

Genetic characterization of an X-STR decaplex system in the State of Mato Grosso, Brazil: distribution, forensic efficiency and population structure

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Abstract Studies with X-STR loci show population genetic substructure, which makes necessary the characterization of such markers in the different geographical and/or ethnic populations. Therefore, this study assessed the distribution and forensic efficiency of an X-STR decaplex system in the population of the State of Mato Grosso, as well as analysed the population structure of this State based on the aforementioned system. All X-STR markers were in Hardy–Weinberg equilibrium and linkage equilibrium, and the DXS6809 was the most informative marker. The power of discrimination value in females and males was 0.9999999995 and 0.9999994, respectively. Analysis of molecular variance indicated 1.10% ($p < 0.00001$) of heterogeneity among Europeans, Africans, Brazilians and other Latin Americans, and in relation to such groups, the population of the State of Mato Grosso showed lower genetic variation when compared with the Brazilian group (-0.10% , $p = 0.67327$). The genetic distance analysis showed lower values of F_{ST} ($0.0004 \leq F_{ST} \leq 0.00331$), with non-significant p value ($p > 0.00024$), between the populations of Mato Grosso and Mato Grosso do Sul, Paraná and the Southeast region of Brazil (except for one sample of Rio

de Janeiro). F_{ST} values with significant p values ($p \leq 0.00024$) were obtained between the population of Mato Grosso and Iberian, African and some Latin American populations. The X-STR decaplex system proved to be extremely useful in the population of the State of Mato Grosso, and the data obtained does not show the need for a specific forensic database for this State in relation to the Brazilian populations compared in this study, except for population of Rio de Janeiro.

Keywords X-STR · Population structure · Mato Grosso · Brazil

Introduction

The genotype analysis of X-chromosomal short tandem repeat (X-STR) markers is broadly used in some complex cases of biological relationships [1]. This is due to the inheritance pattern of the X chromosome, by which males can only inherit the mother's genetic material and transmit it only to their daughters. Thus, in some investigations, X-STRs have a higher mean exclusion chance (MEC), as well as a more efficient haplotype typing than autosomal markers [1]. If a mother–son relationship is being questioned, for example, X-STR analysis allows for a nearly twofold greater power of exclusion than that obtained for autosomal STRs (AS-STRs) with moderate heterozygosity [2]. In addition, X-STRs can differentiate pedigrees involving two non-inbred individuals (half-siblings, grandparent–grandchild and avuncular), as well as some situations of incest, which are indistinguishable by the analysis of unlinked autosomal markers [3]. Among other applications, there are tests in which the alleged father is absent or unavailable, which can be more easily solved with X-STRs than AS-STRs if the alleged paternal grandmother is analysed. In her absence, her profile

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will also be better determined for X-STRs when analyzing the siblings of the alleged father [1].

Several X-STR loci have been reported for forensic purposes, and 55 loci are characterized in the ChrX-STR.org 2.0 database [4]. Only three kits were available for the analysis of these markers: Mentype® Argus X-UL (Biotype AG), Mentype® Argus X-8 (Biotype AG) and finally, the Investigator Argus X-12 (Qiagen), considered the latest technology in X-STR testing [5]. However, in Brazil, population genetic data was reported for 42 X-STRs [6, 7], especially markers of the 10-plex system implemented by Gusmão et al. [8] and the 12-plex system developed by Ribeiro-Rodrigues et al. [9, 10]. This data is observed in 20 of the 27 Brazilian States (including Federal District); the Northeast and Midwest were the least studied regions and there is no information for the State of Mato Grosso (Midwest).

Therefore, this study characterized the X-STR 10-plex system [8] in the population of the State of Mato Grosso-Brazil (Supplementary Fig. S1), and assessed the genetic structure of this population both inside and outside Brazil, based on that system. This is a pioneer genetic investigation using the X-STRs in the population of the State of Mato Grosso and plays an important role in understanding its formation history.

Materials and methods

Sampling

A total of 274 unrelated subjects (100 females and 174 males), residents of the State of Mato Grosso (Brazil), were analysed. This study was approved by the Research Ethics Committee of the Medical School of São José do Rio Preto—FAMERP (CAAE 39088514.8.0000.5415). All participants were informed of the study and signed an informed consent document.

