Research Article

Elif Mertoglu, Gonul Filoglu*, Tolga Zorlu and Ozlem Bulbul

Estimation of the Y-chromosomal short tandem repeat (Y-STR) mutation rates in Turkey
Türkiye’de Y-Kromozomal STR (Short Tandem Repeat) Mutasyon Oranının Belirlenmesi

Abstract

Background: The Non-recombining region of the Y-chromosome (NRY) is transferred from father to son in an unchanged form without recombination in meiosis. Since Short tandem repeats on Y-chromosome (Y-STRs) in this region do not have any recombination, these regions are identical in all male individuals who are related to the father except for mutations. Therefore, these regions gain importance in identification for the forensic sciences or determination of paternity. In determination of paternity, if mismatches are observed between father and child, population-specific mutation rates should be used to determine whether it is a mutation or a true exclusion. Therefore in this study, we aim to determine the mutation rates of 17 Y-STR loci in Turkey.

Material and methods: 17 Y-STR loci were typed by using AmpFlSTR® Yfiler™ Kit in 90 volunteer father-son pairs. Mutation rates were calculated and compared with other populations.

Results: The mutations were found between three father-son pairs at DYS439 and DYS458 loci. In addition, a duplication in DYS389 II loci* 30, 31 was observed. The average mutation rate was determined as $1.96 \times 10^{-3}$ for Turkish population.

Conclusion: This investigation will contribute to minimize the possibility of false exclusion of the father-son and kinship relations.

Keywords: DNA analysis; Mutation; Y chromosome; Y-STR; Forensic genetics.

Özet

Amaç: Y kromozomunun rekombinasyona uğramayan bölgesi, non-recombining region Y (NRY) mayozda rekombinasyona uğramadığından babadan oğula değişmeden aktarılır. Bu bölgede bulunan Y kromozomuna bağlı kısa ardışık tekrar dizileri (Y-STR) rekombinasyona uğramadığından baba tarafından akraba tüm erkek bireyler arasında mutasyonlar haricinde birbirinin ardından. Bu yüzden bu bollger, adlı bilimlerde kimliklendirdimede veya babalık tayininde önem kazanmaktadır. Babalık tayininde, babanın ve çocuk arasındaki uyumsuzluk gözlenmiş durumunda, bunun mutasyondan mı yoksa gerçek bir dışlama mı olduğunu belirleyebilmek için toplumlara özgü mutasyon oranları belirlenmelidir. Bu çalışmanın amacı Türkiye’de 17 Y-STR lokusuna ait mutasyon oranlarının belirlenmesidir.

Gereç ve Yöntem: Bu çalışmada, 90 gönülü baba-oğul çiftinde 17 Y-STR lokusu AmpFISTR® Yfiler™ kit kullanılarak tiplendirildi. Mutasyon oranları hesaplandi ve diğer popülasyonlarla karşılaştırıldı.

Bulgular: Üç baba-oğul çifti arasında DYS439 ve DYS458 lokuslarında uyumsuzluk (mutasyon) gözlenirken; DYS389 II lokusunda bir duplikasyon (30,31) gözlandı. Türkiye popülasyonu için ortalama mutasyon oranı $1.96 \times 10^{-3}$ olarak belirlendi.
Sonuç: Bu çalışma ile baba-oğul ve akrabalık ilişkilerinin araştırılmasında karşılaştırılabilecek yanlış dışlama olasılığı ortadan kalkmış olacaktır.

Anahtar Kelimeler: DNA analizi; Mutasyon; Y kromozomu; Y-STR; Adli genetik.

Introduction

Short tandem repeats on Y chromosome (Y-STRs) transferred from father to all male offspring without recombination in a largely intact manner. If there is no mutation available, all paternal lineage have the same Y-STR haplotype [1, 2]. Y-STR analysis is also a valuable tool to trace familial relationships among males, to help identify missing persons, and to assess paternal relationships when the alleged father is not available. Much like autosomal STR analysis, Y-STR analysis can be used to exclude an individual from a paternal lineage in addition to being able to add statistical weight to an individual being from a paternal lineage. Autosomal STR analysis may not be possible if the sample contains an admixture of body fluids from the perpetrator. Thus, Y-STR primers can be used to determine the profile of a male perpetrator in such admixed samples [3–5].

