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ORIGINAL ARTICLE

Mitochondrial DNA diversity in a population from Santa Catarina (Brazil): predominance of the European input

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Abstract The state of Santa Catarina (Brazil) is known to have represented a cultural crossroads in South America due to several historic migrations mainly from Europe and Africa. We set out to scrutinize whether the genetic imprint of these migrations could be traced through analysis of the matrilineal gene pool of the Catarinenses. The entire control region of the mitochondrial DNA was studied in 80 healthy and maternally unrelated individuals. The analysis of haplogroup distribution revealed that this population is extremely heterogeneous, showing the coexistence of matrilineal lineages with three different phylogeographic origins. European lineages are the most frequent due mainly to the impact of relatively recent migratory waves from Europe. In spite of this, Native American lineages and African lineages incorporated with the slave trade are also present in noticeable proportions. The strikingly high variability generated by intense gene flow is mirrored in a high sequence diversity (0.9930) and power of discrimination (0.9806). Thus, analysis of the entire mitochondrial DNA control region emerges as a valuable tool for forensic genetic purposes in this highly admixed population, an attribute common to several present-day Latin American populations.

Keywords Mitochondrial DNA · Control region · Haplogroup · Hypervariable segment · Diversity parameters

Introduction

The diversity of the human mitochondrial genome is the result of the gradual accumulation of successive mutations in matrilineal lineages during and after the spread of anatomically modern humans out of Africa. Over time, such a process of molecular divergence of the mitochondrial DNA (mtDNA) has given rise to monophyletic units known as haplogroups [1], which tend to be restricted to specific population groups and/or geographical areas [2]. However, there are regions that have been colonized over the course of history by diverse human groups with different geographical origins, which are thereby characterized by different female genetic lineages. Certain regions of Brazil provide a case in point: They contain some of the most heterogeneous populations in the world as a result of ethnic contributions over the past five centuries from European colonists, African slaves, and Native Americans [3–5].

Santa Catarina, a state in southern Brazil, is a fine example. Since the discovery of America, this area has received continual, large-scale waves of migration, mainly from Europe. The earliest colonists were Portuguese, many

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of them originating from the Azores. From the nineteenth century onwards, migrants also began to stream into the area from many other places, notably Germany and Italy [6, 7]. Moreover, between the mid-sixteenth century and 1855, around four million African slaves were shipped to Brazil [4, 6]. Obviously, such a demographic history should be reflected in substantial mitochondrial DNA diversity in the target population.

The property of the matrilineal lineages of being geographically specific is very attractive from the evolutionary and forensic viewpoints, bearing in mind that this is an advantageous attribute in reconstructing the human demographic history and in determining the ancestry of individuals from genetically admixed human groups. On the other hand, the findings of a sizeable number of recent mtDNA studies in worldwide populations are indicative that the analysis of the entire mtDNA control region can yield higher values of the power of discrimination [8–14], which confers additional interest to mitochondrial genome from a forensic perspective. The present study is intended to characterize the diversity of the matrilineal lineages of the current population of Santa Catarina (Brazil) by analyzing the entire mitochondrial DNA control region. With this analysis, we seek to contribute new mtDNA haplotype data, taking into account that the improvement of databases constitutes a major goal for consolidating the use of mtDNA for forensic purposes. We further analyzed the haplogroup distribution in the population of Santa Catarina to corroborate, from the perspective of female genetic lineages, the ancestry composition of this highly mixed Brazilian population.

Materials and methods

Genomic DNA was extracted with Chelex-100 resin from blood samples collected in 80 healthy, unrelated individuals residing in the state of Santa Catarina, Brazil (Electronic supplementary material (ESM) Fig. S1).

The entire mitochondrial DNA control region was PCR-amplified using the primer sets L15988 and H616 as described in a previous study [15]. PCR reactions were carried out in a BioRad iCycler (Bio-Rad Laboratories Inc., Hercules, CA, USA), and the amplified products were cleaned by vacuum ultrafiltration using MultiScreen® PCRμ96 plates (Millipore, Billerica, MA, USA). PCR products were sequenced using the BigDye® Terminator Cycle Sequencing Kit v3.1 (Applied Biosystems, Foster City, CA) on an ABI3130 Genetic Analyzer (Applied Biosystems). Sequencing was performed using primers L15988 and L12, and samples with poor resolution downstream of C-stretches were resequenced using primer H285 and/or H616 [15].

