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Evaluation of rapidly mutating Y-STRs in Pakistani population

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ABSTRACT

Y-chromosomal Short Tandem Repeats have been widely used in forensic investigations, identification of males for criminal justice purpose and population genetics. Commercially available Y-STRs kits allow the identification of male pedigrees and has a limited application in forensic genetics because of its limitation in differentiating closely related male individuals. Recent research with the Rapidly Mutating Y-STRs (RM Y-STRs) have revealed that these loci deliver significantly higher discrimination capacity and haplotype diversity in worldwide populations when compared with the conventional Y-STRs. Although a number of RM Y-STRs have found their way in most updated commercial kits, there are still some loci that are not yet used in such kits. The aim of this study is to develop RM Y-STR haplotypes frequency database for the Pakistani population, in order to appraise the resolution power of these loci. A total of 212 unrelated males from the Pakistani population were typed with 13 RM Y-STRs which comprise DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a + b, DYS626, DYS627, DYF403S1a + b, DYF404S1, DYS449, DYS547 and DYS612. 211 unique haplotypes were identified, out of which 1 haplotype was shared between two individuals, accounting for 0.9952 discrimination capacity (DC). Haplotype diversity was found to be 0.999925. Gene diversity (GD) values of all the loci were higher than 0.5, where the highest GD values were observed at DYF399S1, DYF403S1a and DYF404S1; with values of 0.99419, 0.98252 and 0.93061 respectively. Results of our study revealed that these 13 RM Y-STRs produced significantly stronger discriminatory power in Pakistani populations.

1. Introduction

Y chromosomal short tandem repeats (Y-STRs) are extensively used in forensic genetics, specifically in cases of sexual assault, paternity testing and missing person investigation. Y-STRs show paternal inheritance due to the non-recombining nature of the Y chromosome which allows to distinguish lineages of human populations but have less abilities to differentiate between related males who belong to the same paternal lineage and conclusive identification cannot be drawn on the individual level [1]. A number of studies have revealed that Y-STR haplotype diversity, as measured with current Y-STR sets, can be enriched and male differentiation can be enhanced by including carefully chosen additional Y-STRs. A panel of rapidly mutating Y-STRs, composed of 13 markers including DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a + b, DYS626, DYS627, DYF403S1a + b, DYF404S1, DYS449, DYS547 and DYS612, with mutation rates above 1×10^{-2} was reported [2], whereas most Y-STRs, including all of those currently used in forensics, have mutation rates in the order of 1×10^{-3} or lower.

2. Materials and methods

2.1. Sample collection and extraction

For the purpose of this study, blood samples were randomly collected on FTA® paper from 212 unrelated male individuals that belong to different areas of Pakistan.

2.2. Multiplex development

PCR amplification of 13 RM Y-STRs was performed using a Veriti™ Thermal Cycler in a 15 µl reaction volume comprising 7 µl of Platinum® PCR Multiplex Master Mix, 1.5 µl of the 13 RM Y-STRs primer mix [3], 6.5 µl of PCR grade water and a 0.5 mm disk of FTA® stained with

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Table 1
Gene Diversity (GD), Haplotype Diversity (HD) and Discrimination Capacity (DC) values calculated for the set of 13 RM Y-STRs.

Locus	GD
DYF387S1	0.92524
DYF399S1	0.99419
DYF403S1a	0.98252
DYF403S1b	0.81691
DYF404S1	0.93061
DYS449	0.84480
DYS518	0.84574
DYS526a	0.74725
DYS526b	0.85321
DYS547	0.83426
DYS570	0.78739
DYS576	0.81865
DYS612	0.79540
DYS626	0.84803
DYS627	0.81825
Average GD	0.85616
Haplotype Diversity (HD)	0.99995
Discrimination Capacity (DC)	0.99520

blood. The following cycling conditions were used: 95 °C for 10 min; followed by 12 cycles of 94 °C for 30 s, 58 °C for 45 s, 72 °C for 60 s; then 20 cycles of 94 °C for 30 s, 55 °C for 45 s, 72 °C for 60 s; and a final cycle of 72 °C for 45 min. Three male controls which include TaqMan, 007 and 9948 positive controls, were used in this study, where 1 µl of each was used as DNA template. Prior to electrophoresis, 1 µl of the amplified products was added to 0.4 µl of GeneScan™ 600 LIZ® Size Standard v. 2.0 and 9.6 µl of Hi-Di™ formamide. Samples were then electrophoresed on an ABI 3500 Genetic Analyzer using 36 cm capillaries and POP-4 polymer, and the fragment analysis was performed with GeneMapper® IDX v.1.4.

2.3. Statistical analysis

Sequencing information was subjected to various forensic statistical analyses to obtain information about gene diversity, match probability, haplotype diversity, power of discrimination and allele frequencies. STRAF (STR Analysis for Forensics) was used for the computation of all these relevant parameters based on population studies [4].

3. Results and discussion

Various forensic statistical analysis parameters were studied, the results of which have been summarized in Table 1. Gene diversity (GD) values, which estimates the probability that two alleles drawn at random from the population will be different, were higher than 0.5 at all loci. The highest GD values have been observed at DYF399S1, DYF403S1a and DYF404S1 with values of 0.99419, 0.98252 and 0.93061 respectively. The lowest GD value was observed at DYS526a with the value of 0.7475. All of the 13 RM Y-STR loci were found to be highly polymorphic, indicating their great potential for forensic individual identification.

