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Research Article

Mutation Rate Analysis of 26 Rapidly Mutating Y-STRs in a Tanzanian Male Population

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Abstract

Y-chromosome short tandem repeats (Y-STRs) are widely used in forensic investigations, including sexual assault cases, paternity testing, and disaster victim identification. Despite their usefulness, conventional Y-STRs with low mutation have limitations in distinguishing males within the same paternal lineages. The development of Rapid Mutating Y-Chromosome Short Tandem Repeats with a mutation rate of 10^{-2} has enhanced the capacity of differentiating related males. This study aimed to characterize the mutation rate and pattern of 26 Rapidly Mutating Y-Chromosome Short Tandem Repeat (RM Y-STR) loci in Tanzania where population-specific data are lacking for local casework. 138 DNA confirmed unrelated father-son pairs from consented individuals in Dar es Salaam were analyzed. Genomic DNA was extracted using the Chelex 100 method, amplified with the Microreader™ 26 RM-Yplex Amplification Kit, and analyzed by capillary electrophoresis. Mutation events were identified by comparing allele profiles between father and son across all loci. A total of 34 mutations were observed, with the most occurring at a single locus following a stepwise mutation model with a 59% gain and a 41% loss of alleles. DYF399S1 exhibited the highest locus-specific mutation rate (6.5×10^{-2}; 95% CI: 3.0×10^{-2}–1.22×10^{-1}), while the overall mutation rate across loci was 1.0×10^{-2} (95% CI: 6.0×10^{-3} to 1.3×10^{-2}), consistent with global estimates. These results establish RM Y-STRs as a powerful forensic tool for discriminating paternally related males in Tanzania, filling a critical data gap for local casework.

Keywords

Rapidly Mutating Y-STRs, Forensic Genetics, Mutation Rate, Tanzanian Population

1. Introduction

The use of forensic science as an accurate technique for individual identification and analysis of biological relationships is currently critical for delivering justice and resolving complex criminal and civil (paternity) cases [1]. As forensic methodologies continue to advance, the relevance and applications of Y-chromosome short tandem repeat (Y-STR) profiling are expanding, reinforcing its significance in modern forensic investigations, as previously reported [2-4]. Y-chromosome short tandem repeat (Y-STR) profiling is an indispensable tool in modern forensic science, providing definitive resolution in complex investigations. It is uniquely powerful for confirming

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paternal lineage, isolating the male contributor in sexual assault casework, and identifying male victims in mass disaster scenarios [2]. The haploid nature of the Y chromosome, which is paternally inherited and largely exempt from recombination, is the foundation of this utility. However, this characteristic means that conventional Y-STR markers lack the discriminatory power of their autosomal counterparts [5, 6]. Often failing to distinguish between male relatives or even unrelated males from a common ancestral line. This limitation is compounded by the low mutation rate of standard Y-STRs (~ 1×10^{-3} per meiosis), which results in a high prevalence of shared haplotypes within populations [5]. Consequently, while conventional Y-STR analysis presents a critical resolution gap, driving the need for more advanced typing systems to achieve conclusive individualization in forensic casework [6]. Following their discovery, studies in 2012 and 2014 provided the first empirical evidence that rapidly mutating Y chromosome short tandem repeats (RM Y-STRs) could effectively differentiate between paternally related males, a limitation of traditional Y-STRs [5, 7].

The limitations of conventional Y-STRs in discriminating between male relatives, due to their low mutation rates, have been successfully addressed by the advent of Rapidly Mutating Y-STRs (RM Y-STRs). Previous studies established that a core set of 13 RM Y-STRs could differentiate a significant proportion of paternally related males, a finding consistently validated across diverse global populations [8-11]. Ralf and colleagues use an in-silico screening method to discover 12 new RM Y-STRs, when these markers were tested using 1,616 confirmed father–son pairs, they distinguished 27% of male relatives separated by one meiosis, 47% by two meiosis, and 61% by three meiosis, with a set of 25 RM Y-STRs, high differentiation rates of 44%, 69%, and 83% for the same degrees of relatedness [12]. The forensic utility of these expanded panels is particularly significant in populations with high endogamy, as recently demonstrated in Africa, where a 13 RM Y-STR panel resolved over 58% of related male pairs that standard kits failed to separate [13].

