

# Ancient DNA and MTHFR mutation: insights into human evolution and health

## *Antik DNA ve MTHFR mutasyonu: insan sađlığına ilişkin bulgular*

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### Abstract

**Purpose:** Scientists have discovered more information on ancient genes, migration patterns, and human population evolution by studying DNA extracted from old samples. This research aimed to synthesize current understanding of the relationship between MTHFR mutation and ancient DNA, exploring possible implications for our understanding of the evolutionary and genetic underpinnings of disorders.

**Materials and methods:** Genomic DNA isolation from petrous bone and teeth samples was performed using the Ancient DNA extraction from bones and teeth kit. In the obtained genomic DNAs, the gene-specific region was amplified by PCR, and the polymorphic locus located in this region was observed on high-resolution agarose gel. The MTHFR mutation was examined by genotyping based on DNA sequence analysis.

**Results:** MTHFR A1298C mutation was detected in three samples and MTHFR C1298C mutation was detected in one sample.

**Conclusion:** Looking at MTHFR changes in ancient DNA helps scientists understand how these gene differences affected human survival.

**Keywords:** Archaeogenetics, MTHFR, ancient DNA, polymorphism, stratonikeia.

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### Öz

**Amaç:** Bilim insanları eski örneklerden çıkarılan DNA'yı inceleyerek eski genler, göç kalıpları ve insan popülasyonunun evrimi hakkında daha fazla bilgi keşfettiler. Bu araştırma, MTHFR mutasyonu ile eski DNA arasındaki ilişkiye dair mevcut anlayışı sentezlemeyi ve bozuklukların evrimsel ve genetik temellerine dair anlayışımız için olası çıkarımları keşfetmeyi amaçladı.

**Gereç ve yöntem:** Petroz kemik ve diş örneklerinden genomik DNA izolasyonu, kemik ve dişlerden Antik DNA çıkarma kiti kullanılarak gerçekleştirildi. Elde edilen genomik DNA'larda, gen-spesifik bölge PCR ile çoğaltıldı ve bu bölgede bulunan polimorfik lokus yüksek çözünürlüklü agar jelinde gözlemlendi. MTHFR mutasyonu, DNA dizi analizine dayalı genotipleme ile incelendi.

**Bulgular:** Üç örnekte MTHFR A1298C mutasyonu ve bir örnekte MTHFR C1298C mutasyonu tespit edildi.

**Sonuç:** Eski DNA'daki MTHFR değişikliklerine bakmak, bilim insanlarının bu gen farklılıklarının insanların hayatta kalmasını nasıl etkilediğini anlamalarına yardımcı olur.

**Anahtar kelimeler:** Arkeogenetik, MTHFR, antik DNA, polimorfizm, stratonikeia.

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## Introduction

### Molecular archaeology and anthropological studies of health

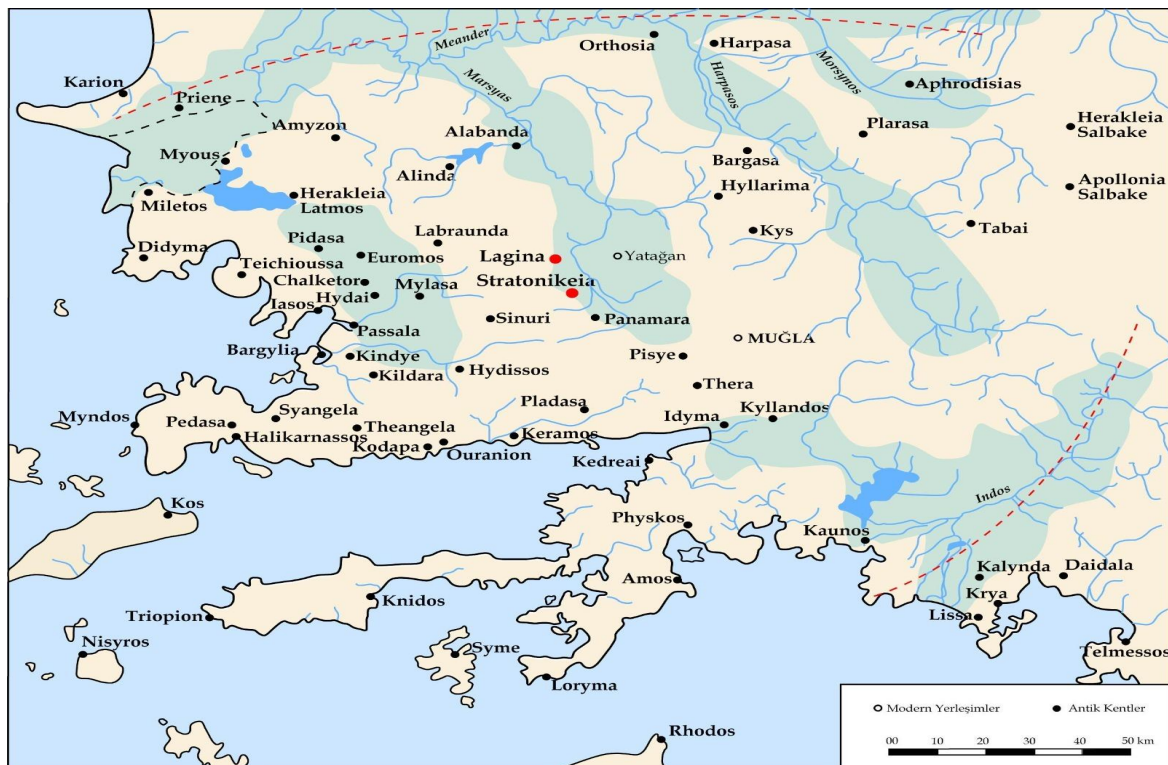
Molecular archaeology is a branch of archaeology that emerged with the advent of DNA science. This developing field focuses on obtaining DNA or mtDNA (mitochondrial DNA) from ancient samples, studying the genetic variations and evolution of the natural archaeological species obtained, and determining the blood type or gender of human or animal remains [1, 2].