The mitochondrial DNA control region was edited from np 16024 to np 576 (1,122 bp). Hypervariable segments HVS-I, HVS-II, and HVS-III were also analyzed separately for some calculations. In this way, the HVS-I region was edited from np 16024 to 16365 (342 bp), HVS-II from np 73 to 340 (268 bp), and HVS-III from np 438 to 574 (137 bp). The sequences obtained were aligned and compared with the revised Cambridge Reference Sequence (rCRS) [16] using the Clustal X v2.0 package [17]. Arlequin v3.11 software [18] was used to calculate diversity parameters such as nucleotide diversity, sequence diversity, and mean number of pairwise differences. Likewise, parameters of interest from the forensic genetics viewpoint were also computed, namely, random match probability and power of discrimination. Because length variations of the mtDNA sequences are misleading for interpretation purposes [19], C-stretch variation and heteroplasmy of HVS-I and HVS-II were excluded from statistical analyses.

Mitochondrial haplotypes found in the population examined were assigned to haplogroups, taking into account the polymorphic positions of the control region, according to the "Phylotree.org–Global Human mtDNA phylogenetic tree" (available at http://www.phylotree.org/) [20]. The same web site, which includes information of recent contributions to the mitochondrial DNA phylogeny [21–23], was consulted to refine the classification of some haplogroups, notably those belonging to lineages A2, A2a, B2, B4b, C1a, C1b and D1. Finally, we compared the haplogroup frequencies of European-specific clades via an exact chi-square test by the Monte Carlo method using previously published data from Portugal [24], Germany [25], and Italy [26].

Results and discussion

Polymorphism of the entire mtDNA control region

The haplotypes obtained for our sample set from Santa Catarina are given in ESM Table S1. For the 80 individuals analyzed, 64 different haplotypes were identified when the entire mtDNA control region (including mtDNA fragments between the hypervariable regions) was considered. Of them, 53 haplotypes (66.3% over total sample) were unique and the most frequent was shared by four individuals (5.0%). Polymorphism of the hypervariable segments HVS-I, HVS-II, and HVS-III was analyzed both separately and jointly (see ESM Table S2), finding that the nucleotide positions with the highest frequency of substitutions were 16223 (45.3%) in HVS-I, 73 (46.8%) and 152 (43.5%) in HVS-II, and 16519 (41.5%) in the region between HVS-I and HVS-II.

Within-population diversity parameters

Holland and Parsons [27] pointed out that within-population diversity parameters can significantly contribute to forensic databases as they provide valuable information on the power of discrimination of mtDNA for use in forensic casework. Diversity parameters for HVS-I, HVS-II, HVS-III, and for the entire mtDNA control region in Santa Catarina population are presented in Table 1. Both the nucleotide diversity (π_n) and the mean number of pairwise differences (π) were higher in HVS-I region, perhaps reflecting the conspicuous difference in the number of polymorphic positions: 69 (HVS-I) vs. 41 (HVS-II) and 26 (HVS-III). As expected, sequence diversity (H) was slightly higher in HVS-I (0.9684±0.0124) than in HVS-II (0.9484±0.0136) as a result of both a greater number of different haplotypes (51 in HVS-I vs. 37 in HVS-II) and a higher proportion of single haplotypes over total sequences included in the sample (46.3% vs. 27.5%). The same arguments could account for the lower values of random match probability in HVS-I, as well as its slightly higher PD value. Conversely, HVS-III showed the lowest values of sequence diversity (H=0.7725±0.0474) and power of discrimination and the highest value for random match probability.

The combined analysis of the three hypervariable segments logically provided an intermediate nucleotide diversity (π_n) value, whereas the sequence diversity (H), the mean number of pairwise differences (π), and the power of discrimination (PD) increased substantially, reaching a PD value of 0.9806 for the whole control region. Consequently, the random match probability value obtained for the entire control region was relatively low.

