FROM TUTANKHAMUN TO ÖTZI: THE USE OF MODERN SCIENTIFIC METHODS IN MUMMY RESEARCH

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INTRODUCTION

The interest in mummies in Europe dates back to the 13th-16th century, when the drug mumia, Egyptian mummy material broken into pieces or pulverized, became a widely used medicine. The beginning of the scientific study of mummies can be closely linked with the work of Thomas Joseph Pettigrew, who documented and described a detailed unwrapping of an Egyptian mummy for the first time (Pettigrew, 1834). In recent times, mummy research has significantly changed from a more descriptive approach to a modern scientific discipline, in which methods from various different technologies from the fields of medicine, molecular biology, microbiology, chemistry and physics are applied to mummies and mummified tissues (Aufderheide, 2003).

The different methods are now widely used to study mummies from all over the world and they allow the reconstruction of living conditions, including dietary conditions and diseases, geographical origin, as well as information on their ancestry and genetic family relationships (Lynnerup, 2007). In several studies molecular evidence for the presence of infectious diseases in ancient populations was achieved which has provided deep insights into the evolution of such diseases (Zink et al., 2003). Moreover, the preservation of soft tissues in mummies has also permitted a broader study of cancer (David and Zimmerman, 2010) and vascular diseases (Allam et al., 2011) in ancient times. By the use of a nanotechnological approach, new insights into the preservation of collagen in skin samples and the mummification process of natural mummies were achieved (Strasser et al., 2006, Janko et al., 2010). Medical imaging techniques, such as radiology, computed tomography or magnetic resonance imaging are widely used in the scientific study of mummies, as they permit a non-invasive examination of ancient human remains (Panzer et al., 2010, Papageorgopoulou et al., 2010, Rühli et al., 2007, Shin et al., 2010).

In this work, the latest results of our study of the Egyptian king Tutankhamun and the Tyrolean Iceman, commonly known as "Ötzi", are presented. The new findings are based on the use of modern scientific technologies and demonstrate the possibilities of their application.

LIFE AND DEATH OF TUTANKHAMUN AND THE ROYAL FAMILY

One of the greatest remaining unsolved mysteries in Egyptology is the genealogy of the famed pharaoh Tutankhamun (Carter, 1927). In 1922, Howard Carter uncovered the almost undisturbed tomb and the royal mummy of this nineteen-year-old boy from the late 18th dynasty, now popularly known as King Tut (Fig. 1). This burial trove remains one of the most remarkable discoveries in Egyptology to date, capturing the public imagination in an unprecedented way, and Tutankhamun's life, 3300 years

Figure 1: The mummified head of Tutankhamun, located in his tomb in the Valley of the Kings, Luxor.

ago, continues to be a subject of fascination. However, despite the wealth of artifacts found, the tomb contained very little information about Tutankhamun's origins and family. Some names of key figures from the period did appear amongst the artifacts, but no one inscription definitively tells us who the pharaoh's parents were. Furthermore, few other mummies from the Amarna period have been positively identified. Many Egyptologists believe that Tutankhamun was born to the pharaoh Akhenaton and his great royal wife Nefertiti, or his second wife Kiya, but even these claims are highly debated.

On Tut's paternal side, most Egyptologists turn to the skeletonized mummy found in tomb KV55, considered by many to be the mummy of Akhenaton (Baker, 2008). There is much archaeological evidence to support this assertion, although previous anthropological studies identified this mummy as a man in his early twenties, leading to the assumption that he could be the enigmatic Smenkhkare (Harrison, 1966). Little is known about this pharaoh other than that he appears to have ruled for a brief period around the time of Akhenaton's death. With both identifications, this mysterious KV55 mummy is a good candidate for Tutankhamun's father.

The mother of Tutankhamun may be one of the two female mummies found in tomb KV35 in the Valley of the Kings, Luxor (Fig. 2a, b). These two mummies were named according to their respective ages at death, the Younger Lady and the Elder Lady (Harris and Wente, 1980). Besides the style of their mummification, which is consistent with royal females of the 18th Dynasty, there is no clear evidence about the identity of these mummies. However, the Younger Lady mummy has been claimed at times to be Nefertiti or Kiya, either of Akhenaton's two wives, which makes her eligible to be Tutankhamun's mother. Some scholars also believed that the beautifully preserved Elder Lady may be the mummy of Nefertiti, or of Queen Tiye, the royal wife of the

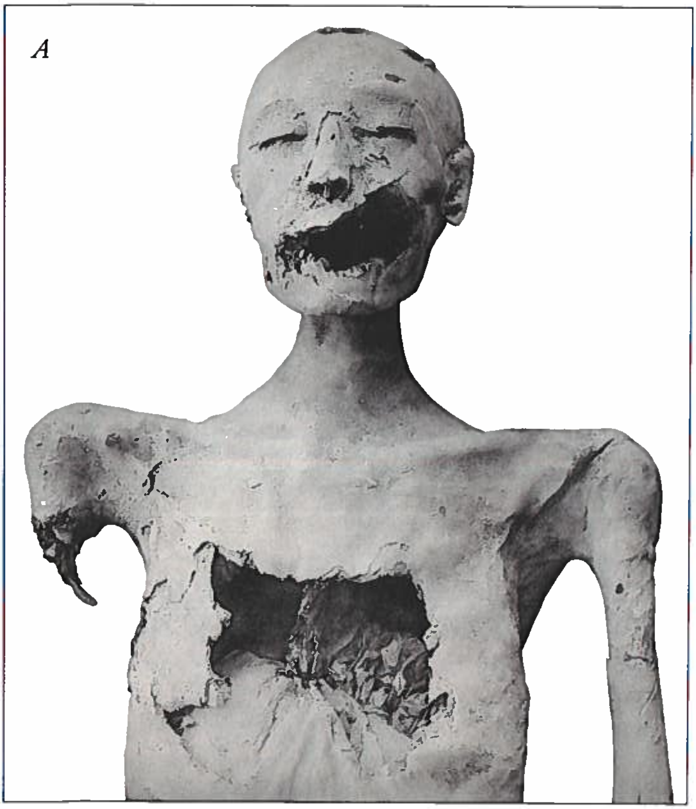
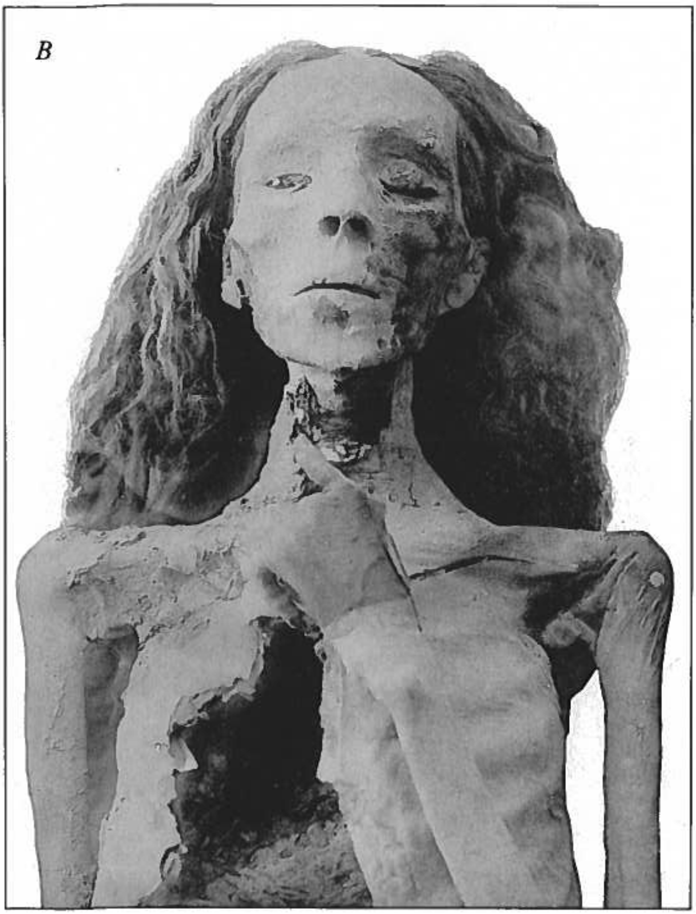