However, the accurate application of these powerful markers is contingent upon population-specific mutation rate data. Existing mutation rate estimates for Tanzania are based on a limited set of 17 markers, with a reported average of 2.35×10^{-3} [14] and it is hypothesized that the rate for the full 26 RM Y-STR panel would be significantly higher [15].

Therefore, establishing a robust, population-specific mutation rate dataset is a critical prerequisite for leveraging RM Y-STRs in Tanzanian forensic practice, particularly for resolving complex kinship and lineage investigations. Therefore, to enable precise kinship analysis and male lineage tracing in the Tanzanian population, this study reports the first comprehensive evaluation of mutation rates across all 26 RM Y-STRs, analyzing 138 confirmed father-son pairs and generating an essential dataset for applied genetic science.

2. Materials and Methods

2.1. Study Area

The cross-sectional study was conducted in Dar es Salaam, the largest commercial city and primary economic hub of Tanzania, with a population of 5,383,728 according to the 2022 National Census https://www.nbs.go.tz/statistics/topic/census-2022. The city's high population density and concentration of diverse ethnic groups from across Tanzania ensure that the recruited male participants provide a representative sample for generating nationally relevant genetic data. All laboratory analyses were carried out at the Government Chemist Laboratory Authority (GCLA) in Dar es Salaam.

2.2. Sample Collection and DNA Extraction

DNA samples from 138 unrelated individuals were randomly collected from Kigamboni and Kinondoni Municipalities in the Dar es Salaam region. Sampling was conducted through random home visits after obtaining ethical approval, during which participants provided informed consent after agreeing to participate in the study. Mother samples were collected to confirm the father-son pair biological relationship. Each sample was assigned a unique, anonymized identifier to ensure integrity and prevent cross-contamination. All personally identifiable information was irreversibly delinked to protect participant confidentiality. Samples were then transported to the Human DNA Laboratory at the Government Chemist Laboratory Authority (GCLA) for drying and subsequent genetic analysis. DNA extraction was performed using the Chelex 100 method.

2.3. DNA Quantification and Standardization

Genomic DNA was quantified using the Quantifiler Trio DNA Quantification Kit (Thermo Fisher Scientific) on a 7500 Real-Time PCR System, in accordance with the manufacturer's protocol. Following quantification, samples with high DNA concentration were diluted to standardized working concentrations based on an internally validated standard operating procedure: 0.1 ng/μL for autosomal STR amplification and 1.0 ng/μL for the 26 Y-chromosomal STR (RM Y-STR) amplification [16].

2.4. Polymerase Chain Reaction (PCR Amplification)

Autosomal STR analysis was performed for all collected samples using the AmpF/STR Identifiler Plus PCR Amplification Kit (Thermo Fisher Scientific) on a Veriti Thermal Cycler (Thermo Fisher Scientific). Amplification followed the manufacturer's protocol and targeted 15 autosomal STR loci (CSF1PO, D13S317, D16S539, D18S51, D19S433, D21S11, D2S1338, D3S1358, D5S818, D7S820, D8S1179, FGA, TH01, TPOX, and vWA) along with the amelogenin locus for

sex determination. Subsequently, father-son paired samples were amplified using the 26 RM-Yplex Amplification Kit (Microreader™) on a Veriti™ Thermal Cycler (Applied Biosystems). The PCR for the 26 RM-Y-STR loci DYS570, DYS576, DYS612, DYS626, DYS627, DYS685, DYS688, DYS712, DYS518, DYS526b, DYS547, DYS724, DYS1007, DYS1003, DYS1010, DYS1012, DYR88, DYS449, DYF387S1, DYF399S1, DYF403S1a, DYF403S1b1, DYF403S1b2, DYF404S1, DYF1000, DYF1001, and DYF1002 was carried as previous reported [16].

