



Y-STR Mutation Patterns in North Indian Male Relatives at 16 Loci: A Preliminary Study

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Article History

Received: December 29, 2025

Accepted: May 12, 2026

Published: May 25, 2026

Abstract

Y-chromosomal short tandem repeats (Y-STRs) are transmitted through the paternal lineage, from father to son. Due to their relatively low mutation rates across generations, Y-STRs are widely used in forensic science and paternal ancestry analysis. However, mutations at Y-STR loci can complicate kinship analyses and may lead to false exclusions in father–son or extended paternal lineage testing. In this preliminary study, putative Y-STR mutations were investigated in 292 paternal lineage cases from the North Indian population. The analyzed cases included sibling pairs, grandparentage cases, uncle–nephew pairs, first cousins, and second cousins. A total of 29 mutations were identified across 11 Y-STR loci among the 16 loci examined. All observed mutations involved single-step repeat changes, consistent with the stepwise mutation model. The highest mutation rates were detected at DYS458 (0.006) and DYS385 (0.005), whereas the lowest rates (0.001) were observed at loci including DYS19, DYS390, and DYS389II. These findings are consistent with previously reported locus-specific mutation rates. They also support the value of more rapidly mutating Y-STR loci in improving the resolution of closely related paternal lineages. Overall, these findings provide baseline estimates of Y-STR mutation rates in the North Indian population and emphasize the importance of population-specific data in forensic applications and genealogical research.

Keywords:

Y-chromosomal short tandem repeats; Y-STR mutation; Y-STR loci; Y-STR markers; forensic genetics

1. Introduction

Y-chromosomal short tandem repeats (Y-STRs) are widely used in forensic genetics, paternity testing, and anthropological studies due to their paternal inheritance pattern and the absence of recombination across most regions of the Y chromosome [1,2]. Approximately 95% of the Y chromosome comprises the non-recombining region (NRY). Mutational changes at Y-STR loci represent an important source of informative haplotypic variation for forensic analysis and the inference of recent paternal relatedness [2,3]. This makes Y-STR profiling useful for assessing paternal relationships, identifying male contributions in mixed DNA samples, and tracing paternal family lineages. The primary mechanism underlying STR mutations is polymerase slippage during DNA replica-

tion. This process typically results in the gain or loss of a single repeat unit [4,5]. Although such mutations occur infrequently, their characterization is important for accurate forensic genetic interpretation. Y-STR markers can assist in differentiating paternal lineages; however, failure to account for mutational events may result in false exclusions in father–son paternity testing [1,6]. Accordingly, accurate knowledge of mutation rates across different Y-STR loci is essential for robust forensic interpretation and the development of population-specific reference databases. Recent large-scale pedigree analyses and forensic reviews have emphasized the importance of population-specific mutation rate data for reliable kinship assessment and likelihood ratio estimation [1,7]. The present study investigates Y-STR mutations in 292 paternal lineage cases

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from the North Indian population. By comparing Y-STR profiles among close and distant paternal relatives, this study provides insights into mutation patterns, locus-specific variation, and their forensic relevance in genetic investigations.

2. Materials and Methods

2.1. Study Population

This study is a retrospective analysis of anonymized data derived from routine casework conducted between August 2016 and December 2024 at an ISO 15189-accredited laboratory in North India. A total of 292 paternal lineage cases from the North Indian population were included. Institutional Ethics Committee approval was obtained before data analysis and manuscript preparation. Written informed consent was obtained from all participants at the time of sample collection as part of routine casework.

2.2. Sample Collection and DNA Extraction

Peripheral blood (3 mL) was collected in EDTA vacutainer tubes (BD Vacutainer®, USA) as part of routine casework for Y-STR analysis. No additional sample collection or experimental intervention was performed specifically for this study. DNA extraction was performed using a Macherey-Nagel solid-phase extraction kit (Germany) according to the manufacturer's instructions, followed by DNA quantification using a Qubit® 2.0 Fluorometer (Invitrogen, USA).

2.3. PCR Amplification and Genotyping

Genotyping was performed using the Yfiler™ PCR Amplification Kit (Thermo Fisher Scientific, USA) with approximately 1 ng of template DNA. PCR cycling was performed on a Veriti Thermal Cycler under standard conditions: initial denaturation at 95 °C for 11 min, followed by 30 cycles at 94 °C for 1 min, 61 °C for 1 min, and 72 °C for 1 min, with a final extension at 60 °C for 80 min. PCR products were analyzed on a SeqStudio Genetic Analyzer after mixing with Hi-Di™ formamide and LIZ® size stan-

dard. Allele calling was performed using GeneMapper® v5 software.

2.4. Statistical Analyses

Mutation rates were estimated using a direct counting approach, where the number of observed mutational events was divided by the total number of meioses analyzed at each locus. Per-locus and overall mutation rates were reported to three decimal places. Exact 95% confidence intervals were estimated using the Clopper–Pearson method under a binomial distribution framework. Data tabulation and calculations were performed using Microsoft Excel.

