Int J Legal Med (2008) 122:87–89
DOI 10.1007/s00414-007-0173-7

SHORT COMMUNICATION

Mitochondrial control region sequences from northern Greece and Greek Cypriots

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Received: 7 September 2006 / Accepted: 2 April 2007 / Published online: 11 May 2007

Abstract Entire mitochondrial control region data were generated for population samples of 319 unrelated individuals from northern Greece and 91 unrelated individuals from Cyprus. The samples from northern Greece have been previously typed for 15 nuclear short tandem repeat (STR; Kovatsi et al., Forensic Sci. Int. 159:61–63, 2006).

Electronic supplementary material The online version of this article (doi:10.1007/s00414-007-0173-7) contains supplementary material, which is available to authorized users.

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Keywords mtDNA · Haplogroups · Greece · Cyprus · Population data

Introduction

Mitochondrial DNA (mtDNA) testing in the forensic context requires appropriate population databases to determine the relative rarity of the questioned profile. For many populations, however, large forensic mtDNA databases that cover the entire control region and adhere to strict guidelines in terms of their generation and maintenance are not available. A number of recent publications reflect large-scale efforts to augment global forensic mtDNA databases with high-quality, entire control region data [1–6]. In line with these efforts, we present entire control region data from two populations: Greeks from northern Greece and Greek Cypriots.

Materials and methods

Bode buccal swab (Bode Technology Group, Springfield, VA, USA) samples were collected from 319 unrelated individuals who reside in the northern region of Greece (see Electronic supplementary material for regions of sample collection and associated population sizes). Buccal swab punches were extracted using the Qiagen QIAmp DNA kit on a Qiagen 9604 robotic platform, using a custom-automated protocol.

Blood samples were collected in ethylenediaminetetraacetic acid (EDTA) vacutainer tubes, after written informed consent, from 91 unrelated Greek Cypriots visiting the Thalassaemia Center to undergo a pre-marital β-thalassaemia

Table 1 Diversity measures for northern Greek and Cypriot populations

	Northern Greeks	Cypriots
N	319	91
H-genetic diversity	0.998	0.994
Haplotypes	250	74
Poly sites	226	144
Pairwise differences	9.85	10.76
Empirical RMP	0.30%	0.63%

Statistics are based on the entire control region sequence (16024–16569, 1–576). Random match probabilities (*RMP*) were calculated empirically.

screening test. DNA was isolated from purified leukocytes using an organic phenol/chloroform procedure. The 91 individuals were residents of the major cities of the island of Cyprus.

The polymerase chain reaction (PCR) set-up was performed on a Corbett CAS-1200 using primers F15971/R599, as described in [1]. Sequencing reactions covered the entire control region (16024-576) and were prepared on a Tecan Genesis workstation. Sequencing products were separated on an Applied Biosystems 3100. Sequencher version 4.1.4Fb19 was used to align electropherograms, with sequences numbered according to the revised Cambridge reference sequence [7, 8]. The guidelines of [9, 10] were largely followed for the consistent placement of gaps, although novel length variants were designated with nomenclature based on phylogenetic information [11]. A redundant approach to data generation and data analysis was used to ensure data quality. Additional details of the approach can be found in [1] and [6]. Sequence data in electronic form are available from the authors upon request. We would recommend that investigators wishing to perform further analysis on these sequences contact the authors for automated electronic transfer of the finalized data, or download directly from GenBank. The GenBank accession numbers are DQ418040-DQ418130 (Cyprus) and DQ418131-DQ418449 (Greece).

Summary statistics and analysis of molecular variance (AMOVA) values were generated using custom software (LISA, Future Technologies, Fairfax, VA, USA) and Arlequin 2.0 [12]. Haplogroups were assigned to control region sequences using in-house computer programs, as described in [3]. In brief, the data were compared to assembled mtDNA control region sequences with known haplogroup affiliations, and the haplogroup status estimated by a nearest neighbor search was confirmed by checking the presence or absence of certain haplogroup diagnostic sites. The mtDNA haplotypes from this study were affiliated with haplogroups based on the patterns of shared haplogroup-specific or haplogroup-associated polymorphisms in the control region, as reported in [13–23].

Point heteroplasmies were denoted by the appropriate International Union of Pure and Applied Chemistry (IUPAC) code and evaluated for the relative proportion of minor vs major component molecule. A lower apparent threshold of approximately 10% minor to major component was set to designate a site as heteroplasmic.

Results and discussion

Mitochondrial DNA control region haplotypes were determined for populations of northern Greece and Greek Cypriots. Haplotype summaries for the two populations are available as Electronic supplementary material. Summary statistics are presented in Table 1. When heteroplasmic samples consistent with the haplotype are included, the single most common Greek haplotype was observed eight times in the database of 319 individuals. The most common Greek Cypriot haplotype was observed five times in the Cyprus database of 91. Of the total number of control region haplotypes, six were shared between the Greeks and the Greek Cypriots. Yet, based on entire control region data, AMOVA results indicate that the two populations are somewhat differentiated. Of the total variation found among the two populations, 1.6% can be attributed to inter-population differences (p=0.00).

Polymorphisms were observed at a total of 260 control region sites. In addition, 39 point heteroplasmies (38 transitions, 1 transversion) were identified, occurring at 23 different positions. A large proportion (23%) of the point heteroplasmies occurred at position 16093.

HVI data from the 319 Greeks and 91 Greek Cypriots in this study were compared to a sample of 54 Greeks for which HVI/HVII data have been previously reported [24], as well as 167 eastern Mediterranean and 83 Greek HVI sequences [25, 26]. Only the largest common region sequenced among the various data sets was used in this analysis (16090–16365). In addition, an unusual transversion at position 78 in the data set of [24] was ignored. No significant differences were observed among the Greek data reported in this paper and any of the other Greek and eastern Mediterranean populations. All pairwise F_{st} values were less than 0.005 and non-significant at the 0.05 level. All Greek and eastern Mediterranean populations did, however, differ slightly from the 91 Greek Cypriots reported in this paper. While pairwise F_{st} values were marginal (all less than 0.02), the differences were significant at the 0.05 level in all cases.

Both the Greek and Greek Cypriot samples examined in this study exhibit low random match probabilities and high genetic diversities, suggesting their utility for mtDNA testing purposes. These databases will serve as a framework within which to assess the relative rarity of mtDNA types in these populations.

Acknowledgment We thank Jennifer O'Callaghan, Heather Williams, Kimberly Watson and Rebecca Just for assistance with data analysis and database confirmation. The opinions and assertions contained herein are solely those of the authors and are not to be construed as official or as views of the US Department of Defense or the US Department of the Army.

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