DNA extraction and marker genotyping

Genomic DNA was extracted from a piece of Whatman FTA® card (4 mm²) containing peripheral blood. For this purpose, 5% suspension of Chelex 100 resin (Bio-Rad) was used, as indicated by GE Healthcare [11], with minor modifications: 200 µL of Milli-Q water was used and centrifugation lasted 2 min.

Amplification of the 10 X-STR markers was performed in a Swift Maxi thermocycler (ESCO, LOBOV Científica), according to Martins et al. [12]. However, 1.5 µL of the solution with the extracted DNA was used. The PCR products were separated by capillary electrophoresis using a 3500 Genetic Analyzer (AB-TFS: Applied Biosystems, Thermo Fisher Scientific), and with GeneScan 600 LIZ Size Standard (AB-TFS) and POP-4™ polymer

(AB-TFS). Genotyping was performed with GeneMapper® ID-X Software version 1.2 (AB-TFS) through comparison with the reference sample 9947A (Promega Corporation).

Statistical analysis

The Arlequin software version 3.5.1.3 [13] was used to determine allele frequencies, exact test for Hardy–Weinberg equilibrium in female samples and exact test for linkage disequilibrium in male samples. Statistical parameters of forensic evaluation, including mean exclusion chance in trios involving daughters (MEC_T), mean exclusion chance in father/daughter duos (MEC_D), mean exclusion chance in deficiency cases involving mother, daughter and paternal grandmother (MEC_{PGM}), power of discrimination in females (PD_F) and power of discrimination in males (PD_M), were calculated using the ChrX-STR.org 2.0 database [4].

Analysis of molecular variance (AMOVA) and an estimate of genetic distance (F_{ST}) between pairs of populations were performed with the Arlequin software version 3.5.1.3 [13]. The genetic distances (F_{ST}) were visualized in a two-dimensional graph obtained from the analysis of multidimensional scaling (MDS) using the STATISTICA TRIAL 13.0 (StatSoft Inc., <http://www.statsoft.com.br>).

Results and discussion

The allelic profiles typed for 10 X-STRs loci in the population of the State of Mato Grosso are shown in Supplementary Tables S1.1 and S1.2, and the allele frequency distribution and forensic statistical parameters to evaluate the efficiency of this system in that population were determined for the total sample (Table 1).

Considering the studied population and other 23 populations who were analysed using the X-STR decaplex system [8, 12, 14–17], the alleles 13.2 (DXS7132), 34.1 (DXS6809) and 8.2 (DXS9898) were observed only in the State of Mato Grosso (Brazil). Also, according to these studies (including this study), intermediate alleles were absent at the loci DXS8378, DXS6789, DXS7133 and GATA31E08; only the alleles 8.2 and 14.2 were mentioned for GATA172D05 [17] and DXS7423 [16], respectively; and various intermediate alleles were reported for DXS9898, DXS9902, DXS7132 and DXS6809, with the last two loci holding the largest number of such alleles (DXS7132: 13.2, 13.3, 14.3, 15.3, 16.3, 17.3, 18.3; DXS6809: 30.1, 31.1, 31.3, 32.1, 33.1, 34.1, 34.2, 35.1, 38.1, 39.1), which implies greater attention to the allelic designation of these markers.

Only the marker DXS9898 showed significant deviation from Hardy–Weinberg equilibrium (Table 1; $p = 0.02418$), but it did not remain significant after Bonferroni correction (significance level of 0.005). The marker DXS6809 showed

Table 1 Allele frequency distribution and forensic statistical parameters for X-STR decaplex system in the population of the State of Mato Grosso (Brazil) as well as observed (Ho) and expected (He) heterozygosity in females with corresponding *p* values for Hardy–Weinberg equilibrium test (*p*-HWE)