3. Results

A total of 292 paternal lineage cases were analyzed, comprising 66 sibling and grandparentage cases, 133 uncle–nephew cases, 70 first-cousin cases, and 23 second-cousin cases. Across these cases, 29 mutational events were identified at 11 of the 16 Y-STR loci examined, as shown in [Table 1](#). No mutations were found at five loci: DYS389I, DYS392, DYS437, DYS438, and DYS448. This suggests relative stability of these five loci in the studied dataset; however, larger datasets would be required to confirm locus-specific stability in the wider population. All observed mutations were single-step changes, consistent with the stepwise mutation model, as summarized in [Table 2](#). The observed proportion of mutation-positive cases appeared to increase with greater genetic distance. A progressive increase in the proportion of mutation-positive cases was observed with increasing meiotic distance (siblings < uncle–nephew < first cousins < second cousins), consistent with the stepwise accumulation of mutations across generations. The highest observed proportion was noted in second-cousin pairs (13.04%), as shown in [Table 1](#). This observed trend across relationship categories is illustrated in [Figure 1](#). Per-locus mutation rates ranged from 0.001 to 0.006, as presented in [Table 3](#), with the highest rates observed at DYS458 (0.006) and DYS385 (0.005), followed by DYS391, GATA-H4, and DYS635. The distribution of per-locus mutation rates is illustrated in [Figure 2](#).

Table 1: Distribution of observed Y-STR mutations among studied cases.

Relationship Type	No. of Cases	Total Meioses	Mutations Observed	% of Cases with Mutation
Siblings & grandparentage	66	2112	5	7.58%
Uncle-Nephew	133	6384	13	9.77%
First cousins	70	4480	8	11.43%

Table 1: *Cont.*

Relationship Type	No. of Cases	Total Meioses	Mutations Observed	% of Cases with Mutation
Second cousins	23	2208	3	13.04%
Total	292	15,184	29	—

Percentages represent the proportion of cases with at least one mutation within each relationship group. Total meioses were calculated by multiplying the number of cases by the relevant meiotic distance and the number of Y-STR loci examined.

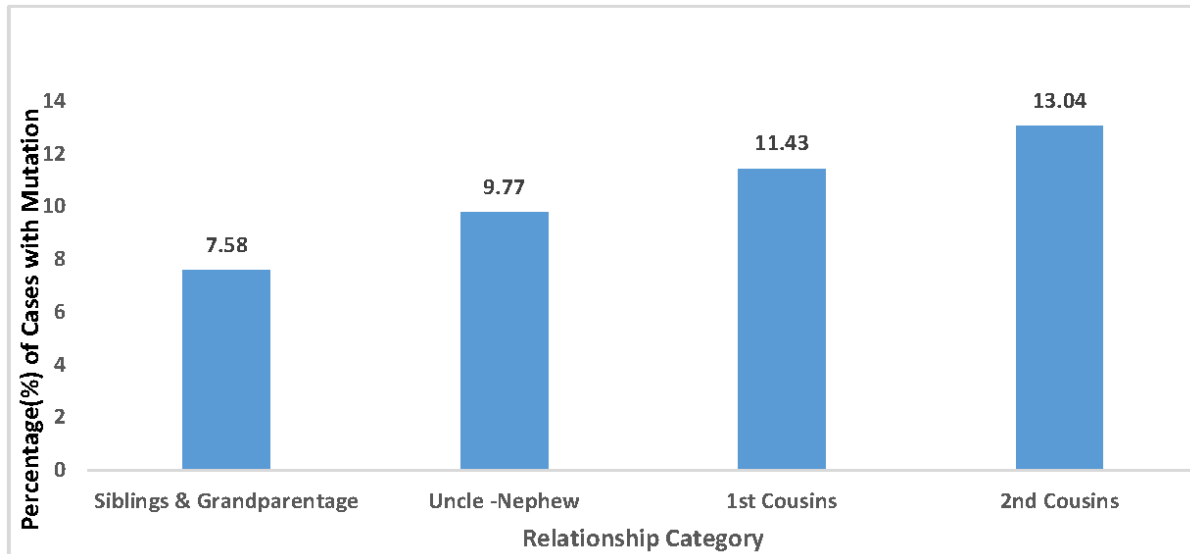


Figure 1: Mutation frequency across relationship categories.

