Population and mutational assessment of novel repeats in 13RM Y-STRs in unrelated males born in Gilgit, Pakistan

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ABSTRACT

Because they are totally transferred to the future generations until mutations occur, Y chromosome genetic markers are commonly utilised in forensics for the classification of male lineages for criminal justice purposes. The mutation rate of Rapidly Mutating Y-STRs (RM Y-STRs) markers is high. That is not seen in other Y-STRs markers, and they appear to be effective in distinguishing paternally related men. This study aimed to estimate the population and mutational parameters of 13 RM Y-STRs in 13 unrelated males born in Gilgit, Pakistan. Repeat there was no population substructure and strong discriminating capacity in the counts. In this population, there were higher mutation rates with the unusual structure of repeats. More research is needed to better characterize these loci in diverse Pakistani groups.

Keywords:
Mutation rates
Rapidly mutating Y-STRs
Y chromosome

1. Introduction

Y-STRs (Y-chromosomal short tandem repeats) are STRs (also known as microsatellites) from the non-recombining region of the human Y chromosome that are used to determine paternity when the putative father is not present, as well as in forensic genomics (Chen et al., 2015). In the forensic field, short tandem repeats are the most commonly employed polymorphism markers (STRs) (Zhang et al., 2015; Collins et al., 2004). In many human populations, for example, Y-STR is used to differentiate lines; however, it is less effective in distinguishing between related males who share the same paternal lineage and cannot be identified on an individual level (Kayser et al., 2004). Consanguineous marriages are frequent in Pakistan, with first and second cousins accounting for 61% of all marriages. First cousin marriages are more common on the paternal side than on the maternal side (32%), (21%). Second cousins account for 8% of all marriages, while other relatives account for 7% and non-relatives account for 33%. There are a variety of consanguinity tendencies in both urban and rural areas. In urban regions, 40% of first-cousin marriages occur, but in rural areas, 57% occur (http://www.pbscensus.gov.pk). Due to these developments, genetic distinction between related and unrelated males in the Pakistani population is difficult (Adnan et al., 2015). Sexual offences against the human population, paternity tests, and missing person reports all use Y-chromosome STRs. Because Y-STRs have paternal ancestry, each community has a significant structure that aids in understanding population history (Karafet et al., 2008). In these cases, the most latest Y-STR panels (Yfiler and PPY23) have proven to be beneficial (Adnan et al., 2018:...
Adnan et al., 2019: Ye et al., 2015: Gibson-Daw et al., 2017: Rapone et al., 2016: Redd et al., 2002). This is primarily due to male-specific Y-chromosome markers being uniparental inherited and lacking recombination. According to several studies, Y-STR haplotype diversity can be increased using current Y-STR sets, and male lineage differentiation can be improved by carefully selecting extra Y-STRs (Hanson and Ballantyne, 2004, 2007; Vermeulen et al., 2009). A panel of 13 rapidly mutating (RM) YSTRs (DYF399S1, DYF387S1, DYS570, DYS576, DYS518, DYS526a + b, DYS626, DYS627, DYF403S1a + b, DYF404S1, DYS449, DYS547, and DYS612) has been reported. Most Y-STRs, including all currently used in forensics, have mutation rates in the order of $1 \times 10^{-3}$ or below, and mutation rates in the order of $1 \times 10^{-3}$ or lower have been observed. This RM Y-STR panel differentiates between males who are closely related and males who are distantly related extremely effectively (Ballantyne et al., 2012). The study aims to develop a 13 R-M Y-STRs database for the Pakistani population and to increase the global database for 13 R-M Y-STRs. Polymorphism is detected in Y-STRs through data analysis. Burki (2015), Hall (1999), Tamura et al. (2013) and Weightman (2002) discuss such type of results in the published works.
2. Methods

2.1. Sampling, DNA extraction, and quality estimation

Blood samples were collected from 13 unrelated healthy male individuals from different areas of the Gilgit population. Informed consent was signed by all members who contributed to the study. DNA was isolated from preserved blood using commercially available kit i.e. Genomic DNA kits (Thermo Fisher Scientific Inc., Waltham Massachusetts, USA) according to the manufacturer’s instructions. A NanoDrop™ 1000 spectrophotometer was used to determine parameters such as concentrations and purity for extracted DNA products.

