Ancient DNA: Cardiovascular Disease Prediction from Mummies

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Abstract

The impact of human health disorders on history has been an area of insufficient study, with genetic evidence pivotal in addressing this dilemma. The detection of genetic evidence of pathogens through ancient DNA in mummies has revealed insights into the presence of cardiovascular diseases (CVD) in ancient populations. This review article provides an extensive analysis of studies focused on ancient DNA analysis for predicting CVD from remains of ancient individuals, particularly mummies from various regions across the world. The introduction section explains the multifactorial nature of CVD and the importance of understanding the hereditary susceptibility to this disease. Notably, genome-wide association studies (GWAS) have identified numerous loci related to atherosclerotic (AS) cardiovascular disease, shedding light on the genetic basis of its pathogenesis. The review further discusses the significance of noninvasive procedures, such as computed tomography (CT) scans, in identifying evidence of AS cardiovascular disease in mummified remains. The subsequent sections delve into specific findings from Egyptian, Peruvian, North American, European, and African mummies, each revealing unique insights into the prevalence and characteristics of CVD in ancient populations. A particular focus is placed on the Tyrolean Iceman, whose well-preserved state has enabled comprehensive genetic analyses, leading to the identification of specific single nucleotide polymorphisms associated with CVD risk. In conclusion, the review highlights the potential for future studies to further explore genetic predispositions to CVD in ancient populations, leveraging advancements in ancient DNA analysis techniques and complete genome sequencing. The challenges and opportunities associated with analyzing DNA from mummies, especially in warmer regions, are acknowledged, emphasizing the need for continued research in this fascinating field. Overall, this review emphasizes the potential of ancient DNA analysis in broadening our understanding of the genetic underpinnings of CVD and its prevalence in ancient populations, contributing to both historical and medical knowledge.

Keywords: Cardiovascular Disease (CVD), Genetic predisposition, Ancient DNA (aDNA), Mummified remains, Polygenic risk score (PRS)

Significance | Understanding CVD's genetic roots through ancient mummies reveals its historical prevalence and informs contemporary health.

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Introduction

Atherosclerosis (AS) is a multifactorial disorder and a leading cause of mortality globally. The inflammation caused by AS leads to alterations in the arterial tunica intima, primarily due to the buildup of fats that transform into plaques. This process can induce thrombosis, reduce blood circulation, and increase the risk of

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myocardial infarction (MI) or cerebrovascular accident (Madaudo et al., 2024). According to epidemiologists, in addition to traditional cardiovascular (CV) risk factors such as obesity, tobacco use, hyperlipidemia, and high blood pressure, approximately half of the risk of AS is inherited. As a result, the analysis of hereditary susceptibility to AS cardiovascular disease (CVD) has become a crucial area of research (Sharifi-Rad et al., 2020; Powell-Wiley et al., 2021).

To identify novel loci and understand the genomic role in AS pathogenesis, four research groups have utilized genome-wide association studies (GWAS). To date, over 1,790 loci related to AS CVD have been discovered, although the majority of single nucleotide polymorphisms (SNPs) appear to have a minimal impact. Susceptibility to AS CVD results from the accumulation of alleles for hereditary diseases, weighted by their impact magnitude, which can be indicated by a weighted polygenic risk score (PRS) (Vollenbrock et al., 2022; Kessler et al., 2016). However, the etiology is not fully understood, and only about 50% of identified SNPs can be linked to a pathological pathway. Research is ongoing, and recently, 95 novel loci have been identified (Park et al., 2019; Owen et al., 2023).

Over the past 15 years, the use of computed tomography (CT) and other noninvasive procedures has led to the discovery of increasing evidence of AS CVD in ancient remains. Mummies from various regions, including America, Greenland, Europe, North Africa, and East Asia, have demonstrated that CVD was present in individuals with diverse living conditions. It is now believed that CVD has been affecting humans for more than 5,000 years (Wurst et al., 2024).

However, it remains unclear whether our ancestors had the same genetic AS CV risk as people do today. Only two mummies have been found to possess certain SNPs related to coronary heart disease (CHD) associated with calcified plaque in blood vessels. These mummies include the Iceman, an Italian glacial mummy from 3350 BC, and a 17th-century mummy from Korea. One reason for the lack of information is that the relationship between genetic makeup and phenotype may only be established in mummified remains (Binder et al., 2023).