Figure 2: Two female mummies, Younger Lady (A) and Elder Lady (B), found in tomb KV35 in the Valley of the Kings, Luxor.

long ruling pharaoh Amenhotep III (Fig. 3) and the mother of Akhenaton.

Apart from Tutankhamun himself, the only mummies whose identity is 100% certain and who are known to be members of King Tut's same family group, are the mummies of his putative paternal great-grandparents, Thuya and Yuya. These two nobles



were also found in an almost undisturbed tomb in the Valley of the Kings, clearly identified, and provide a good control group for genetic analyses. To further investigate Tutankhamun's family tree, the two foetuses found in his tomb were also examined, and the two female mummies from the tomb of KV21, claimed to be possibly his children and his wife respectively.

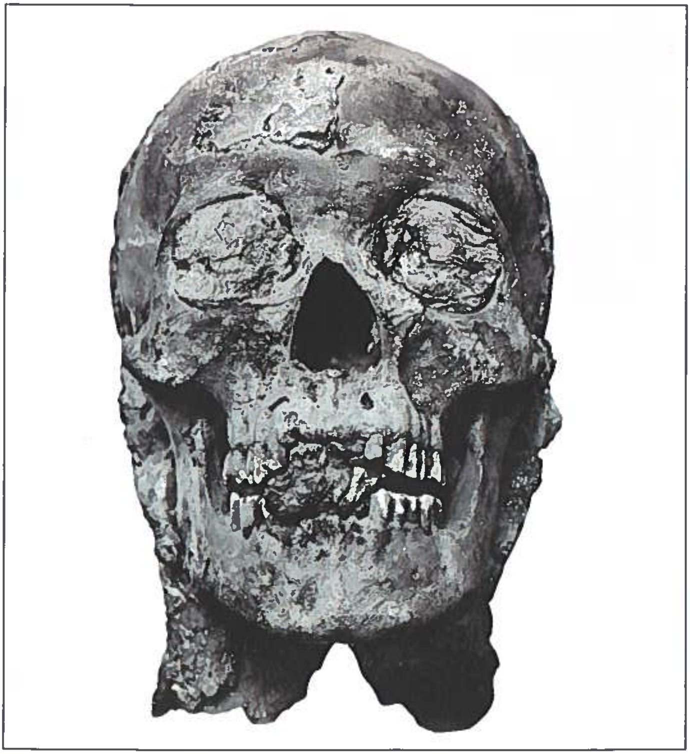


Figure 3: Mummified head of Amenhotep III.

The aim of this project was to perform a multidisciplinary approach (i.e., by using means of genetic, archaeological, anthropological and Egyptological research) in order to determine the degree of kinship between King Tutankhamun and his putative family members. As most of the archaeological and Egyptological data are still subject to debate, we established thorough genetic fingerprints of King Tutankhamun and his putative family members, i.e. the mummies of Yuya and Thuya, Amenhotep III, KV35 Elder Lady, KV35 Younger Lady, KV55, both foetuses found in the tomb of KV62, and the two mummies A and B from KV21. By conducting such a detailed ancient DNA study, we identified the mummies' origins and shed light on King Tutankhamun's family bonds.

Eleven mummies of the 18th Dynasty (c. 1550-1295 BC) of the New Kingdom (c. 1570-1070 BC) underwent a detailed anthropological and radiological study in order to determine the preservation status, the individual's age and sex and also to reveal any evidence for diseases or the cause of death. For the radiological analysis, the mummies were scanned using a multidetector CT unit "emotion 6" by Siemens medical system. This information was further used to identify the exact locations to take tissue samples for later DNA extraction in the laboratories. Subsequently, the mummies were sampled by taking small bone punch biopsies from at least four different areas of the corpses. The samples were stored in sterile tubes and transferred to dedicated ancient DNA laboratories for further processing. The ancient DNA extractions of the bone samples and all further analytical steps, such as, for example, PCR amplification, cloning and sequencing, were all performed according to strict and widely accepted guidelines (Richards et al., 1995). Along with these precautions, detailed contamination monitoring protocols for the PCR experiments were included in the research (mock and negative controls, separated working areas etc.). Moreover, all laboratory staff involved were tested for their Y-chromosomal and autosomal markers. For the authentication of the results all analytical steps were repeated at least five times; in addition, a subset of the data was independently replicated in a newly equipped lab exclusively dedicated to ancient DNA work.

The analyses of the ancient Egyptian mummy samples included laborious optimization strategies by applying several different DNA extraction and purification protocols. Inhibition PCR experiments were performed in a third lab located at the National Research Centre Cairo to titrate for the proper amount of amplifiable ancient DNA. After the successful extraction of DNA

we performed an intensive genetic testing of nuclear DNA loci including sixteen Y-chromosomal (AMPFLSTR Yfiler PCR Amplification kit, Applied Biosystems) as well as eight autosomal microsatellite markers (AMPFLSTR Minfiler PCR Amplification kit, Applied Biosystems).
In order to test for Plasmodium falciparum DNA, PCR primers were designed that specifically amplify small Stevor, Ama1, and Msp1 gene fragments, thereby yielding amplicons in the range of c. 100-250 bp. PCR products and cloned DNA fragments were sequenced (Fig. 4).