The high sequence diversity estimated for the whole control region (H = 0.9930±0.0035) implies that in this population, mtDNA analyses could be very informative in terms of forensic genetic identification due to an increased probability of differentiating between two given maternal lineages. This was corroborated by the relatively high value estimated for the power of discrimination (0.9806). Obviously, the heterogeneous geographic origin of the immigrants who arrived to Santa Catarina would be the causative factor of the high sequence diversity observed, as can be inferred from the comparison of this parameter between the population examined (sequence diversity for HVS-I+HVS-II, 0.9892+0.0042) and several Central and South American groups characterized by a great predominance of Native American mtDNA lineages: Guahibo (0.947), Cayapa (0.860), Mapuche (0.955), and Yanomama (0.974) from South America and Huetar (0.901) and Ngöbe (0.897) from Central America [28]. The sequence diversity estimate considering the entire control region (H = 0.9930±0.0035) was also higher in Santa Catarina than those of other Latin American populations featuring a high admixture level of the maternal lineages, such as Argentina (North Argentina, 0.906; Central Argentina, 0.937; South Argentina, 0.878) [9] and the city of Caracas, Venezuela (0.9863) [29].

mtDNA haplogroup composition in Santa Catarina

Mitochondrial DNA haplogroups identified in the population sample from Santa Catarina and the corresponding frequencies are listed in Table 2. The most common clade within this population was haplogroup H (27.5%), followed by haplogroups B, C, and T (8.8% each).

The haplogroups identified in the sample examined clearly showed the coexistence of matrilineal lineages with three different phylogeographic origins (ESM Fig. S2): haplogroups N, I, R0a (also known as preHV), H, HV, HV0 (also known as preV), J, T, U, and K are typically Eurasian [30, 31]; haplogroups A, B, C, and D are characteristic of Native American populations [1]; and the African haplogroup L [32].

As Santa Catarina received major migratory flows from Europe, particularly from Germany, Italy, and Portugal [6, 7], such European input is expected to be perceptible in the mtDNA gene pool of this Brazilian population. The analysis of the mtDNA haplogroup distribution strongly supports this notion: In all, mtDNA lineages of European ancestry accounted for 63.8% of total haplogroup diversity in Santa Catarina (Table 2). Accordingly, haplogroup H was the most representative female genetic lineage in Santa Catarina collection, as occurs in most European or European-descendant populations [31, 33, 34]. Haplogroups K and T, which are also typically European [31, 35], were observed at notable frequencies. Other European lineages observed at a frequency of 5% or below, such as haplogroups I, U, R0a, HV0, N, and J, also contributed to the predominance of European-specific haplogroups in the population examined. The 63.8% of mtDNA lineages of European ancestry found in our study is notably higher than the overall frequency reported by Alves-Silva et al. [3] for the Brazilian population (39.0%) and, at the same time, is very similar to the frequency estimated in the same study for the southern part of the country (66.0%).

Although most of the mtDNA haplogroups identified in Santa Catarina were of European origin, frequencies of these specific lineages differed significantly (p<0.05) from those of the European populations that have historically contributed to the gene pool of the region (Italy, Germany, and Portugal; see Table 2), as indicated by the results of an exact chi-square test by the Monte Carlo method: Italy (χ^2=30.7, df=13, p=0.0102), Germany (χ^2=30.3, df=13, p=0.0032), and Portugal (χ^2=56.4, df=12, p<0.0001). As discussed above, the high degree of genetic admixture of Santa Catarina, mirrored in the coexistence of female genetic lineages with

Table 1 Diversity measures estimated from the analysis of the entire mtDNA D-loop region in a population sample from Santa Catarina (Brazil)

Parameters	HVS-I	HVS-II	HVS-I+II	HVS-III	HVS-I+II +III	16024-576
Sequence diversity (*H*)	0.9684± 0.0124	0.9484 ± 0.0136	0.9892 ± 0.0042	0.7725 ± 0.0474	0.9921 ± 0.0035	0.9930 ± 0.0035
Mean number of pairwise differences (π)	6.8462 ± 3.2567	4.3943 ± 2.1926	11.2405 ± 5.1559	2.3687 ± 1.3041	13.6092 ± 6.1782	15.003481 ± 6.7796
Nucleotide diversity (π_n)	0.0200 ± 0.0106	0.0163 ± 0.0090	0.0184 ± 0.0094	0.0159 ± 0.0097	0.0179 ± 0.0090	0.0132 ± 0.0066
Random match probability	0.04374	0.06343	0.02312	0.23718	0.02031	0.01937
Power of discrimination (PD)	0.95626	0.93656	0.97687	0.76282	0.97969	0.98063

different geographic origins underlies the differences in European-specific haplogroup distribution.

