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Population and mutation analysis of 17 Y-STR loci from Rio de Janeiro (Brazil)

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Abstract The 17 Y chromosome STR loci DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA C4, GATA H4 and GATA A10 were analyzed in a male sample of 126 unrelated individuals from Rio de Janeiro. No shared haplotypes were observed, demonstrating the usefulness and informative power of these Y-STRs in male lineage identification in Rio de Janeiro. Pairwise haplotype analysis showed no significant differences in the comparison of Rio de Janeiro with Iberian samples from different regions of Portugal and Spain, as well as with other Caucasian samples from South America, namely Costa Rica, Buenos Aires (Argentina) and São Paulo (Brazil). The same set of Y-STRs was also typed in 119 father/son pairs and among 2,023 allele transfers, 8 mutations were observed with an overall mutation rate of 0.003955±0.001396 per locus/meiosis across the 17 loci. Except in one case, all mutations were single step. For DYS438 a four-step mutation was found which has never been reported before, where allele 10 mutated to allele 6.

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Introduction

During the last decade, the advantages of studying Y-chromosome-specific markers in population and forensic genetic fields have been emphasized by many authors (Jobling et al. 1997; Prinz et al. 2001; Kayser et al. 2001; Roewer et al. 2001). The Y-STR analysis can be very useful in paternity tests in which the alleged father is missing or deceased. In these cases, reference individuals from the same patrilineage can be analyzed. The investigation of Y-STRs is also very useful in analyzing body fluid mixtures present in criminal cases. By typing Y markers, even a minor male DNA component in a mixed male/female stain of a sexual assault can yield a male-specific profile which can be compared with the DNA of suspects (Betz et al. 2001).

The determination of Y-STR haplotype frequencies in different populations is a major point for the correct interpretation of the genetic profile matches in paternity and forensic casework and information on mutation rates is also crucial in kinship analysis.

For the Brazilian population there are few Y-STR haplotype data (Da Costa et al. 2002; Vallinoto et al. 1999) and mutation studies have not yet been reported.

The aim of the present study was to type 17 Y-STR loci using 2 multiplex PCR-based systems in order to establish a Y-STR haplotype database for the Rio de Janeiro population and also to compare our results with those from other historically related populations studied for the same set of markers. We also aimed to study the overall mutation rate of these markers by the analysis of father/son pairs whose relationship was previously confirmed by autosomal STR typing.

Material and methods

DNA samples

A sample of 245 healthy males, submitted for routine paternity testing, was selected from the Rio de Janeiro (Brazil) population. These 245 samples comprised 126 unrelated individuals from which in 119 cases it was also possible to type the respective son. 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 PCR amplification kit (AB Applied Biosystems) and GenePrint FFFL kit (Promega Corporation), following the manufacturer's instructions.

Genomic DNA was extracted from peripheral blood samples using the salting out method according to Miller et al. (1988).

Multiplex amplification

The 16 Y-STRs (17 loci) were amplified in 2 PCR multiplex reactions. The minimal haplotype plus DYS437, DYS438 and DYS439 (or DYS438 and DYS439) were typed with the commercial kits PowerPlex Y System (Promega Corporation) or Y-PLEX12 commercial kit (Reliagene, New Orleans, LA), following the amplification conditions recommended by the manufacturers. DYS460, DYS461, GATA A10, GATA C4 and GATA H4 plus DYS437, DYS438 and DYS439 were typed in a single multiplex, as described by Gusmão and Alves (2005) with primer sequences described in Sánchez-Diz et al. (2003). Primer concentrations in the multiplexed PCR were 0.04 μM (DYS437), 0.8 μM (DYS438), 0.2 μM (DYS439), 0.22 μM (DYS460), 0.12 μM (DYS461, GATA C4 and GATA H4) and 0.18 μM (GATA A10). PCR amplification was performed in a GeneAmp PCR system 2400 thermocycler (AB Applied Biosystems), with 5–10 ng of genomic DNA in a 12.5 μl reaction volume comprising 1.5 mM $MgCl_2$, 200 μM dNTPs, 1× Gold buffer and 1 U of Taq Gold polymerase (AB Applied Biosystems). Thermal cycling conditions were the same as for PowerPlex Y System amplification.