Kinship analyses

The optimization protocols for extraction and purification of DNA, PCR amplification, sequencing and fragment length analyses yielded results for all mummies under investigation. The genetic data clearly identified the mummies of KV35 Elder Lady, and KV55. Our results –in conjunction with archaeological data-

Figure 4: DNA data analysis performed by the German-Egyptian team in the ancient DNA laboratory in Cairo, Egypt.

provide substantial evidence that the mummy found in KV55 is indeed Akhenaton, and that the KV35 Elder Lady is King Tut's paternal grandmother, Queen Tiye. Moreover, the KV55 mummy and the KV35 Younger Lady mummy can be safely interpreted as the father and mother of King Tut. This is demonstrated by the following results. The established Y-chromosomal profiles show identical patterns in Amenhotep III, KV 55 and Tutankhamun. This provides evidence that these individuals share the same paternal lineage. Control mummies examined along with King Tut's putative family members yielded different Y-specific alleles. Fine analysis of the genetic relationship between the mummies was achieved by a genetic fingerprint typing exploring autosomal alleles. We obtained complete fingerprint profiles of all individuals except for one of the KV62 foetuses and both mummies from KV21, who yielded partial data sets. By evaluating the segregation of alleles through the familial generations we reconstructed the most plausible royal pedigree, a five-generation kindred (Fig. 5). Yuya and Thuya are the parents of the KV35 Elder Lady indicating that she is most likely Queen Tiye, the royal wife of Amenhotep III. Both Amenhotep III and the now identified Queen Tiye are the parents of the mummy found in KV55 and also the Younger Lady found in KV35. Anthropological and radiological analysis of the KV55 mummy showed that he was much older than previously assumed, which provided evidence that this mummy could be the pharaoh Akhenaton, and not Smenkhare. Further support for this claim was found on the sarcophagus of KV55; gold sheets that were once attached to the lid of the coffin identify the pharaoh Akhenaton, the sun god. The proof that Amenhotep III and Queen Tiye are the parents of KV55, combined with this anthropological and archaeological evidence, clearly indicates that the mummy in KV55 is Akhenaton.

The KV35 Younger Lady could be Nefertiti or Kiya, or possibly the sister of Akhenaton, who would have been the right age to give birth to Tutankhamun. Specifically, we should also mention the eldest sister of Akhenaton, Sitamun, as a possible identity for

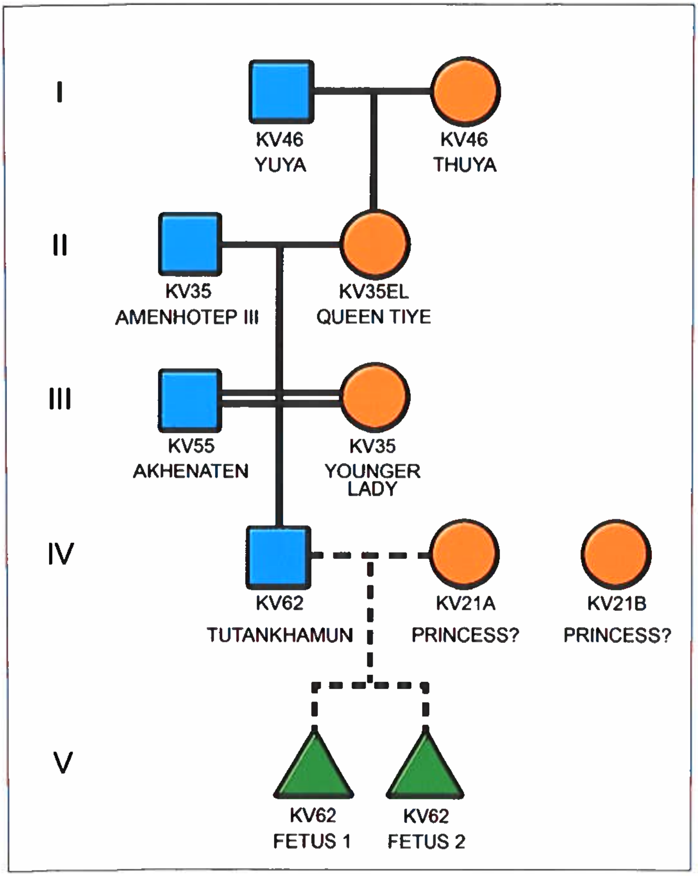


Figure 5: Pedigree showing the genetic relationships of the tested 18th Dynasty mummies. Quadrants define males, circles illustrate females, and triangles stand for still-birth. A double line represents an interfamilial marriage (here it is a 1st degree brother-sister relationship). Dotted lines indicate insufficient data, thus, the relationship is meant to be a proposal. Note that foetus 1 and foetus 2 may be daughters of Tutankhamun, however, the mother is not known to date. The few data obtained from KV21A are not sufficient to define her as the wife of the boy pharaoh, Ankhensenamun,

KV35 Younger Lady. Daughter of Amenhotep III and Queen Tiye, Sitamun was the most well known offspring, and could also be Tutankhamun's mother. Consequently Akhenaton and KV35 Younger Lady are the parents of Tutankhamun. Additionally, Tutankhamun might be the father of at least one of the foetuses found in KV62.

Gynecomastia and Syndromes

The most prominent feature exemplified by the El Amarna art of Akhenaton and to a lesser degree in Tutankhamun is their feminine appearance in some of the busts and statuettes (Fig. 6a, b), reasonably suggesting some form of gynecomastia as the underlying disease (Paulshock, 1980).

However, an examination of the presence of putative breasts in the mummies of Tutankhamun and his father Akhenaton (KV55) is impossible, because KV55 is a mummified skeleton with no soft tissue remains and the entire frontal part of the chest wall including ribs in Tutankhamun is missing. Although the pelvic bones of Tutankhamun are absent, the pelvis of KV55 is present but fragmented and does not show any feminine features. We cannot therefore support a diagnosis pointing to any form of gynecomastia or femininity.