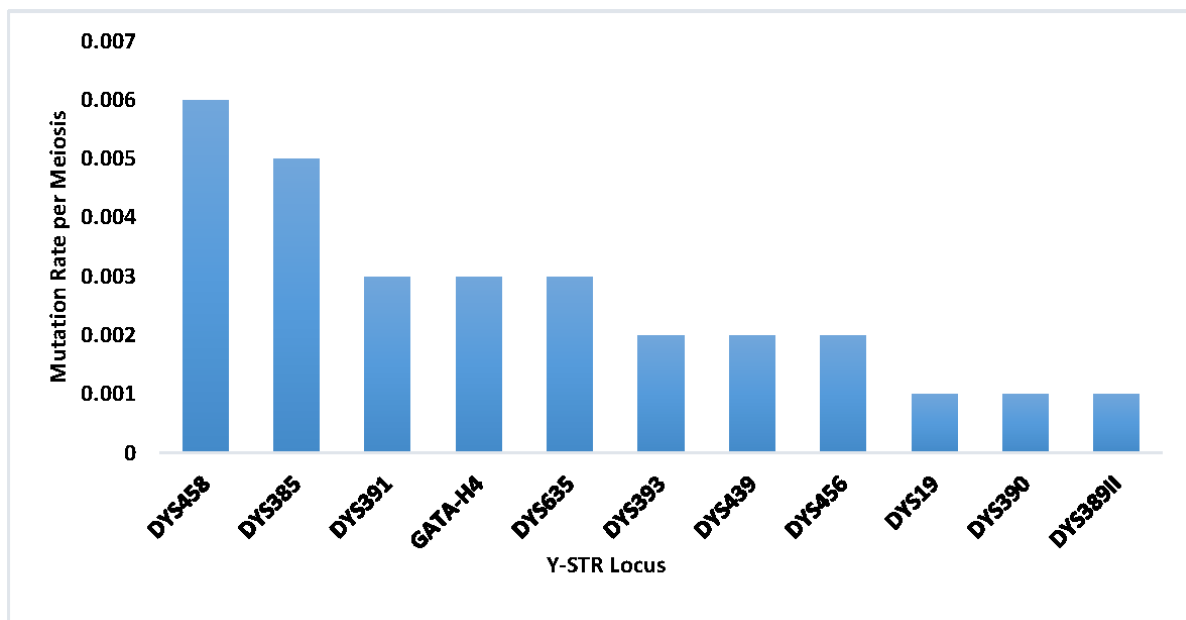


Figure 2: Per-locus Y-STR mutation rates observed among the studied population.

Table 2: Summary of mutation events by locus among studied cases.

Y-STR Locus	No. of Mutations	Mutation Type	Example Allele Shift
DYS385	5	Single-step	16→15, 17→16, 15→16, 13→14, 19→18
DYS391	3	Single-step	10→11, 11→10
DYS458	6	Single-step	16→17, 18→19, 17→16, 18→17, 18→19
DYS19	1	Single-step	18→19
DYS393	2	Single-step	14→13, 13→14
GATA-H4	3	Single-step	13→12, 12→13
DYS439	2	Single-step	11→10, 12→13
DYS635	3	Single-step	20→21, 22→23
DYS456	2	Single-step	13→14, 16→17
DYS390	1	Single-step	24→25
DYS389II	1	Single-step	32→31
Total	29	—	—

All mutations were single-step changes, consistent with the stepwise mutation model. Example allele shifts are provided for representative cases.

Table 3: Per-locus mutation rates with exact 95% confidence intervals.

Y-STR Locus	Mutation Count (x)	Mutation Rate	95% CI (Clopper–Pearson)
DYS458	6	0.006	0.0023–0.0137
DYS385	5	0.005	0.0017–0.0123
DYS391	3	0.003	0.0007–0.0092
GATA-H4	3	0.003	0.0007–0.0092
DYS635	3	0.003	0.0007–0.0092
DYS393	2	0.002	0.0003–0.0077
DYS439	2	0.002	0.0003–0.0077
DYS456	2	0.002	0.0003–0.0077
DYS19	1	0.001	0.0000–0.0059
DYS390	1	0.001	0.0000–0.0059
DYS389II	1	0.001	0.0000–0.0059

* Mutation rates are reported to three decimal places, while confidence intervals are presented to four decimal places.

4. Discussion

This study provides preliminary data on Y-STR mutational patterns in the North Indian population and contributes to available data on paternal lineage markers. All the Y-STR mutations observed in this study were single-step changes, consistent with the stepwise mutation model of STR evolution [5,8]. No mutations were observed at five Y-STR loci: DYS389I, DYS392, DYS437, DYS438, and DYS448. These loci showed relative stability in the present dataset, supporting their usefulness in paternal lineage assessment; however, this finding should be interpreted cautiously because of the limited sample size. The predominance of single-step mutations is also consistent with findings reported across multiple populations. This suggests that Y-chromosomal STRs may follow broadly similar mutational mechanisms across diverse human populations [1,2].