2.2. PCR amplification

Thirteen single copy or multi-copy primers were selected from the rapidly mutating RM-YSTRs reviewed from the literature and STRBase database (Balassa et al., 2021; Rutberg et al., 2001). UCSC genome browser provides In-Silico PCR online tool (Kuhn et al., 2008; Sabit, et al., 2021) which is used for retrieving PCR amplicon lengths for related primers. Selected primers were used for PCR amplification to gain amplified products for sequencing. Primer sequences and library length used for PCR amplification are given in Table 1. PCR amplification was done using a 25 μl reaction mixture containing: 6 μl of master mix (Go Taq Green, Thermo Fisher Scientific), 2 μl of genomic sample DNA, 1.5 μl of each primer (Forward and Reverse), and double distilled water to level the value of reaction mixture. The amplification was run on SimpliAmp Thermal Cycler (Applied Biosystems, Foster City, CA, USA) under the following conditions: Initial denaturation of 15 min at 95 °C followed by 40 cycles of 1 min at 95 °C and 1 min at 60 °C. Then the final extension of 10 min was set at 72 °C. PCR products were separated and visualized by Gel electrophoresis. 4 μl of PCR product and 3μl of DNA loading dye (TFS) was subjected to 1.5% of agarose gel. A voltage gradient of 100 was applied for 30–35 min. DNA bands were visualized by using UV light.

2.3. Sanger sequencing

Following the manufacturer’s instructions, the PCR product was purified using the kit method (Gene JET Genomic DNA Purification Kit). Sanger sequencing of PCR amplicons was done through MACROGEN Korea.

Fig. 4. Multiple sequence alignment for RM-YSTR Primers using MEGA7 showing repeats among various sequences against reference. (A) DYS626, (B) DYS627, (C) DYS526. Gap in alignment highlights variation in STR Repeat. Visualization in Black color view via BioEdit.
2.4. RM Y-STR nomenclature

The RM Y-STR nomenclature used in this study is in agreement with the DNA commission’s recommendations from the International Society of Forensic Genetics (ISFG) (Gusmao et al., 2006). The allele designation and repeat structure of each locus are shown in Table 2.

2.5. Data analysis

Sequencing outputs were subjected to various analysis steps to study STR repeat variation. Biological sequence alignment editor (Bio-Edit 7.2.6.1) is an open-source alignment tool that was used for refining and trimming unnecessary parts in sequences. Repeat motifs were estimated by direct counting.

3. Results

We studied 13 RM Y-STR loci in 13 unrelated males from Pakistan’s Gilgit province in this study. RM Y-STR markers were discovered to be DYF387S1, DYF399S1, DYF403S1, DYF404S1, DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627 (all with mutation rates > 1 × 10⁻²). Nine of the 13 RM Y-STR markers were single-copy (however, six of them included multiple Y-STR loci in one amplicon). Five markers are included in multicopy systems (DYS526 with two copies, DYF387S1 with two copies, DYF404S1 with two copies, DYF399S1 with three copies, and DYF403S1 with four copies). Table 2 provides the observed allele ranges of the 13 RM Y-STRs, as well as other variables, as well as detailed information on the repeat structure and chromosomal position. Figs. 1-6 depicts the graphical representation of the obtained results.

3.1. Repeat motif counting

Every two unrelated male individual samples had each of the 13 RM Y-STR markers applied. The tetranucleotide markers DYS570 and DYS576 are also simple tetranucleotide markers. DYS570 and DYS576 have the structures (TTTC)n and (AAAG)n, respectively. For DYS570, the number of repeats was (TTTC)3–16, while for DYS576 it was (AAAG)2–20. The structures reported for DYS449, DYS518, DYS526, DYS547, DYS570, DYS576, DYS612, DYS626, and DYS627 (all with mutation rates > 1 × 10⁻²). Nine of the 13 RM Y-STR markers were single-copy (however, six of them included multiple Y-STR loci in one amplicon). Five markers are included in multicopy systems (DYS526 with two copies, DYF387S1 with two copies, DYF404S1 with two copies, DYF399S1 with three copies, and DYF403S1 with four copies). Table 2 provides the observed allele ranges of the 13 RM Y-STRs, as well as other variables, as well as detailed information on the repeat structure and chromosomal position. Figs. 1-6 depicts the graphical representation of the obtained results.
repeat-structured tetranucleotide complex locus (GAAA). The DYF399S1 marker has the same number of repetitions (GAAA) 3N11(GAAA)16 in both samples. The DYS626 locus is a tetranucleotide amplicon with a complicated structure. A length polymorphism was discovered downstream from the major (GAAA) polymorphism in the repeat unit (GAAA). The structure of this

Table 1
In PCRs, RM Y-STRs primer sequences and library length were used.