In mummified subjects, soft tissue is often preserved well enough to allow for the examination of organs and blood vessels for disorders that do not affect the skeleton. Another reason for the limited information is that most of the existing genetic data from ancient subjects is pseudohaploid due to poor coverage of genotyping. Ancient DNA (aDNA) is often highly fragmented and contaminated, leading to low endogenous DNA content in samples, usually less than 1%, resulting in insufficient sequencing depth (Green & Speller, 2017).

Egyptian mummies

Many cultures throughout history have made mummies. However, in Ancient Egypt, the scale, resources expended, and technological advances in mummification were far superior to others. Some of these mummies have remarkably preserved soft tissues. Whereas embalming techniques in Ancient Egypt changed throughout more than 3,000 years of practice, the heart and arteries are very well preserved in many Egyptian mummies. Computed tomography (CT) scans of the mummies showed a striking level of preservation of the blood vessels, with density consistent with atherosclerotic calcification (AS CAC) indicated in major arteries (Oras et al., 2020).

The Horus Team reported that the CT scans of seventy-six mummified bodies have been systematically checked for coronary artery calcification (CAC). Even though the mummies were in varying states of preservation, cardiovascular disease (CVD) was evident. AS calcifications were found in twenty-nine of the seventy-six bodies. Additionally, CAC was observed in all vessels, including carotid arteries and coronary vessels. The mummified bodies of long-lived subjects demonstrated a higher frequency and gravity of the disease. The patterns of CAC were found to be similar to those of AS disorder in CT scans of modern people (Allam et al., 2011; Thompson et al., 2013).

A CT scan of Princess Ahmose-Merieth-Amon's mummified body revealed particularly severe CAC. In the mummy of Egyptian scribe Hatiay from the Eighteenth Dynasty, severe CACs were found in carotid sinuses and in the superficial femoral arteries (SFAs). Bodies mummified with different methods, from various periods over up to two thousand years, also showed CACs. CT scans confirm that these lesions are indeed AS carotid artery calcifications. AS in mummies was also verified histologically (Thompson et al., 2024).

The diet of the ancient Egyptians has been well studied. Available wall paintings and papyri show that the elite diet included a lot of protein and fat. It was a developed agricultural society with regular consumption of domestic goats, cattle, poultry, fish, and grain crops. This society also had a strict hierarchy. David and colleagues noted that ancient Egyptian priests ate especially well, consuming animal sacrifices, and suggested that these individuals had a higher risk of AS due to their rich diets, similar to how a high-fat diet (HFD) is now considered a cardiovascular risk factor. It is believed that most Egyptian mummified bodies belonged to wealthy individuals (Halawa, 2023). Significant resources were spent on mummification in the final centuries of pharaonic rule in Egypt, with only the richest individuals being mummified using the best available methods. A relative lack of exercise and a HFD could have been widespread among Egyptians whose mummified bodies survive today. Although tobacco use was not yet introduced, the usual level of exercise among common people at the time was likely higher than it is now. Infectious or parasitic chronic inflammation

was likely common and could have promoted atherosclerosis (Madaudo et al., 2024).

Ancient Peruvian Mummies

The Horus Team studied Peruvian mummified bodies and discovered evidence of cardiovascular disease (CVD) in computed tomography scans of fifty-one mummies (200 to 1500 AD) kept in the Puruchuko Museum. In nearly all of these mummies, the level of preservation could not be determined until a computed tomography scan was performed because they were still in bundles. Mummified bodies from Peru are usually preserved in a crouching position (Thompson, Mahadev, Sutherland, & Thomas, 2023). There were very few soft tissues in some of them, as they were poorly preserved; however, atherosclerosis (AS) was not difficult to detect. Some AS coronary artery calcifications (CACs) were detected on computed tomography in thirteen of fifty-one subjects (25%), including: coronary arteries (CA), n = 2 (4%); aorta, n = 7 (14%); distal arteries of the lower extremities, n = 7 (14%); iliofemoral, n = 8 (16%). Similar to Egyptian mummies, these CACs were more severe in subjects with longer life spans, as predicted with AS (Allam et al., 2011; Thompson, Allam, Lombardi, et al., 2013). In mummies from Peru, CACs were found in iliac arteries, abdominal aorta, and vessels of the arch of the aorta, and they are very similar to the AS CACs in modern people. Ancient Peru was as developed as Ancient Egypt. The people of Peru had a hierarchy and a complex farming system. They consumed fish, deer, birds, corn, peppers, potatoes, and cassava. However, the lack of written records makes research into their lifestyle difficult, highlighting the significance of bioarchaeological studies in this area (Sharifi-Rad et al., 2020). Ancient Peruvians typically had adobe houses and cooked outside using fires. It is accepted that they did not use tobacco, as it was unknown to them until the Spanish introduced it in the sixteenth century. However, a new study showed the presence of nicotine in Chilean mummies' hair, leading to the conclusion that these people could have used tobacco (Venter et al., 2001).

