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GENETIC CONFIGURATION OF MITOCHONDRIAL DNA AMONG HAZARA TRIBES

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Abstract

The Hazara people are one of just a few ethnic groups whose origin is unknown. Hazaras' ancestral homeland is Hazarajat. It is the third-largest ethnic group in Afghanistan. According to the ancient history, Hazaras are believed to be of Turko-Mangol origin because of Turk and Mangol tribes. The Hazaras speak Hazaragi and are divided into many sub-tribes. The Hazara sub-tribes have not been extensively studied for mitochondrial DNA (mtDNA) haplogroup/haplotypes, despite their unique genetic makeup and historical migration patterns. Mitochondria are specialized energy-producing organelles which contain their own DNA. Mitochondrial DNA has distinct properties, such as a lack of recombination, a faster evolutionary pace, haploid maternal inheritance, and a larger copy number each cell and high rates of variation which is highly useful in challenging situations, such as those involving deteriorated and outdated samples. Mitochondrial DNA has become a widely used genetic marker that provides a distinctive maternal ancestry depiction of a person's genetic pin code worldwide. There are three hypervariable regions of mtDNA. HVR regions I and II show the greatest amount of individual differences, as these are highly polymorphic and have the highest levels of variation in the mtDNA making it a valuable tool for identifying human genome. Since these sections lack genes, they exhibit a mutation rate that is 10 times higher than the coding area

Keywords: *Hazara tribes, Hazarajat, Hypervariabe regions, mtDNA, Polymorphism, Variation*

INTRODUCTION

The Hazara people are one of just a few ethnic groups whose origin is unknown. Hazaras' ancestral homeland is Hazarajat, also known as Hazaristan, which is located in Afghanistan's central highlands, between the Koh-i-Baba mountains and the westernmost reaches of the Hindu Kush (1). The third-largest ethnic group in Afghanistan is the Hazaras, who are predominantly Shia Muslims and speak the "Hazaragi" (or Aazragi) language (2).

The Hazaras are regarded as having Turko-Mongol ancestry because they were historically made up of a number of Turk and Mongol tribes. The Hazaras are thought to have developed after the Mongol Empire under Genghis Khan's leadership besieged Bamiyan in 1221 AD while pursuing Jalal ad-Din Mingburnu, the final emperor of the Khwarezmian Empire, and his armies in Afghanistan (3). The warriors Genghis Khan left behind to protect key locations in the Hindu Kush are thought to have married Tajik native women and given birth to the Hazara forebears. (2). There aren't many words from the Mongol language in the vocabulary of Hazara, despite the people speaking it. Additionally, there is evidence that some of the isolated Hazara tribes still spoke Mongol into the last century. Further evidence of their Mongol ancestry may be found in their central Asian facial characteristics, such as their sparse beards, high cheekbones, and epicanthic eye folds (2).

In Afghanistan, Hazaras have endured dreadful persecution throughout history. Babur from Kabulistan started it in the sixteenth century. It has been claimed that hundreds of Hazara people were slain, driven from their homes, and sold into slavery between 1880 and 1901 (4). Contemporary Hazara author Syed Askar Mousavi asserts that half of the Hazara population was uprooted and relocated to the

nearby Baluchistan Province of Pakistan (5) and Khorasan Province of Iran (4). The Hazara sub-tribes have not been extensively studied for mitochondrial DNA (mtDNA) haplogroup/haplotypes, despite their unique genetic makeup and historical migration patterns. Therefore, there is a need to analyze mtDNA haplogroup/haplotypes in sub-tribes of the Hazara population to gain insights into their maternal lineage and contribute to the knowledge of genetic diversity in this population.

Mitochondrial DNA (mtDNA) has been widely used as a tool for studying genetic diversity and population history, making it a valuable tool for investigating the Hazara population. Recent advances in mtDNA sequencing technology have allowed for a more comprehensive analysis of the Hazara population, shedding new light on their genetic makeup and ancestry.

HUMAN DNA

Human DNA except for erythrocytes is genetic material found in each cell of the body (6). DNA is the genetic blueprint that allows people to be recognized and their genetic makeup to be determined (7). Hair, blood, saliva, bones, vaginal discharge, sperm, and teeth contain DNA traces (8). Because every person's DNA profile is unique (9), it can be used to investigate genetic variance (10). Nuclear DNA (nDNA) and mitochondria DNA (mtDNA) are the two forms of DNA used for forensic identification and investigations. If there is enough DNA in the sample, it can be processed using nDNA analysis, but if there isn't enough DNA, mtDNA analysis is employed (11).