The information in the obtained DNA can be used for the reconstruction of life, cultural activities, coexistence, etc., with a unique precision that cannot be achieved by traditional archaeological methods [2]. As technology advances, our knowledge of DNA and our understanding of ancient humans, animals, and plants opens a biological window into their lives [2-4]. Genetic materials can be obtained from biological samples such as skeletons, tissues, hair, tooth remains, and even fossils in some cases [5]. Interestingly, a very small amount of residue is sufficient for DNA testing [5]. The analysis of ancient genetic material offers

archaeology and anthropology some substitute and innovative ways to understand and interpret the past [6, 7]. Molecular archaeology has opened new horizons in answering archaeological and anthropological questions related to human evolution and population and has provided information about the ways of domestication of animal and plant species [5, 6].

### The ancient city of Stratonikeia

The ancient city of Stratonikeia, located in Eskihisar, Yatağan District, Muğla Province, is situated on the northern slope of Kadikulesi Hill and the adjacent plain (Figure 1). Due to this topographical structure, both hillside and plain settlements are observed here [8]. Although findings from this site date back to the 3<sup>rd</sup> millennium BC, according to studies conducted so far, the architectural data related to the settlement consist of the fortification walls on Kadikulesi Hill, dating back to the Archaic Period. In the 4<sup>th</sup> century BC, the old fortification walls were repaired, and a new fortification wall was built extending into the plain, expanding the settlement area and securing a wider area within the city. It is understood that a regular city plan was made during this period of establishing a new and central settlement [8].



**Figure 1.** The ancient city of Stratonikeia

During the Hellenistic and Roman Imperial Periods, the settlement area and city center from the Classical Period continued in the same way, connected to the fortification wall, and many new structures were built during these periods [8]. The Eastern Roman Period settlement continued until the late 12<sup>th</sup> century AD. Based on structures such as the Selçuk Bath and Şaban Ağa Mosque, it is understood that the settlement center shifted to the northwest of the city during the Menteşe Beylik Period. During the Ottoman and Republican Periods, the settlement area from the Menteşe Beylik Period expanded, and more monumental religious and civil structures were built. During this expansion process, a part of the settlement also extended to the outer sections of the northern and western parts of the fortification wall [8]. Today, there is a settlement only within the area enclosed by a fortification wall. As all of this clearly shows, there are remains of settlement texture and urbanization from antiquity to the present day. Thus, we can see ruins and finds from at least nine different periods in Stratonikeia, from the Bronze Age to the Republic period. Fragments of animal and human skeletons from the same periods are also found. The samples we discuss here are samples from approximately the 4<sup>th</sup> century AD and later. These were unearthed in tombs and other excavation sites [8].

