Int J Legal Med (2012) 126:299–302
DOI 10.1007/s00414-011-0656-4

ORIGINAL ARTICLE

Mitochondrial diversity in Amerindian Kichwa and Mestizo populations from Ecuador

Miriam Baeta · Carolina Núñez · Cecilia Sosa · Miguel Bolea · Yolanda Casalod · Fabricio González-Andrade · Lutz Roewer · Begoña Martínez-Jarreta

Received: 28 November 2011 / Accepted: 9 December 2011 / Published online: 22 December 2011

Abstract This study presents mitochondrial DNA (mtDNA) data from 107 unrelated individuals from two of the major ethnic groups in Ecuador: Amerindian Kichwas (n=65) and Mestizos (n=42). We characterized the diversity of the matrilineal lineages of these Ecuadorian groups by analyzing the entire mtDNA control region. Different patterns of diversity were observed in the two groups as result of the unique historical and demographic events which have occurred in each population. Higher genetic diversity values were obtained for the Mestizo group than for the Amerindian group. Interestingly, only Native American lineages were detected in the two population samples, but with differences in the haplogroup distribution: Kichwa (A, 49%; B, 3%; C, 8%; and D, 40%) and Mestizo (A, 33%; B, 33%; C, 10%; and D, 24%). Analysis of the complete mtDNA control region proved to be useful to increase the discrimination power between individuals who showed common haplotypes in HVSI and HVSII segments; and added valuable information to the phylogenetic interpretation of mtDNA haplotypes.

Keywords mtDNA population data · Ecuador · Native American · Control region

Electronic supplementary material The online version of this article (doi:10.1007/s00414-011-0656-4) contains supplementary material, which is available to authorized users.

M. Baeta (✉) · C. Núñez · C. Sosa · M. Bolea · Y. Casalod · B. Martínez-Jarreta
Laboratory of Forensic Genetics, Faculty of Medicine, University of Zaragoza, Zaragoza, Spain
e-mail: mbaeta@hotmail.com

F. González-Andrade
Science and Technology Department, Ministry of Public Health, Quito, Ecuador

L. Roewer
Department of Forensic Genetics, Institute of Legal Medicine and Forensic Sciences, Charité-Universitätsmedizin Berlin, Berlin, Germany

Introduction

Mitochondrial DNA (mtDNA) analysis is a very useful tool for population studies and forensic applications because of its maternal inheritance, rapid rate of evolution, absence of recombination, and high copy number per cell. Most studies have focused on the noncoding region of mtDNA, known as the control region or D-Loop. This region is highly polymorphic and can be used to study short-term evolutionary events and for human forensic identification especially in cases where DNA is degraded or in low quantities [1, 2]. However, the standard typing of the control region, based on the hypervariable segments HVSI and HVSII, can result in a rather limited power of discrimination. In order to overcome this drawback, the analysis of the entire control region and/or the typing of single nucleotide polymorphisms in the coding region seems to be effective approaches [3, 4].

MtDNA studies have been used to reconstruct the human demographic history as well as to infer the ancestry of individuals [5]. In this sense, the study of American human groups has awakened a growing interest because of their complexity and diversity [6]. This is the case of the Ecuadorian population, a multicultural and pluri-ethnic population with three main groups: (a) Mestizos, an admixed population of Spanish and Amerindian descendants (72%); (b) Amerindian natives with more than 13 major groups

(7%), being the Kichwa (Quechua speaking) the most numerous group; and (c) African-derived populations (7%) [7].

Previous studies, based on autosomal and Y chromosome STRs [8, 9], have evidenced variable genetic contribution of European, Amerindian, and African ancestry in the Ecuadorian groups, as a result of the European colonization and the arrival of African slaves during colonial times. Moreover, a certain level of sex-biased genetic admixture has been detected in the Ecuadorian Mestizo population, which has been suggested to be the reflection of the preferential mating that occurred between European male settlers and Native American females. In order to provide the female side of the history, some mtDNA population studies have been carried out, but they have focused on small groups as Waorani [10, 11] and Cayapa [12].

In this work, the study of the mtDNA control region from the two main Ecuadorian ethnic groups has been carried out with the following aims: (a) to study the genetic structure of these groups through mtDNA; (b) to infer the demographic and historical processes that may have influenced the genetic diversity of these groups; and (c) to contribute to mtDNA databases of Ecuadorian populations for forensic purposes.

Materials and methods

The population sample included 107 maternally unrelated individuals born and living in Ecuador: 65 self-identified Amerindian Kichwas from the Amazonian provinces of Pastaza, Orellana, and Napo (Kichwa del Oriente) and 42 Mestizos from different regions of the country. All donors gave their informed consent prior to inclusion in the study.