It has also been suggested by some authors that Akhenaton and other family members may have suffered from Marfan syndrome (Braverman et al., 2009). A criterion for Marfan syndrome is the presence of dolichocephaly (Pyeritz and McKusick, 1979) which we tested in our mummies by establishing the cephalic indices for 15 mummies. Many scholars believe that dolichocephaly, a condition in which one has an abnormally long head, is present in individuals of the 18th Dynasty. Dolichocephaly is also a feature that is quite frequently seen in busts and statuettes of the El Amarna period (e.g., Nefertiti, Akhenaton, Tutankhamun). Technically, dolichocephaly is defined as a skull with a cephalic index (CI) of 75 or less.

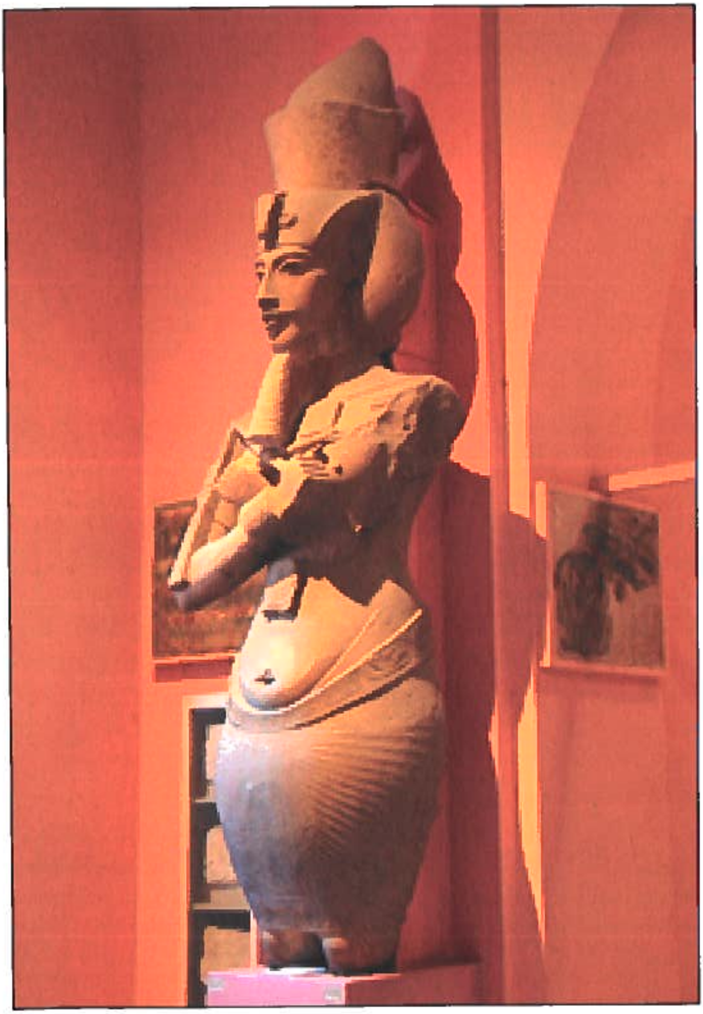


Figure 6a: Examples of El Amarna art (ca. 1351-1334 BC) showing Akhenaton. JE49529, colossus of Akhenaton, Karnak, Precinct of the Aten, 18th Dynasty, reign of Akhenaton. The king wears a pleated kilt which hangs low on a swollen belly and wears a double crown that symbolises dominion over Upper and Lower Egypt..

Figure 6b: JE49528, colossus statue that once lined a colonnade in the Precinct of the Aten at Karnak temple, 18th Dynasty, reign of Akhenaton.

With the exception of Yuya (CI = 70.3), it resulted that none of the mummies of the Tutankhamun lineage satisfies the criterion for dolichocephaly. Alternatively, Akhenaton has a CI of 81.0 and Tutankhamun has a CI of 83.9, which defines these skulls as brachycephalic.

The diagnosis of Marfan syndrome is based on a combination of the major and minor clinical features (De Paepe et al., 1996). The presence of either two major features or one minor feature, or of one major feature and four minor features supports a diagnosis of Marfan syndrome. Following this classification, we could not find evidence to strengthen a Marfan diagnosis.

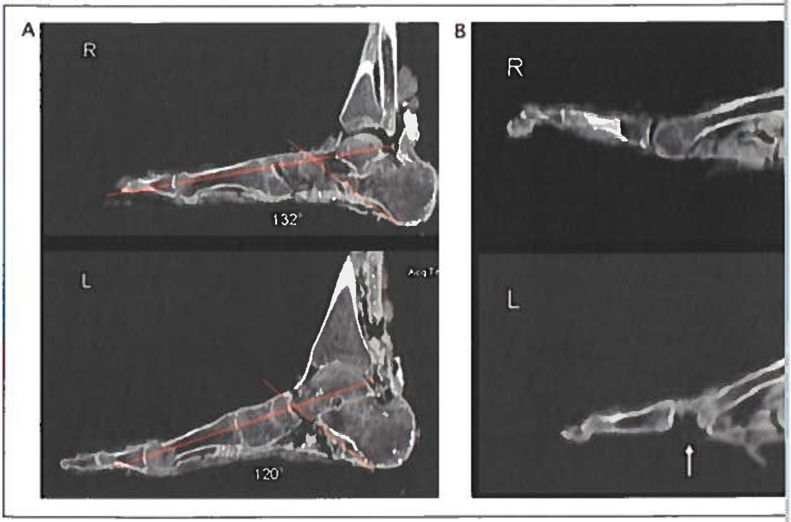


Figure 7: Feet pathology in Tutankhamun.
A) Axial CT cross section with sagittal CT reconstruction of the feet. The right foot arch is flat compared to the left displaying features of a flat foot.
B) Axial CT reconstruction of the second metatarsal of the right and left foot: The second metatarsal bone head shows evidence of bone destruction with loss of bone substance and soft tissue. The second toe

Radiological Findings

Although previous X-ray analyses revealed much about the life of the pharaoh, they also left many questions open and left room for speculation over the years. The actual investigation was designed to confirm or refute the conclusions of previous examinations and focused on details that earlier studies may have overlooked. We specifically searched for life-threatening features that might be discussed in connection with a cause of death or that could have directly caused the king's death. Inspection of the entire body did not reveal any new information, except for the detailed examination of the king's feet.

of the left foot lacks the second phalanx (oligodactyly). The right foot is without pathological findings.
C) CT reconstruction of both forefeet: The right foot shows no pathological findings. The second toe of the left foot misses the second phalanx (oligodactyly). This toe is anteriorly displaced. The ungual phalanx is subluxated, the first toe is splayed. The bone necrosis of the second metatarsal head can be unambiguously identified.