	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DXS7423	DXS6809	DXS7132	DXS9902	DXS6789
<i>N</i>	374	374	371	374	374	374	374	374	374	372
6					0.15775					
7		0.00535	0.00539	0.00802	0.02139					
8			0.00809	0.01604	0.13369	0.00535				
8.2		0.00267								
8.3		0.14171								
9	0.00267	0.01070	0.41507	0.13904	0.15508				0.02139	
10	0.35027	0.02941	0.16712	0.04011	0.24866				0.03743	
11	0.38235	0.13904	0.36119	0.16310	0.20588			0.01337	0.32353	
12	0.23529	0.29411	0.03504	0.30481	0.07487	0.00802		0.09091	0.41979	
12.1									0.01604	
13	0.02674	0.27273	0.00270	0.21390	0.00267	0.05882		0.29947	0.17112	
13.1									0.00267	
13.2								0.00267		
13.3		0.01070								
14	0.00267	0.08556	0.00270	0.10963		0.32086		0.30749	0.00802	0.00269
15		0.00802	0.00270	0.00535		0.44652		0.17914		0.11828
15.3								0.00535		
16						0.10695		0.06417		0.09677
16.3								0.02139		
17						0.05348		0.01337		
17.3								0.00267		
18										0.00806
19										0.03226
20										0.32258
21										0.23118
22										0.13441
23										0.04839
24										0.00538
27							0.00267			
28							0.02941			
29							0.03743			
30							0.06417			
31							0.12299			
31.1							0.00267			
32							0.15241			
32.1							0.00267			
33							0.28342			
34							0.16578			
34.1							0.00535			
35							0.09358			
35.1							0.00267			
36							0.02406			
37							0.01070			
Ho	0.64000	0.68000	0.77778	0.82000	0.85000	0.68000	0.81000	0.79000	0.67000	0.76768
He	0.67889	0.79729	0.68036	0.79191	0.83648	0.68216	0.83995	0.77236	0.67688	0.78808
<i>p</i> -HWE	0.51971	0.02418	0.59864	0.78354	0.78811	0.42638	0.34331	0.75156	0.13044	0.80871

Table 1 (continued)

	DXS8378	DXS9898	DXS7133	GATA31E08	GATA172D05	DXS7423	DXS6809	DXS7132	DXS9902	DXS6789
PD_F	0.82828	0.92664	0.82702	0.93309	0.94444	0.84547	0.95555	0.91270	0.84773	0.93236
PD_M	0.67503	0.79119	0.66799	0.80143	0.82290	0.67982	0.83794	0.77044	0.68764	0.79759
MEC_T	0.60891	0.76143	0.60523	0.77395	0.79871	0.62780	0.81975	0.73584	0.63293	0.77093
MEC_D	0.46197	0.63429	0.45931	0.64937	0.68038	0.48328	0.70979	0.60358	0.48819	0.64625
MEC_{PGM}	0.39634	0.59584	0.40087	0.61234	0.64569	0.43451	0.68365	0.56152	0.43441	0.61123

N number of chromosomes, PD_F power of discrimination in females, PD_M power of discrimination in males, MEC_T mean exclusion chance in trios involving daughters, MEC_D mean exclusion chance in father/daughter duos, MEC_{PGM} mean exclusion chance in deficiency cases involving mother, daughter and paternal grandmother

the highest forensic efficiency values, whereas DXS7133, DXS8378 and DXS7423 were the least effective markers (Table 1). These results were obtained in 17 (for DXS6809) and 14 (for DXS7133, DXS8378 and DXS7423) of the 23 populations analysed using the same genetic system [8, 12, 14–17]. The power of discrimination value in females and males was 0.9999999995 and 0.9999994, respectively. For the evaluation of biological relationships, the overall MEC_T , MEC_D and MEC_{PGM} values were 0.999998, 0.9999 and 0.9997, respectively. The results show that the X-STR decaplex system is highly informative for the population of the State of Mato Grosso (Brazil), as described in other Brazilian populations [8, 12, 15].

Analysis of linkage disequilibrium (LD) (Supplementary Table S2) showed allelic association ($p \leq 0.05$) only at pairs DXS9898/DXS7132 ($p = 0.0149$), DXS6809/DXS9902 ($p = 0.0491$) and DXS9898/DXS6789 ($p = 0.0142$), whose loci are spaced by more than 7.65 Mb (Rutgers Map v.3, http://compgen.rutgers.edu/download_maps.shtml). However, LD did not remain after Bonferroni correction (significance level of 0.0011), and these allelic associations were not consistent with other admixed populations of the Brazilian Midwest, Southeast and South, which were evaluated based on the same X-STR system [8, 12].