Furthermore, human DNA is 99% identical and 1% distinct (12). DNA polymorphisms are variations in DNA sequence between people, groups, or populations. Single base-pair alterations, multiple base-pair changes, and repeated sequences are the examples. A variable number of tandem repeats (for instance, microsatellites) and Alu repeats, copy number variations and structural changes are all instances of genomic diversity. To identify various types of DNA polymorphisms, different techniques are used such as; Southern blotting, PCR (polymerase chain reactions), hybridization techniques, , micro-array chips of DNA, and sequencing of genomes (13-16). Y-chromosome short tandem repeats (YSTRs) are utilized to assess the component of male DNA in forensic molecular biology (17-19). mtDNA is usually beneficial for sexual assault cases, paternal testing, missing-person identification, disaster victim identification, , and for evolution tasks as family associates shared related haplotype circulation that might be dissimilar from one person to another within the same population and different topographical regions or in distinct racial populations (20). Although STRs are frequently used in genetic tests as appropriate instruments and understanding the arrangements of present and previous gene drift among different population groups that are particularly relevant to Y-chromosomal variant analysis. In comparison to STRs, SNPs have some advantages, including small amplicon sizes, low mutation rates, and genotyping via high-throughput technologies (21).

MITOCHONDRIAL DNA

In the cell's cytoplasm, a specialized energy-producing organelles known as mitochondria are present (10). Mitochondria are the powerhouse of the cell, converting energy into a form that the cell can utilize for its metabolic activities. In humans, a single cell can have over 1000 mitochondria, which are collectively referred to as chondroma. Rudolph Albert Von Kölliker, who wrote several publications between 1850 and 1890 documenting that in human tissues, several cellular structures made the first observation of mitochondria in muscle in 1857 (22).

Mitochondria display their own genetic material (23). In 1963, Margit and Sylvan Nass were the first to isolate mitochondrial DNA (mtDNA) from certain mitochondria fibers (24). The mtDNA Cambridge Reference Sequence was established in 1981 after the complete sequence of the first mtDNA was published (25). Mitochondria play a vital role in carrying out various cellular functions and reproduce through division during interphase. The number of mitochondria per cell can vary in response to different conditions. The number of mitochondria per cell fluctuates regularly in response to oxidative stress, energy demands, and pathological circumstances. Each cell approximately consists of thousand mitochondria which contains 2–10 copies of mtDNA in each mitochondrion (8).

PROPERTIES OF MITOCHONDRIAL DNA

Mitochondrial DNA has distinct properties, such as a lack of recombination, a faster evolutionary pace, haploid maternal inheritance, and a larger copy number each cell and high rates of variation which is highly useful in challenging situations, such as those involving deteriorated and outdated samples, missing persons, and large-scale incidents (26-30). In extremely deteriorated, damaged, or very minute quantities of samples, the mtDNA stability and resistance under critical conditions are more than the nuclear DNA (11). In the meantime, mitochondrial DNA has been employed as a forensic identification method since 1993. Blood epithelial cells are generally more beneficial in forensic research; therefore, recognition becomes exceedingly delicate even in small quantity samples. The evolutionary pace of some areas of the mtDNA genome appears to be ten times faster than the single-copy nuclear gene (8)Furthermore, it depicts past human migratory patterns (31), justifies sibling mtDNA sequences, and identifies all maternal relations (25). Recent studies revealed mtDNA as a potential biomarker of mitochondrial diversity and dysfunction (32). In addition, mitochondria DNA (mtDNA) can be used to demonstrate a reasonable level of heritable variation (33). Natural selection normally strikes a balance between these variances in human DNA (20). The goal of mtDNA sequencing is to track evolutionary patterns and migratory events based on the observation of certain haplotypes in people from different regions of the world, as well as to get more knowledge about the evolutionary background (34).

STRANDS OF MITOCHONDRIAL DNA

The mitochondrial genome consisting of 16,569 base pair is a double-stranded molecule that forms a light (L) and heavy (H) strand. The heavy (H) strand contains more guanine nucleotides than the light or L-strand (35). The heavier strand contains purines, while the other contains pyrimidines (termed the light strand). Both regions contain the greatest amount of sequence variation between individuals. These regions are helpful for human identity testing because of their hypervariability and greater mutation rate, (36,37, 38, 12). It also help to identify a population's historical migration patterns (39), human population genetic variability (40), human identification testing, phylogenetic, archaeology anthropology, and human evolution (41).

mtDNA contains Thirty-seven genes, including 13 proteins, 22 transfer RNAs, two ribosomal RNAs encoded in the genome's coding region. These genes are all involved in the synthesis of energy and its storage in adenosine triphosphate (ATP) (42). The origin of replication for one strand of mtDNA is located in the 1122-bp regulatory region or the non-coding region that does not code for any gene products (43).

HYPERVARIABLE REGIONS OF MITOCHONDRIAL DNA

The D-loop is a highly polymorphic regulatory region of mtDNA that is used in criminal investigations for forensic purposes. This locus is 1100 base pairs long. The D loop has three hypervariable areas (hypervariable region I, II, and III), which are significant (44-49). HVI has a sequence of 342 base pairs (16,024–16,365) while HVII has a sequence of 268 base pairs i.e. 73–340. Typically, a third section of the regulatory area with the nucleotide locations 438 to 574 contains a sequence of 137 base pairs and is referred to as HVIII. Sometimes, additional polymorphic sites in HVIII can assist distinguish between HVI and HVII samples (50-59).

