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Research paper

The mitochondrial DNA makeup of Romanians: A forensic mtDNA control region database and phylogenetic characterization

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ABSTRACT

To evaluate the pattern of Romanian population from a mitochondrial perspective and to establish an appropriate mtDNA forensic database, we generated a high-quality mtDNA control region dataset from 407 Romanian subjects belonging to four major historical regions: Moldavia, Transylvania, Wallachia and Dobruja. The entire control region (CR) was analyzed by Sanger-type sequencing assays and the resulting 306 different haplotypes were classified into haplogroups according to the most updated mtDNA phylogeny. The Romanian gene pool is mainly composed of West Eurasian lineages H (31.7%), U (12.8%), J (10.8%), R (10.1%), T (9.1%), N (8.1%), HV (5.4%),K (3.7%), HV0 (4.2%), with exceptions of East Asian haplogroup M (3.4%) and African haplogroup L (0.7%).

The pattern of mtDNA variation observed in this study indicates that the mitochondrial DNA pool is geographically homogeneous across Romania and that the haplogroup composition reveals signals of admixture of populations of different origin. The PCA scatterplot supported this scenario, with Romania located in southeastern Europe area, close to Bulgaria and Hungary, and as a borderland with respect to east Mediterranean and other eastern European countries.

High haplotype diversity (0.993) and nucleotide diversity indices (0.00838 ± 0.00426), together with low random match probability (0.0087) suggest the usefulness of this control region dataset as a forensic database in routine forensic mtDNA analysis and in the investigation of maternal genetic lineages in the Romanian population.

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1. Introduction

Romania is located in Southeastern Europe, bordering the Black Sea, between Bulgaria and Ukraine, Hungary, Serbia, and Moldova and in early history its territory was an important place for human settlement in Europe. The discovery of anatomically modern human fossils dating 41 kya in Pestera cu Oase in southwestern Romania provides evidence of early modern humans in the lower Danubian Corridor and are signals of the first settlement of Europe by anatomically modern humans during the Paleolithic [1,2].

At present the Romanian population is made up of 88.9% Romanians, 6.5% Hungarians, 3.3% Roma and 1.3% other populations (the ethnic groups for which the number of persons exceeded 20,000 are: Ukrainians, Germans, Turks, Russians – Lipovans and Tatars), according to the 2011 census [3].

From the historical point of view the Romanian population is a mixture of local and surrounding populations. In brief, Romania can be divided in four major historical regions, each with its particular surrounding population's influence: Moldavia, Transylvania, Wallachia and Dobruja.

Moldavia is a historical region located between the Carpathian Mountains, the Danube River, the Black Sea, and the Dniester River, and comprises 1/3 of today Romania, entire Moldova Republic and small parts of Ukraine; in the past this region was the Eastern European border to Mongol, Tatar and Ottoman invasions. Transylvania is a historical region located in the Intra-Carpathian area between today Hungary, Serbia and Ukraine and here the

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Austro-Hungarian Empire had an important influence. Wallachia is a historical region situated on the northern side of Danube River and the present structure of the Wallachian population is the result of many historical events such as Roman Empire conquests, the Slav migration from the north, and the Turkish south-east influence. Finally, Dobruja is a historical region located between the lower Danube River and the Black Sea including the Danube Delta, Romanian coast and the northernmost part of the Bulgarian coast. It comprises Northern Dobruja, which is part of Romania, and Southern Dobruja, which belongs to Bulgaria. In the past this region was conquered by Greeks, Romans, Tatars, Turks and Slavs for its economic and strategic value [4].

At present the information concerning the genetic variation of Romanians from the perspective of autosomal markers has shown that the dominant influences were Slavic, Italian, Greek and Turkish [5], while genetic studies made on Y-STR markers suggest that the Slavic influences were dominant [6].

From a mitochondrial DNA perspective, the Romanian maternal lineages were investigated in order to provide an insight into understanding the genetic structure of the Balkans [7]. Although the linguistic and cultural diversity found in the region could have acted as an important genetic barrier, Balkan populations have been shown to be genetically homogenous. Linguistic and other cultural differences were probably introduced into genetically homogeneous groups and/or these cultural barriers were not strong enough to prevent genetic flow between populations. The mtDNA haplogroup distribution found in the Balkans was similar to that found in other European populations and sequences belonging to the H haplogroup were the most prevalent, with frequencies around 40–50%.