Mutation rates at commonly used Y-STR loci may show broad similarities across populations; however, regional validation remains essential for accurate kinship analysis [1,3]. Even small population-specific differences

in mutation rates can influence likelihood ratio calculations and the interpretation of kinship in relationship testing [3,6]. Therefore, estimates of mutations from regional populations help improve the statistical standardization and reliability of paternal lineage testing in Indian forensic casework. Among loci with observed mutations, the mutation rate in this study ranged from 0.001 to 0.006 per meiosis. This range is consistent with previously reported findings from studies of European and East Asian lineages, including large-scale father–son comparative datasets [1,2,9]. Moreover, haplogroup-stratified analyses have demonstrated modest inter-haplogroup variability in mutation behavior, underscoring the importance of region-specific datasets for improved forensic standardization [3,7,8]. Loci showing higher mutation frequencies, such as DYS458 and DYS385, may provide greater discriminatory value for distinguishing closely related paternal lineages [10–12]. Conversely, more stable Y-STR loci remain important for supporting paternal ancestry assessment. Loci such as DYS19 and DYS389II exhibited low mutation rates, indicating greater consistency for paternal lineage verification but lower discriminatory power

for differentiating closely related male individuals. More mutations were observed in cases involving more distant paternal relatives, such as first cousins and second cousins. An increase in mutation frequency with greater meiotic separation was observed across the relationship categories, including sibling/grandparentage cases, uncle–nephew pairs, first cousins, and second cousins. The observed trend supports the expected pattern of gradual mutation accumulation across generations. Although the current sample size limits formal modeling of mutation rates, the observed pattern is consistent with extended lineage studies reporting variability in Y-STR mutation behavior across paternal lineages and haplogroups [2].

From a forensic casework perspective, locus-specific mutation rate estimates are essential for accurate statistical interpretation in paternal lineage testing. Y-STR loci with higher mutation rates can improve discrimination among closely related males, particularly in complex kinship investigations involving extended paternal relationships. However, even single-step mutations may result in apparent mismatches in father–son comparisons. Incorporating empirically derived mutation rates into likelihood calculations can reduce the risk of false exclusions and improve the reliability of forensic conclusions [3,6,13]. Future studies with larger sample sizes and detailed haplogroup characterization are required to refine population-specific Y-STR mutation rate estimates for India. Additionally, high-resolution Y-STR sequencing may help clarify specific mutational patterns [5]. Such advances are expected to enhance the forensic utility of Y-STR markers and support anthropological and genealogical studies in North Indian populations.

5. Conclusions

This study provides preliminary estimates of Y-STR mutation rates for selected loci in the North Indian population. All observed mutational events were single-step changes. The highest mutation rates were observed at DYS458 and DYS385, broadly aligning with reports from other populations. These findings highlight the importance of considering locus-specific mutation rates when differentiating closely related paternal lineages and interpreting possible mutational mismatches in kinship testing. Establishing population-specific Y-STR mutation data may enhance the reliability of forensic casework and genealogical applications in India.

6. Limitations

This study has certain limitations. First, haplogroup testing was not performed as part of routine casework; there-

fore, haplogroup-specific variation in mutation rates could not be assessed. Given that Y-STR mutational behavior may vary among haplogroups, this limits the generalizability of the findings.

List of Abbreviations

CI	Confidence Interval
DNA	Deoxyribonucleic Acid
EDTA	Ethylenediaminetetraacetic Acid
ISO	International Organization for Standardization
NRY	Non-Recombining Region
PCR	Polymerase Chain Reaction
STR	Short Tandem Repeat
Y-STRs	Y-chromosomal Short Tandem Repeats

Author Contributions

Conceptualization: V.C.M., V.R.; Methodology: D.C., A.R.; Formal analysis: V.C.M., D.C., R.S.; Data curation: D.C., D.D.; Writing-original draft, writing-review & editing: V.C.M., D.D., A.R., R.S.; Visualization: V.R.; Supervision: V.R., V.C.M.; Project administration: V.C.M., V.R. All authors have read and agreed to the published version of the manuscript.

Availability of Data and Materials

The data supporting the findings of this study are included within the article.

Ethics Committee Approval and Consent to Participate

This study was approved by the Institutional Ethics Committee of Chimera Transplant Research Foundation (CH/002/2025; Approval Date: 22 March 2025). Written informed consent was obtained from all participants during routine casework procedures. The study involved the analysis of anonymized data derived from previously collected casework samples, with no additional sample collection or intervention performed specifically for research purposes.

Human Rights Statement

The study was conducted in accordance with the Declaration of Helsinki.

Conflicts of Interest

The authors declare no conflicts of interest.

Funding

The study did not receive any external funding and was conducted using only institutional resources.

Acknowledgments

The authors are grateful to our laboratory colleagues for their kind support.

AI Declaration

While preparing this manuscript, the authors used Grammarly to improve readability and language. After using this tool, the authors reviewed and edited the content as needed and take full responsibility for the content of the publication.

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