The methylenetetrahydrofolate reductase (MTHFR) enzyme is one of the important enzymes involved in folate metabolism, which is at the crossroads of many biochemical pathways such as homocysteine methylation, DNA methylation, nucleotide synthesis, and methionine synthesis [9]. The gene for the MTHFR enzyme is located in chromosome 1 (1p36.3). Human MTHFR cDNA was first isolated in 1994, and it was shown that this cDNA is 2.2 kb in size and consists of 11 exons and 10 introns. The A1298C polymorphism is the conversion of adenine to cytosine (A1298C) at position 1298, which corresponds to exon 7 of the MTHFR gene. This conversion causes the conversion of glutamate to alanine (Glu429Ala) at codon 429 [9-12]. The region where the change occurs corresponds to the carboxy-terminal region of the enzyme and forms the S-adenosylmethionine regulatory region. The glutamate-alanine change here causes disruption of the three-dimensional (3D) conformational structure of the enzyme. In this context, the 1298 CC genotype has been shown

to reduce MTHFR enzyme activity by 40%. The 1298C allele frequency was found to be 17-19% in the Asian population and 27-36% in Western Europe, while its rates in African and South American populations were reported to be low [10, 13, 14].

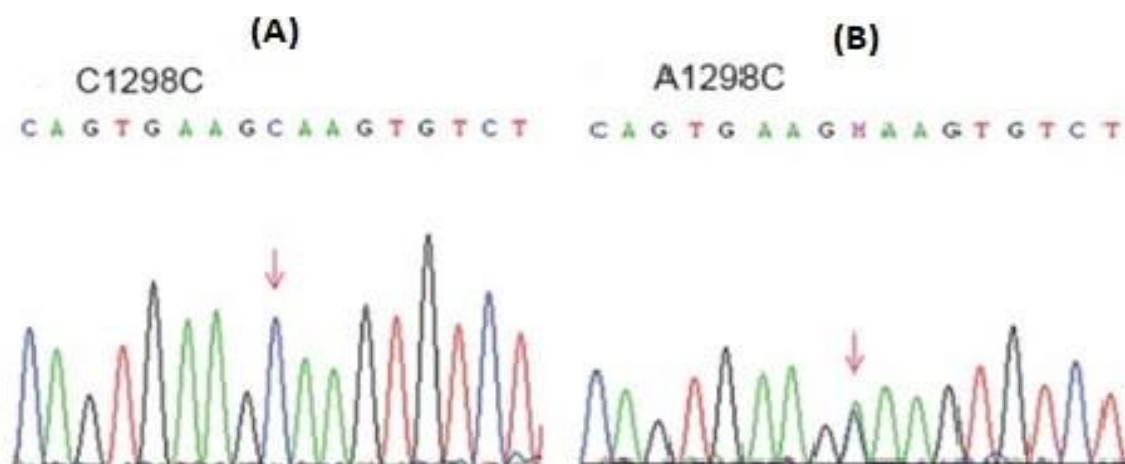
Comparative studies of the DNA of modern populations and ancient DNA allow us to obtain information about ancient times. In this study, we aimed to determine past disease-causing mutations and other gene mutations by performing molecular analyses on samples obtained from the ancient city of Stratonikeia. These studies are considered to be social, archaeological, and evolutionary archaeogenetic studies based on both the detection of disease-causing mutations and the investigation and comparison of polymorphic nucleotide sequences on genes in populations.

## Materials and method

### Genomic DNA isolation

Genomic DNA isolation from petrous bone and teeth samples was performed using the Ancient DNA extraction from bones and teeth kit (Bone DNA Extraction Kit, Promega). These were first placed under UV irradiation to crosslink any surface-contaminating DNA from soil bacteria or handling. To ensure there was no contamination, the teeth material from inside was removed by a dental pick while the surface of the teeth was left intact. Then, the teeth particles (inside) were placed into a sterile tube for extraction. The standard phenol-chloroform DNA extraction method was used. In the obtained genomic DNAs, the gene-specific region was amplified by PCR, and the polymorphic locus located in this region was observed on high-resolution agarose gel, and genotyping was performed based on DNA sequence analysis. MTHFR A1298C primers were Forward primer: 5'-CAAGGAGGAGCTGCTGAAGA-3'; Reverse primer: 5'-CAACTCCAGCATCACT-3' [15].

This study was conducted within the scope of archaeological excavations. Prof. Dr. Bilal Söğüt served as the excavation director, and Prof. Dr. Aylin Köseleler was a member of the excavation team. Since the study was based on archaeological materials and did not involve living human subjects or identifiable personal data, ethical committee approval was not required.



