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SHORT COMMUNICATION

Sub-Saharan Africa descendents in Rio de Janeiro (Brazil): population and mutational data for 12 Y-STR loci

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Abstract A male sample of 135 African descendents from the Rio de Janeiro population were typed for the 12 Y-chromosome short tandem repeat (STR) loci included in the PowerPlex Y System. A high haplotype diversity was observed (0.9971), with 91% of haplotypes being unique, demonstrating the usefulness and informative power of this Y-STR set in male lineage identification. Samples with shared haplotypes were additionally typed with the Yfiler kit, which includes five extra markers. The haplotype diversity when using the 17-Yfiler loci increased to (0.9998) with 97% unique haplotypes. The same set of Y-STRs was also typed in 135 father/son pairs and three single-step mutations were observed: one at DYS19 and two at DYS385. Genetic distance analysis showed highly significant differences in all pairwise comparisons between this sample of African descendents and the general population from Rio de Janeiro, as well as with Iberian and African samples from Portugal, Mozambique, Angola and Equatorial Guinea. Comparisons with samples from other regions in Brazil showed that heterogeneity does exist, indicating that a Y-haplotype database for the whole country should take into account the population sub-structure. Moreover, a strong European influence was detected, and thus, a Y-chromosome STR profile proves a rather poor indicator for the ethnic origin of an individual in Rio de Janeiro.

Keywords Y-chromosome haplotypes · STRs · Brazil · Africans · Mutation

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Introduction

In the last few years, analysis of STRs located on the Y-chromosome has been shown to be very useful in some forensic and kinship analysis, namely in detecting male profiles in mixtures of body fluids from sexual assaults (e.g. [9, 17, 18]).

Because of the lack of recombination between Y-chromosome-specific markers, they are transmitted as haplotypes in the same way as single locus alleles. Because of the lower effective number of Y-chromosomes in a population, Y-haplotypes/haplogroups tend to present a higher proportion of variation between populations than the observed for other markers located on autosomes or X-chromosome. Therefore, the DNA Commission of the

International Society of Forensic Genetics (ISFG) recommended the use of regional Y-STR haplotype databases, and pooling data from different regions is only valid after verifying that no population sub-structure exists [15].

A large amount of Y-haplotype data is, therefore, essential for two main reasons: (a) in match probability calculation, because it is not valid to multiply the allele frequency of each locus, a large number of haplotypes are needed to allow reliable frequency estimates; (b) population sub-structure analysis is highly dependant on the amount of data available.

Although Brazil is a large country harbouring a high multiplicity of population groups characterised by different percentages of European, sub-Saharan African and Amerindian ancestry, until now, few Y-haplotype data are available concerning Brazilian populations [8, 12, 13, 27]. Therefore, much more data on many more regions are needed to evaluate population sub-structure.

In a previous study, a random sample from Rio de Janeiro was analysed for Y-STR haplotypes diversity [12], and a significant difference was found between Portugal, the main source of European immigrants, and Brazil, which was explained by the presence of haplotypes from African ancestry. In the present study, we aimed to study a sample of 135 unrelated individuals, self-declared Afro-Brazilians ("Negros"), living in Rio de Janeiro and to compare our results with those from the Rio de Janeiro general population. For these 135 unrelated men, their respective sons were also typed for the same Y-STR markers to estimate the overall mutation rate.

Materials and methods

A sample of 270 healthy men, involved in routine paternity testing, who declared to have sub-Saharan African ancestry, was selected from the Rio de Janeiro (Brazil) population. This sample comprised 135 unrelated individuals and their respective sons. The biological relationship had been confirmed for all father/son pairs by using autosomal STRs, with paternity index values above 10,000. Autosomal STRs were typed using the AmpFlSTR Identifiler polymerase chain reaction (PCR) amplification kit (AB Applied Biosystems, Foster City, CA) and GenePrint FFFL kit (Promega Corporation, Madison, WI), following the manufacturer's instructions.

Genomic DNA was extracted from peripheral blood samples using the salting out method according to Miller et al. [21].

In PCR amplification with the commercial kits PowerPlex® Y System (Promega) and YFiler (Applied Biosystems), the conditions recommended by the manufacturers were followed. Amplicons were typed in an ABI 3100 Sequencer (Applied Biosystems) using the Genemapper 3.2 Analysis software. Allele designations were based on comparison with the allelic ladders provided by the manufacturers. To follow the ISFG recommendations [15] a correction factor of 16 was added to the GATA H4 alleles generated by the YFiler kit [22].

Haplotype frequencies and diversities were estimated according to Nei [23] using the ARLEQUIN Software Ver 3.01 [11], which was also used for genetic distances computations, assuming the stepwise mutational model (sum of squared size differences, Rst; [25]); therefore, DYS385 was not considered, and the number of repeats in DYS389I was subtracted from DYS389II. The neighbour program, implemented in PHYLIP 3.65 software package (http://evolution.genetics.washington.edu/phylip.html), was used to draw unrooted neighbour-joining trees based on genetic distance values among populations. The tree was visualised with the Treeview software [24].

