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New Perspective on Tappeh Hesar

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Abstract

Since the last decades, scientists have tried to answer where human beings originated and how spread all over the world. In this way, ancient human remains can play a fundamental role to answer these questions. Tappeh Hesar is a prehistoric site located in the Damghan city (northeastern Iran). This place history dates back to more than 4 000 years. Due to the city's key position in trade and communications, it has always been an attractive site for archaeologists. In this study ancient DNA was extracted from one human skeletal remains by considering all precautions. All mitochondrial hypervariable segments (HVS-I, HVS-II and HVSIII) were analyzed using sequencing. After comparison HVS sequences with revised Cambridge Reference Sequence (rCRS), the consensus sequences showed three transitions in HVS-II. Haplogroup H32 was determined for this sample. Haplogroup H is a Eurasian haplogroup which likely originated in Southwest Asia ~25–30 thousand years ago (kya) and entered Europe ~23–28 kya. Nearly half of Europeans have this haplogroup and there is a considerable frequency of haplogroup H in Middle East. Evidences indicate long-term presence of this haplogroup at western Asia.

Introduction

These days' evolutionary history and movement of populations is much debated. Archaeological records can be used to date divergence events within the phylogeny, but Molecular anthropology can play a key role in human evolution explanation to determine humans and other organisms' phylogeny. Mitochondrial DNA (mtDNA) due to its high copy number, lack of recombination and maternal inheritance is a proper tool for inferring the evolutionary origin of populations P [1,2]. In 1984, Higuchi and his colleagues successfully extracted ancient mtDNA from a 150-year-old quagga -zebra like species- for the first time [3]. One year later, in 1985 Pääbo isolated DNA sequence from a 2400-year-old Egyptian mummy [4]. In 1987 Cann, et al. published that common ancestor mtDNA existed 140–290 kya in Africa [5]. People first arrived in Asia about 60–70 kya [6]. Then they spread all around the world; thus, Analyses of ancient DNA in this area can give a better sense of population origins.

Tappeh Hesar is one of the most important archaeological sites in Iran. This place is located in northeastern Iran at the south of Alburz Mountains near the Damghan city, some 361 km east of Tehran [7,8]. This prehistoric place is located at an optimal altitude for exploiting the farming and cattle/sheep herding. Because of these conditions, this location was constantly used as a residential area for more than 4000 years. The original excavation was undertaken for the University Museum of the University of Pennsylvania in 1931 and 1932 by Dr. Erich F Schmidt. Based on analysis of burials a range of time between fifth to second millennium B.C has been determined for this place [9]. Ancient artefacts made from copper belong to copper age, transition phase between the Neolithic and the Bronze Age, and is evidence of civilization in this area [8,10]. Investigation of ancient human skeletons in this region can help to find out the origin of the Iranian population and human movement pathway. In this framework, hypervariable segments in the mitochondrial control region have the potential to study evolutionary events [2,11].

In order to increase our understanding about genetic background of people who resided in this prehistoric place, we sequenced hypervariable regions of one skeletal remains. This study shows a view of people who lived more than 4000 years ago on this site.

Materials and Methods

Sample collection

Extracted human remains from Tappeh Hesar in 1997 were preserved in Cultural Heritage, Handcrafts and Tourism Organization of Semnan province. For genetic analysis, skeletal remains from a single grave were performed with permission from the Cultural Heritage Organization. Sampling was performed from left tibia.

DNA extraction

Bone sample preparation and ancient DNA extraction were carried out in two separated clean rooms. All laboratory staff wore specific lab coats which had been designed for ancient DNA works.

Table 1: Primers used for PCR amplifications of mitochondrial hypervariable segments.

Target region	Primer sequence (5′–3′)	Product length (bp)
HVS-I	CTC CAC CAT TAG CAC CCA AAG	283
	GGCTTTGGAGTTGCAGTTGATG	
	CAT CAA CTG CAA CTC CAA AGC C	184
	GAT TTC ACG GAG GAT GGT GG	
HVS-II	GGTCTATCACCCTATTAACCAC	125
	GACAGATACTGCGACATAGG	
	CCTATGTCGCAGTATCTGTC	168
	GATGTCTGTGTGGAAAGTGG	
	CCACTTTCCACACAGACATC	129
	CTGGTTAGGCTGGTGTTAGG	
HVS-III	CCTAACACCAGCCTAACCAG	266
	GATGTGAGCCCGTCTAAAC	

To avoid contamination by contemporary DNA, strict precautions were performed. Laboratory surfaces were decontaminated frequently by bleach and Ultraviolet Light (UV) exposure. Sterile materials and solutions were used [12]. Negative controls were accomplished for each DNA extraction and Polymerase Chain Reaction (PCR).