The right foot has a low arch (angle after Rocher: 132°, normal value 126°). There are no pathological findings on the bone structure of the right foot. The tarsal, metatarsal and phalanges bones are completely preserved (Fig. 7).

The medial longitudinal arch of the left foot was slightly higher than normal (angle after Rocher: 120°), with the forefoot in supine and inwardly rotated position akin to an equinovarus foot deformity. Despite the evidence of significant bone degradation, the second metatarsal head displayed a distinctly altered structure with areas of increased and decreased bone density. On the plantar surface there is a crater-shaped bone and a soft tissue defect in that area. The study further showed a widening of the metatarsal-phalangeal joint space with a normal articulating surface of the first phalanx. The third metatarsal head was only slightly deformed, the bony structure, however, showed signs that resemble bone necrosis. The remaining metatarsal heads of the left foot appeared to be of normal structure. The second and third toes on the left foot are in abduction. The second toe is shortened because it lacks the second phalanx. The first phalanx directly articulates with the ungual phalanx. These findings show that the boy pharaoh suffered from a juvenile aseptic bone necrosis of the second and third metatarsal bone of his left foot (Köhler's disease II, Freiberg-Köhler syndrome). A widening of the metatarsal-phalangeal joint space as well as secondary changes of the second and third metatarsal heads indicate that the disease was still flourishing. Bone and soft tissue loss at the second metatarsal-phalangeal articulation could further indicate that an acute inflammatory condition on the basis of an ulcerative osteoarthritis and osteomyelitis was apparent.

There is some clear evidence that the pharaoh may have suffered from the impairment for quite some time. The walking disability caused by the syndrome can be substantially aided by the use of a cane (Fig. 8). Howard Carter discovered 130 sticks and staves, counting both whole and partial examples, in the king's tomb. There is a very interesting inscription on one of these staves, which records that it was cut by the king himself from a bed of reeds, during a visit to a temple.

Infectious Diseases

We considered different kinds of life-threatening diseases as the cause of death in the 18th Dynasty individuals, since the several macroscopic inspections, X-ray and CT examinations in the past did not yield conclusive data. We successfully identified DNA of Plasmodium falciparum in the mummies of Tutankhamun, Yuya and Thuya. Since we applied primers that are highly specific for the P. falciparum genome, we may safely conclude that our positively typed mummies suffered from malaria tropica, the most severe form of malaria.

Cause of Death

King Tutankhamun suffered from multiple disorders, and some of them might have reached the cumulative character of an inflammatory, immune-suppressive thus weakening syndrome. We may envisage a scenario with a young but frail king who needed canes to walk safely because of the bone-necrotic and sometimes painful Köhler's disease II, plus oligodactyly in the right foot and the clubfoot on the left. A sudden leg fracture possibly introduced by a tumble (perhaps as a consequence of the obvious walking impairment) resulted in a life-threatening condition when a malaria tropica infection became additionally apparent.

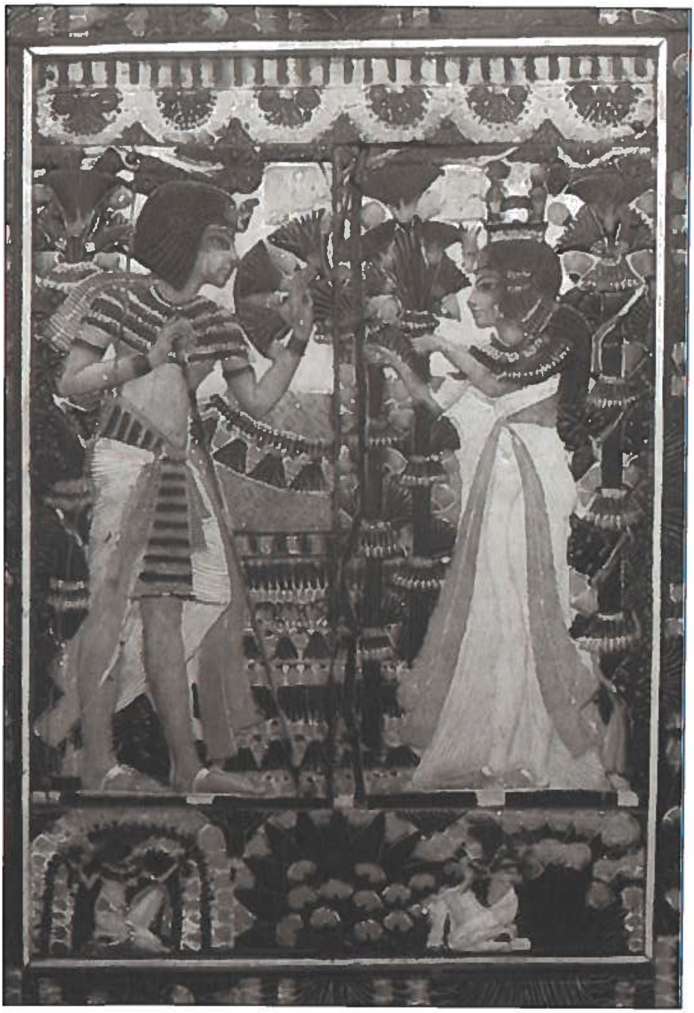


Figure 8: Private illustration showing the king essentially resting upon a cane while he is accompanied by his wife Ankhensenamun. Relief in KV62

NEW INSIGHTS INTO THE LIFE AND DEATH OF THE ICEMAN

On 19th September 1991, the naturally mummified body of the Iceman, also referred to as "Ötzi", was discovered by two hikers in the Ötztal Alps at an altitude of 3210 m. The glacier mummy lived around 3300 B.C. and died at an age of approximately 46 years in the high alpine area. The Iceman is now kept together with his well-preserved clothing and equipment at the Archaeological Museum in Bolzano (Fig. 9). Since his discovery in 1991,