2.5. Capillary Electrophoresis

Capillary electrophoresis of the PCR amplicons for autosomal 15 STR loci was prepared using 9.6 µL HiDi formamide, 0.4 µL Liz size standard 600, and 1µL PCR products. The plates containing the prepared samples were heated at 95°C for 5 minutes and snap-cooled for 5 minutes before loading into the instruments. Similarly, for 26 RM Y-STRs loci, the PCR amplicons were prepared using 8.5 µL HiDi formamide (Thermo Fisher Scientific), 0.5 µL Microreader size standard QD 650 (Beijing Microreader Genetics, China), and 1ul PCR products. The plates containing the prepared samples were heated at 95°C for 3 minutes and snap-cooled for 5 minutes before loading into the instruments. Sample analysis was performed using the 3500xl Genetic Analyzer (Thermo Fisher Scientific), equipped with a 24-capillary array (36 cm in length), employing POP-4 polymer (Applied Biosystems) for capillary electrophoresis. Genotyped data were analyzed using GeneMapper ID-X Software v1.7.

2.6. Quality Control

Positive control (genomic DNA 9948), Negative control (Deionized water), and Blank were used as controls to monitor the analysis process. Blank controls were used from the first step of the extraction process to assess the procedure and any contamination, while Positive and Negative controls were added in the amplification process to monitor the PCR process. These controls were run concurrently with the samples, and their assessment was carried out during the interpretation of the results.

2.7. Data Analysis

The 15 Autosomal STRs and 26 RM Y-STRs DNA profiles from the Gene Mapper ID Analysis Software v 1.7 were recorded in a table of locus-specific alleles using a Microsoft Excel spreadsheet 2021. The mutations were counted directly, and the locus-specific mutation rate was calculated as the number of observed mutations divided by the number of father-son pairs for each Y-STR marker. 95% Confidence interval (CI) was estimated using the exact binomial probability distribution [17]. The differentiation rate was calculated as the number of father-son pairs differentiated by at least one RM Y-STR marker divided by the total number of father-son pairs [18].

3. Results

3.1. Mutation Analysis

Analysis of 15 autosomal STR markers using AmpF/STR Identifiler plus PCR amplification kit (Thermo Fisher Scientific) revealed a single germline mutation in sample KN12, specifically at the D21S11 locus, where a mismatch was observed between the father and son. The mutation is a one-step mutation, with the father carrying alleles 28,31, the child 27,31, and the mother 30,31 (Figure 1). In subsequent analysis using 26 RM Y-STR markers, the current study identified germline at the DYS1003 locus, where the father has allele 64, and the child has allele 63, and at the DYF403S1b2 locus, where the father has allele 52, and the child has allele 53. Among the 138 father-son pairs analyzed using 26 RM Y-STRs, 34 mutations were identified, whereby 32 of the mutations observed were one-step mutations, and 2 were multi-step mutations in a single germline transmission.