Typing method

Amplicons were typed in an ABI 310 sequencer (AB Applied Biosystems) using the Genescan 2.1 Analysis software. Allele designations were based on comparison with the allelic ladders provided in the commercial kits by both manufacturers, or with the help of home-made ladders obtained by mixing previously sequenced samples for the most common alleles. Minimal haplotype STR alleles were named as in the YHRD (Y-STR Haplotype Reference Database; http://www.yhrd.org) and the others according to Gusmão et al. (2002a).

Sequencing

Before the sequencing reaction, samples carrying mutations were amplified in a singleplex reaction according to Gusmão et al. 2002a. PCR amplified fragments were purified with Microspin S-300 HR columns (Pharmacia, Uppsala, Sweden). A dideoxy cycling sequencing reaction was carried out using ABI Big Dye Terminator v.3.0 kit (AB Applied Biosystems, Foster City, CA). The products were purified using an ethanol-based protocol and run in an ABI Prism 3100 Genetic Analyzer instrument (AB Applied Biosystems, Foster City, CA) following the manufacturer's instructions.

Statistical analysis

Gene, haplotype frequencies and diversities were estimated according to Nei (1987) using the ARLEQUIN Software ver. 2.000 (Schneider et al. 2000). Analysis of molecular variance (AMOVA) was summarized in the form of R_{st}values and assessed for statistical significance using a Monte-Carlo test as implemented in the ARLEQUIN software ver 2.000 (Schneider et al. 2000). In population comparisons DYS385 was not considered and the number of repeats in DYS389I was subtracted from DYS389II.

Results and discussion

Table 1 shows the haplotype distribution in a sample of unrelated males from Rio de Janeiro for the 17 Y-chromosome STR loci, DYS19, DYS385, DYS389I and II, DYS390, DYS391, DYS392, DYS393, DYS437, DYS438, DYS439, DYS460, DYS461, GATA C4, GATA H4 and GATA A10. No shared haplotypes were obtained out of the 126 individuals studied. The sample yielded an overall haplotype diversity of 1.000±0.0010, demonstrating the usefulness and informative power of these Y-STRs in male lineage identification in Rio de Janeiro.

The presence of some non-European male lineages can explain the increase in haplotype diversity when compared with the Portuguese sample (Gusmão et al. 2002b). At least seven haplotypes are most likely of sub-Saharan African origin since four of them (37SP, 84SP, 107SP and 126SP, in Table 1) have the "Bantu founder haplotype" (DYS19*15; DYS390*21; DYS391*10; DYS392*11 and DYS393*13) described by Thomas et al. (2000) and the remaining are its one step neighbors (13SP, 14SP and 79SP).

The present haplotype data were compared with previously published population data from Rio de Janeiro (Da Costa et al. 2002) for a smaller set of markers (DYS19, DYS389 I and II, DYS390, DYS391 and DYS392). Genetic distance analysis resulted in an unexpectedly high significant pairwise Φ_{st}value between these two samples (Φ_{st}=0.07672, P=0.00000). According

Table 1 Y chromosome haplotype distribution in 126 individuals from Rio de Janeiro population

