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Mutation rate of 7 X-STRs of common use in population genetics

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ABSTRACT

Microsatellite loci analysis is a relevant tool in population genetic studies and also in paternity testing. In this field the X-chromosome STR loci are widely used for female offspring testing but their mutation rates are scarcely known.
In this work we performed a mutation rate analysis of seven STR markers located in the X- chromosome specific region, in mother-son pairs and father-mother-daughter trios. Among 1015 allele transfers, we found only one change for a mother-daughter transmission in the DXS9898 locus, consisting of a loss of one single repeat. This finding allowed us to estimate a general mutation rate of $\mu=9.85 \times 10^{-4}$ ($2.49 \times 10^{-4} – 5.49 \times 10^{-2}$) which can be applied to population studies. This value is consistent with other reports on autosomal STR loci, suggesting that similar molecular mechanisms are acting on X and autosomal repetitive markers.

Key words: X chromosome, STR, mutation rate, population genetics

RESUMEN

La genética de poblaciones recurre frecuentemente al análisis de loci microsatélites para estudios evolutivos, como también para pruebas de paternidad. Sin embargo, a pesar de la importancia de los loci STR ubicados en el cromosoma X, sus tasas mutacionales aún no han sido estudiadas en profundidad. En el presente trabajo realizamos un análisis de la tasa mutacional de 7 marcadores STR ubicados en la región específica del cromosoma X, en dúos madre-hijo y tríos padre-madre-hija. De un total de 1015 transferencias alélicas, hallamos una única mutación en una transmisión de madre a hija para el marcador DXS9898, la cual consiste en la pérdida de un repetido. Los resultados obtenidos nos permitieron estimar una tasa mutacional conjunta de $\mu=9.85 \times 10^{-4}$ ($2.49 \times 10^{-4} – 5.49 \times 10^{-2}$) la cual puede utilizarse en estudios poblacionales. Asimismo, el valor obtenido es consistente con datos reportados para loci STR autosómicos, lo cual sugeriría que los mecanismos que actúan en loci repetidos de autosomas y cromosoma X son similares.

Palabras clave: Cromosoma X, STR, tasa de mutación, genética de pobblaciones

INTRODUCTION

The mutation rate of short tandem repeats is usually higher than that of other genetic markers, what makes STR loci interesting for micro-evolutionary studies since particular characteristics can emerge from a short number of generations, allowing to define substructures, migration routes, and genetic distances among closely related populations (Kaessmann et al. 2002, Katoh et al. 2002, Xu and Fu 2004).

Mutations can change either the number of repeats of the core sequence and/or the sequence of one section of the marker (Hammer et al. 1995, Brinkmann et al. 1996). Changes in the number of repeats likely occur when slippage of DNA strands escapes the mechanisms of repair during DNA replication. The nacent strand displacement is followed by an incorrect pairing to the template strand. As a consequence, the allele increases or decreases its size usually in one repeat and, less frequently in two or more repeats

(Chen et al. 2009). In either case the mutation mechanism is known as stepwise mutation model (Weber and Wong 1993, Klintschar and Wiegand 2003).

Several studies have been published on Y chromosome and autosomal STR mutation rates (Huang et al. 2002, Zhivotovsky et al. 2004, Gusmao et al. 2005, Goedbloed et al. 2009), On the other hand, the usefulness of X-STR analysis is widely recognized for evolutionary studies and paternity testing, when the offspring is a female, though their mutation rates are scarcely known.

The aim of this work was to contribute to define a general mutation rate for STR loci located in the X-specific region of the X chromosome (not recombining with Y chromosome). The allele transfer to daughters was analyzed in both the mother and father, while for sons, only the allele received from the mother was tested.

MATERIALS AND METHODS

This work is part of a larger population genetic project approved by the Ethics Committee at the Multidisciplinary Institute of Cell Biology (IMBICE). All blood or salive sample donors were informed about their role in the project and asked to sign their consent in an appropiate form. Samples were coded with numbers and letters for their anonymous handling.

The fidelity of transmission was checked by analysis y of 16 STR autosomal markers in mother-son pairs, and father-mother-daughter trios from 72 families from several provinces of Argentina, mainly residents at Buenos Aires and Misiones. The average parental age of the families tested was 29.62 ± 1.13 years old.

DNA was obtained either from blood or cotton swabs using conventional protocols (Sambrook et al. 1989, Walsh et al. 1991).

X-STR loci included in the analysis and the source of the published primer sequences are detailed in Table I. These markers are of common use for population genetic analysis and paternity testing (Hering and Szibor 2000, Edelmann and Szibor 2001, Edelmann et al. 2002).