For the DXS6809–DXS6789 markers, which are closely linked (0.35 cM), an allelic association was reported in one sample of the German population [18]. However, such association could not be observed in the population of Mato Grosso, other admixed populations in Brazil [8, 12] and populations in Latin America [8], Iberian countries [8, 16, 19], Italy [20], Africa [14, 17, 21] and Japan [22].

The linkage equilibrium found in Europeans [8, 16, 19] and Africans [14, 17] (considering the Bonferroni correction) for the X-STR decaplex system suggests that LD was not inherited from these groups during the formation of the population of Mato Grosso (Brazil). Moreover, the absence of LD for these loci in the studied population shows that mixing of different racial groups did not result in significant allelic association, which increased the power of discrimination to the multiplex system and allows the use of allele frequency

database for such markers, including DXS6809 and DXS6789 loci.

Analysis of molecular variance (AMOVA) was carried out with the allelic profiles obtained from the X-STR decaplex system in four European populations (Portugal: northern and central regions [8]; Spain: Galicia and Cantabria [8]), one African population (Uganda: Karimojong [14]), nine Brazilian populations (Mato Grosso—this study; Mato Grosso do Sul [8], São Paulo [8], Rio de Janeiro [8], Paraná [8], São Paulo [12], Rio de Janeiro [12], Vitória [12] and Belo Horizonte [12]) and seven other Latin American populations (Argentina: Buenos Aires [8], Cordoba [8], Rio Negro [8], Entre Rios [8] and Misiones [8]; Colombia: Antioquia [8]; Costa Rica [8]). Asian and Native American populations with available data about allelic profiles for such X-STR system could not be found in the literature. This analysis was performed in five tests (Table 2), and the results showed that most of the genetic variability (above 96.60%; $p < 0.00001$) was due to differences within the populations. However, there was significant variation ($p \leq 0.05$) among the populations within the studied groups and among some groups, 1.10% ($p < 0.00001$) of heterogeneity was observed among the groups of European, African, Brazilian and other Latin American countries. The State of Mato Grosso, in relation to the different geographical groups (tests 2 to 5), showed lower genetic variation when compared with the Brazilian group (−0.10%), followed by Latin American (0.19%), European (0.52%) and African (3.40%).

Genetic distances, based on the number of different alleles (F_{ST}), were estimated for the X-STR decaplex system (Supplementary Table S3), using the same populations described in the AMOVA analysis. Low genetic distance values $0.0004 \leq F_{ST} \leq 0.00331$ with non-significant p value ($p > 0.00024$ after Bonferroni correction) were obtained for the population of Mato Grosso in relation to the Brazilian populations, except for one sample of Rio de Janeiro to which further studies are needed to evaluate this genetic differentiation. Internationally, the population of Mato Grosso showed increased genetic similarity with the Argentine population ($0.00437 \leq F_{ST} \leq 0.00581$, except Entre Rios), followed by

Table 2 Analysis of molecular variance (AMOVA) for X-STR decaplex system in different population groups

Test	Population	Source of variation	Variation (%)	<i>p</i>
Test 1	European	Among groups	1.10	<0.00001*
	African	Among populations within groups	0.33	<0.00001*
	Latin American ^a	Within populations	98.57	<0.00001*
Test 2	Brazilian ^b	Among groups	0.52	0.20079
	Mato Grosso, Brazil	Among populations within groups	0.17	0.00089*
	European	Within populations	99.31	<0.00001*
Test 3	Mato Grosso, Brazil	Among populations	3.40	<0.00001*
	African	Within populations	96.60	<0.00001*
Test 4	Mato Grosso, Brazil	Among groups	0.19	0.38386
	Latin American ^a	Among populations within groups	0.51	<0.00001*
		Within populations	99.30	<0.00001*
Test 5	Mato Grosso, Brazil	Among groups	−0.10	0.67327
	Brazilian ^c	Among populations within groups	0.32	<0.00001*
		Within populations	99.78	<0.00001*

* Significant *p* values for a level of 0.05

^a Latin American: Buenos Aires [8], Cordoba [8], Rio Negro [8], Entre Rios [8], Misiones [8], Antioquia [8] and Costa Rica [8]

^b Brazilian: Mato Grosso [this study], Mato Grosso do Sul [8], São Paulo [8], Rio de Janeiro [8], Paraná [8], São Paulo [12], Rio de Janeiro [12], Vitória [12] and Belo Horizonte [12]