North American Mummies

The Horus Team inspected the results of computed tomography scans of ten mummified bodies from North America. Five of these bodies belonged to the ancestors of the Puebloans, who lived approximately a thousand years ago in what is now Utah. The other five belonged to the Unangan people from Alaska. They did not live in ancient times but rather around the nineteenth century, living by hunting and gathering. AS lesions were found in 2 of the Puebloan bodies and three of the five Unangan bodies. One of the Unangan individuals, a woman who lived to the age of 50, had severe CAC (Thompson, Sutherland, Allam, et al., 2024).

The culture of these people was not as developed as that of Egyptian and Peruvian societies. It is believed that the mummified ancestors of the Puebloans lived by hunting and gathering or gathering and farming. They likely did not consume the same food as people today or the elite in Ancient Egypt (Thompson et al., 2024). The Unangan people did not have a developed farming system and lived mostly by hunting and gathering. Their diet included eggs, birds, shellfish, fish, urchins, whales, and seals, with almost no fruits or vegetables. Despite likely not eating foods that would cause atherosclerosis, they could have been adversely affected by considerable smoke from fires, as they lived in underground homes and cooked inside (Sharifi-Rad et al., 2020).

Europe: Tyrolean Iceman

In 1991, tourists discovered a mummy from the Copper Age, dating back to approximately 3350 BC, in the Ötztal Alps. This mummy, often called the Iceman or Ötzi, was carefully inspected at the South Tyrol Museum of Archaeology. The mummified body belonged to a man who lived to around forty or fifty years old. A computed tomography scan of this mummy showed evidence of AS CACs (Thompson et al., 2024). The Iceman's lifestyle has been carefully investigated. His diet included meat from deer and chamois, herbal bread, barley, blackthorn, wild berries, and wheat bran. His bowels also contained pollens, indicating that wheat might have been cultivated. This man was an omnivore, and his diet was very diverse (Nerlich, Egarter Vigl, Fleckinger, Tauber, & Peschel, 2021).

Since the Iceman was discovered high in the mountains, it is concluded that he engaged in regular exercise, supported by the computed tomography scan findings showing a skeleton structure indicative of this (Ruff et al., 2006). The Iceman's diet did not imply a higher risk of AS; thus, the genomic assessment of this mummified body is especially promising. Genetic analysis revealed several SNPs associated with coronary heart disease (CHD) and AS. For example, he was homozygous for the rs10757274 and rs2383206 minor alleles, important loci for CHD (Wang et al., 2023). Additionally, the ET-B heterozygote variant rs5351, a male AS risk factor, was identified, along with SNPs in CHD-related genes such as vitamin D receptor, T-box 5, and bradyb1 (González Rojo et al., 2022; Tomei et al., 2020).

Notably, the Iceman's lungs showed blackening from soot, suggesting smoke inhalation. He also had a threadworm infection along with several other diseases, which left marks on his nails. The Horus team had already suggested that smoke could lead to atherosclerosis and that parasitic and other infections could cause inflammation promoting the development of AS. These risk factors were indeed found in the Iceman (Rawla & Sharma, 2023).

DNA studies

Shortly after the Iceman was discovered, the first genetic analyses of his mtDNA and hypervariable region (HVR) began. In subsequent years, the entire mitochondrial genome was thoroughly investigated. A group of researchers examined a sample of the Iceman's bone to study the nuclear genome (Wang et al., 2023). The

sequencing identified approximately forty percent of the reads that mapped uniquely to the reference, covering more than eighty percent of the human genome in total. Comparison with the previously identified mtDNA confirmed the authenticity of the Iceman's DNA (Venter et al., 2001).