Compared with previously published results, 5 samples manifested up to 6 alleles at the DYF399S1 locus, which was found to be an aberrant result. These samples additionally exhibited deletions at five different single allelic markers including DYF403S1b, DYS576, DYS627, DYS570 and DYS449. Although a large number of deletions were present in these samples, all of them were differentiated by only two markers (DYF399S1 and DYF403S1a), signifying the power of RM Y-STRs in forensic applications, especially when it comes to closely related individuals.

4. Conclusion

RM Y-STRs show high discrimination capacity (0.9952) which significantly increases the ability to differentiate between related males in forensic caseworks. 212 haplotypes of 13 RM Y-STRs were identified,

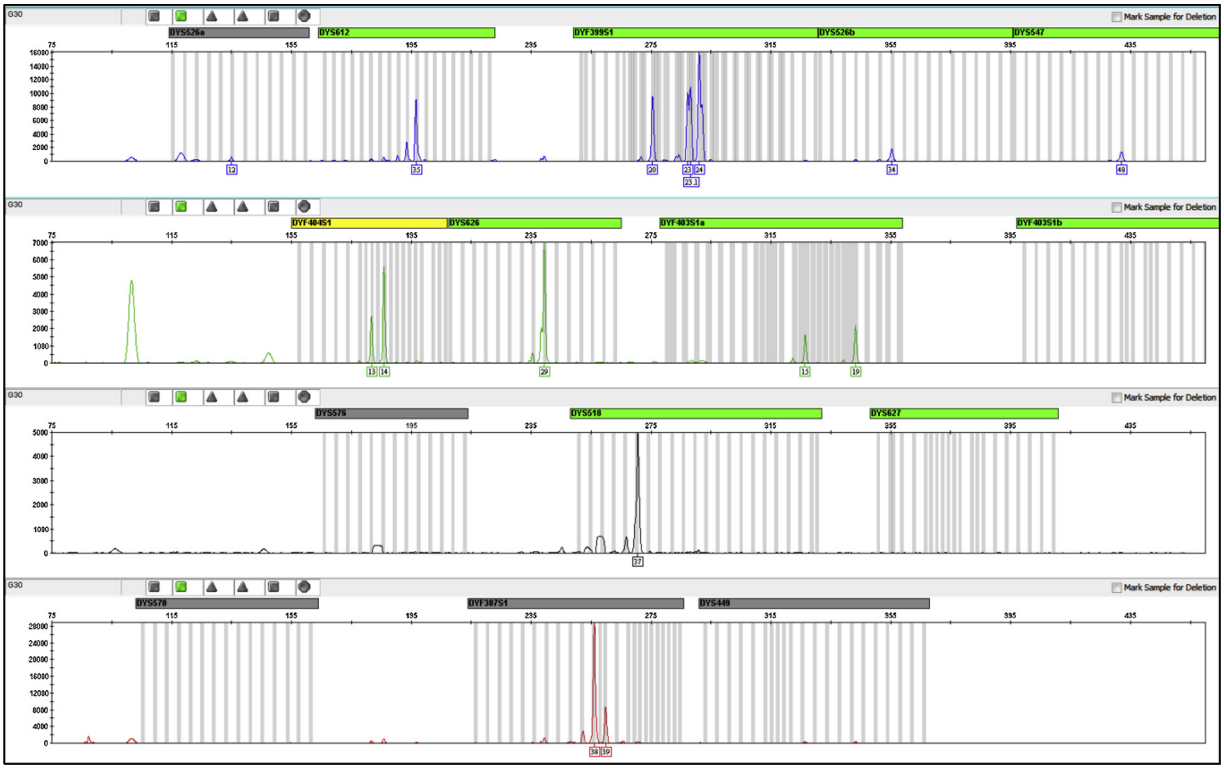

Fig. 1. RM –Yplex haplotype showing the atypical number of alleles detected at DYF399S1 along with deletion detected at DYF403S1b, DYS570, DYS627, DYS576 and DYS449.

out of which 1 haplotype was shared by two individuals, accounting for 99.52% unique haplotypes. Haplotype diversity was found to be 0.999955. All of the 13 RM Y-STR loci were found to be highly polymorphic which indicated their great potential for forensic individual identification. Gene diversity (GD) values of all the loci were higher than 0.5, with the lowest being 0.7475 at DYS526a. On the other hand, the highest GD values were observed at DYF399S1, DYF403S1a and DYF404S1 with values of 0.99419, 0.98252 and 0.93061 respectively. In comparison with previously published results, five samples manifested up to 6 alleles at locus DYF399S an example shown in Fig. 1. These samples also exhibited deletions at five different single allelic markers including DYF403S1b, DYS576, DYS627, DYS570 and DYS449; yet two markers, which include DYF399S1and DYF403S1a, were able to differentiate all of those samples. The results of our study revealed that the 13 RM Y-STRs produced significantly stronger discriminatory power in Pakistani populations.

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