International Congress Series 1288 (2006) 115–117

www.ics-elsevier.com

Mitochondrial DNA variability in populations from East Timor (Timor Leste)

L. Souto [a,b,*], A.M. Rocha [b], A. Pires [b], E. Ferreira [b], M. Kayser [c], A. Amorim [d], F Côrte-Real [e], D.N. Vieira [e]

[a] *Centro de Biologia Celular, Departamento de Biologia, Universidade de Aveiro*
[b] *Departamento de Biologia, Universidade de Aveiro, Aveiro, Portugal*
[c] *Department of Forensic Molecular Biology, Erasmus University Medical Centre Rotterdam, The Netherlands*
[d] *IPATIMUP, Instituto de Patologia e Imunologia Molecular da Universidade do Porto and Faculdade de Ciências, Universidade do Porto, Porto, Portugal*
[e] *Delegação de Coimbra, Instituto Nacional de Medicina Legal and Faculdade de Medicina da Universidade de Coimbra, Coimbra, Portugal*

Abstract. In this study we continue the genetic characterization of human populations from East Timor, as previously started for autosomal STRs and Y STRs, with a preliminary report on mitochondrial DNA (mtDNA) diversity. Individual samples ($n=133$) collected from all the districts of East Timor and representing different linguistic groups were studied for the hypervariable region 1 (HVS1) sequence and the 9-bp deletion (intergenic region COII-tRNA lys). 57 haplotypes were found (haplotype diversity=0.9727; nucleotide diversity=0.017864; mean number of pairwise differences=7.52) and the 9 bp deletion reached 16.7%. We found typical sequences of non-Austronesian (Papuan) speaking populations from New Guinea, along with a significant frequency of haplogroup B (namely B4a). In addition our overall genetic data (mtDNA, STRs) may serve as a starting-point to create a genetic database for forensic applications in East Timor. © 2006 Published by Elsevier B.V.

Keywords: MtDNA; Mitochondrial DNA; HVI; East Timor; Timor Leste; Southeast Asia

1. Introduction

Following the previous characterization of the population of the new country República Democrática de Timor Leste by autosomal [1] and Y-chromosomal STRs [2]

* Corresponding author. Departamento de Biologia, Universidade de Aveiro, Campus Universitário de Santiago, 3810-193 Aveiro, Portugal. Tel.: +351 234 370350; fax: +351 234 426408.
E-mail address: lsouto@bio.ua.pt (L. Souto).

0531-5131/ © 2006 Published by Elsevier B.V.
doi:10.1016/j.ics.2005.11.060

the present study shows the results of mitochondrial DNA. Besides its use in the forensic practice in Timor Leste, the analyse of MtDNA variants is interesting in respect of studying the human population history of East Timor as it may reveal whether the high linguistic diversity, where two major language groups, Austronesian and non-Austronesian (or Papuan) languages are found, is reflected in the East Timor's genetic diversity.

Besides HVI region sequencing, the presence of the COII/tRNA^{Lys} intergenic 9 base pair deletion is also relevant. Although not exclusive to Asian countries and Pacific islands, its frequency and association with some HVI patterns, has been extensively used as an indication of Austronesian or Papuan (non-austronesian) influence.

2. Materials and methods

133 blood samples on FTA® cards from unrelated individuals of several locations and distinct language groups in East Timor were extracted by chelex and phenol–chloroform standard procedures. The HVI control region (16050–16400) was amplified using primers L15997/H16401, following conditions detailed in Pereira et al. [3] and analysed in an ABI 310 automatic sequencer (Applera).

Sequences were hand aligned and compared with the revised Cambridge Reference Sequence CRS [4]. The software package Arlequin 2.0 [5] was used for statistical calculations, namely molecular diversity indexes and mismatch distributions.

For the 9 bp deletion analyses, we followed the PCR conditions described in Redd [6] and amplicons were separated in agarose gels.

3. Results and discussion

The 9 bp deletion was found with a frequency of 16.7% in the entire East Timor sample analysed. This value is between the values found for other Lesser Sunda islands (14.8–22.6%) and close to the frequency found in the island of Sumba (16%) which is next to Timor [7], and clearly far from the high values generally mentioned for pacific populations (from 40% to 100%) [8] (Table 1).