One recent study [8] evaluated whether Carpathian Mountain represent a genetic barrier in East Europe. In this study, regarding the mtDNA haplogroup and haplotype distributions, the populations living outside the Carpathian range (South of Romania) displayed some degree of genetic differentiation compared to those living within the Carpathian range (North of Romania). However, this differentiation can be mostly attributed to the demographic movements from East to West that differently affected North and South Romania, suggesting that the Carpathian mountain range represents a weak genetic barrier in South-East Europe.

In order to evaluate the pattern of mitochondrial lineages in the Romanian population and to contribute forensically relevant mtDNA data, we generated a high-quality mtDNA control region dataset from a Romanian population sample. Moreover, to define the matrilineal relationships between Romanians and other European populations, the results were compared with surrounding or historically related populations.

2. Materials and methods

2.1. Study populations

A total of 407 Romanians buccal swab samples (62.4% male and 37.6% female) were collected from the general population belonging to four major historical regions: Moldavia (n = 105), Transylvania (n = 94), Wallachia (n = 168) and Dobruja (n = 13). For 27 individuals the regional allocation was not possible. Written informed consent was obtained from each participant.

2.2. Generation of mtDNA sequences, data analysis, alignment and notation

The analysis and interpretation of mtDNA control region were carried out following the guidelines for mitochondrial DNA typing [9].

DNA was extracted from buccal swabs using the DNA IQ™ Reference Sample Kit for Maxwell® 16 (Promega). PCR amplification and sequencing spanning the entire control region (16024-576) were performed according to [10]. Briefly, the entire control region was amplified twice with two different primer sets and each PCR product was sequenced using five different sequencing primers. Sanger-type sequences were generated by 3130 Genetic Analyzer. All sequences were imported into SeqScape v2.5 (Life Technologies) and aligned relative to the revised Cambridge Reference Sequence for human mitochondrial DNA (rCRS; [11]) using the phylogenetic alignment rules [12]. C-stretches were noted by reporting the dominant LHP (length heteroplasmy) variant in the data [9,13]. To ensure high data quality at least a double reading of each site and twice independent evaluation of raw data were performed. The final consensus haplotypes were based on redundant sequence coverage over all positions.

2.3. Quality control and haplogroup assignment

A phylogenetic approach as *a posteriori tool* for detecting potential errors in the dataset was performed by a quasi-median network analysis (QMN), through the application NETWORK available on the EMPOP website and applying the "EMPOPspeedy" filter.

The haplogroup affiliation of the samples was inferred according to Phylotree, build 16 [14], by using EMMA, the new software tool for haplogroup assignment that has been implemented in EMPOP ver.3 [15].

The data will also be made available for forensic searches via EMPOP (http://www.empop.org) under accession numbers EMP00674 (Moldavia), EMP00675 (Transylvania), EMP00676 (Wallachia) and EMP00677 (Dobruja).

2.4. Statistical analysis

The random match probability was calculated as the sum of squared haplotype frequencies based on mtDNA CR sequences, while the haplotype diversity was calculated according to Tajima [16]. The mean number of pairwise difference and nucleotide diversity were calculated using Arlequin 3.5 software packages [17]. All the analyses were performed using entire mtDNA control region (16024-576), excluding C-stretch length variation at 16193, 309 and 573, with a total of 1135 usable sites.

In order to define the matrilineal relationships between Romanian and other European populations a principal component analysis (PCA) was performed by PASW Statistics 17.0. The haplogroups and subhaplogroups included in the PCA are listed in Table S1, as well as the populations taken into account for comparison.

3. Results and discussion

In the dataset of 407 Romanian sequences we observed 277 (68%) distinct haplotypes of which 220 (79%) were unique (Table S2). The most common haplotype 16519C, 263G, 315.1C, (haplogroup R0) was shared by twenty-five individuals (6%), while the second most common haplotype 16126C, 16189A, 16223T, 16278T, 16519C, 73G, 195C, 263G, 315.1C (haplogroup X2e1b) was shared by ten individuals (4%). Other frequent haplotypes belonged to haplogroups J1c (seven individuals, 16069T, 16126C, 16261T, 73G, 263G, 295T, 315.1C, 462T, 489C) and HV (six individuals, 16311C, 263G, 315.1C).

The number of polymorphic control region positions was 216, 198 of which were substitutions and the remaining 18 were insertions and deletions.

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