**Figure 2.** DNA sequencing of MTHFR gene (A) MTHFR C1298C mutation, (B) MTHFR A1298C mutation

## Results

We determined MTHFR A1298C mutation in three samples and MTHFR C1298C mutation in one sample (Figure 2).

## Discussion

Scientists have gained a lot of insight into how humans evolved, moved around, and got used to different places by looking at ancient DNA (aDNA) [2, 16]. This aDNA comes from stuff like bones and teeth that archaeologists and paleontologists dig up. It gives us a peek into the genes of things that lived a long time ago. By getting this ancient DNA out and reading it, researchers can now see how ancient groups of people dealt with changes in their environment, sicknesses, and eating different foods. This has made a big difference in what we know about our past. Scientists are interested in looking at changes in the MTHFR gene. This gene plays a big role in breaking down amino acids, which are like the Lego blocks for proteins. It's also super important to help our bodies use folate and make DNA. By studying how common these MTHFR changes were in old populations and what they did, we can learn a lot about their health, how they adapted to different foods, and what kinds of things in their environment might have caused these changes over time.

The MTHFR gene makes an enzyme that's key for turning homocysteine into methionine [17, 18]. This process is super important for DNA methylation and keeping your body working right. But when there are changes in

the MTHFR gene, the enzyme doesn't work as well. This leads to less 5-methyl THF and more 5,10-methylene THF and homocysteine in the blood. The MTHFR enzyme sits at a crossroads where folate, methionine, homocysteine, DNA making, and methylation all meet [9, 18]. So when the gene for this enzyme has changes, it can cause too much homocysteine in the blood, homocystinuria, and problems with folate use. These issues can trigger a bunch of health problems. The main ones are hardening of the arteries and blood clots. But it can also lead to cancer, nerve problems, slow growth, weak muscles, stroke, diabetes, birth defects, not enough blood flow, blood cancer, bad headaches, schizophrenia, feeling down, pregnancy blood pressure problems, Alzheimer's, heart problems at birth, Down syndrome, and cleft palate [19].

Looking at MTHFR changes in ancient DNA helps scientists figure out how these gene differences affected the well-being and survival of early humans. This research gives them a chance to see how our ancestors' bodies dealt with stuff back then.

1. Nutrition Habits: Folate comes from leafy greens, fruits, and beans. It plays a big role in baby-making and growth. Older groups of people who ate lots of these foods faced different pressures on their MTHFR gene than those who didn't eat much folate. Changes in the MTHFR gene might have helped or hurt depending on what people ate.

2. **Health Stuff:** When MTHFR doesn't work well, it can lead to high levels of homocysteine in the blood. This can make heart problems more likely and cause trouble during pregnancy. By looking at ancient DNA, scientists can guess how common these health issues were in the past and how they might have affected people's ability to stay alive and have kids.

3. **Population Dynamics:** The way MTHFR mutations are spread out in old populations gives us a peek into genetic variety and how populations are set up. For instance, some modern groups of people have the A1298C mutation more often. This hints at past pressures that picked certain traits and how people moved around long ago.

DNA studies of populations show different amounts of MTHFR changes over time. The A1298C change has gone up and down, showing how diet, moving, and environment affected people. These studies help track how old Europeans got used to eating farm food with lots of folate compared to their ancestors who hunted and gathered food. Looking at MTHFR changes in ancient remains from native people in the populations gives a special view on how these groups got used to different places and foods. The gene info can also show the health problems these people faced and how they changed to deal with them.

The latest improvements in aDNA extraction and sequencing tech are going to help us understand ancient MTHFR mutations better. In the future, scientists will look into:

1. **Whole Genome Studies:** They will be able to sequence more ancient genomes to get a clear picture of how common MTHFR mutations were and how they affected health in different times and places.

2. **Testing How They Work:** They will be able to do experiments on ancient MTHFR variants to figure out what they did, which will tell us more about how these changes messed with metabolism and health back then.

3. **Mixing with Ancient Site Info:** They will be able to combine gene info with stuff they find at dig sites and info about the environment. This will give us a full view of how ancient people lived, changed, and got better at surviving.

Looking at ancient DNA and MTHFR changes gives us a cool peek into our ancestors' genes. Scientists are figuring out how ancient genomes work, which shows how people's health, ability to adapt, and differences came to be. As we get better at this stuff, the secrets in ancient DNA will tell us more about how humans changed over time and what happened in the past. It's like putting together a big puzzle of human history, but with genes instead of pieces.

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**Conflict of interest:** The authors declare that there is no conflict of interest.

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