Id	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 437	DYS 438	DYS 439	DYS 460	DYS 461	GATA A10	GATA C4	GATA H4
1SP	14	13	29	22	11	13	13	11, 14	14	12	12	11	13	17	23	27
2SP	14	13	29	24	11	13	13	11, 14	15	11	11	10	12	15	23	28
3SP	14	12	28	22	10	11	13	13, 14	16	10	13	10	12	15	22	27
4SP	15	13	28	23	9	11	12	13, 16	14	9	11	11	13	14	23	27
5SP	14	13	29	24	11	13	13	11, 14	14	12	12	11	12	15	24	27
6SP	15	13	32	22	10	11	12	13, 15	14	9	12	9	12	15	22	27
7SP	16	14	30	24	11	13	13	11, 15	15	12	12	11	12	15	23	28
8SP	14	13	30	24	11	13	13	11, 14	15	12	12	11	12	13	25	28
9SP	14	13	29	24	11	13	14	11, 14	14	12	12	10	12	16	24	28
10SP	14	13	29	24	10	13	13	11, 14	14	12	11	11	12	13	23	28
11SP	14	13	29	24	10	13	13	11, 15	15	12	13	11	12	15	24	28
12SP	13	12	29	24	10	11	12	12, 19	14	10	13	10	11	14	21	26
13SP	15	13	30	22	10	11	13	14, 14	16	10	12	10	12	14	21	27
14SP	15	12	29	22	10	11	13	12, 14	16	10	12	11	11	14	21	27
15SP	14	13	26	24	10	13	13	14, 16	14	9	11	10	11	14	21	27
16SP	16	13	28	22	11	11	13	11, 12	15	10	13	11	11	15	21	28
17SP	14	13	30	24	10	13	13	11, 14	15	12	12	11	11	15	23	28
18SP	14	14	31	23	10	14	12	15, 17	16	11	12	10	11	14	21	27
19SP	12	13	29	24	11	13	13	12, 14	15	12	11	11	12	14	23	28
20SP	14	13	30	23	11	13	12	12, 14	14	12	13	11	12	15	23	28
21SP	17	13	30	21	11	11	14	16, 17	13	11	13	11	12	13	21	27
22SP	15	12	26	24	11	13	13	12, 14	15	12	10	10	12	15	24	28
23SP	14	14	30	24	11	13	13	11, 11	15	13	13	11	12	14	23	28
24SP	14	13	29	25	11	13	13	11, 14	14	12	12	10	12	15	24	27
25SP	16	14	31	21	10	11	14	15, 18	14	11	11	10	13	14	21	28
26SP	16	11	28	22	10	11	12	12, 14	15	10	12	10	12	16	25	27
27SP	15	12	29	22	11	11	12	13, 16	16	9	11	10	13	13	21	27
28SP	15	14	31	22	10	12	14	13, 17	14	10	11	11	12	15	21	27
29SP	13	14	30	23	9	11	13	13, 14	14	10	10	11	13	14	21	28
30SP	14	12	29	23	10	11	13	13, 14	16	10	11	11	12	15	23	27
31SP	14	13	28	24	11	13	13	12, 15	15	12	11	11	12	15	23	28
32SP	14	13	29	25	10	13	13	11, 14	15	12	12	10	11	14	23	28
33SP	14	13	29	23	11	13	13	11, 14	14	12	12	11	12	15	23	27
34SP	14	14	29	24	11	13	13	11, 14	15	12	14	11	14	15	24	28
35SP	14	13	29	24	11	13	13	11, 14	15	11	12	11	13	15	24	28
36SP	14	13	29	25	10	13	13	11, 14	15	12	12	10	12	15	23	28
37SP	15	13	30	21	10	11	13	16, 17	14	11	11	10	12	14	21	28
38SP	14	13	29	23	11	13	13	11, 14	15	12	12	11	12	14	23	28
39SP	15	14	31	23	10	12	15	15, 15	14	10	11	11	12	15	22	27
42SP	14	13	28	24	11	13	13	11, 14	15	12	12	10	12	14	23	28
43SP	14	13	30	23	10	15	13	1114	14	12	11	11	12	15	23	28
44SP	13	13	30	24	10	11	13	16, 16	14	10	11	9	12	15	22	28
45SP	14	13	29	24	11	13	13	11, 14	15	12	12	11	12	17	24	28
46SP	13	13	29	23	10	13	14	15, 16	15	9	12	10	11	14	20	28
47SP	14	13	28	24	10	14	12	13, 14	15	12	13	10	12	15	25	28
48SP	14	14	30	24	11	13	13	12, 14	15	12	12	10	12	15	23	27
49SP	15	14	31	24	10	13	13	11, 15	15	12	12	10	12	14	23	28
50SP	15	13	30	24	11	11	13	14, 15	15	10	12	10	12	14	23	27
51SP	15	13	31	23	10	14	13	14, 19	14	9	11	11	12	15	21	28
52SP	14	13	30	23	11	13	13	11, 14	15	12	12	11	12	15	23	28
53SP	14	13	31	23	10	11	12	13, 18	14	10	11	11	11	14	19	27
55C	15	13	29	24	9	11	12	13, 16	14	9	12	10	12	15	23	28
56SP	14	13	30	24	11	13	13	12, 14	15	12	12	11	12	15	23	28