DNA was extracted from blood stains on FTA cards (Whatman Inc., Clifton, NJ) using the Chelex extraction procedure [13]. The entire control region was amplified from positions 16024 to 576 using the primer sets L15988 and H616 as described in a previous study [14]. DNA products were then purified with ExoSAP (USB) and sequenced using the same set of primers L15988/H616 as well as L16363 [15], L29 [16], and H7 [17]. Sequencing extension reactions were performed using BigDye Termination v3.1 Cycle Sequencing Kit (Applied Biosystems, Foster City, CA) following the manufacturer's conditions. Purification of products was completed with IBIAN ® Dye Clean-up (IBIAN Technologies, Spain). Automated DNA sequencing was carried out on the ABI Prism™ 310 Genetic Analyzer (Applied Biosystems).

The sequences were aligned and compared to the revised Cambridge Reference Sequence [18] using the SeqScape software (Applied Biosystems). Statistical analyses were performed using Arlequin v3.11 software [19], ignoring C-stretch variation. Diversity estimations were calculated for the entire mtDNA control region (16024 to 576), although some calculations were carried out for HVSI (16024-16365), HVSII (73-340), and HVSIII (438-574). Exact tests of population differentiation and pairwise *F*st were also performed. Haplogroups were defined on the basis of entire control region sequences, according to the phylogenetic tree of global human mtDNA v12 (available at http://www.phylotree.org/).

Sequences will be searchable via the EMPOP database under accession numbers EMP00421 for the Kichwa and EMP00422 for the Mestizo individuals.

Results and discussion

Diversity in the two Ecuadorian populations

The haplotypes obtained for Kichwa and Mestizo individuals are provided in Table S1. The analysis of the complete mitochondrial control region resulted in 39 distinct haplotypes in the entire Ecuadorian sample, with four sequences common to both ethnic groups. Within the Kichwa subset, 13 different haplotypes were described, with five of them being unique to single individuals. The most frequent sequence was shared by 40% of the Kichwa individuals. In the Mestizo subset, 30 different haplotypes were observed, and 23 of these sequences were present in just one individual. The most frequent sequence in the Mestizo sample set was observed in 14.29% of the cases.

It is noteworthy that when the analysis was restricted to the HVSI and HVSII regions, a lower number of haplotypes could be differentiated when compared to the entire control region analysis, in Kichwa (11 vs 13) and Mestizo (29 vs 30). The analysis of the HVSIII did not allow further differentiation among individuals who shared identical HVSI–HVSII haplotypes. Thus, it was the analysis of non-HVS regions that increased the total number of haplotypes detected.

Other parameters characterizing within-population diversity based on the complete mtDNA control region sequences are listed in Table 1.

Haplotype diversities obtained for the entire control region were 0.8029±0.0373 and 0.9733±0.0144 for the Kichwa and Mestizo groups, respectively. These differences are explained by the higher number of different sequences

Table 1 Diversity measures for control region data from Kichwa and Mestizo populations

	Kichwa	Mestizo
Number of individuals	65	42
Different haplotypes	13	30
Number of polymorphic sites	47	83
Haplotype diversity	0.8029±0.0373	0.9733±0.0144
Random match probability (%)	0.2095	0.0499
Power of discrimination	0.7905	0.9501

and proportion of single haplotypes over total sequences found in Mestizos.

These results are consistent with general patterns described in other Mestizo and Amerindian populations [3, 12, 20, 21]. Mestizo groups usually exhibit high diversities because they are composed of lineages from different geographical and ethnic origins, which results in a high heterogeneity in their genetic pool. On the other hand, Amerindian populations normally present a lower diversity range, with values that are especially lower in groups that have suffered restricted gene flow and high inbreeding levels over long periods [10, 22]. The intermediate diversity values described for the Kichwa group seem to indicate that this group did not experience important bottlenecks or genetic drifts and maintained its heterogeneity through gene flow from other groups.

Furthermore, to evaluate the usefulness of mtDNA analysis for forensic purposes in these populations, the probability of randomly selected sequences matching (RMP) was estimated, which is inversely proportional to the power of discrimination of a genetic system. In a logical reflection of the data on diversity parameters, the RMP value was smaller in Mestizo population (5%) than in Kichwa (20.95%). Accordingly to these results, the power of discrimination of the mtDNA in forensic identity testing would be more limited for the Amerindian population, where it would more probable to find two individuals with the same haplotype.

Comparison of the two Ecuadorian populations

The exact test of population differentiation based on haplotype frequencies showed significant differences for mtDNA sequences between the two Ecuadorian populations ($P<0.001$). The same results were obtained through Fst values (Fst=0.0667; P=0.0037±0.0007).