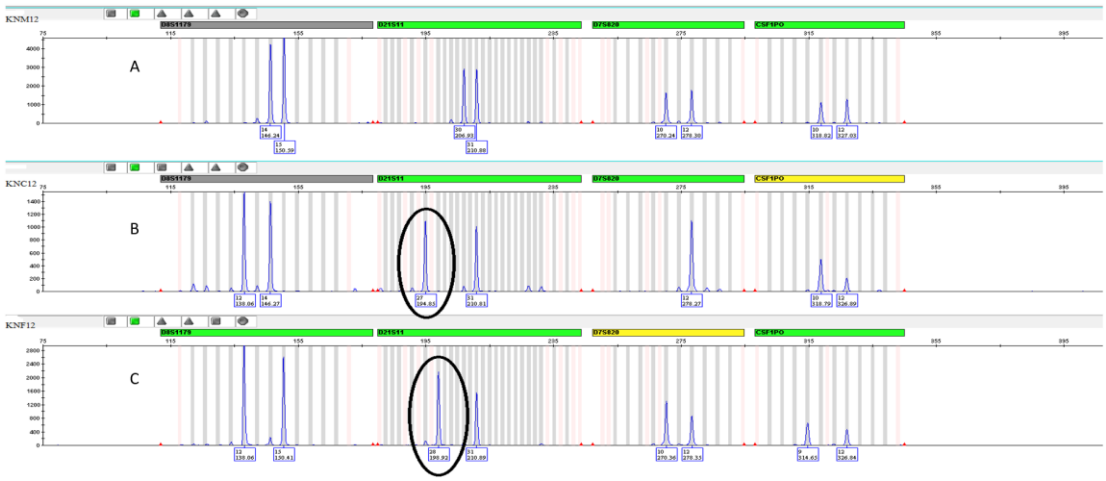

Figure 1. *Electropherograms illustrating a mutation at locus D21S11 in sample KN12, showing a match between the Mother (A) and Child (B), and a mismatch between the Child (B) and Father (C).*

3.2. Locus-Specific Mutation Characteristics of 26 Rapid Mutating Y-STRs

Analysis of 26 RM Y-STRs in confirmed father-son paired samples revealed 34 mutations. Loci with observed mutation in a single germline transmission were 16, and those with no mutation were 11; the mutations observed were 20 (59%), which were gains, and 14 (41%) were losses. Additionally, 96% of one-step germline mutations were observed compared to 6% of multi-step mutations. A multi-step mutation was identified at the DYS570 marker in sample KG07, in which the paternal allele was 17, and the corresponding allele in the son was 14, with a loss of three repeats. The samples were re-amplified and re-analyzed independently using both the original amplification kit and the Yfiler™ Plus PCR Amplification Kit, and the same allelic pattern (17 in the father and 14 in the son) was consistently observed, confirming that this result was not due to a technical artifact.

Loci with the highest mutation event were DYF399S1 with 9 mutations and DYS576 with 4 mutations. Loci with few mutation events were DYF403S1b2, DYS612, DYS712, DYS1003, DYS626, DYF404S1, DYF1000, DYS526b and DYS688 each shows one mutation. Loci with no mutation events were DYS724, DYF403S1b1, DYS627, DYS518, DYS1007, DYS685, DYS1010, DYR88, DYS547, DYS449 (Table 1).

In addition, at least one mutation was detected in 28 of these father-son paired samples, yielding a differentiation rate of approximately 20%. Among the 28 father-son pairs exhibiting differentiation in this study, 22 showed a single-locus mutation (78.57%), while 6 displayed mutations at multiple loci (21.43%).

Table 1. *Locus-Specific Mutation Characteristics of 26 RM Y-STRs.*

Loci	No of pairs	Mutation Observed	Gain	Loss	One-step mutation	Two-step mutation	Three-step mutation
DYS570	138	2	0	2	1	0	1
DYS724	138	0	0	0	0	0	0
DYF403S1 b1/b2	138	1	1	0	1	0	0
DYS627	138	0	0	0	0	0	0
DYS612	138	1	0	1	1	0	0
DYS518	138	0	0	0	0	0	0
DYF403S1a	138	3	2	1	3	0	0
DYS712	138	1	0	1	1	0	0
DYS1007	138	0	0	0	0	0	0
DYS1003	138	1	0	1	1	0	0
DYF399S1	138	9	6	3	8	1	0
DYS626	138	1	0	1	1	0	0
DYS685	138	0	0	0	0	0	0
DYS1010	138	0	0	0	0	0	0
DYS1001	138	3	2	1	3	0	0
DYF1012	138	0	0	0	0	0	0
DYF404S1	138	1	1	0	1	0	0
DYF387S1	138	2	1	1	2	0	0
DYS449	138	0	0	0	0	0	0
DYS547	138	0	0	0	0	0	0
DYR88	138	0	0	0	0	0	0
DYS576	138	4	3	1	4	0	0
DYF1000	138	1	1	0	1	0	0

Loci	No of pairs	Mutation Observed	Gain	Loss	One-step mutation	Two-step mutation	Three-step mutation
DYS526b	138	1	0	1	1	0	0
DYF1002	138	2	2	0	2	0	0
DYS688	138	1	1	0	1	0	0
TOTAL	3588	34	20	14	32	1	1
			59%	41%	94%	6%	

