Legal Medicine 17 (2015) 134–139

Contents lists available at ScienceDirect

Legal Medicine

journal homepage: www.elsevier.com/locate/legalmed

Announcement of Population Data

Genetic characterization of the Makrani people of Pakistan from mitochondrial DNA control-region data

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ARTICLE INFO

Article history:
Received 17 June 2014
Received in revised form 26 September 2014
Accepted 30 September 2014
Available online 13 October 2014

Keywords:
Mitochondrial DNA
mtDNA control region
Haplotyping
Hypervariable segment (HVS)
Makrani population
Pakistan

ABSTRACT

To estimate genetic and forensic parameters, the entire mitochondrial DNA control region of 100 unrelated Makrani individuals (males, $n = 96$; females, $n = 4$) living in Pakistan (Turbat, Panjgur, Awaran, Kharan, Nasirabad, Gwadar, Buleda, Karachi and Burewala) was sequenced. We observed a total of 70 different haplotypes of which 54 were unique and 16 were shared by more than one individual. The Makrani population showed a high genetic diversity (0.9688) and, consequently, a high power of discrimination (0.9592). Our results revealed a strongly admixed mtDNA pool composed of African haplogroups (28%), West Eurasian haplogroups (26%), South Asian haplogroups (24%), and East Asian haplogroups (2%), while the origin of the remaining individuals (20%) could not be confidently assigned. The results of this study are a valuable contribution to build a database of mtDNA variation in Pakistan.

Population: The mitochondrial DNA control-region blue print aimed to articulate the geographic foundation and immigration narration of populations [1]. Pakistan is situated in the western part of the Indian subcontinent, with Afghanistan and Iran to the west, India to the east and the Arabian Sea to the south (Fig. 1). Pakistan is hypothesized to be on the frequently voyaged route which was followed by modern humans out of Africa, and potentially is one of the first regions outside Africa where modern humans settled [2,3]. On the basis of culture and language, present-day Pakistani people are usually divided into 16 ethnic groups of miscellaneous pedigrees [4]. The Makrani people live at the Makran coast of Baluchistan [5]. Baluchistan consists of inter-mountainous area, central Makran and Makran coast. Along the coast, various Makrani Baloch are predominant [6]. The Makrani people, also known as Siddi or Sheedi, are living not only in Pakistan but also in India. They are partially descended from Bantu people from Southeast Africa who were brought to the Indian subcontinent mostly as slaves. The dissection of Indian Siddis' mtDNA revealed the presence of sublineages L2a and L0, similar to those of Bantu [7,8]. Furthermore, Y-chromosome studies revealed a high proportion of African-specific haplogroups [7,9]. Higher level of disparity was reported with respect to analogy between mtDNA and Y-chromosomal loci in Makrani population. This discrepancy was based on higher frequencies of African mtDNA haplogroups L3d, L3b, L2a and L1a. Out of these haplogroups L3d is most prevalent with an estimated frequency of 18.2% [5]. However, a study of Y-chromosome variation among Makrani people provided evidence for a dominance of Western Eurasian lineages relating them to other Pakistani populations [9]. Here we present the largest mtDNA survey so far of Pakistani Makrani by studying a relatively large sample of 100 individuals.

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[1] Part of Ph.D. thesis of first author.

http://dx.doi.org/10.1016/j.legalmed.2014.09.007
1344-6223/

Fig. 1. Map of Pakistan showing sampling areas for Makrani population.

DNA extraction, amplification and sequencing: Blood samples (3–5 ml) were collected from 100 healthy, unrelated Makrani individuals with oral and written consent of the participants according to the declarations of Helsinki. DNA was extracted from blood samples using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions. The genomic DNA was quantified using NanoDrop™ 1000 Spectrophotometer. The primers given in Table 1 were used for the amplification and sequencing of the entire mtDNA control region (http://forensic.yonsei.ac.kr/protocol/mtDNA-CR.pdf). PCR was performed in a 50 µl volume containing 1–2 ng of genomic DNA, 0.4 µM of each primer, and AmpliTaq Gold® 360 Master Mix (Applied Biosystems, Foster City, CA, USA) was used according to the manufacturer's instructions. The amplification program consisted of pre-denaturation at 95 °C for 11 min, followed by 35 cycles consisting of denaturation step at 95 °C for 30 s, annealing at 56 °C for 30 s, and extension at 72 °C for 90 s, with a final extension at 72 °C for 7 min. PCR products were purified using ExoSAP-IT® (USB, Cleveland, OH, USA).

Sequencing of the entire mtDNA control region spanning nucleotide positions 16,024–16,569 and 1–576 was done using Big Dye Terminator Cycle Sequencing v3.1 Ready Reaction Kit (Applied Biosystems) according to the manufacturer's instructions, or using a commercial sequencing facility (http://www.base-asia.com).

Data analysis: All samples were sequenced bi-directionally and evaluated twice as recommended by [10] using the sequence analysis software Geneious (Version 7.0.3, Biomatters Ltd, New Zealand) [11] by two independent researchers. MitoTool [12], mtDNA profiler [13] and HaploGrep [14], making use of PhyloTree Build 16 (http://www.phylotree.org) [1] as classification tree, were used to assess the quality of mtDNA data [12,13].

The Makrani mtDNA sequences were assigned to haplogroups according to PhyloTree [1] and published data [15–18]. The population statistical parameters like, haplotype diversity, random match probability and power of discrimination were statistically calculated according to [19,20]. The recommendations and guidelines from the International Society for Forensic Genetics (ISFG) regarding the mtDNA population data reporting were followed in this study [10].

Results: In this study, we report the population data of 100 samples from Makrani population for the entire mtDNA control region (spanning positions 16,024–16,569 and 1–576). Seventy different haplotypes (of which 54 unique haplotypes) were observed in this sample size with 149 variable sites in the control region. The detected mtDNA haplotypes, their respective

Table 1
List of Primers used in this study.

Sr. No.	Primer name	PCR	Sequencing	Sequence (5′ → 3′)
1	F15975	Yes	Yes	CTC CAC CAT TAG CAC CCA AA
2	F16327	No	Yes	CCG TAC ATA GCA CAT TAC AGT C
3	F155	No	Yes	TAT TTA TCG CAC CTA CGT TC
4	R16410m	No	Yes	GAG GAT GGT GGT CAA GGG A
5	R042	No	Yes	AGA GCT CCC GTG AGT GGT TA
6	R635	Yes	Yes	GAT GTG AGC CCG TCT AAA CA
7	F403	No	Yes	CCG CTT CTG GCC ACA GCA CT
8	R389	No	Yes	CTG GTT AGG CTG GTG TTA GG
9	F16524	No	Yes	AAG CCT AAA TAG CCC ACA CG