Table 1 (continued)

Id	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 437	DYS 438	DYS 439	DYS 460	DYS 461	GATA A10	GATA C4	GATA H4
57SP	14	12	28	24	11	14	13	11, 14	15	12	12	11	12	15	23	28
59SP	14	13	29	24	11	13	13	11, 11	15	12	11	10	12	14	23	28
60SP	14	12	28	24	10	11	13	13, 15	14	10	11	10	12	15	21	27
61SP	14	12	28	22	10	11	12	14, 14	16	10	12	10	12	15	24	27
62SP	16	14	31	24	10	11	14	11, 14	14	11	10	11	11	17	23	29
63SP	17	13	28	23	11	12	14	15, 15	15	10	11	11	12	15	20	27
64SP	14	13	30	23	11	13	13	11, 14	15	12	12	11	11	15	24	28
65SP	14	14	30	23	11	13	13	11, 14	15	12	12	11	12	15	23	28
66SP	13	14	30	24	10	14	13	15, 16	14	10	12	10	13	14	23	29
67SP	15	13	30	23	10	11	12	13, 20	14	10	11	10	12	15	20	28
68SP	14	14	30	23	10	11	12	13, 13	14	10	11	11	13	14	20	27
69SP	16	13	30	25	11	11	13	11, 14	14	11	11	12	11	16	23	27
71SP	14	13	29	24	10	13	12	17, 18	14	11	10	11	11	14	21	26
72SP	13	13	30	24	10	11	13	16, 17	14	10	11	9	12	16	22	29
73SP	13	14	30	24	9	11	13	14, 14	14	10	10	11	13	14	22	28
74SP	14	13	29	24	11	13	13	12, 12	15	12	10	10	12	15	23	28
75SP	14	13	29	23	11	13	13	11, 11	15	11	12	10	12	14	23	25
77SP	14	13	29	24	10	13	13	11, 15	15	12	12	11	12	15	23	29
78SP	15	12	28	22	9	11	13	16, 16	16	10	12	10	13	14	20	26
79SP	15	13	31	21	11	11	13	15, 17	15	11	11	10	13	14	21	28
80SP	13	12	30	25	10	11	13	18, 18	14	10	11	10	14	15	22	27
81SP	14	13	29	24	11	13	13	11, 14	15	12	12	11	12	15	23	28
82SP	14	14	30	24	11	13	14	11, 14	15	12	12	11	12	15	23	29
83SP	16	13	29	24	12	13	13	11, 14	15	12	12	10	12	15	24	27
84SP	15	13	30	21	10	11	13	17, 17	14	12	12	10	13	15	21	27
85SP	14	13	29	24	11	13	13	11, 14	15	13	12	11	12	15	23	28
86SP	14	13	29	24	11	13	13	11, 16	15	13	13	10	12	16	23	28
87SP	15	13	31	24	10	11	13	18, 19	14	10	12	11	12	14	20	28
88SP	14	13	31	21	11	11	13	16, 17	14	11	11	10	13	14	21	28
89SP	14	14	30	24	11	13	13	11, 14	14	12	12	10	13	15	24	27
90SP	14	15	31	24	11	14	13	11, 15	15	12	12	11	12	16	23	28
92SP	13	14	30	24	9	11	13	13, 14	14	10	10	10	14	14	22	28
93SP	13	13	31	24	10	11	13	17, 17	14	10	12	10	13	14	21	27
94SP	14	14	31	23	11	13	14	11, 15	15	12	12	11	12	14	23	28
95SP	15	13	29	24	10	13	13	10, 14	15	12	12	11	12	15	24	28
96SP	14	13	29	24	11	13	12	11, 14	15	12	11	11	13	15	23	28
97SP	14	12	28	25	10	11	13	14, 20	14	11	11	10	14	15	23	27
98SP	14	13	29	24	11	14	13	11, 14	15	12	12	11	12	16	24	28
99SP	15	13	30	25	10	13	13	11, 13	15	12	12	11	12	15	23	28
100SP	13	14	30	24	9	11	13	13, 14	14	10	10	11	14	14	21	28
101SP	13	14	31	23	9	11	14	12, 17	14	10	12	10	12	14	21	28
102SP	13	13	29	24	9	12	13	13, 14	14	10	10	11	12	14	22	27
103SP	13	12	28	22	10	15	11	12, 16	15	10	12	9	13	14	21	27
104SP	13	14	30	24	9	11	13	14, 14	14	10	10	11	13	14	21	28
105SP	14	13	29	24	11	14	12	11, 14	15	12	13	12	11	15	23	28
106SP	14	14	30	24	11	13	13	11, 15	14	12	12	11	12	15	24	28
107SP	15	13	31	21	10	11	13	15, 15	14	11	12	10	13	14	22	28
108SP	14	12	28	22	10	11	13	13, 15	16	10	12	10	12	16	22	27
109SP	14	14	30	25	10	14	13	11, 14	15	12	12	10	12	14	23	29
110SP	14	13	30	24	11	13	12	13, 14	14	12	13	11	12	15	23	28
111SP	14	13	29	24	11	13	14	11, 14	16	12	14	11	12	15	23	28
112SP	15	13	31	24	11	13	13	11, 13	14	12	13	11	12	14	23	29
113SP	14	13	29	23	10	13	13	11, 14	15	12	11	11	12	15	24	28