3.3. Mutation Rate Estimates from Father and Son

The estimated mutated ranged from 7.2×10^{-3} (95% CI 2.0×10^{-4} to 4.06×10^{-2}) for DYF403S1b2, DYS612, DYS712, DYS1003, DYS626, DYF404S1, DYF1000, DYS526b and DYS688 to 6.5×10^{-2} (95% CI 3.0×10^{-2} to 1.22×10^{-1}) for DYF399S1. The high mutation rate was observed at DYF399S1 with a mutation rate of 6.5×10^{-2} (95% CI 3.0×10^{-2} to 1.22×10^{-1}) followed by DYS576 with mutation rate of 2.9×10^{-2} (7.7×10^{-3} to 7.36×10^{-2}), DYF403S1a, DYS1001, with mutation rate of 2.2×10^{-2} (95% CI 4.3×10^{-3} to 6.32×10^{-2}), DYS570, DYF387S1, DYF1002 mutation rate of 1.4×10^{-2} (1.7×10^{-3} to 5.23×10^{-2}). Lowest mutation rate was observed at DYF403S1b2, DYS612, DYS712, DYS1003, DYS626, DYF404S1, DYF1000, DYS526b and DYS688 with mutation rate of 7.2×10^{-3} (95% CI 2.0×10^{-4} to 4.06×10^{-2}. The loci without mutation in this study were DYS724, DYF403S1b1, DYS627, DYS518, DYS1007, DYS685, DYS1010, DYR88, DYS547, DYS449, and DYF1012.The average mutation rate was 1.0×10^{-2} (95% CI 1.8×10^{-2} to 1.22×10^{-1}). The details are presented in Table 2.

Table 2. Mutation Rate of 26 RM Y-STRs of the Tanzanian Population.

Loci	No of pairs	Mutation Observed	Gain	Loss	Mutation Rate	Binomial 95%C1
DYS570	138	2	0	2	1.4×10^{-2}	1.7×10^{-3} to 5.23×10^{-2}
DYS724	138	0	0	0	0	0 to 2.7×10^{-2}
DYF403S1b1	138	0	0	0	0	0 to 2.7×10^{-2}
DYF403S1b2	138	1	1	0	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYS627	138	0	0	0	0	0 to 2.7×10^{-2}
DYS612	138	1	0	1	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYS518	138	0	0	0	0	0 to 2.7×10^{-2}
DYF403S1a	138	3	2	1	2.2×10^{-2}	4.3×10^{-3} to 6.32×10^{-2}
DYS712	138	1	0	1	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYS1007	138	0	0	0	0	0 to 2.7×10^{-2}
DYS1003	138	1	0	1	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYF399S1	138	9	6	3	6.5×10^{-2}	3.0×10^{-2} to 1.22×10^{-1}
DYS626	138	1	0	1	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYS685	138	0	0	0	0	0 to 2.7×10^{-2}
DYS1010	138	0	0	0	0	0 to 2.7×10^{-2}
DYS1001	138	3	2	1	2.2×10^{-2}	4.3×10^{-3} to 6.32×10^{-2}
DYF1012	138	0	0	0	0	0 to 2.7×10^{-2}
DYF404S1	138	1	1	0	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}

Loci	No of pairs	Mutation Observed	Gain	Loss	Mutation Rate	Binomial 95%C1
DYF387S1	138	2	1	1	1.4×10^{-2}	1.7×10^{-3} to 5.23×10^{-2}
DYS449	138	0	0	0	0	0 to 2.7×10^{-2}
DYS547	138	0	0	0	0	0 to 2.7×10^{-2}
DYR88	138	0	0	0	0	0 to 2.7×10^{-2}
DYS576	138	4	3	1	2.9×10^{-2}	7.7×10^{-3} to 7.36×10^{-2}
DYF1000	138	1	1	0	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYS526b	138	1	0	1	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
DYF1002	138	2	2	0	1.4×10^{-2}	1.7×10^{-3} to 5.23×10^{-2}
DYS688	138	1	1	0	7.2×10^{-3}	2.0×10^{-4} to 4.06×10^{-2}
TOTAL	3726	34	20	14	1.0×10^{-2}	6.0×10^{-3} to 1.3×10^{-2}

3.4. Effect of Father's Age on 26 RM Y-STRs Mutations Rate Analysis

The current study reports the relationship between paternal age and Y-STR mutation occurrence. Collected age data was used to analyse the father's age at the time of the son's birth and the mutation rate (Table 3).

