in a multiplex fashion. Manufacturer of commercial kits provide allelic ladder that assist the analyst in assigning accurate allele designations (Griffiths et al., 1998). The development of allelic ladder is based on variations observed in different populations and most of the DNA profiles are usually represented in it. However, when large population samples are typed, a small number of individuals found to harbor variant alleles absent from the allelic ladder. Forensic DNA typing laboratories should be aware of the existence of rare alleles, so that the variants are taken into account and dealt carefully in casework interpretation. If these variants are typically omitted from genetic analyses, this may greatly reduce the discriminatory power of STR test batteries, where the DNA is degraded, inconsistencies at single genetic locus or in situations where tested parties are of mixed racial origin. It is therefore important that forensic science community shares information on the occurrence of these variants and reduces complications during STR typing. In this study, we report 5 variant alleles at AmpF*l*STR SGM Plus loci in a sample population of Bangladesh.

MATERIALS AND METHODS

Samples and DNA extraction

Genomic DNA from 442 unrelated individuals was isolated by Chelex method (Walsh et al., 1991). Whole blood and buccal cells were used as a DNA source. Individuals included in this study were selected randomly from samples submitted for casework in this laboratory. Extracted DNA was quantified by using QunatiBlot® Human DNA Quantitation kit (Applied Biosystems, Foster City, CA, USA).

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Table 1. Characteristics of STR loci resulted variant alleles.

STR locus	Chromosome location	Repeat motif	GeneBank accession	Allele range	Allele size range(bp)
D16S539	16q24-qter	GATA	G07925	5-15	237-274
D19S433	19q12-13.1	AAGG	G08026	9-17.2	106-140
D21S11	21q21	[TCTA][TCTG]	AP000433	24-38	187-243

PCR amplification

About 1-2 ng of template DNA was used for each amplification process. Ten STR loci namely D3S1358, vWA, D16S539, D2S1338, D8S1179, D21S11, D18S51, D19S433, TH01 and FGA were co-amplified using AmpF/STR® SGM Plus™ PCR amplifi-cation kit (Applied Biosystems, Foster City, CA, USA). As one of the steps to confirm the variant alleles, the DNA samples were also amplified by PowerPlex-16 PCR amplification kit (Promega Corpo-ration, WI Madison). The PCR reaction was carried out in a GenAmp PCR System 2700 (Applied Biosystems, Foster City, CA, USA). Thermal cycling parameters were setup according to the manufacturer's protocol.

STR typing

PCR amplified fragments were separated on ABI Prism 3100-*avant* Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using POP-4 polymer and Data Collection Software version 1.0 or 1.1. Data were sized using GeneScan Software version 3.7 through the use of GS500-ROX internal size standard. Tabular data from GeneScan were converted to genotype calls using Genotyper version 3.7 NT with the help of Kazam macro. In cases where samples were PCR amplified using PowerPlex 16 PCR amplification kit, Power Typer macro was used.

Variant allele confirmation

All variant alleles were confirmed by re-extraction and amplification. Allelic variants at loci D16S539 and D21S11 were further confirmed by PowerPlex 16 PCR amplificationi kit (Promega Corporation, WI Madison). Alleles not recognized by allele calling software were approximated by comparing allele size with the mean sizes of the flanking ladder alleles given by the manufacturer (Applied Biosystems Corporation). Variant allele designations were also calculated by using the following equations (Gill et al., 1996):

$$\delta_1 = S_Y - L_Y, \quad \delta_2 = S_{OL} - L_X, \quad c = |\delta_1 - \delta_2|$$

Where δ_1 = Size difference (in base pairs) between sister allele Y (S_Y) and ladder allele Y (L_Y), δ_2 = Size difference between the variant (S_{OL}) and the smaller ladder allele adjacent to the variant X (L_X), and c = absolute size difference between the variants and the ladder allele.

Variant alleles that fell outside the allele range of the allelic ladder was extrapolated by comparing variant allele size to the size of the smallest or largest ladder alleles

RESULTS AND DISCUSSION

This study screened a total 442 individuals from Bangladeshi population for variant alleles not included in the allelic ladder provided by the manufacturer. Of 442 individuals, 211 were randomly selected people and 231 were selected from casework samples where children were discarded from the study since their alleles are identical by decent with those of their parents. A total 5 variants occurred at 3 of 10 SGM Plus loci as single observation. All these variants were heterozygotes, paired with well-characterized sister alleles represented in allelic ladder in each case.

Allele '7' at D16S539 was absent from both the allelic ladder provided with AmpF/STR® SGM Plus™ and PowerPlex 16 PCR amplificationi kit. The STRBase Web site (http://www.cstl.nist.gov/biotech/strbase) lists three different reports of this D16S539 variant but no published report is available. This study also observed two variant alleles (allele 29.3 and 34.1) at D21S11 locus. Allele 29.3 has previously been reported in Caucasians and African Americans whereas allele 34.1 was reported in Caucasians, African Americans as well as in Hispanics (Catherine et al., 2005). In STRBase web site allele 29.3 and 34.1 has been reported separately 29 and 14 times, respectively. These two alleles also fell within the allelic range of the allelic ladder in SGM Plus and PowerPlex-16 (Table 2).

At D19S433 locus we identified two variant alleles as 11.2 and 18. Allele 11.2 was within the allelic range, but allele 18 was outside the allelic range. Though these alleles were absent in the allelic ladder they were automatically called by the Genotyper software used in ABI prism 3100 avant Genetic analyzer. Since locus D19S433 is absent in PowerPlex-16, they were confirmed by re-extraction and amplification as well as with the help of equation used by Gill et al. (1996). The STRBase web site lists only one report for allele 11.2 and three for allele 18. A literature search shows that these two alleles have not been reported in any publications.

In conclusion, this study identified 5 unusual variant alleles in a database of nuclear STR profiles from Bangladesh, based on AmpF/STR® SGM Plus™ PCR amplification kit. All these alleles have been separately reported in STRBase. Out of the five alleles, no published report is available regarding three alleles reported here. Dissemination of this information will assist forensic DNA analysts and other human identification laboratories in an Awareness of anomalies due to variant off-ladder alleles encountered during analysis.

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Table 2. List of observed variant alleles.

STR locus	Variant	No. of allele	Frequency observation	Alleles included in SGM Plus	Alleles included PowerPlex-16
D16S539	7	1	0.113	5,8,9,10,11,12, 13,14,15	5,8,9,10,11,12, 13,14,15
D19S433	11.2 18	2 1	0.226 0.113	9,10,11,12,12.2, 13,13.2,14,14.2, 15,15.2,16,16.2, 17,17.2	
D21S11	29.3 34.1	1 2	0.113 0.226	24,25,26,27,28, 28.2,29,29.2,30, 30.3,31,31.2,32, 32.2,33,33.2,34 34.2,35,35.2,36, 37,38	24,25,25.2,26,27, 28, 28.2,29,29.2, 30, 30.3,31,31.2, 32, 32.2,33,33.2, 34, 34.2,35,35.2, 36,37,38

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