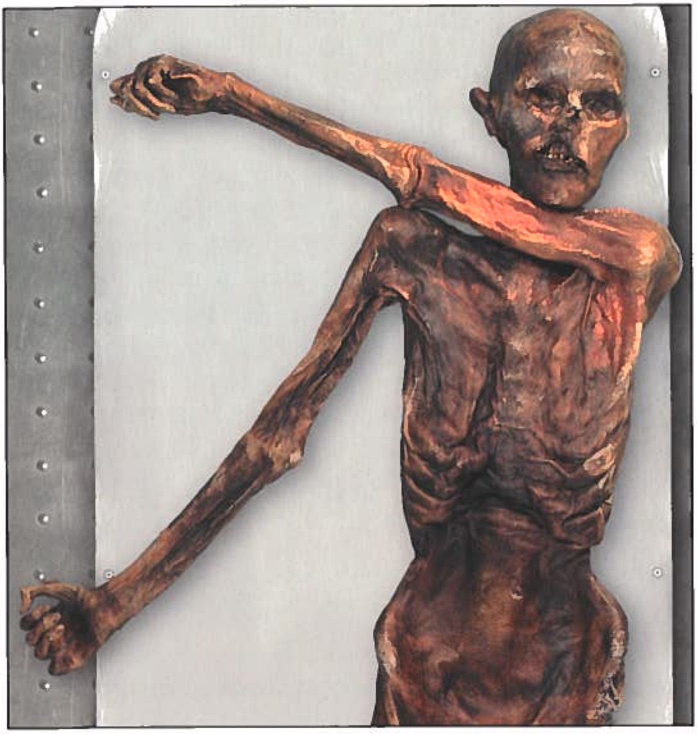


Figure 9: The neolithic glacier mummy from the Ötztal Alps in the laboratory cell of the South Tyrolean Archaeology Museum.

the mummy has been intensively studied and many articles, including overviews and detailed reports, have been published (Barfield et al 1992, zur Nedden und Wicke 1992, Spindler 2000). Nevertheless, the research still continues in various fields, such as archaeology, prehistory, anthropology and medicine. In the past, a variety of methods was applied for the study of the glacier mummy, including radiology and computer tomography (Murphy et al., 2003) of his body, histology (Nerlich et al., 2003), isotope analysis (Müller et al. 2003), palaeobotany (Oeggl et al., 2007) and genetic analysis of his mitochondrial DNA (Ermini et al., 2008).

In 2007, the EURAC-Institute for Mummies and the Iceman was founded and all available scientific data on the Iceman was collected and critically reviewed. The Institute furthermore developed scientific studies related to the Iceman. Recently, the Iceman has been studied with different technologies, including nanotechnology of soft tissue and bone samples (Janko et al., 2010), spectroscopy of blood remnants at his clothing, a re-evaluation of radiological data (Gostner et al., 2011) and a detailed genetic analysis of his nuclear DNA. Additionally, a photographic high-resolution scan of the entire body of the Iceman was performed and made available online (see iceman.photoscan.edu) and a preservation system with the use of nitrogen was developed. These studies produced new insights into his life and the circumstances of his violent death. In the following, the most important new findings are presented.

New radiological findings

The Iceman was repeatedly investigated with radiological methods and several articles have already been published (zur Nedden and Wicke, 1992; zur Nedden et al., 1994; Murphy et al., 2003; Gostner et al., 2004). However, further investigations using new

Figure 10: Three-dimensional reconstruction of the shoulder region, dorsal view: Numerous grouped radio-opaque inclusions are visible in the superficial tissue layers, predominantly situated in the shallow depressions in the body surface.

technology, as well as a re-evaluation of the imaging data can still reveal important findings. As an example, the arrowhead located in the mummy's left shoulder region was not discovered until ten years after the first X-ray images were taken (Gostner and Egarter Vigl, 2002). An improved multislice CT scan technology allowed the researchers to obtain detailed images of the damage caused to the blood vessel by the arrowhead (Pernter et al., 2007).

At the outer layer of various body regions and the directly underlying tissue, numerous radio-opaque particles of up to 2 mm in size are visible (Fig. 10). These grain- or strip-shaped radio-opaque skin inclusions have hitherto received little attention in radiological evaluations. They consist of vivianite, a compound that develops in mummies when iron salts from the surrounding area enter the body tissues (Tiefenbrunner 1992). These inclusions show a special distribution pattern: On the rear side they are mainly to be found within the upper body half, primarily in shallow recesses. On the front side they can be found within the right cheek and in the vicinity of the anterior iliac horns. They are almost completely missing in the other body regions such as in the anterior thoracic wall, within the abdominal wall and in the lower extremities. They apparently penetrated the front side of the body through direct contact with the ground, on the reverse side probably through the melt water, little or not at all from the ice. If so, the assumption seems reasonable that the corpse had lain there for a very long time and always in the same position, furthermore that the ice within the rock pit melted only superficially, causing the uppermost body parts to come into contact with water. Other macroscopically visible dots and patches, such as those in the vicinity of the tattoos, are not visible in radiographic images. These were found to contain charcoal particles (Van der Velden et al., 1994)

In the region of the skeletal anomaly at the lumbosacral junction, a degenerative discopathy with spondylarthrosis is present on the level of L4-L5 and L5-S1. The prevertebral calcification adjoining L5-S1 does not pertain to the spine, is not of vascular nature and due to its size cannot be situated within the intestinal lumen. It most probably constitutes a dysontogenetic calcification or a calcified lymph node.

The knee joint of the Iceman shows slight degenerative, osteoarthritic changes in the form of calcifications of tendons and their entheses (Fig. 11). The so-called enthesopathies (Resnick and Niwayama, 1983) in the knee present the image of a man accustomed to strenuous walks in high altitude terrains, rather than that of a valley dweller who rarely wandered into the mountains, as enthesopathies are indicators of the affected person's habits and activities (Stirland, 1998). The signs of enthesopathy at the knee joints and lower legs support the "new image of the Iceman"

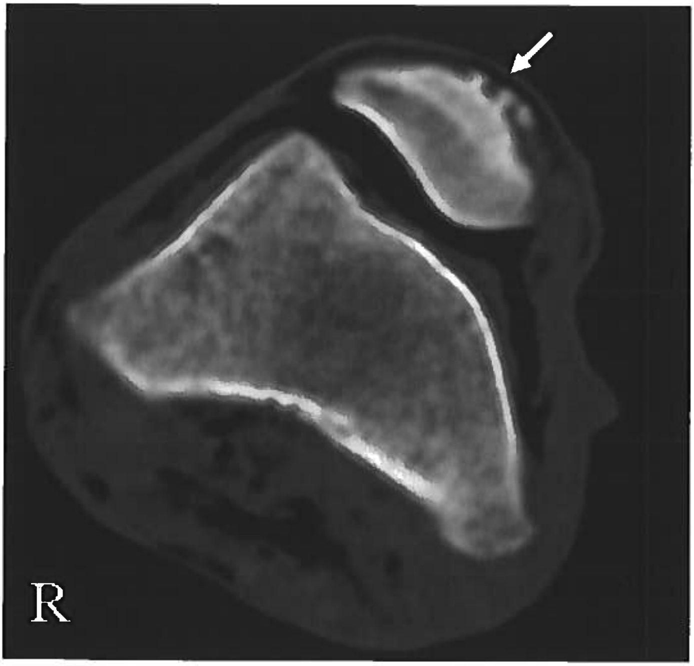


Figure 11: CT section image through the right patella: Along the anterior margin, the cortical bone appears bilaterally thickened and roughened with calcium deposits upon the right patella (arrow), constituting an enthesopathy. Several points of decomposition are visible within the bone substance.