Mitochondrial haplogroup composition

Mitochondrial haplogroup identified in the two population and the corresponding frequencies are shown in Table 2.

Results showed that both populations were entirely composed of Native American mitochondrial lineages. The four major Native American haplogroups (A, B, C, and D) were represented in both ethnic groups, but with a different frequency distribution. In the Kichwa sample, the most frequent haplogroups were A2* and its sublineage A2p with 49.23%; followed by D1f, identified in 40% of the population. Haplogroup C1* and its sublineages C1b and C1d were found in 7.69% of the individuals, whereas B4b was restricted to 3.08% of the Kichwa population.

In Mestizos, haplogroup A2* (and A2p) and haplogroup B (including B4b and B2h) were both detected in 33.33% of the population. Lower frequencies of haplogroups D1f and C1b lineages were present, with 21.43% and 9.52%, respectively. Also the minor Native American haplogroup D4h3a was found in 2.38% of Mestizo individuals.

Table 2 Mitochondrial DNA haplogroup percentages observed in Kichwa and Mestizo populations

Haplogroup	Kichwa	Mestizo
A2[a]	21.54	26.19
A2p	27.69	7.14
B4b	3.08	30.95
B2h	–	2.38
C1[a]	3.08	–
C1b	1.54	9.52
C1d	3.08	–
D1f	40.00	21.43
D4h3a	–	2.38

[a] Further analysis of the coding region should be performed for a more accurate classification

The lack of European and African contribution in maternal lineages can be explained through the historical background of these ethnic groups. In the Amerindian Kichwa population, no significant European or African contributions were expected to be found, as this group basically comprises individuals from different Amerindian tribes, which shared the same linguistic affiliation (Quechua). On the other hand, the Mestizo population mainly originated as a result of the admixture between European men and Native American women. Therefore, while male admixture is dominated by European Y chromosomes [8], the female gene pool shows the high influence of ancestral Native American mitochondrial lineages. This pattern is the result of the strong sexual asymmetry in the origin and ongoing history of the Mestizo population in Ecuador, which has also been described for other admixed populations from America [23, 24].

Furthermore, in this study, the comparison of the two Ecuadorian populations revealed genetic heterogeneity of the mtDNA gene pool, as demonstrated by the differences in the frequency of haplogroups and the presence of particular haplotypes. From the forensic perspective, the determination of population substructure in Ecuador supports the use of local population databases which reflect the genetic diversity existing among populations and/or ethnic groups of the country, whenever possible. The implementation of a single genetic database that wrongly assumes the homogeneity of the population could lead to erroneous interpretation of the weight of the evidence. The forensic field should not ignore population stratification, as assumptions about the homogeneity of pseudo-ethnic groups such as the "Hispanics" can have important repercussions on the statistical evaluation of the evidence in paternity testing and forensic casework.

Conclusions

The results of the present study corroborate the strong Amerindian influence in the Ecuadorian populations, which can be still observed in mitochondrial lineages despite the centuries of European presence and the extinction of a large number of Amerindian ethnic groups. Moreover, the results validate the usefulness of the analysis of the entire mtDNA control region in these populations, since it increases the discrimination power of mtDNA testing for forensic applications and adds valuable information to the phylogenetic interpretation of mtDNA haplotypes.

Acknowledgments We would like to thank Jorge González-Solórzano, Omar Vacas, and Sarah Zweynert for the support in the fieldwork. This research paper is part of a research initiative and network called EQGen Project, Ecuadorian Ethnogenetics.

Conflict of interest None.