We found 13% of samples that could be clearly assigned to the mtDNA haplogroup B (mostly to B4a). This haplogroup is considered to be widely spread in East Asia and its presence in Southeast Asia is interpreted as Austronesian genetic influence from East Asia [9]. Two of those sequences (12%) displayed the so-called "Polynesian motif", a specific B4a haplotype associated with the Austronesian expansion into the Pacific [10].

Table 1
Descriptive statistics of Control Region (HVI) sequences in 133 individuals from East Timor

Haplotype diversity	0.9727 ± 0.0049
Mean number of pairwise differences	7.520734 ± 3.533959
Nucleotide diversity	0.017864 ± 0.009293
Observed substitutions	60
Transitions	51
Transversions	9
Insertion/deletions	0
Polymorphic sites	57
Haplotypes	57

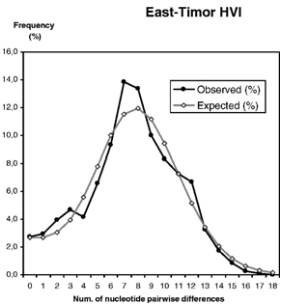

Fig. 1. Mismatch distribution in Timor Leste.

In our sample we also detected, among others (as typical F1a and M7c), 15% of sequences that can be assigned to haplogroup Q, which has been suggested to be of New Guinean origin prior to the Austronesian arrival in Melanesia [11]. However, in order to confirm the mtDNA haplogroup designation from HVS1 sequence data, additional RFLP analyses of SNPs from the mtDNA coding region will be performed.

The shape of the mismatch distribution (Fig. 1) is consistent with a relatively recent population expansion episode (less than 12 generations ago, τ Arlequin calculations, data not shown). The unimodal shape is in accordance with studies in east Indonesia [11].

Although preliminary, our genetic data corroborate the linguistic data as for the presence of both Papuan and Austronesian influences in the peopling of East Timor.

References

[1] L. Souto, et al. Population data on 15 autosomal STRs in a sample from East Timor. Forensic Science International (in press).
[2] L. Souto, et al. Y-chromosome STR haplotypes in East Timor: Forensic evaluation and population data. Forensic Science International (in press).
[3] L. Pereira, M.J. Prata, A. Amorim, Diversity of mtDNA lineages in Portugal: not a genetic edge of European variation, Ann. Hum. Genet. 64 (6) (2000) 491–506.
[4] R.M. Andrews, et al., Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA, Nat. Genet. 23 (1999) 147.
[5] S. Schneider, D. Roessli, L. Excoffier, Arlequin ver. 2000: A software for population genetics data analysis, Genetics and Biometry Laboratory, University of Geneva, Switzerland, 2000.
[6] A.J. Redd, et al., Evolutionary History of the COII/tRNA^{Lys} Intergenic 9 base Pair Deletion in Human Mitochondrial DNAs from the Pacific, Mol. Biol. Evol. 12 (4) (1995) 604–615.
[7] H.Y. Handoko, J.K. Lum, R. Gustiani, Length variations in the COII-tRNA(Lys) intergenic region of mitochondrial DNA in Indonesian populations, Hum. Biol. 73 (2) (2001) 205–223.
[8] Y. Yao, W.S. Watkins, Y.P. Zhang, Evolutionary history of the mtDNA 9-bp deletion in Chinese populations and its relevance to the peopling of east and southeast Asia, Hum.Genet. 107 (2000) 504–512.
[9] P Forster, et al., Phylogenetic star contraction applied to Asian and Papuan mtDNA evolution, Mol. Biol. Evol. 18 (10) (2001) 1864–1881.
[10] J.A. Trejaut, et al., Traces of Archaic Mitochondrial Lineages Persist in Austronesian-Speaking Formosan Populations, PLoS Biol. 3 (8) (2005) e247.
[11] A.J. Redd, M. Stoneking, Peopling of sahul: mtDNA Variation in Aboriginal Australian and Papua New Guinean Populations, Am. J. Hum. Genet. 65 (1999) 808.