| RM Y-STR | Forward Primer (5'-3') | Reverse Primer (5'-3') | Library length (bp) |
|----------|------------------------|------------------------|---------------------|
| DYS576   | TGGGCTGAGGAGTGCAATC    | GGCAGTCTCATTCTCGCAG    | 191                 |
| DYS570   | GAACGTGCTCTGAAGCTCAG   | TCAGCATTGACAAAGCAGCA   | 256                 |
| DYS3175  | GCTGGTGGAGAGCTCAGGAAG | GGCAGTCTGAGAAAACTGTA   | 261                 |
| DYS399S1 | GGGTTTCCAGAGTACGAGT   | CACTTTTGGGACATCTTC    | 289, 302, 293       |
| DYS626   | GCCAGGGCACTCGAGCGG    | AGAAGATTTTTTGGGACATTT | 253                 |
| DYS627   | CATGCTGACAGGCGAGGT   | GCCATAGGCAAAGCGGAG     | 337                 |
| DYS526a+b| TCTGTCGACACTGATCCAAA  | GCCGTTACTGCCCCAGAAGGT  | 370                 |
| DYS5518  | GCGAACAAGTGAAAACGC    | TCAGCTCTCATTGCGTACG    | 279                 |
| DYS612   | CCCCATGCCCACTGAAAGTA   | GTGACGAAAAGCAGAAAAAG   | 264                 |
| DYF404S1 | GCCCTGGAATTTCCACCACTA | CCAATGTCGACAAAGCTAGC   | 197, 189            |
| DYS449   | TCTGACGCTGACAGCTTCTA  | CCTGGAAATGGGAGTTGTCTG  | 355                 |
| DYS5547  | TCCATGCTTACTCGAAAAATAC | TGCACAGCATAAAGCTTCTC   | 438                 |
| DYF403S1 | CAAAT TCATGTGAGATAGTA | ACAGGCGAGATCCATCTA     | 312, 316, 437       |

Fig. 6. Multiple sequence alignment for RM-YSTR Primers using MEGA7 showing repeats among various sequences against reference. (A) DYS449, (B) DYS547, (C) DYS403S1. Gap in alignment highlights variation in STR Repeat. Visualization in Black color view via BioEdit.
locus results (GAAA)$_n$(GAAA)$_m$(GAAA)(GAAG)(AAAG)(GAAA)(GA AA). The total number of repeats in our sample was (GAAA)$_{14-16}$ - N$_{24}$(GAAA)$_n$(GAAA)$_m$(GAAA)$_n$(GAAA)$_m$(GAAA)$_m$(GAAA)$_m$. A previous research of 51 populations from around the world found a similar genetic structure (HGDP-CEPH) (Ballantyne et al., 2012). The repetition motif of each locus is (AGAA)$_n$(AGAA)$_m$(AGAA)$_n$(AGAA)$_m$(AGAA)$_m$(AGAA)$_m$. The hypothesised structure for locus DYF526 (a + b) is (CCTT)(CTTC)(TCTC)(CTTC)N(TCTC)N(TCTC)N(TCTC). DYS547 Yq11.221 (AAAG)$_{11-12}$(GAAG)$_{1}(AAAG)$_{12}$(GAAG)$_{2}$(AAAG)$_{1}$(GAAG)$_{2}$(AAAG)$_{2}$(GAAG)$_{2}$. DYS547 is the reported structure for DYS627, and (CAAT)(AAAG)(GAAG) is the reference assembly represented as (CCTT)$_{n}$(CTTC)$_{n}$(TCTC)$_{n}$(TCTC)$_{n}$(TCTC)$_{n}$(TCTC)$_{n}$. DYS547 in this population will be confirmed further by sequencing. For DYS518 the reference assembly contains an array of AAAG followed by GAAG. However, we observe GGAG in place of GAAG and novel repeat motif AAGG also found in our samples. DYS547 in the Gilgit population group of Pakistan. These RM loci have shown their full potential for identification in the Gilgit population. Further study is needed to identify the haplotype frequencies and diversities for these RM Y-STRs to assess their full potential for identification in the Gilgit population.

5. Conclusion

This study offered loci polymorphism for 13 RM Y-STR makers in the Gilgit population group of Pakistan. These RM loci have proven to be ideal in the identification and discrimination of unrelated as well as paternally related male individuals in forensic investigations. The variations in the identification power of Loci DYF518 and DYS576 in this population will be confirmed further by sequencing. It can be proposed soon that currently used Y-STR kits will be replaced by RM Y-STR because of their higher diversity and discriminating power. Further study is needed to identify the haplotype frequencies and diversities for these RM Y-STRs to assess their full potential for identification in the Gilgit population.

Consent for publication

Yes, all patients included in this research gave written informed consent to publish the data contained within this study. If the patient was less than 16 years old, deceased, or unconscious when consent for publication was requested, written informed consent
for the publication of this data was given by their parent or legal guardian.

6. Availability of data and material

The Availability of data and materials section I was used the raw data in this study and all data is contained within the manuscript and no additional files.

Competing interests: The authors declare no conflict of interest.

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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