Table 3. Comparison of Father's Age in Father- Son Pairs With and Without RM Y-STR Mutations.

Parameter	Fathers with Mutation (n = 28)	Fathers without Mutation (n = 43)
Mean age (years)	35.2	34.1
Standard deviation (SD, years)	5.98	10.60
Youngest father (years)	20 (KG10)	20 (TM10)
Oldest father (years)	50 (KN24)	53 (KN02, KN05, KG19)
Statistical test	Independent-samples t-test	
P-value	0.59	
Interpretation	No significant association	

4. Discussion

4.1. 26 Rapid Mutating Y-STRs Locus-Specific Mutation Characteristics

Y-STR mutations are known to occur predominantly through a stepwise model, largely governed by replication slippage [19]. Our results are consistent with this principle, demonstrating a preponderance of one-step mutations over multi-step events. This observation is in agreement with prior work, including a study of Tanzanian father-son pairs analyzed with 17-Y-STRs and other investigations utilizing 26 RM Y-STR kits in various populations [20-22].

The high prevalence of single-locus mutations (79%) observed in the current study conforms to the canonical Y-STR mutational pattern, wherein alterations predominantly follow a stepwise model at individual loci [23]. Additionally, 59% of the mutations observed were gain and 41% were loss of alleles, which is in agreement with other findings [15]. This trend is consistent with that of Wang et al. [21], who reported slightly more allele gains (89) than allele losses (80) in Chinese population, although the difference was not statistically significant. In contrast, Neuhuber et al. [15] observed more allele contractions (175) than expansions (158), likewise without statistical significance. Together, these findings indicate that both allele gains and losses occur at comparable frequencies in RM Y-STRs, with no consistent directional bias across studies. Several factors influence the mutation rates of Y-STRs, including

the length of individual repeat units, the overall number of repeats, and the structural complexity of the repeat motif [23, 24].

The mutation rates of individual RM Y-STR loci observed in this study revealed considerable variability, underscoring the distinct mutational pattern of each locus within the Tanzanian population. Some loci exhibited notably higher mutation rates, suggesting their enhanced potential for differentiating between closely related male individuals, while others showed more stable inheritance patterns across generations.

In the current study, we report the RM Y-STR markers with high mutations at DYF399S1 and DYS576 loci, which exhibited 9 and 4 mutations with a mutation rate of 6.5×10^{-2} (95% CI 3.0×10^{-2} to 1.22×10^{-1}) and 2.9×10^{-2} (95% CI 7.7×10^{-3} to 7.36×10^{-2}), respectively. Similar findings reported high germline mutation at the DYF399SI locus using a different population. For example, a study involving 501 father-son pairs from the Han population in Beijing reported that DYF399S1 exhibits the highest mutation rate of 4.59×10^{-2} (95% CI 2.93×10^{-2} to 6.81×10^{-2}) [11]. Chinese Yi Population: a study involving 260 father-son pairs, the mutation rate of DYF399S1 was 3.85×10^{-2} (95% CI 1.86×10^{-2} to 6.96×10^{-2}) [18]. In comparison, the mutation rate of DYF399S1 observed in Tanzanian population is considerable higher than those reported in these population, though statistically not significance (P value 0.3782) for Han population and (P value 0.3225) for Chinese Yi population while in Pakistan the mutation rate was 1.79×10^{-1} higher than the current study and statistically significance with (P value 0.003) tested using Fisher's exact test [25]. A study conducted in the Japanese population using 178 father-son pairs found that the mutation rate was 10.67×10^{-2} higher than in this study but not statistically significant (P value 0.234) [26]. In the combined Chinese Han population, the mutation rate at the DYF399S1 locus was 5.45×10^{-2}, compared to 7.36×10^{-2} in the international combined dataset [17]. These differences highlight the population-specific nature of germline mutation rates at RM Y-STR loci such as DYF399S1, and high mutational frequency is likely due to the complex, multi-copy, and repetitive structure of the locus, which is a known molecular factor influencing mutations in RM Y-STRs [23]. In addition to this, a study of 542 father–son pairs in the Korean population, a total of 57 mutations were observed in locus DYS712 with a mutation rate of 10.52×10^{-2}, which was higher than 38 mutations for DYF399S1 with the rate of (70.1×10^{-3}) [27]. Study on Chinese population using 367 father son pairs DYS712 exhibited a mutation rate of $4.67 \times 10^{-2,}$ [17] different from this study in Tanzania population where DYS712 exhibited the mutation rate of 7.2×10^{-2} these variations can be contributed to small sample size which can influence the precision of mutation rate estimates [22].