External surface of the bone sample was removed to approximately 1mm in depth by a sterile dental drill. Then, bone sample submerged in 5% bleach for 10 minutes and each side of sample irradiated by UV for 20 minutes. After that, bone pulverized using freezer mill. 300mg of Bone powder was decalcified overnight with 0.5 M EDTA (pH 8.0). Afterwards, decalcified powder was incubated for 3 hours in 20 μl of 20 mg/ml Proteinase K (CinnaGen) and 5 μl of 1 M DTT (Sigma-Aldrich). After centrifugation, an equal volume of chloroform was added to the sample. Finally, DNA extracted using EZhigh™ DNA extraction kit (Texas BioGene).

PCR amplification and sequencing

In this study, sequences of HVS-I, HVS-II and HVSIII were analyzed. Since ancient DNA fragments are usually short, six primer pairs were designed for all hypervariable regions. The primers are listed in table 1. The PCR amplifications were carried out in a total reaction volume of 25 μl containing Multiplex PCR Kit (Amplicon), 0.2 μM of each primer and 15 μl of DNA sample. PCR conditions were primary incubation at 95 ^{0}C for 3 minutes, followed by 35 cycles of 94 ^{0}C for 30 seconds, 58 ^{0}C for 30 seconds, 72 ^{0}C for one minutes and final extension at 72 ^{0}C for 10 minutes. Gel electrophoresis was used to analyze reaction quality. PCR products were purified using fragment DNA purification kit (Gene all), and sequenced in both directions by means of Big Dye Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems). Sequence reactions were performed on 3130xl Genetic Analyzer (Applied Biosystems). Sequence alignments were analyzed using the DNA Baser Sequence Assembler version 4.31.

Results

All mitochondrial hypervariable regions were successfully amplified and sequenced at least three times on separated runs. HVS-I, HVS-II and HVSIII sequences were compared with the rCRS. Comparison of HVS-I and HVS-III sequences with the rCRS showed no significant difference. On the other side, three different transition variants, including m.73A>G, m.152T>C and m.263A>G were observed at HVS-II. Haplogroup H32 was defined manually base on phylotree [13]. Furthermore, EMMA algorithm in the EMPOP database was used to define the haplogroup. 14990 full mt genomes from Gen Bank and 3925 virtual haplotypes from Phylotree have been used in this software to estimate haplogroups [14]. Two other web-based bioinformatic platforms MITOMASTER (www.mitomap.org) and MitoTool (www.mitotool.org) with different scoring system for determining mitochondrial DNA haplogroups [15,16] was used to confirm our recognition.

Discussion

Genetic studies provide brilliant insights into human evolutionary. Over the past decades, there have been several studies about the human origins and movement [17,18]. According to the genetic and archeological records, south and west Asia regions have fundamental role in determining human migration model [19,20]. Genetic analysis of human remains can be helpful in better understanding of this issue. The major concern in these studies is DNA authenticity. Contamination with contemporary DNA is a crucial problem, which can greatly affect the results. In this study standard precautions followed to avoid contamination. DNA typing of human bone remains belonging to a 4000 years old sample was performed. Based on all hypervariable regions sequence, haplogroup H32 was observed. Haplogroup H is a descendant of haplogroup HV and a branch of the mega-haplogroupR. Some studies proposed that this haplogroup originated in the Middle East ~25–30 thousand years ago (kya) [21]. Previous studies suggest that haplogroup H entered Europe from the Near East ~23–28kya around Last Glacial Maximum and re-expanded from a European glacial refuge ~15kya [22]. This haplogroup is one of the most predominant haplogroups in Europe with wide geographic distribution and high frequency, about one half of the European population (44.5-48.2%); besides, it is common among the population of Middle East and northern Africa [21,23,24]. The frequency of this haplogroup in Iran (17%) is lower than Europe, but it has a high frequency rather than other countries in this area, such as Central Saudi Arabians (10%), Syrians (13.6%) and Iraqis (9.5%) [23,25]. In 2010, five mummies excavated from the salt mines of Chehrabad, located in the Zanjan city, north-west of Iran. Mitochondrial DNA amplification was possible for four mummies. Haplogroup determination was performed for three samples. According to HVS-I sequence, one mummy was inferred to belong to haplogroup H. This sample in Chehrabad has similar haplogroup with the sample examined in this study. The results indicate a long-term presence of this haplogroup in Iran [26].

Obviously, further researches on ancient samples are needed to provide a clear insight into human movement and evolution.

Supplementary Material

See supplementary material for the sequence alignment of the three hypervariable regions.

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