^c Brazilian: include all Brazilian populations reported for Brazilian^b group, except Mato Grosso [this study]

Iberians ($0.00559 \leq F_{ST} \leq 0.00663$, except Cantabria), as well as greater genetical distance from the African population ($F_{ST} = 0.03404$). Significant *p* values ($p \leq 0.00024$ after Bonferroni correction) were observed when comparing the population of Mato Grosso with the Iberian, African and some Latin American populations (Cordoba, Buenos Aires, Costa Rica and Antioquia). This demonstrates a need for specific allelic databases for different populations. Although the genetic distance to Entre Rios ($F_{ST} = 0.01008$; $p = 0.00574$) was greater than the Argentine estimates, it was not statistically significant. Pairwise analysis of F_{ST} revealed some significant genetic differences, which were not observed in the AMOVA test.

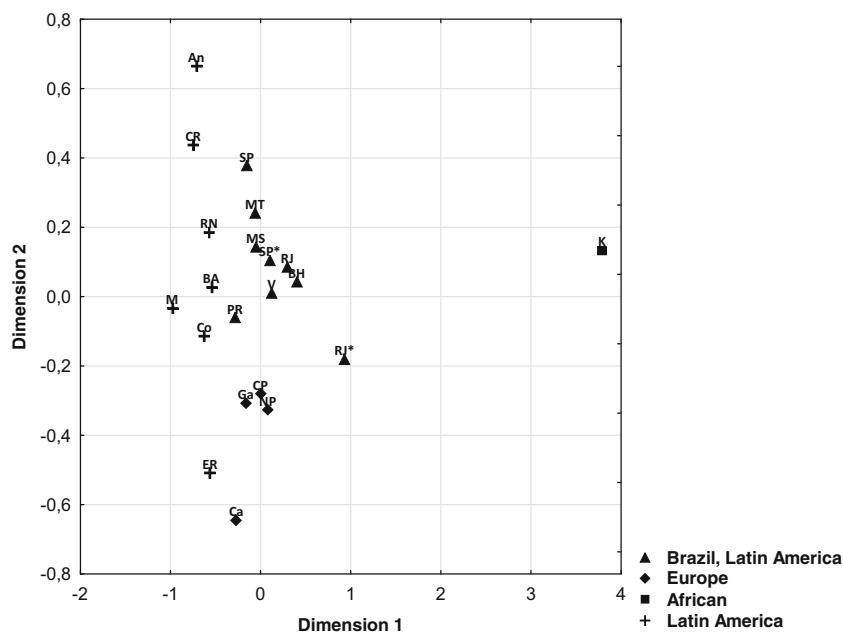
Multidimensional scaling analysis (MDS) based on the matrix of F_{ST} is shown in Fig. 1, which shows that the population of Mato Grosso is grouped into the Brazilian populations, especially Mato Grosso do Sul (belonging to the Midwest region of Brazil, where Mato Grosso is located). It is also closer to the Argentine group, followed by the Iberian group, and very distant from the African population, which is consistent with the data obtained from the AMOVA and F_{ST} analyses.

Considering the X-STR decaplex system in the Brazilian Midwest, the population of Mato Grosso shows similar F_{ST} results to those of Mato Grosso do Sul [8, 12], especially within a national context, as observed in the MDS graph. In a study with 12 X-STRs in 16 Brazilian States of all five geopolitical regions of the country [19], Mato Grosso do Sul

also showed no significant differences with the other States. Goiás, a Midwest State, differed from Rondonia only. When all Brazilian regions were compared among each other, the Midwest did not differ from the other regions and showed more similarities to the Northwest and Southeast of Brazil, followed by the South and finally the North, confirming the data obtained in this study. However, statistically significant differences are found among some Brazilian populations for the X-STR decaplex [8, 12] and 12-plex systems [19], which demonstrate that a national database should not be used for such markers in Brazil.