4.2. Estimation of Mutation Rate across the 26 RM Y-STR Loci

The overall mutation rate estimated for the 26 RM Y-STR loci in this study was 1.0×10^{-2} (95% CI: 6.0×10^{-3} to 1.3×10^{-2}, confirming the high mutability inherent to this marker set compared to conventional Y-STRs. This rate aligns with the established mutation spectrum for RM Y-STRs, which typically ranges from 10^{-3} to 10^{-2} per generation [24].

Our finding is highly consistent with previous reports from diverse populations. For instance, Yuan *et al.* [28] reported a nearly identical rate of 1.02×10^{-2} using 13 RM Y-STRs, and Lang *et al.* [29], observed a rate of 1.05×10^{-2} in a Chinese Han cohort. When compared to studies employing similar 30 RM Y-STR panels, our estimated rate falls within the expected global range. It is slightly equal with rates reported in Chinese (1.84×10^{-2}) and European (1.7×10^{-2}) populations, yet lower than those observed in Korean (2.3×10^{-2}) and Japanese (2.6×10^{-2}) populations [15, 21, 22, 27]. The observed inter-population variation in mutation rates may be attributed to underlying biological differences, such as distinct haplogroup distributions, which are known to influence Y-STR mutability [15].

Mutations detected in this study showed that 28 pairs were differentiated, corresponding to a differentiation rate of approximately 20%. This rate is slightly lower than that observed in the European population, 26.9% [23] and the Pakistani population is 24.3% [8]. However, it is higher than the rates reported for the Chinese Yi population, 18.8% [18] and the Chinese Han population 18.96% [30]. The differentiation rate improved when using RM Y-STRs, thus proving their ability to differentiate male relatives, which is consistent with previously reported mutation rates in similar studies. [8, 22, 24, 31]. The differentiation rate observed in this study contributes to understanding mutation dynamics in specific populations and may aid in refining statistical models for relationship testing.

4.3. Effect of Father's Age on 26 RM Y-STRs Mutations

The present study investigated the relationship between paternal age and the occurrence of Y-STR mutations. Though statistically it is not significant, the average age among males with at least one germline mutation is marginally older than that of those without a mutation. The Chi-square analysis shows variation in RM Y-STR mutation frequencies across paternal age groups, with the highest proportion of mutations observed among fathers aged 31–45 years (47.65%). The results suggest no statistically significant association between paternal age group and the occurrence of Y-STR mutations in this dataset (p=0.236). This may be attributed to the limited sample size, particularly in the oldest age group (46-60 years), which reduces the statistical power. These findings are not in agreement with other studies that reported a significant difference in paternal age at the time of the son's birth and the number of Y-STR mutations observed [23]. Though previous studies have reported conflicting results, the difference may be

contributed to by several factors, including participant selection and sample size used [14]. Gusmão et al. [31] reported an increased mutation rate with advancing paternal age. Ralf et al. [20] examined the effect of paternal age at conception using a total of 1,500 fathers, categorized into four age groups: Group 1 (n = 432, <24 years), Group 2 (n = 378, 24–29 years), Group 3 (n = 324, 30–36 years), and Group 4 (n = 366, 37–66 years). The results revealed a significant difference, with Group 4 showing the highest Y-STR mutations.