These studies provided valuable insights into the Iceman's origin and health. For instance, it was discovered that his eyes were brown, contrary to earlier beliefs that they were blue. Additionally, the Iceman was found to suffer from lactose intolerance, a genetic marker associated with the development of European farming culture (Keller et al., 2012). There is also evidence suggesting that the Iceman and modern people from the Tyrrhenian Sea area share a common origin. Genetic research revealed risk factors in the Iceman's DNA, including single nucleotide polymorphisms (SNPs) related to health disorders. Notably, the Iceman was highly susceptible to coronary heart disease (CHD) (Sharifi-Rad et al., 2020). This is significant because a computed tomography scan of the Iceman showed coronary artery calcification (CAC) in the coronary arteries, iliac artery, and distal aorta, all indicative of atherosclerosis (AS). Genetic predisposition may have exacerbated the progression of CAC. Other cardiovascular risk factors like obesity, tobacco use, lack of exercise, and high-fat diet (HFD) were likely absent, suggesting that the Iceman had a toned body, sufficient exercise, and a balanced diet (Powell-Wiley et al., 2021). Although tobacco was not known at the time, the blackening of his lungs implies exposure to smoke from fires. It is currently unknown if fire smoke has effects similar to tobacco on AS cardiovascular disease (CVD), though animal trials suggest a relationship between wildfire exposure and AS progression (Mallah et al., 2023).

In genome-wide association studies (GWAS) on the Iceman, researchers identified homozygosity for the rs10757274 minor allele at the chr9p21 locus, a SNP considered a significant indicator of myocardial infarction (MI) and CHD risk. This SNP alone was found to increase CHD risk by forty percent (Aleyasin et al., 2017; Nawaz et al., 2015). Meta-analysis of six cohort studies further indicated that the rs10757274 SNP is a crucial risk factor for acute ischemic stroke (AIS) and sudden cardiac death (SCD). The Iceman's genome also showed homozygosity for the rs2383206 minor allele at the chr9p21 locus, another CHD risk factor. The presence of both chr9p21 SNPs nearly doubles the risk of CHD. Additionally, the Iceman's genome contained the ET-B heterozygote variant rs5351 on chromosome 13, which has been associated with AS risk (Xu et al., 2020). Other SNPs related to CHD included mutations in the vitamin D receptor, T-box 5, and bradyb1 genes (Zaharan et al., 2018; Shimizu et al., 2018). Keller and colleagues (2012) identified new mutations in these genes that induce alterations in their respective stop codons.

African

In 2010, an intact skeleton (UCT 606) was discovered in Saint Helena Bay. The remains belonged to a man of no more than 150 cm in height, who likely died around the age of fifty or older due to worn teeth and considerable osteoarthritis (OA) (Oras et al., 2020). The absence of cavities and the wear on dental tissue suggest a diet based on hunting and gathering. A pathological bone growth in the right internal acoustic canal indicated a disorder known as "surfer's ear," suggesting significant time spent in cold waters (Dewar et al., 2020). Radiocarbon (14C) and stable carbon-13 (13C) isotope analysis of the rib showed that the man lived approximately 2305–2355 years ago (sample ID: UGAMS 7255), with a δ13C value of −14.6‰. A large number of shells in the grave suggests a maritime lifestyle. Dewar and colleagues (2020) reported that the calibrated date, adjusted for a 52.5% sea diet, lies between 2241 and 1965 years ago. This timeframe coincides with the introduction of cattle breeding in Western Cape; however, archaeological evidence indicates that this man lived before this introduction (Binder et al., 2023).

DNA extraction from an internal canal of one tooth and a rib was successful. Paired-end sequencing of mitochondrial genomes with the Illumina GAIIx system provided a coverage of 103.1 for the tooth and 20.8 for the rib, with no mitochondrial DNA covered by fewer than 2 reads (Green & Speller, 2017). The consensus sequences from both tooth and rib were identical. Krause, Briggs, and colleagues evaluated contamination with modern mitochondrial DNA, finding zero out of 391 sequences from the rib and four out of 1678 from the tooth matched modern DNA, indicating contamination rates of 0.6% and 1%, respectively (Oras et al., 2020; Pääbo et al., 2015). Additional studies confirmed DNA integrity through misincorporated nucleotides indicative of ancient DNA (Briggs et al., 2009; Krause et al., 2010). The mean DNA fragment lengths were at the minimum reported range for ancient remains, confirming the authenticity of the samples (Briggs et al., 2009; Krause et al., 2010; Krause et al., 2010).