According to Moura et al. [24], the populations in the Midwest (MW) and Southeast (SE) of Brazil have similar genetic composition for autosomal markers (MW 64% European (E), 24% African (A) and 12% Native American (NA); SE 67% E, 23% A and 10% NA), and it is also closer to the genetic composition of the Northeast (NE 58% E, 27% A and 15% NA). These findings have also been reported by Manta et al. for 46 AIM-Indels [25]. Ancestry estimates based on 24 X-Indel markers in four Brazilian regions confirm similarities between the Southeast and the Northeast (SE 38% E, 35% A and 27% NA; NE 37% E, 36% A and 27% NA) [26]. Although the Midwest has not been analysed, it is believed that the ancestry composition for the X chromosome is similar to these regions, as observed in autosomal studies [24, 25]. These findings are in agreement with the data obtained for the population of Mato Grosso in this study and for other populations of the Brazilian Midwest [8, 12, 19].

Fig. 1 MDS plot based on the pairwise F_{ST} genetic distance matrix, supplementary Table S3, (stress = 0.10)



Argentina, Costa Rica and Antioquia have a tri-hybrid population and, for autosomal markers, they show marked European ancestry, followed by Native American ancestry, and a smaller percentage of African ancestry. The African ancestry is very small in Argentina (65% E, 4% A, 31% NA [27]) and in Costa Rica (58% E, 4% A, 38% NA [28]), compared with the percentages in Antioquia (44.3% E, 21.1% A, 34.6% NA [29]). For markers of X chromosome, the population of Antioquia carries genetic heritage of 37.5% E, 29% A and 33.5% NA, showing greater European contribution, which is very close to the Native American data [30]. In Brazil, as mentioned above, X ancestry data for the Northeast and Southeast also show greater European contribution, but this value is very close to that of the African population [26]. Statistics show that the number of permanent immigrants in Brazil until 2012 includes Bolivians (50,240 immigrants) and Argentinians (42,202 immigrants) as the main representatives from Latin America, with fewer Colombians (5915 immigrants) and Costa Ricans (only 411 immigrants) [31]. The significant number of immigrants from Argentina into Brazil is one of the factors that may explain the greater genetic similarity of the population of Mato Grosso with Argentinians, in relation to Costa Rica and Antioquia.

Genetic data for the X-STR decaplex system in the population of Mato Grosso confirm its history of colonization. This region was initially occupied by indigenous people. In the fifteenth century, the Treaty of Tordesillas, signed between Spain and Portugal [32], divided the newly discovered lands outside Europe and this territory came under the Spanish rule. However, the Spanish did not settle in this area immediately, as they were attracted by the riches found in the mines in Peru. In the late seventeenth century, explorers

(called “Bandeirantes”) hailed from Sao Paulo and led expeditions to the west of the country, reaching Minas Gerais, Goiás and Mato Grosso. They extended the territorial control over the Brazilian Midwest and discovered large gold deposits. At that point, Mato Grosso started to receive a large number of Spanish and Portuguese settlers [32–34], as well as settlers from other populated areas of the Portuguese colony, such as São Paulo de Piratininga, São Vicente and the Northeast coast (https://pt.wikipedia.org/wiki/Minera%C3%A7%C3%A3o_no_Brasil).

In order to meet the increasing demand for mine workers, African slaves were brought to Mato Grosso, especially from Guinea, Angola, Mozambique and the Democratic Republic of Congo [35]. After the mining boom, the State directed the focus to the agricultural sector. As of 1960, substantial investments from the States of Rio Grande do Sul and Paraná for the cultivation of wheat and soybean brought progress to the region, which began to stand out in the Brazilian agricultural sector [36]. In the 1970s and 1980s, an intense migration flow was observed in this promising region, driven by incentive programs for agricultural modernization and interaction of Mato Grosso to other markets [36]. Therefore, the Midwest was the last region to be colonized by individuals from all other Brazilian regions, mainly the Northeast and Southeast [37].

In conclusion, the X-STR decaplex system proved to be extremely useful for biological relationships tests and forensic cases involving the population of the State of Mato Grosso and the data related to population genetic structure does not show the need for a specific forensic database for this State in relation to the Brazilian populations compared in this study, except for the population of Rio de Janeiro.

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Compliance with ethical standards This study was approved by the Research Ethics Committee of the Medical School of São José do Rio Preto—FAMERP (CAAE 39088514.8.0000.5415). All participants were informed of the study and signed an informed consent document.

Conflict of interest The authors declare that they have no conflict of interest.

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