References

1. Budowle B, Allard MW, Wilson MR, Chakraborty R (2003) Forensics and mitochondrial DNA: applications, debates, and foundations. Annu Rev Genomics Hum Genet 4:119–141
2. Núñez C, Sosa C, Baeta M, Geppert M, Turnbough M, Phillips N, Casalod Y, Bolea M, Roby R, Budowle B, Martinez-Jarreta B (2011) Genetic analysis of 7 medieval skeletons from the Aragonese Pyrenees. Croat Med J 52:336–343
3. Palencia L, Valverde L, Alvarez A, Caine LM, Cardoso S, Alfonso-Sanchez MA, Pinheiro MF, de Pancorbo MM (2010) Mitochondrial DNA diversity in a population from Santa Catarina (Brazil): predominance of the European input. Int J Leg Med 124:331–336
4. Alvarez-Iglesias V, Jaime JC, Carracedo A, Salas A (2007) Coding region mitochondrial DNA SNPs: targeting East Asian and Native American haplogroups. Forensic Sci Int Genet 1:44–55
5. Lee C, Mandoiu I, Nelson C (2011) Inferring ethnicity from mitochondrial DNA sequence. BMC Proc 5:S11
6. Achilli A, Perego UA, Bravi CM, Coble MD, Kong QP, Woodward SR, Salas A, Torroni A, Bandelt HJ (2008) The phylogeny of the four pan-American MtDNA haplogroups: implications for evolutionary and disease studies. PLoS One 3:e1764
7. INEC (2010) Instituto Nacional de Estadística y Censos. Ecuador. http://www.inec.gov.ec/
8. Gonzalez-Andrade F, Roewer L, Willuweit S, Sanchez D, Martinez-Jarreta B (2009) Y-STR variation among ethnic groups from Ecuador: Mestizos, Kichwas, Afro-Ecuadorians and Waoranis. Forensic Sci Int Genet 3:e83–e91
9. Gonzalez-Andrade F, Sanchez D, Gonzalez-Solorzano J, Gascon S, Martinez-Jarreta B (2007) Sex-specific genetic admixture of Mestizos, Amerindian Kichwas, and Afro-Ecuadorans from Ecuador. Hum Biol 79:51–77
10. Baeta M, Núñez C, González-Andrade F, Sosa C, Casalod Y, Bolea M, Zweynert S, Vacas Cruz OW, González-Solorzano J, Geppert M, Roewer L, Martínez-Jarreta B (2009) Mitochondrial analysis revealed high homogeneity in the Waorani population—the last nomadic group of hunter-gatherers from Ecuador. Foren Sci Int Gene Supp Ser 2:313–314
11. Cardoso S, Alfonso-Sánchez MA, González-Andrade F, Valverde L, Odriozola A, Pérez-Miranda AM, Peña JA, Martínez-Jarreta B, de Pancorbo MM (2008) Mitochondrial DNA in Huaorani (Ecuadorian amerindians): a new variant in haplogroup A2. Foren Sci Int Gene Supp Ser 1:269–270
12. Rickards O, Martinez-Labarga C, Lum JK, De Stefano GF, Cann RL (1999) mtDNA history of the Cayapa Amerinds of Ecuador: detection of additional founding lineages for the Native American populations. Am J Hum Genet 65:519–530
13. Walsh PS, Metzger DA, Higuchi R (1991) Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10:506–513
14. Cardoso S, Zarrabeitia MT, Valverde L, Odriozola A, Alfonso-Sánchez MA, De Pancorbo MM (2010) Variability of the entire mitochondrial DNA control region in a human isolate from the Pas Valley (northern Spain). J Forensic Sci 55:1196–1201
15. Handt O, Krings M, Ward RH, Pääbo S (1996) The retrieval of ancient human DNA sequences. Am J Hum Genet 59:368–376
16. Vigilant L, Pennington R, Harpending H, Kocher TD, Wilson AC (1989) Mitochondrial DNA sequences in single hairs from a southern African population. Proc Natl Acad Sci USA 86:9350–9354
17. Vanecek T, Vorel F, Sip M (2004) Mitochondrial DNA D-loop hypervariable regions: Czech population data. Int J Legal Med 118:14–18
18. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N (1999) Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet 23:147
19. Excoffier L, Laval G, Schneider S (2005) Arlequin ver. 3.0: an integrated software package for population genetics data analysis. Evol Bioinformatics Online 1:47–50
20. Bobillo MC, Zimmermann B, Sala A, Huber G, Rock A, Bandelt HJ, Corach D, Parson W (2010) Amerindian mitochondrial DNA haplogroups predominate in the population of Argentina: towards a first nationwide forensic mitochondrial DNA sequence database. Int J Legal Med 124:263–268
21. Saunier JL, Irwin JA, Just RS, O'Callaghan J, Parsons TJ (2008) Mitochondrial control region sequences from a U.S. "Hispanic" population sample. Forensic Sci Int Genet 2:e19–e23
22. Schmitt R, Bonatto SL, Freitas LB, Muschner VC, Hill K, Hurtado AM, Salzano FM (2004) Extremely limited mitochondrial DNA variability among the Ache Natives of Paraguay. Ann Hum Biol 31:87–94
23. Stefflova K, Dulik MC, Pai AA, Walker AH, Zeigler-Johnson CM, Gueye SM, Schurr TG, Rebbeck TR (2009) Evaluation of group genetic ancestry of populations from Philadelphia and Dakar in the context of sex-biased admixture in the Americas. PLoS One 4: e7842
24. Nuñez C, Baeta M, Sosa C, Casalod Y, Ge J, Budowle B, Martinez-Jarreta B (2010) Reconstructing the population history of Nicaragua by means of mtDNA, Y-chromosome STRs, and autosomal STR markers. Am J Phys Anthropol 143:591–600