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Announcement of Population Data

Mitochondrial DNA control region sequences study in Saraiki population from Pakistan ☆

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ABSTRACT

The analysis of mitochondrial DNA (mtDNA) control region was carried in 85 unrelated Sariki individuals living in the different provinces of Pakistan. DNA was extracted from blood preserved in EDTA vacutainers. Hypervariable regions (HV1, HV2 & HV3) were PCR amplified and sequenced. Sequencing results were aligned and compared with revised Cambridge reference sequence (rCRS). The sequencing results showed presence of total 63 different haplotypes, 58 of them are unique and 05 are common haplotypes shared by more than one individual. The most common haplotype observed was (W6) with a frequency 12.9% of population sample. The Saraiki population was detected with genetic diversity (0.9570) and power of discrimination (0.9458). This study will be beneficial for forensic casework.

Introduction: The endogamy of Pakistani populations generates a high degree of genetic differentiation [1]. Mitochondrial DNA control region has been found most suitable to express the geographic origin as well as history of the populations [2–5]. In this way (mtDNA) haplogroup typing has become a vital tool to study human evolutionary history [6] which also provide exploratory leads towards identifying unknown suspects when conventional autosomal short tandem repeat (STR) profiling fails to provide a match [7]. In Pakistan, most of the ethnic groups are prehistoric with characteristics language and strictly endogamy. Anthropologists manifest the prolongation of Saraiki Civilization nearer to the Indus valley on the Western side and Harappa Civilization on its Eastern shores. The Saraiki culture represents historic pre-Aryan people of a Semite origin [8]. The word "Semite" is used to refer the peoples of ancient Southwestern Asia. In Pakistan, most of the ethnic groups have their prehistoric basis and language is an important characteristics marker. The Saraiki ethnic group also distinguishes itself on the basis of Saraiki language. In Pakistan Saraiki area stretches from central Punjab to adjacent areas of Sindh, Khyber Pakhtunkhwa (KPK) and Balochistan. Furthermore, consanguineous marriages are the customs of Saraiki peoples which make them an important subject of the study. Saraikis as being a separate sub population of Pakistan as well as having significant strength in Punjab, Sindh and Khyber Pakhtunkhwa, mtDNA data base would be very useful for forensic identity purposes.

The aim of this study is to report mtDNA control region profiles of 85 unrelated Saraiki individuals from Pakistan.

Methodology: Blood samples were collected from 85 unrelated male and female Saraiki volunteers living in different areas of Pakistan (Fig. 1). Saraiki individuals were identified based on their ethnicity, first language and birthplace of mother and father and this information was recorded in the consent form. These blood samples were preserved in EDTA vacutainers. Sampling was done by following all ethical aspects.

DNA extraction: DNA was extracted from preserved blood using a QIAamp® DNA Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer's instructions.

PCR amplification and sequencing: Genomic DNA quantification was determined by Nano Drop™ 1000 Spectrophotometer (Thermo Scientific, USA). Two primers F15975/R635 were used

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Fig. 1. Map of Pakistan showing study areas for Saraiki population.

for the amplification of entire mtDNA control region [9] utilizing 1–2 ng of genomic DNA. The concentration of primers was 0.4 μM each in PCR reaction mix. PCR amplification was performed in 50μL volume using Ampli Taq Gold® 360 PCR Master Mix (Applied Biosystems, Foster City, CA, USA). The PCR amplification consists of pre-denaturation at 95 °C for 11 min., followed by 35 cycles consisting of denaturation at 95 °C for 30s, annealing at 56 °C for 30 s, extension at 72 °C for 90 s, with final extension at 72 °C for 7 min. Sequencing of entire mitochondrial control region ranging from nt 16,024 to 16,569 and 1 to 576 was done by Big Dye® Terminator v3.1Cycle sequencing kit (Applied Biosystems) according to manufacturer instructions using ABI 3100 Genetic Analyzer (Applied Biosystems) as well as commercial facilities were also availed for sequencing. For mtDNA control region bidirectional sequencing same set of forward and reverse primers were used as for PCR amplification Table 1.

Analysis of the data: All the samples were sequenced bidirectionally and aligned using sequence analysis software Geneious (Version 7.1.5, Biomatters Ltd, New Zealand). In both directions sequences and results were compared with rCRS [10,11]. Two researchers checked the sequences for verification and individual profile of each sample was generated. Mito tool and HaploGrep were used to access the quality of mtDNA data [12]. HaploGrep software was also used to find out the haplogroups of the donors and the assignment of haplogroups was confirmed by published studies [13,14]. Haplotype diversity and power of discrimination was statistically calculated according to Tajima [15].

Results: The polymorphic sites found during the sequencing analysis of the control region are given as haplotypes data in Table 2. The statistical data of resulted mtDNA diversity in the Saraiki population samples is given in Table 3.

Other remarks: The observed mutations in this population samples compared to rCRS were, transition (75.58%) transversion (4.67 %), insertion (15.56%) and deletion (4.17 %). Sixty-three different haplotype were identified in 85 samples with 140 polymorphic sites. Out of 85, 58 of them are unique and 5 haplotypes were shared by more than one individual. The most frequently observed haplotypes in this population were West Asian haplotype W6 and East Eurasian haplotype M5c1, each of these constitute the 12.9% and 11.7% population respectively Table 4. In this study, the South Asian haplogroups have clear dominance (29.4%) including U2b2 (9.4%), M2a1a (1.1%), R9 (1.1%), M4 (1.1%), U2c'd (2.3), U2 + 152 (1.1%), M18a (2.3%), HV2a (1.1%), R31 (1.1), U8c (1.1%), U4a2a (1.1%), M30 + 16234 (1.1%). The second major types of haplogroups are West Eurasian and South Asian, which cover the 20% population including U7a (8.2%), U7 (7.0%), U2a1a (3.5%) and relevant subgroups. East Eurasian and South Asian haplogroup also represented by the 20% population including M5c1 (11.7%), M5a2a1a (3.5%), M5 (2.3%), L3e'i'k'x (2.3%), M5b2 (1.1%) and relevant subgroups. Third most prevalent type of haplogroup was West Asian to claim the 16.4% population including I (1.1%), W6 (12.9%), X2 (1.1%), X2d (1.1%). West Eurasian haplogroups cover 2.3% population including, R2 (2.3%) and H2a2a1g (1.1%). South West Asian and East Asian haplogroups both represent 5.8% population each. Only one individual (1.1%) related to South Asian/African haplogroup. We compared the genetic diversity of mtDNA control region of our Saraiki population with other eleven distinct ethnic groups of Pakistan and observed that Pathan population is the diverse

Table 1
Primers sequences used for PCR amplification and sequencing of mtDNA control region.

Sr. No.	Primer	Sequence (5′ → 3′)	TM (°C)
1	Forward primer (F15975)	CTCCACCATTAGCACCCAAA	56
2	Reverse primer (R635)	GATGTGAGCCCGTCTAAACA	55.6

TM, melting temperature.