(Lippert et al. 2006 and 2007), as they demonstrate that the man spent a great deal of time on mountain wanderings.

The CT scans clearly show that the unnatural position of the left arm is not due to glacier movements. This can be demonstrated by the configuration of skin folds and muscles, the intact shoulder joints and the rotated scapula (Fig. 12). The haematoma in the soft tissue of the left shoulder can be continually followed through the arrow wound channel into the superficial

Figure 12: Three-dimensional CT reconstruction of the shoulder soft tissues: The muscles and the skin folds correspond exactly to the positions of the right and left arms.

tissue layers without interruption or tearing. Therefore, any post mortem displacements of these parts can be effectively ruled out. The fact that the cervical spine and the connection to the skull show no damage whatsoever proves that the head was not moved in a frozen state. In conclusion, the Iceman was in this position, in which he was to be found more than 5000 years later, within a short time of his death (Lippert et al. 2007).

The stomach and the Iceman's death

Within the upper abdomen, a transversely situated, inhomogeneous structure is visible that could be identified as the stomach

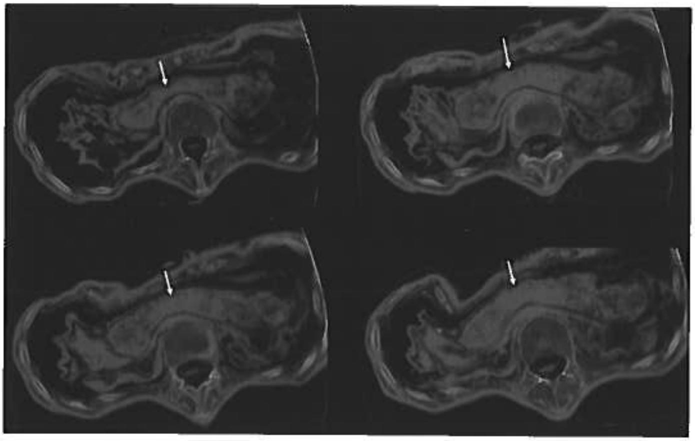


Figure 13a: In the axial CT images of the abdomen a tubular, inhomogeneous organ is visible below the diaphragm, which can be identified as the full stomach due to its topographical site (arrows), to the right of the stomach the extensively shrunken liver can be seen.

with no further digestion processes having begun (Fig. 13 a, b). The shape and topographical site of this organ show that it is in fact the stomach, and not as previously assumed, the colon transversum (Murphy et al., 2003).

It is full of a meal, with largely inhomogeneous content, of which the Iceman must have partaken shortly before death, possibly near to or at the spot in which he was killed. Furthermore, 3 gallstones are visible. Several colon segments are clearly visible and contain small particles of calcium density, which are in all probability residues of a meat-rich meal with bone remnants. Up to now, the colon contents were presumed to contain the Iceman's last meal (Oeggl, 1999). The gallstones, in addition to the previously identified atherosclerosis, suggest a diet rich in animal fats and proteins and lend weight to the theory of the Iceman as a herdsman. As these findings appear somewhat contradictory to the stable isotope and general morphological results, further interdisciplinary studies within this field of research may help

Figure 13b: Sagittal CT reconstruction through the upper abdomen. The organ in the upper abdomen shows the typical form and topographic site of the stomach (asterisks). Below, intestinal loops are visible.

to shed more light on possible cardiovascular and/or metabolic anomalies.

The diet of the Iceman could also have played a certain role, in particular with regard to the degenerative changes in his knee indicating that he did not lack physical exercise. In a previous study, based on the stable carbon and nitrogen isotopes from the Iceman's hair, as well as the high degree of dental abrasion, Macko and colleagues drew the conclusion that the Iceman maintained a predominantly vegetarian (or even vegan) diet (Macko et al., 1999). This has already been contradicted by the fact that the colon contents were shown to contain meat remnants (Dickson et al., 2000). The presumption that a high cholesterol level would have been the most probable cause of the atherosclerosis and gallstones points toward a much higher long-term level of animal product intake than assumed on the basis of the aforementioned isotopic analyses.

The full stomach also gives further insight into the last hours of the Iceman's life. In contrast to previously reconstructed scenarios in which the Iceman was fleeing from his pursuers (Spindler, 2000), he apparently considered the situation safe enough to rest and eat a large meal after the strenuous ascent. Shortly afterwards, he moved a short distance away from his place of rest and was killed by a surprise ambush from behind. This new finding necessitates a reconsideration of the currently supported scenario, in which the Iceman was fleeing for his life after the first skirmish, which caused the cut wound to the hand (Nerlich et al. 2003).

The fractures of the Iceman

The multiple injuries to the bones and soft tissues have already been reported several times (Murphy et al. 2003, Gostner et al. 2004, zur Nedden und Wicke 1997, Lippert et al. 2006, Lippert et al. 2007). Additionally, we detected a spiral fracture of the right humerus, with neither displacement of the fragments nor signs of soft tissue swelling, haematoma or callus formation (Fig. 14). The skin surrounding the fracture site is intact. As this fracture is only visible after specific post-processing of CT images, it was overlooked in the earlier evaluations. Only the left-sided rib fractures show any callus formation. The other fractures show no vital signs whatsoever, so that the radiological examinations cannot