In forensic genetics the specific mutation rates of the alleged population should be known due to high mutation rate and high polymorphism of the Y-STR loci. With the increase in the number of Y-STRs loci analyzed, the presence of a single genetic inconsistency between a child and his biological father (paternal exclusion) has become relatively frequent, and cases with double paternal genetic inconsistencies have been reported more and more often [6, 7]. Mutations on germ cells of father may cause an incompatibility between father-son Y-STR profiles (one or more loci). Such mutations may usually occur either at one locus or multiple loci [8, 9]. In order to prevent any false exclusion resulting from mentioned mutations, mutation rates should be assessed. Y-STR mutation rates based on father-son pairs can be obtained by population studies as well as deep pedigrees [10]. In forensics, the well-known Y-STR database and Y-chromosome haplotype reference database (YHRD) have the biggest mutation rate database for many populations. They regularly update the data from various researchers and published data [11].

Y chromosomal mutation rate has been an essential parameter for many studies including evolutionary and population genetics, medicine and forensics. General approaches for estimating the Y-STR mutation rates are divided into three groups: (1) genealogical line (pedigree analysis), (2) evolutionary rate and (3) ancient DNA. The genealogical analysis approach is the most-frequently used approach in forensic studies by direct counting of the mutations between father and son pairs [12–14].

The purpose of this study was to determine the specific mutation rates in Turkey by investigating the 17 Y-STR loci (routinely used in any forensic laboratory) of Turkish father-son pairs. Thus, the false exclusion ratio which may occur in paternity tests based on Y-STR can be minimized by determining the population-specific mutation rate.

Materials and methods

We analyzed 90 healthy father-son pairs from all geographic regions of Turkey. Buccal swabs were collected after informed consents were received. The present study was approved by Ethical Committee of Cerrahpaşa Faculty of Medicine, Istanbul University, Turkey (Number: 1940). DNA extraction was carried out using the DSBIO DNA Extraction Kit. DNA was quantified by The Quanti-IT™ dsDNA HS Assay Kit on Qubit® Fluorometer (Invitrogen, Carlsbad, CA, USA). Amplification of DYS19, DYS385 a/b, DYS389 I, DY389 II, DYS390, DYS391, DYS392, DYS393, DYS387, DYS437, DYS438, DYS439, DYS448, DYS456, DYS458, DYS635 and Y GATA H4 was performed using the AmpF/STR® Yfiler® PCR amplification kit (AB) on a GeneAmp 9700 thermal cycler (Thermo Fisher Scientific, TFS) according to manufacturer’s instructions [15]. Amplification conditions were as follows: an initial denaturation at 95°C for 11 min; 28 cycles of 94°C for 1 min, 59°C for 1 min and 72°C for 1 min, and a final extension of 60°C for 80 min. Electrophoresis was performed using the ABI 310 (TFS) at 15 kV for 30 min at 60°C. Data were analyzed using GeneScan version 3.7 Analysis Software (TFS) to assess the quality of PCR amplification and to assign the specific alleles to each fragment that was analyzed. Mutation rate between the father-son pairs were calculated with a formula of (μ); (μ = x number of mutations/N number of meiosis). Mutation rates and confidence intervals (CI) (95%) were determined as per binominal standard deviation [16]. Obtained mutations were compared with those available at the YHRD [11].

In our study, PCR and Y-STR analyses were repeated when there were incompatibilities and duplications detected between alleged father-son pair. Negative and positive controls were employed for the incidence of contamination and erroneous typing.
Results

In this study, 17 Y-STR loci of 90 father-son pairs were typed and compared. Mutations were found in three father-son pairs. Among the three mutations, two repeat losses and one repeat gains were observed. Both repeat losses (fathers with allele 13 and sons with allele 12) were detected on DYS439 loci (Figures 1 and 2). No mutations

Figure 1: Electropherogram of mutation between an alleged father and his son in the DYS439 locus (Electropherograms of father-son were overlapped).
Mutation rates and average of the mutation ratio were calculated in reference to the mutations observed between father-son pairs (Table 1). Observed mutations were calculated and compared via the data on YHRD (Table 2).
One pair displayed a duplication (alleles 30 and 31) at DYS389 II in both the father and son demonstrating that these allele patterns could be inherited (Figure 4).