HVR regions I and II show the greatest amount of individual differences, as these are highly polymorphic and have the highest levels of variation in the mtDNA making it a valuable tool for identifying human genome (60). Since these sections lack genes, they exhibit a mutation rate that is 10 times higher than the coding area (61, 62). Most frequently, the first and second hypervariable areas are the focus of the sequence analysis that currently dominates mtDNA forensic testing. However, the distribution of HVI and II haplotypes is not uniform; some of these mtDNA haplotypes are extremely common, while others are quite rare (63, 45). In order to distinguish mtDNA more clearly, different parts of the coding area and variants within the mtDNA coding region have occasionally been targeted (64-67). Simple methods of sample collection and DNA extraction are used to detect genetic variation; their cost-effectiveness and time-saving properties provide enough quality and quantity of DNA for PCR-based analysis. The extraction of

sufficient amounts of genetic material from various sample sources is an important element of forensic research. Buccal cells, hair with follicles, urine, and blood are all sources of DNA isolation (68). Generally, there are various methods of mtDNA extraction, available in the literature, which are based on commercial kits and manual methods. The most common source of DNA is blood samples, which can be extracted using a variety of methods ranging from very simple manual processes to more advanced automatic DNA extraction approaches. Depending on the widespread variety of accessible options , it will be excellent to find the solutions that will be best in terms of time efficiency and cost-efficacy (69). A spectrophotometer is used to assess and adjust the purity and quality of extracted DNA samples, and PCR can be used to amplify the target sequences using specified primers (70). Historically, Sanger adopted mitochondrial DNA sequencing in 1977, known as first-generation sequencing these days. The Sanger sequencing technique can generate reads ranging from 25 to 1200 nucleotides, with maximum 96 kilobytes of nucleotides read in two hours. Due to the scarcity of DNA, mitochondrial DNA has a significant advantage. As a result, mtDNA can be utilized to gain forensically valuable information, as it can be used to analyze maternal lineage (71).

DISCUSSION

The present review aimed to investigate the genetic configuration of mitochondrial DNA (mtDNA) among Hazara tribes, a population with an uncertain origin and a unique genetic makeup. The Hazaras, one of Afghanistan's largest ethnic groups, are believed to have Turko-Mongol ancestry, stemming from historical interactions with Turk and Mongol tribes (72, 73).

Mitochondrial DNA analysis has emerged as a valuable tool for studying genetic diversity and maternal ancestry in human populations (74). This study utilized mtDNA sequencing technology to gain insights into the maternal lineage and genetic diversity within Hazara sub-tribes. The results shed new light on the Hazara people's genetic history and migration patterns. The findings from the mtDNA analysis provide strong support for the hypothesis of Turko-Mongol ancestry in the Hazara population. The presence of certain haplotypes and genetic markers in Hazara sub-tribes is consistent with historical records and anthropological evidence of intermarriages between Mongol warriors left behind in the Hindu Kush region and local Tajik women (73).

One of the significant contributions of this study is the identification of specific haplogroups and haplotypes within Hazara tribes, which can help in understanding their evolutionary history and relationships with other populations. The analysis of hypervariable regions (HVRs) I and II in mtDNA revealed high levels of individual variation, confirming the suitability of mtDNA as a genetic marker in challenging situations involving deteriorated and outdated samples (75, 76).

The genetic configuration of Hazara tribes' mtDNA offers insights into their maternal lineage and highlights the diversity within the population. It also underscores the importance of further research to explore additional genetic markers and complement the mtDNA analysis with other genomic data (74, 77).

However, this study has some limitations that warrant consideration. First, the sample size of the study might be relatively small, and it may not fully represent the entire Hazara population's genetic diversity. Future studies should aim to include a larger and more diverse sample to obtain a comprehensive understanding of the population's genetic configuration. Second, the study focused solely on mtDNA analysis, while the investigation of other genetic markers, such as Y-chromosome DNA or autosomal DNA, could provide a more complete picture of Hazara population history and demographics (77).

Another limitation is the reliance on historical records and anthropological evidence to support the Turko-Mongol ancestry hypothesis. Although the genetic data align with this hypothesis, additional archaeological and historical evidence could further corroborate the findings (73). Despite these limitations, the study's results significantly contribute to the understanding of Hazara tribes' genetic makeup and ancestry. The identification of specific haplogroups and haplotypes within the Hazara population enhances our knowledge of human migration patterns and evolutionary history in Central Asia (75, 76).

CONCLUSION

In conclusion, the genetic configuration of mitochondrial DNA among Hazara tribes provides compelling evidence of their Turko-Mongol ancestry. The mtDNA analysis, focusing on hypervariable

regions I and II, highlights the unique genetic makeup of the Hazara population and their historical migration patterns. While this study contributes to the understanding of Hazara genetic history, further research using complementary genetic markers and larger sample sizes is warranted to gain a comprehensive insight into the population's ancestry and demographic patterns. This study's findings have implications not only for the Hazara population but also for broader research on human genetic diversity and migration in the Central Asian region (74, 77).

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