Table 1 (continued)

Id	DYS 19	DYS 389I	DYS 389II	DYS 390	DYS 391	DYS 392	DYS 393	DYS 385	DYS 437	DYS 438	DYS 439	DYS 460	DYS 461	GATA A10	GATA C4	GATA H4
114SP	14	13	29	23	10	13	13	11, 15	15	12	12	11	12	14	24	28
115SP	14	14	30	24	11	13	13	12, 14	15	12	12	10	12	14	23	29
116SP	14	14	30	24	11	13	13	11, 14	15	12	12	11	12	15	24	28
117SP	16	13	29	21	10	11	15	18, 20	14	11	12	10	13	13	21	27
118SP	15	14	32	24	10	11	13	11, 11	14	10	12	11	12	14	17	29
119SP	14	13	29	24	11	13	13	11, 14	15	12	12	11	12	15	24	28
120SP	14	13	30	24	10	10	13	18, 18	14	10	12	10	12	15	19	29
121SP	13	13	29	24	9	11	13	13, 14	14	10	10	11	14	14	21	28
122SP	14	13	29	24	11	13	14	11, 15	15	12	13	11	12	15	23	27
123SP	15	14	30	23	11	13	13	13, 14	15	12	11	12	12	15	24	28
124SP	14	13	29	25	10	13	13	11, 15	15	12	11	10	12	16	23	27
125SP	13	13	30	23	11	11	13	15, 18	14	10	12	9	12	16	21	28
126SP	15	13	30	21	10	11	13	15, 18	14	11	12	10	13	14	22	29
127SP	16	13	30	25	11	11	13	11, 14	14	11	10	11	11	16	23	28
128SP	14	14	29	23	10	13	13	14, 17	14	9	11	10	11	15	21	26
130SP	16	13	31	25	10	12	14	12, 15	15	10	12	10	11	15	21	27
131SP	14	13	30	23	11	13	13	11, 14	15	12	12	11	13	15	23	28
132SP	15	13	30	24	10	12	15	14, 15	15	10	11	11	11	14	20	27
133SP	13	12	29	24	10	15	12	14, 14	14	11	14	12	12	15	23	28
134SP	16	13	30	21	11	11	15	17, 19	14	11	11	11	13	13	21	27