X-STR marker	Location	Repetitive sequence	Reference
DXS8378	Xp22.31	[CTAT]	(1)
DXS9898	Xq21.33-22.3	$[TATC]_2$-ATC-$[TATC]_N$	(2)
DXS7424	Xq22.3	[TAA]	(1)
DXS101	Xq21.33-22.3	$[CTT]_N$-$[ATT]_N$	(3)
HPRTB	Xq26	[AGAT]	(4)
GATA31E08	Xq27.3	[AGAT]	(5)
DXS7423	Xq27-28	[TCCA]	(6)

Reference:
1) Edelmann et al. 2002
2) Hering and Szibor 2000
3) Edelmann and Szibor 2001
4) Szibor et al. 2000
5) Asamura et al. 2006
6) Zarrabeitia et al. 2002

Table I: STR loci analyzed

PCR amplifications were performed in an MPI thermocycler (Argentina) using Taq DNA polymerase from Invitrogen®. Cycling conditions were: 94°C for 2 min, then 35 cycles at 93°C for 45 sec, annealing start at 63ºC for 1 min, each cycle touchdown at 1ºC to annealing end at 60ºC, extension at 72ºC for 1 min, and a final extension at 72ºC for 5 min. Separation of the amplified fragments was performed by denaturing polyacrylamide gel electrophoresis followed by silver staining of the gels.

A mutation was defined when an offspring allele did not match with any of the parental alleles, in such a case, the genotyping process was repeated for confirming the mutation. The mutation rate resulted from dividing the number of unmatched transmissions by total transmissions analyzed, and a confidence interval was calculated online at http://statpages.org/confint.html according to Poisson distribution.

RESULTS AND DISCUSSION

Among the 1015 allelic transmissions analyzed we only found one single repeat change for a mother-daughter transmission of DXS9898, causing one tetranucleotide unit loss (12→11 allele). Autosomes and the other six X-markers showed complete fidelity of transmission (Table II). Whenever the father had a different allele than those

of the mother, drop-out was not observed in any case. Several factors affect the mutation process, including the number of repeats, the motif and length of the core sequence, the flanking regions of the marker, the parental age at birth, and the parental origin of the mutant allele (Crow 2006, Gusmão et al. 2005, Goedbloed et al. 2009). We found only one mutated allele transferred from the mother. Since males have much more germ-line divisions than females, it is usually expected to find a higher amount of germ-line mutations during male divisions (Ellegren 2004). As only one mutation was found, the high stochastic influence in this unique event makes the analysis of such a trend not possible to be performed.

X-STR marker	Number of allelic transmissions	Number of mutations
DXS101	139	0
DXS7424	157	0
DXS7423	173	0
GATA31E08	93	0
DXS8378	206	0
DXS9898	124	1
HPRTB	123	0
TOTAL	1015	1

Table II. Number of mutations and allele transmissions for each separate X-STR marker

On the other hand, long alleles have been reported to show a high trend to loss repeats while short alleles show a higher number of gains vs. losses (Xu et al. 2000, Huang et al. 2002). As we have already mentioned, we found one mutation in an allele 12 changing to allele 11 for DXS9898, where the allelic range in the sample analyzed was 8.3 -13. Our finding confirms the trend of higher loss of repeats in long alleles.

According to our results, a general mutation rate of $\mu=9.85 \times 10^{-4}$ can be estimated for the 7 X-STR loci here studied, with a Poisson confidence interval of $2.49 \times 10^{-4} – 5.49 \times 10^{-2}$. Shin et al. (2005) reported 4 mutations for 18 X-STR markers, resulting in $\mu=1.31 \times 10^{-3}$ ($3.57 \times 10^{-4} – 3.36 \times 10^{-3}$), which supports our finding.

With the exception of a lower rate of recombination, (because it does not recombine in males), the molecular mechanisms affecting sequence changes in X repetitive markers are mostly the same that affect autosomes, as X chromosomes are diploid and recombine in women. Accordingly, an average of 17 mutation rates available at the STRbase (http://www.cstl.nist.gov/div831/strbase/) gives a value of $1,56 \times 10^{-3}$ for autosomal STRs. This value and others reported for autosomal STRs (Weber and Wong 1993, Brinkmann et al 1998, Henke and Henke 1999, Sajantila et al 1999, Xu et al 2000) are similar to our result for X-STRs.

A higher mutation rate is usually reported for STRs located in the Y chromosome (Ge et al. 2009, Goedbloed et al. 2009). In fact, the Y chromosome is exposed to a higher number of germ-line divisions which can be responsible for an increase in the mutation rate of Y-STRs. A second reliable explanation might be the missing of X mutated alleles transmitted in trios analyses, which does not occur in Y-STRs, because of its inheritance mechanism. Analysis of Y-STRs allele transmission is restricted to father-son pairs and shows a straight inheritance of the paternal alleles. Contrarily, biallelic STRs may sometimes bring confusion when defining maternal and paternal origin of the alleles and can mask mutation occurrence.

The present work contributes to the estimation of a general X-STR mutation rate which is consistent with other reports and can be applied to population studies and forensic analysis, whenever these markers are genotyped.

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