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Mutation rate of 12 X-STRs from investigator Argus X-12 kit in Argentine population

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ABSTRACT

The analysis of X-chromosomal markers can be important in different situations where the application of autosomal and Y- chromosomal STR markers are not sufficient to solve the cases. Currently, the Argentine population lacks a representative database on X-chromosomal markers, regarding allele and/or haplotype frequencies, and mutation rates. The absence of this information represents an important limitation for their routine use in laboratories, preventing the achievement of a quantitative, statistically supported evaluation. In order to estimate mutation rates for the twelve X-chromosomal markers included in the Argus X-12 kit, 345 father-daughter duos were genotyped.

The samples were selected from all provinces of Argentina and the biological relationship of paternity was previously confirmed by the analysis of autosomal STR markers for all duos, for which likelihood ratios higher than 10^6 were achieved.

A total of 21 mutations over 4140 allelic transmissions were observed at DXS7132, DXS10134, DXS10079, DXS10146, DXS10101, DXS10103, DXS10074, DXS10148 and DXS10135 loci.

The overall X-STRmutation rate observed was 5.1×10^{-3} (95% CI, 3.1×10^{-3}–7.7×10^{-3}) and all the genotypic configurations were explainable by the gain or loss of a single repeat.

Finally, it should be noted that the overall mutation rate observed in this work resulted higher in comparison with some other reports, likely due to only father-daughter duos had been considered. Indeed, these findings are in agreement with previous works suggesting higher mutation rates for males, due to the higher number of germline divisions they experience.

1. Introduction

X-chromosomal genetic markers are particularly useful in some situations of kinship analysis, especially in deficiency paternity cases. X-STR (short tandem repeats) markers may also be employed in cases of mixed DNA samples, in human identification and to weigh the possibility of an incestuous relationship, in case of daughters [1]. Unless mutation, males transmit their X-chromosome unchanged to the daughters, making the X-STR markers excellent candidates to solve cases where the alleged father is absent but his mother or daughter is available to be tested.

Currently, a representative database on X-chromosomal markers, regarding allele and/or haplotype frequencies, and mutation rates, is lacking for the Argentine population. This represents an important limitation for their routine use in laboratories, preventing the achievement of a quantitative, statistically supported, evaluation.

In this work, overall and marker-specific mutation rates were determined for the 12 X-STR markers included in the Argus X-12 kit (Qiagen GmbH, Hilden, Germany).

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Table 1
STR mismatches observed in a total of 4140 allelic transmissions in duo cases. Overall and marker mutation rates.

X-STR markers	Geographic regions	Daughter		Father	Origin	Mutations	Allelic transmissions	Mutation rate
DXS10103	B	18	19	17	Gain	3	345	0.0087
	A	16	19	18	Gain			
	C	18	20	19	Unknown			
DXS7132	C	14	14	15	Loss	1	345	0.0029
DXS10134	A	35	38	36	Loss	1	345	0.0029
DXS10074	D	16	18	17	Unknown	3	345	0.0087
	D	17	17	18	Loss			
	D	17	19	18	Unknown			
DXS10101	B	30.2	32	31	Gain	2	345	0.0058
	D	32.2	32.2	31.2	Gain			
DXS10135	A	25	31	30	Gain	4	345	0.0116
	A	22	27	23	Loss			
	B	22	24	21	Gain			
	B	20	25	24	Gain			
DXS10146	B	26	39.2	38.2	Gain	2	345	0.0058
	D	29	30	28	Gain			
DXS10079	E	17	21	18	Loss	1	345	0.0029
DXS10148	A	28.1	28.1	27.1	Gain	4	345	0.0116
	B	25.1	30.1	29.1	Gain			
	C	18	28.1	27.1	Gain			
	E	18	25.1	24.1	Gain			
Overall						21	4140	0.0051

2. Materials and methods

2.1. Sample selection

Anonymous DNA extracts obtained from 345 father-daughter duos were selected from all provinces of Argentina and the biological relationship of paternity was confirmed by the analysis of autosomal STR markers for all duos using the Investigator 24plex QS, Investigator IDplex Plus (Qiagen GmbH, Hilden, Germany) or PowerPlex® 16 System kits (Promega Corporation, Madison, USA.) for which likelihood ratios higher than 10^6 were achieved.

The analyzed samples represent all the Argentine provinces and were grouped into 5 regions (1-Jujuy, Salta, Tucumán, Catamarca, La Rioja. 2- Santiago del Estero, Chaco, Formosa. 3- Corrientes, Misiones, Entre Rios 4- San Juan, San Luis, Mendoza, Córdoba, Santa Fe, Entre Rios, Ciudad de Buenos Aires. 5- La Pampa, Provincia de Buenos Aires, Neuquén, Río Negro, Chubut, Santa Cruz, Tierra del Fuego

For most of the samples DNA was extracted using MagNA Pure 96 and MagNa Pure Compact instruments (Roche Molecular Diagnostics) and a smaller part with phenol-chloroform extraction. quantification of samples was performed using De Novix DS-11 Spectrophotometer (De Novix IncWA).

This project was reviewed and approved by two Ethical Committees, COFyBCF (Colegio Oficial de Farmacéuticos y Bioquímicos de la Capital Federal) and CEIH (Comité de Ética Institucional de Halitus), both from Buenos Aires city, Argentina.

2.2. Genetics profiling

2.2.1. X-chomosomal STR typing

A total of 12 X-chromosomal STR markers (DXS10103, DXS8378, DXS7132, DXS10134, DXS10074, DXS10101, DXS10135, DXS7423, DXS10146, DXS10079, HPRTB, DXS10148) were amplified using the Investigator Argus X-12 kit (Qiagen GmbH, Hilden, Germany) according to the manufactureŕs recommended protocol, except that the final PCR reaction volume was 10 ul.

PCR products were separated by capillary electrophoresis in an ABI 3500 or ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) and the electrophoretic data were analyzed using GeneMapper ID-X v 1.2 and Gene Scan Analysis software (Applied Biosystems).

2.3. Determination of mutation rates

All father-daughter genetic profiles were compared for incompatibilites. The mendelian incompatibilities identified between them were considered as mutations.

To identify the mutations (or, rather, mendelian incompatibilities between father and daughter genotypes) genetic profiles were compared between father and daughter (Table 1).

X-STR mutation rates were calculated as the number of mutations divided by the total of allelic transmissions analyzed. Confidence interval (CI) was estimated using the exact binomial distribution via spreadsheet formulas provided at http://statpages.org/confint.htlm

3. Results and discussion

3.1. Overall mutation rates

A total of 4140 allelic transmissions were analyzed in 345 confirmed father-daughter duos for the 12 X-STR markers, and 21 mendelian incompatibilities were observed across 9 of the 12 markers and in all five Argentine populations groups.

Among the 12 markers analyzed, three of them exhibited no mutations in this study, including two with simple repeat structure (DXS8378, HPRTB) and one with complex repeat structure (DXS7423). On the other hand, the highest number of mutations were observed at DXS10135 and DXS10148, both markers with complex repeat structures.

The overall X-STRmutation rate observed was 5.1×10^{-3} (95% CI, 3.1×10^{-3}–7.7×10^{-3}) and all the genotypic configurations were explainable by the gain or loss of one single repeat. In three out of the 21 incompatibilities it was not possible to establish if the origin of the mutation was either due to gain or loss of one repetition unit.

It should be noted that the overall mutation rate was higher in this work than in some previous reports [2,3], most likely because only father-daughter duos have been considered. This higher mutation rate in males is usually attributed to the higher number of germ-line divisions they experience [4,5].

Conflicts of interest

None for declare.

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