to Carvalho-Silva et al. (2001), in the present-day population white Brazilians and the Portuguese populations share the same male gene pool since no significant differences were found for haplogroups defined by 12 Y-SNPs. For this reason, we compared the haplotype distribution generated for these six STRs in Rio de Janeiro and Portugese samples (Gusmão et al. 2002b). The pairwise Φ_{st}value between Portugal and Rio de Janeiro data previously published (Da Costa et al. 2002) was 0.0876, much higher than the one found between Portugal and data from the present work (0.01194). Therefore, although some sampling problems may also exist, the most likely explanation for the significant difference between these two samples from Rio de Janeiro is a systematic error in at least one marker in the data of Da Costa et al. (2002). Indeed, in that work, allele 12 was reported to be the most frequent at DYS392 locus (34%), an extremely high frequency never found in other samples from Brazil or in other populations sharing historic affinities with Brazilians, like Portuguese, sub-Saharan Africans or Amerindians (Gusmão et al. 2002b; Alves et al. 2003; Bortolini et al. 2003).

Population comparison

The present haplotype data were compared with the others published for northern Portugal (Beleza et al. 2003) and Spain (Martín et al. 2004), the only Caucasian data available for the same set of 17 Y-STR loci. AMOVA results revealed that most of the molecular variation was due to variation within populations (99.61%) rather than among them. Pairwise analysis did not reveal significant differences between Rio de Janeiro and Spain (Φ_{st}=0.00416) but a marginally significant *P*-value was found in the comparison with the northern Portuguese population (*P*=0.04505±0.0203, Φ_{st}=0.00864).

Haplotype data for the 8 STRs not included in the "minimal haplotype" (DYS437, DYS438, DYS439, DYS460, DYS461, GATA A10, GATA H4 and GATA C4) were compared with data reported by Gusmão et al. (2003) in 13 different population samples. Pairwise analysis showed no significant differences (*P*>0.05) in the comparison of Rio de Janeiro with Iberian samples from central Portugal (Φ_{st}=0.00784), south Portugal (Φ_{st}=0.00342), Spain general population (Φ_{st}=−0.00167), Spain Cantabria (Φ_{st}=0.00096) and Spain Galicia (Φ_{st}=0.00253) as well as with other Caucasian samples from South America, namely Costa Rica (Φ_{st}=0.00162), Argentina Buenos Aires (Φ_{st}=0.00332) and Brazil São Paulo (Φ_{st}=−0.00818). Although marginally significant (*P*=0.02703), the genetic distance between Rio de Janeiro and north Portugal is small, being less than 1% (Φ_{st}=0.00912). This sample did not show statistically significant Φ_{st} values in the pairwise comparison with a Lara Amerindian sample (Φ_{st}=−0.00388) although, as expected highly significant values were found in the comparison with two non-Caucasian samples, a Chinese sample from Macao (Φ_{st}=0.18740) and an African sample from Mozambique (Φ_{st}=0.21373). The genetic distance between Rio de Janeiro and an African sample from Costa Rica was not statistically significant (Φ_{st}=0.01166, *P*=0.14414) and this can be explained by both haplotype sharing between Costa Rica Africans and Iberian popula-