In contrast, Kayser et al. [32] found no significant difference ($p = 0.215$), with fathers without mutations being slightly older than those with mutations. Similarly, studies conducted in Chinese Yi and Han populations also reported no significant relationship between paternal age at the time of the son's birth and the occurrence of Y-STR mutations. Chinese Yi population study found a slight difference in the average paternal age between individuals with Y-STR mutations (35.07 years) and those without (33.60 years); however, this difference was not statistically significant [18]. In the Han population, the mean age of fathers with mutation events was 34.47, while for those with no mutation events was 32.42; the difference was not statistically significant [30], while the results do not support a strong association between paternal age and Y-STR mutation occurrence, the observed age gap suggests a potential trend that may warrant further investigation. These findings are consistent with some previous studies but differ from others that reported a significant positive correlation. The variability across studies indicates that paternal age may be one of several factors influencing Y-STR mutation rates.

4.4. Mutation Observed at the Autosomal STR Locus D21S11

In addition to RM Y-STRs mutation analysis, a single-step mutation was observed at the autosomal STR locus D21S11, where a mismatch was observed between father and child in one of the father-child pair characterized by a loss of one repeat (Father: 28,31; Child: 27,31; Mother: 30,31). D21S11 is one of the most polymorphic autosomal STR markers due to its complex repeat structure, which promotes replication slippage during meiosis and results in elevated allelic variability [33]. Most mutations reported at this locus are single-step repeat changes, consistent with the observation in the present study [34]. Similar single-step mutations at D21S11 have been reported previously, including a study from Bosnia and Herzegovina that documented a one-repeat gain between a father and child (Father: 30,30; Mother: 30,32.2; Child: 31,32.2) [35]. In the present study, concordance was observed at 14 of 15 autosomal STR loci, and the single-locus mismatch at D21S11 does not exclude paternity, in accordance with established forensic interpretation guidelines [36].

5. Conclusion

This study establishes the first comprehensive mutation rate estimates for 26 RM Y-STR loci in a Tanzanian population, based on an analysis of DNA-confirmed father-son pairs. Among 28 differentiated paternal lineages, 34 mutation events were observed. The loci DYF399S1 and DYS576 were observed as the most mutable, with mutation rates of 6.5×10^{-2} and 2.9×10^{-2}, respectively, aligning with their characterization as highly unstable markers in global populations. The mutational profile was predominantly characterized by one-step, single-locus events (78.57%), confirming the prevalence of the stepwise mutation model for Y-STRs. The minority of multi-locus mutations (21.43%) suggests the potential influence of cumulative mutational processes or haplogroup-specific dynamics. A slight bias towards repeat gains (59%) over losses (41%) was noted, consistent with a multistep mutation mechanism. The overall average mutation rate of 1.0×10^{-2} confirms the enhanced discriminatory power of the RM Y-STR panel and is consistent with established global estimates, though it occupies the lower end of the spectrum reported for some Asian populations. Furthermore, analysis of paternal age revealed no statistically significant correlation with mutation rate within this cohort. Collectively, these findings validate the utility and reliability of RM Y-STRs for high-resolution paternal lineage differentiation and underscore their critical value in advancing forensic and population genetic studies in previously underrepresented regions like Tanzania.

Abbreviation

DNA	Deoxyribonucleic Acid
GCLA	Government Chemist Laboratory
NIMR	National Institute for Medical Research
PCR	Polymerase Chain Reaction
RM	Rapidly Mutating
STRs	Short Tandem Repeats
Y-STRs	Y-chromosomal Short Tandem Repeats

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Author Contributions

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Data Availability Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author on reasonable request.

Conflicts of Interest

There is no conflict of interest.

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