The four Native American haplogroups (A, B, C, and D) appeared well represented in Santa Catarina with a total frequency of 21.3% (Table 2), practically identical to that estimated by Alves-Silva et al. [3] for Southern Brazil (22.0%). These authors found that the frequency of the four Pan-American haplogroups throughout Brazil (33.0%) was slightly higher than the frequency estimated specifically for south Brazil. This finding supports the notion of the southern region of Brazil being a gravitational pole for migration flows from Europe [4]. The entrance of new haplogroups caused by the intense gene flow associated with immigration processes could have led to the dispersal

Table 2 Mitochondrial DNA haplogroup composition (as percentage) in Santa Catarina population sample and other European collections used for comparison: Portugal [24], Germany [25], and Italy [26]

Haplogroup	Santa Catarina (*n*=80)	Portugal (*n*=241)	Germany (*n*=177)	Italy (*n*=395)
L0	2.50	0	0	0
L1	3.75	0.83	0	0.25
L2	7.50	2.49	0	0
L3	1.25	3.73	0	0.25
A	2.50	0	0	0
B	8.75	0	0	0
C	8.75	0	0	0
D	1.25	0	0	0.76
PreHV/R0a	3.75	0	0	0.76
HV	0	0	0.56	4.81
H	27.50	40.66	37.29	38.99
PreV/HV0	3.75	0	3.95	1.52
V	0	7.47	0	2.03
U	5.00	17.43	16.95	11.65
K	6.25	5.39	7.34	10.13
T	8.75	10.79	12.99	13.42
J	1.25	6.64	10.17	7.85
I	5.00	0.83	1.13	1.52
R	0	0	1.13	0
N	2.50	0	0.56	1.52
M	0	0.41	0	0.25
W	0	1.24	3.95	1.01
X	0	2.07	1.13	2.78
Y	0	0	0.56	0
Others	0	0	2.26	0.51

of the Native American lineages out of the study area and, accordingly, to a notable reduction of their frequencies in the Santa Catarina population.

The African haplogroups L0, L1, L2, and L3 were also relatively common in Santa Catarina (15.0%). African haplogroups arrived in the New World with the slave trade from the sixteenth century onwards [36, 37]. The frequency of African haplogroups in Brazilians from Santa Catarina was lower than in the Brazilian population as a whole (28.0%), but similar to that estimated for the whole southern part of the country (12.0%) [3].

In a few cases, it was difficult to unambiguously assign a haplotype to one of the major haplogroups even by analyzing the entire control region. This is particularly important in the case of the Pan-American haplogroup B2, which is not completely classifiable via analysis of control region motifs [22, 23], and in some widespread European-specific haplogroups (e.g., H) that are subdivided into many sublineages [34, 38]. In such cases, analysis of SNPs of the mtDNA coding region has proved to be a valuable tool for refining haplogroup classification, as demonstrated for haplogroup H in several studies [39, 40] and more recently for Native American lineages [9]. For that reason, it would be advisable to develop a SNaPShot approach for the precise classification of some Native American clades such as B2. Yet, analysis of the hypervariable segments HVS-I, HVS-II, and HVS-III provides an accurate classification of some mtDNA sequences into specific subhaplogroups (e.g., L1c1a1b or K1b1a1).

Conclusions

The analysis of the entire mtDNA control region allows high within-population variability to be detected, reflected in high sequence diversity (0.9930), a high power of discrimination (0.9806) and, accordingly, a low random match probability value (0.0194). Likewise, phylogeographical findings based on haplogroup composition confirm the high genetic heterogeneity of the population of Santa Catarina as a logical consequence of the impact of intense gene flow associated with relatively recent migratory waves from Europe. Despite the predominance of the European input, Native American lineages and African lineages which settled much earlier are also present in noticeable proportions. The results above validate the usefulness of the analysis of the mitochondrial DNA control region for forensic genetic purposes in highly admixed human groups, such as some present-day Latin American populations.

The haplotypes reported in the present study will be made available from the EMPOP database (www.empop.org) upon publication. Sequence data are also available under GenBank accession numbers GQ449292–GQ449371.

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Conflict of interest None.

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