**Discussion**

Microsatellites are commonly found on noncoding DNA regions of both autosomal and sexual chromosomes. Due to genetic inheritance features of Y chromosome, they can be used in forensic sciences and population genetics as well as human migration and evolutionary studies [1, 17]. Since recombination does not occur on 95% of Y chromosome (NRY region), these regions are transferred directly from father to son. Thus all male relatives of the alleged father share the same Y-STR profile except any mutations [1, 2]. This unique feature enables the remedy of the paternity cases where alleged father is not available. But any mutations in germ cells of father may cause incompatibilities on several loci between the father and

| Locus     | No. of mutations | Allele transmission | Mutation rate (x10^-3) (No. of mutations/meiosis) | Confidence interval (CI) (x10^-3) |
|-----------|------------------|---------------------|-------------------------------------------------|----------------------------------|
| DYS456    | 0                | 90                  | 0                                              | 0                                |
| DYS389 I  | 0                | 90                  | 0                                              | 0                                |
| DYS390    | 0                | 90                  | 0                                              | 0                                |
| DYS389 II | 0                | 90                  | 0                                              | 0                                |
| DYS458    | 1                | 90                  | 11.1                                           | 0.3–60.4                         |
| DYS19     | 0                | 90                  | 0                                              | 0                                |
| DYS385 a/b| 0                | 180                 | 0                                              | 0                                |
| DYS393    | 0                | 90                  | 0                                              | 0                                |
| DYS391    | 0                | 90                  | 0                                              | 0                                |
| DYS349    | 2                | 90                  | 22.2                                           | 2.7–78                           |
| DYS635    | 0                | 90                  | 0                                              | 0                                |
| DYS392    | 0                | 90                  | 0                                              | 0                                |
| YGATA H4  | 0                | 90                  | 0                                              | 0                                |
| DYS437    | 0                | 90                  | 0                                              | 0                                |
| DYS438    | 0                | 90                  | 0                                              | 0                                |
| DYS448    | 0                | 90                  | 0                                              | 0                                |
| Average mutation ratio | 3              | 1530                | 1.96                                           | 0.4–5.7                          |

**Table 2:** Total mutations and allele transmissions per each Y-STR.

| Locus     | No. of mutations | Allele transmission | Mutation rate (x10^-3)  | Total mutation | Total transmission | Mutation rate (x10^-3) |
|-----------|------------------|---------------------|-------------------------|----------------|--------------------|------------------------|
| DYS19     | 36               | 16090               | 2.24                    | 36             | 16180              | 2.23                   |
| DYS389 I  | 42               | 14339               | 2.93                    | 42             | 14429              | 2.91                   |
| DYS389 II | 59               | 14310               | 4.12                    | 59             | 14400              | 4.10                   |
| DYS390    | 33               | 15612               | 2.11                    | 33             | 15702              | 2.10                   |
| DYS391    | 38               | 15486               | 2.45                    | 38             | 15576              | 2.44                   |
| DYS392    | 8                | 15418               | 0.52                    | 8              | 15508              | 0.52                   |
| DYS393    | 15               | 14264               | 1.05                    | 15             | 14354              | 1.05                   |
| DYS385 a/b| 64               | 26171               | 2.45                    | 64             | 26351              | 2.43                   |
| DYS437    | 13               | 10652               | 1.22                    | 13             | 10742              | 1.21                   |
| DYS438    | 4                | 10673               | 0.38                    | 4              | 10763              | 0.37                   |
| DYS439    | 58               | 10647               | 5.45                    | 60             | 10737              | 5.59                   |
| DYS448    | 11               | 7229                | 1.52                    | 11             | 7319               | 1.50                   |
| DYS456    | 31               | 7229                | 4.29                    | 31             | 7319               | 4.24                   |
| DYS458    | 46               | 7228                | 6.36                    | 47             | 7318               | 6.42                   |
| DYS635    | 35               | 8076                | 4.33                    | 35             | 8166               | 4.29                   |
| YGATA H4  | 25               | 8260                | 3.03                    | 25             | 8350               | 2.99                   |
Such incompatibility may cause the exclusion of the real biological father [5]. Related misinterpretation can be prevented by determination of the Y-STR mutation rates of specific population. For this purpose, we assessed the mutation rates of 17 Y-STR loci in Turkish population.

Buccal swab samples of 90 father-son pairs from all geographic regions of Turkey were typed, and each Y-STR locus pair was compared. The common and more accurate method of estimating mutations rates for forensic researches is direct

Figure 3: Electropherogram of mutation between an alleged father and his son on the DYS458 locus (Electropherograms of father-son were overlapped).
Figure 4: Electropherograms of the relevant father-son pair displaying duplication in DYS389II locus.
counting of the number of genetic transfers (meiosis) between the father and son pairs [18]. Therefore, we used the pedigree method for estimating mutation rates in Turkish population.