Results and discussion

Y-haplotype variability

Table S1 shows the haplotypes observed in a male sample of 135 unrelated Afro-Brazilians from Rio de Janeiro, typed for the 12 Y-chromosome STR loci included in the PowerPlex Y System (Promega): DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438 and DYS439. Out of the 135 individuals studied, five shared haplotypes were obtained, where two were repeated three times (N102/N52/N57 and N38/N67/N130) and three were observed twice (N65/N116, N40/N99 and N19/N51). A high haplotype diversity was observed (0.9971). To compare this value with the one obtained using the five additional markers (DYS448, DYS456,

Table 1 Total number of mutations and allele transmissions per locus calculated when joining our data with those previously reported in [3, 6, 7, 10, 12, 14, 16, 19, 20, 26]

Locus	Mutations	Allele trans.	Freq. ($\times 10^{-3}$)	95% CI $\times 10^{-3}$
DYS19	13	7314	1.7774	0.947–3.038
DYS389I	11	5518	1.9935	0.996–3.564
DYS389II	12	5505	2.1798	1.127–3.805
DYS390	15	6796	2.2072	1.236–3.638
DYS391	23	6744	3.4104	2.163–5.113
DYS392	4	6710	0.5961	0.162–1.526
DYS393	4	5498	0.7275	0.198–1.862
DYS385	24	10207	2.3513	1.507–3.497
DYS437	5	2437	2.0517	0.667–4.781
DYS438	1	2476	0.4039	0.010–2.248
DYS439	12	2451	4.8960	2.532–8.537
Total	124	61656	2.0112	1.673–2.397

DYS458, DYS635 and GATA H4) included in the YFiler kit (Applied Biosystems), all samples with a non-unique haplotype were also typed with the YFiler kit (results in Table S2). The haplotype diversity, when using the 17-YFiler loci, increased to 0.9998 reducing the number of shared haplotypes in the sample to just two (N52 and N102, N65 and N116).

Both kits are, therefore, highly informative in male lineage identification inside this group, with the YFiler improving in 0.27% the haplotype diversity of the PowerPlex Y system.

Population comparison

The present 12-Y-STR haplotype data were compared with those available for the same set of markers. Genetic distance analysis showed highly significant differences in all pairwise comparisons between this sample of African descendents and the general population from Rio de Janeiro [12] as well as with Iberian and African samples from Portugal [5], Mozambique [1], Angola [4] and Equatorial Guinea [2]. These differences can be explained by the European male admixture usually found in African American groups. Rio de Janeiro African descendents presented larger genetic distances from Africans (Rst values varied between 0.139 and 0.184) than from non-Africans (Rst=0.082 in the comparison with Portugal and Rst=0.031 in the comparison with the Rio de Janeiro general population). The smallest distance with Europeans can also be seen in the neighbour-joining tree built with the Rst–distance matrix (Fig. S1a).

The present data were also compared with those previously published from different regions of Brazil using "minimal haplotype" information. A neighbour-joining tree built with pairwise Rst values (Fig. S1b) showed that the sample from Rio de Janeiro African descendents is genetically distant from all the others (Rst≥0.034; P= 0.000). The Rio de Janeiro general population [12], a population from Santa Catarina [8] and a pooled sample from Brazil [13] group together (Rst≤0.007; P≥0.076), closer to Portugal.

Segregation analysis

The set of Y-STR loci included in the PowerPlex Y System was typed in 135 father/son pairs and among 1,620 allele transfers, three mutations were observed. One mutation occurred at DYS19 with allele 16 mutating to 15 (N49 in Table S1). The remaining two occurred at the DYS385 loci; one allele 19 mutated to 18 (N106 in Table S1) and one allele 10 mutated to 11 by gaining one repeat (N28 in Table S1). All mutations were single steps, and we did not find mutations in more than one locus for the same father/son pair. The overall mutation rate in our sample (1.8×10^{-3}; 95% confidence interval (CI) 0.38–5.40) was not significantly different from that previously reported. In Table 1 the mutation rates are given for these 12 Y-STRs and their respective 95% CI, calculated after combining our data with those previously reported in [3, 6, 7, 10, 12, 14, 16, 19, 20, 26].

In summary, the present work demonstrates the high diversity of the African population group in Rio de Janeiro when using PowerPlex Y System or Y Filer kits. The observed haplotypes, as well as the results of genetic distance analysis, demonstrate that a high proportion of European Y chromosomes is expected to be found inside this population group. Therefore, the Y-chromosome STR profile proves to be rather poor as an indicator for the ethnic origin of an individual in Rio de Janeiro.

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