Table 2 Observed mutations in 119 father/son pairs for Y-chromosome STR markers

	Locus*allele	Repetitive sequence structure	Father HP*	Fathers age**	Paternity Index
Father	DYS19*14	$(TAGA)_3TAGG\ (TAGA)_{11}$	2SP	36	40,373
Son	DYS19*15	$(TAGA)_3TAGG\ (TAGA)_{12}$			
Father	DYS390*24	$(TCTG)_8(TCTA)_{11}TCTG(TCTA)_4$	122SP	26	19,095
Son	DYS390*25	$(TCTG)_8(TCTA)_{12}TCTG(TCTA)_4$			
Father	DYS391*10	$(TCTA)_{10}$	32SP	29	46,857
Son	DYS391*11	$(TCTA)_{11}$			
Father	DYS392*11	$(TAT)_{11}$	73SP	49	11,325
Son	DYS392*10	$(TAT)_{10}$			
Father	DYS438*10	$(TTTTC)_{10}$	132SP	22	12,471
Son	DYS438*6	$(TTTTC)_6$			
Father	DYS439*13	$(GATA)_{13}$	11SP	33	19,016
Son	DYS439*14	$(GATA)_{14}$			
Father	GATA H4*27	$(AGAT)_4CTAT(AGAT)_2(AGGT)_3(AGAT)_{10}...\ (ATAG)_4(ATAC)_1(ATAG)_2$	6SP	26	32,236
Son	GATA H4*28	$(AGAT)_4CTAT(AGAT)_2(AGGT)_3(AGAT)_{11}...\ (ATAG)_4(ATAC)_1(ATAG)_2$			
Father	GATA A10*14	$(TCCA)_2(TATC)_{12}$	109SP	48	14,217
Son	GATA A10*15	$(TCCA)_2(TATC)_{13}$			

*Father haplotypes are cited according to Table 1.
**Fathers age at time of birth (years).

tions (Gusmão et al. 2003) and also by the presence of some sub-Saharan haplotypes detected in our sample.

In overall, the present population comparison results suggest a higher contribution of the European gene pool to the present day population of Rio de Janeiro, in accordance with the results obtained by Carvalho-Silva et al. (2001) for Y-chromosome binary markers and the ones obtained by Callegari-Jacques et al. (2003) for autosomal STR loci.

Table 3 Distribution of fathers' age in 5-year intervals

Age group	Number of fathers
15–19	7
20–24	25
25–29	42
30–34	40
35–39	1
40–44	1
45–49	1
Mean age	27.3

Father/son segregation analysis

The same set of 17 Y-STR loci was also typed in 119 father/son pairs and among 2,023 allele transfers, 8 mutations were observed, 1 each for DYS390, DYS391, DYS392, DYS438, DYS439, DYS19, GATA H4 and GATA A10 markers (Table 2). All the mutations were confirmed by sequence analysis and found to have occurred inside the repetitive sequence structure (Table 2).

The mean age at the birth of the child was higher in fathers who were involved in the mutation events (33.6 years; see Table 2) when compared with the mean age for the 119 fathers included in our sample (Table 3).

Except in one case (DYS438), all mutations were single step. For DYS438 a 4-step mutation was found,which has never been reported before, where allele 10 has mutated to 6 repeats. We did not find any mutations occurring in more than one locus for the same father/son pair.

The overall mutation rate across the 17 loci was 0.003955±0.001396 per locus/meiosis. This value, although high, is inside the 95% CI of other mutation rate estimates published for Y-STRs (Kayser et al. 2000; Dupuy et al. 2004; Kurihara et al. 2004). Therefore, for these loci no differences in mutation rates are expected in the context of Rio de Janeiro haplotype background and previous estimates can be applied in kinship analysis.

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