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The ancient mitochondrial DNA was combined with 525 published genomes of lineage and regional significance. As reported by Schuster et al. (2010), Barbieri et al. (2013), and van Oven and Kayser (2009), the dataset includes 491 L0d/L0k haplogroup donor-specific, twenty-six L0a, and 7 L0f mitochondrial DNAs, linked to the revised Cambridge Reference Sequence (CRS) (Schuster et al., 2010; Barbieri et al., 2013; van Oven & Kayser, 2009). Phylogenetic inference, according to Phylotree Build 16, indicated that the Saint Helena skeleton belonged to the L0d2c haplogroup, particularly L0d2c1, based on genome identity and comparison with twelve known L0d2c1 genomes. Mitochondrial DNA from the Saint Helena skeleton is strongly related to two mitochondrial DNAs of Ju-speaking !Xun people, constituting a novel subclade L0d2c1c (defined by C16355T and C10822A), which apparently arose independently of L0d2c1a and L0d2c1b (Schuster et al., 2010). The order of appearance for L0d2c1 is uncertain due to its size, although general results align with mitochondrial DNA comparisons and phylogenetic analysis with a limited coding region (Barbieri et al., 2013). In contrast to the prevalence of L0d2c1c in Ju, Khoe populations represented L0d2c1a and L0d2c1b. In this novel lineage, nine unique variants distinguish the ancient mitochondrial DNA from two modern !Xun mitochondrial DNAs, such as A16399C, A2581G, A11884G, A4824G, T408A, C11431T, C16261T, T16086C, and C11279T, while modern genomes differ at only one site, G3591A (van Oven & Kayser, 2009).

Studies conducted by Weaver et al. (2012), Li et al. (2008), and Henn et al. (2011) demonstrate that while the exact origins of early

modern humans remain to be fully explored, researchers agree that modern humans originated in Africa, with the most genetic diversity observed in southern Africa (Weaver et al., 2012; Li et al., 2008; Henn et al., 2011). Results from Schuster et al. (2010) and Gronau et al. (2011) established that the first complete genome sequence of an ancient Khoesan subject revealed this diversity and assessed divergence as 157-108 ka. This research generated the first whole mitochondrial DNA sequence from an ancient Khoesan subject, using osteological and archaeological evidence to affirm the presence of maternal lineages in South African sea hunter-gatherers before the introduction of cattle breeding in the region (Schuster et al., 2010; Gronau et al., 2011). This suggests that sequencing South African archaeological data could reveal more genomic diversity.

Future studies of DNA in ancient mummies

To date, only the Iceman has been identified as an ancient individual genetically predisposed to cardiovascular disease (CVD). Despite the increasing number of complete genome studies, no additional single nucleotide polymorphism (SNP) studies on CVD risk factors have been conducted. Furthermore, it is challenging to compare bone samples with phenotypes due to the degradation of soft tissues. The Iceman's preserved state offers a unique opportunity for interdisciplinary research on genetic makeup and phenotype in relation to CVD (Thompson et al., 2024).

Horus Team trials have demonstrated a significant frequency of coronary artery calcification (CAC) in mummified bodies from various sites and time periods. Future research will involve detailed SNP analysis of CVD risk factors and the identification of previously unknown gene polymorphisms that may be absent in modern humans. Successful research requires well-preserved DNA, which is likely due to the Iceman's low-temperature preservation. Mummified bodies from warmer regions tend to have degraded DNA, complicating SNP analysis. However, the rapid drying and embalming of mummies in ancient Egypt may have slowed DNA degradation, leading some researchers to speculate about the potential for complete genome sequencing from Egyptian mummified bodies (Binder et al., 2023; Oras et al., 2020; Allam et al., 2011).

Conclusion

The analysis of ancient DNA from mummies has significantly contributed to our understanding of cardiovascular disease (CVD) in ancient populations. The multifaceted nature of CVD, involving genetic and environmental factors, has been underscored through the examination of mummified remains from diverse geographical regions and time periods. The genetic susceptibility to CVD, as evidenced by specific gene variants identified in mummies, provides compelling insights into the ancient roots of this disease. Moreover, the study of the Tyrolean Iceman's genome has offered valuable information regarding the genetic predisposition to atherosclerosis and other CVD-related conditions.

Despite the challenges associated with ancient DNA analysis, the discoveries made thus far warrant continued exploration of mummies to unravel further clues about ancient populations' cardiovascular health. Future research endeavors aimed at elucidating the molecular mechanisms underlying CVD in antiquity have the potential to shed light on the interplay between genetic predisposition and lifestyle factors, thereby offering valuable perspectives for contemporary cardiovascular health. Ultimately, the investigation of ancient DNA in mummies serves as a poignant reminder of the enduring relevance of studying the health of past civilizations and its implications for our understanding of human health today.

Author contributions

A.V.P. prepared the original draft of the manuscript, while V.N.S., T.V.K., T.I.K., I.A.S., D.F.B., A.N.O., and A.V.C. contributed to the writing, review, and editing. All authors have read and approved the final version of the manuscript for publication.

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Competing financial interests

The authors have no conflict of interest.

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