We observed a total of three mutations (two deletions on DYS439 of two father-son pairs and an insertion on DYS458 of one father-son pair). While two of the fathers had allele 13 on DYS439, their sons had allele 12. In one pair, father-son had allele 19.2 and 20.2, respectively (Figures 1–3). Many of the researchers studied on different populations also observed similar mutations on DYS439 and DYS458. These mutations are also in the forms of deletion and insertion. Burgarella and Navascues [10] conducted a meta-analysis study using father-son pair data for 80 loci from 29 published studies. According to their results, DYS458 and DYS439 had one of the highest mutations in meiosis (DYS439: 51 mutations in 9313 individuals; DYS458: 46 mutations in 6684 individuals). Decker et al. [19] detected deletions on DYS439 of an Asian and a Hispanic father-son (allele 13–allele 12) pair and a gain of 1 repeat on DYS458 of another Asian father son (allele 18–allele 19) pair. Mutations of both DYS439 and DYS458 were also observed between the father-son pairs in the studies performed by Lee et al. [20] in Korean, by Sen Shi et al. [21] in Chinese and by Onofri et al. [22] in Italian populations. Moreover, mutations on DYS458 loci were detected on the studies conducted by Boudowle et al. [23] in North American, African-American, Caucasian, Asian, Hispanic and Native American males, and by Hohoff et al. [24] in German population, by Kurihara et al. [25] in Japanese population and by Vieira-Silva et al. [7] in Portuguese population. In addition, mutations on DYS458 loci were found by Farfán and Prieto [26] in Spanish males.

In our study, since no mutations were observed in DYS19, DYS385 a/b, DYS389 I, DYS389 II, DYS389, DYS393, DYS437, DYS438, DYS448, DYS456, DYS635 and Y GATA H4 loci, the rate was determined as zero (0).

The observed mutation rate of DYS458 was detected as (95% CI: 0.3×10⁻²–6.0×10⁻⁷) 1.1×10⁻³. DYS439 had a mutation rate of (95% CI: 2.7×10⁻⁸–78×10⁻¹) 22.2×10⁻³. Average mutation rate of 90 father-son pairs was calculated as (95% CI: 0.4×10⁻³–5.7×10⁻³) 1.96×10⁻³ (Table 1). Observed mutation rates were similar to the other different populations such as Texas population [23] (1.7×10⁻³), Brazilian population [27] (1.8×10⁻³), Northern Portuguese population [7] (1.86×10⁻¹), Northwest German population [24] (2.1×10⁻³) and Japanese population [25] (2.2×10⁻³). Laouina et al. [28] determined that the average mutation rate in the sample group of Moroccan population was (95% CI: 2×10⁻³–5.8×10⁻³) 3.5×10⁻³. Furthermore, Y-STRs mutation occurrence seemed to be four times more frequent than autosomal STRs mutation in this sample. Also, a total of 15 single repeat mutations between 252 fathers and sons were observed. Nine mutations resulted in the insertion of the son, and six resulted in a deletion [28].

The mutation rates of this study were compared with those available at the YHRD (Table 2). It was found that the DYS458 (6.42×10⁻³) and DYS439 (5.59×10⁻³) loci had the highest mutation rate in both YHRD and in our study. Lowest mutation rate was obtained in DYS438 (0.37×10⁻³) and DYS392 (0.52×10⁻³) loci. Additionally, in agreement with our results, the mutation rates for the commonly-used forensic kits Y-filer and PowerPlexY23 sets were observed between ~8.6×10⁻⁵ and ~12.5×10⁻⁵ per locus per year [12].

Some of the mutations of the fathers’ germ cells might not be observed in all of his sons. In some cases, multilocus haplotypes of related persons could differ at a single locus due to mutation. Rolf et al. [29] typed the nine Y-STR loci of three brothers and detected a different haplotype in one of the brothers. Two (duplication) or more (triplication) alleles in different Y-STR loci were reported in many studies [19, 25, 30–32]. In our study, 30–31 duplication in both father and son was observed on DYS389II loci (Figure 4). Ge et al. [33] and Coble et al. [34] detected 30–31 duplication in DYS389II loci in three and one Caucasian male, respectively. Multialleles in a single locus may refer to two or more individuals, and this situation may cause misinterpretations. If there are no other multialleles on two or more loci, duplications and triplications should be considered. Thus, the duplication/triplication frequency of the Y-STRs should be calculated.

Y-STR loci are generally used to support the autosomal STR results when the exclusion is observed in one or two STR loci in paternity testing. In addition, rapidly mutating (RM) Y-STRs were adjunct to the current Y-STR kits. Whenever a non-exclusion is observed with a current Y-STR kit, the RM Y-STR set should be applied to reduce the probability of adventitious matches or male relative involvement [13, 35]. The current Y-STR kits (e.g. PowerPlex Y23) contains additional high mutation rate Y-STRs (e.g. DYS576) [12]. Thus, it is essential to calculate the population-specific average mutation rates in new Y-STRs.

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Conflict of Interest: The authors have no conflict of interest.
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