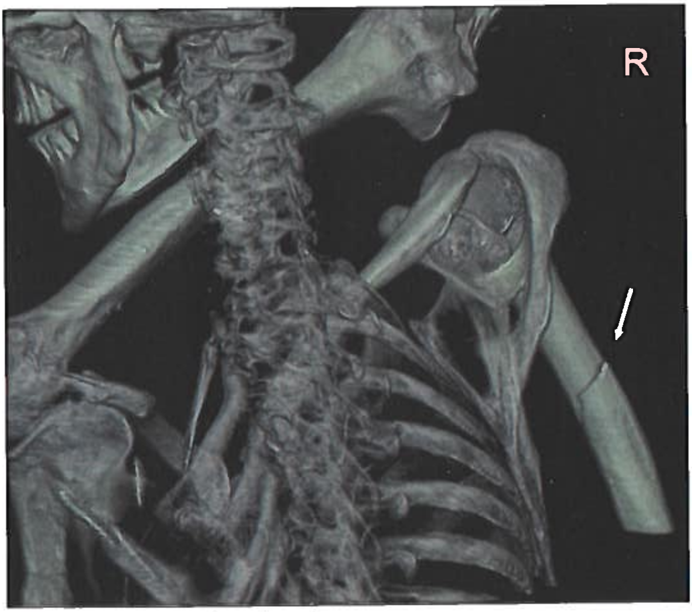


Figure 14: Three-dimensional reconstruction of the right shoulder: Fracture of the right humerus (arrow).

give definitive insight as to whether the injuries were sustained before or after death. As the archaeological value was not recognized until some time after the discovery, heavy equipment was employed in the recovery of the body, causing extensive damage to bones and soft tissue. This indicates that the fracture of the right humerus occurred during recovery, as was the case with the left humerus, and can in all probability be added to the list of damage done during the recovery process.

Evidence for pre- or perimortem fractures could be identified at the skull. The fractures are located at the right side of the neuro- and viscerocranium (Fig. 15). Together with the patchy areas of increased radiological transparency in the posterior cerebral regions (Fig. 16) and the soft tissue swelling in the right facial side they indicate a skull injury shortly before death (Lippert et al. 2007). If water had entered the skull over the subsequent millennia, the expansive pressure of the freezing ice would also have promoted splitting of the cranial bones (Murphy et al. 2003). The fact that this can be observed mainly on the right hand side also indicates a traumatic injury of this side.

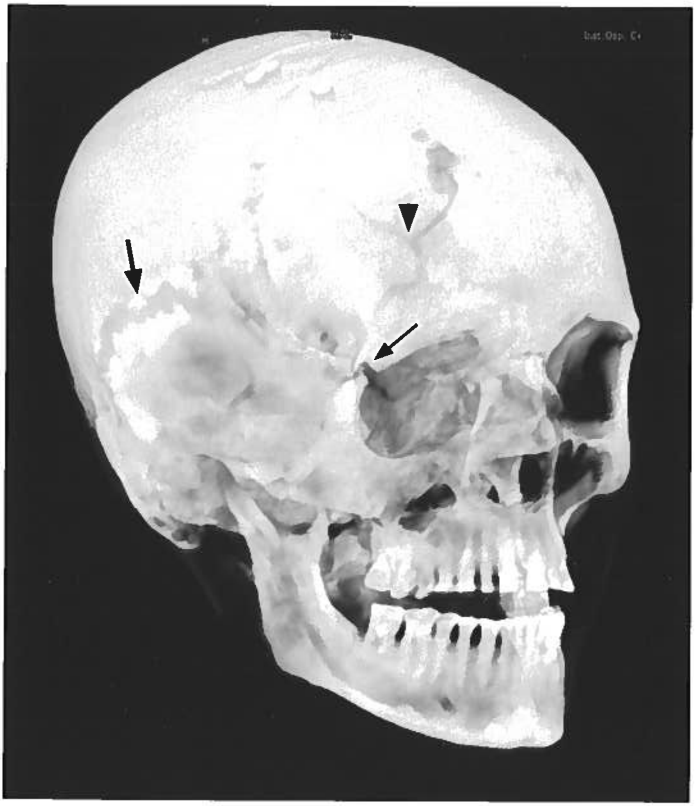


Figure 15: Three-dimensional CT reconstruction of the skull: Fracture at the outer right orbital margin (thin arrow). The cranial suture between the parietal and temporal bone has split (thick arrow). Strongly defined diploe veins (pointer).

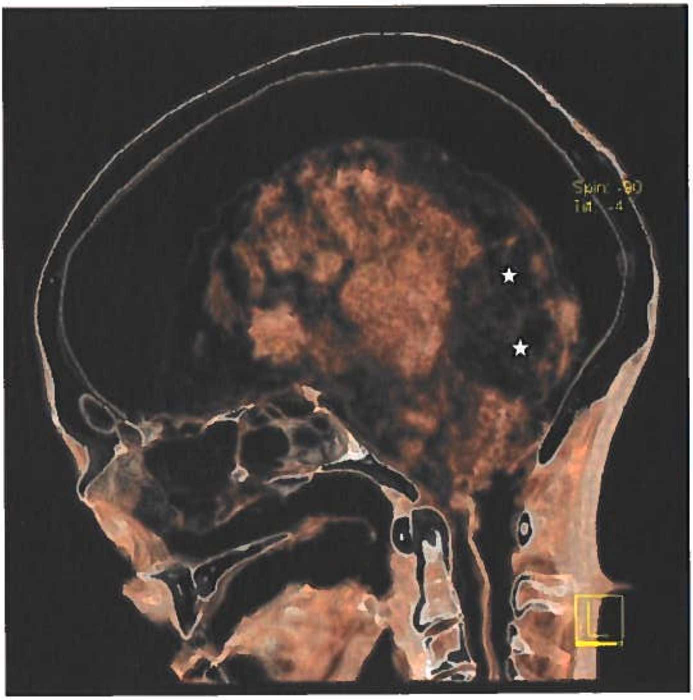


Figure 16: Transverse CT section through the skull shows an irregular area of increased radiographic transparency in the posterior cerebral regions (asterisk). The meninges have become detached from the skull vault and surround the shrunken, inhomogenously disintegrated brain.

The Iceman genes

A few years after the discovery of the Iceman, first studies of his mitochondrial DNA were initiated with the analysis of the HVS1 region (Handt et al., 1994). In later years the entire mitochondrial genome was successfully analysed (Ermini et al., 2008, Rollo et al., 2006). Despite the fact that the mtDNA studies provided conclusive data, no successful amplification of nuclear DNA from the Iceman has been reported until recently. The lack of detection of nuclear DNA and the recent improvements of methods for ancient DNA analyses has led us to initiate a whole-genome sequencing study of the Iceman's genomic DNA. The conservation of the glacier mummy in a cold environment over more than 5,000 years and the excellent preservation of his biomolecules as shown in other studies (e.g. Janko et al., 2010), appeared to be a good precondition for a successful study. For the DNA extraction, a small bone sample was taken from the Iceman's left ilium under sterile conditions in the Iceman's preservation cell at the South Tyrol Archaeological Museum in Bolzano. Follow-

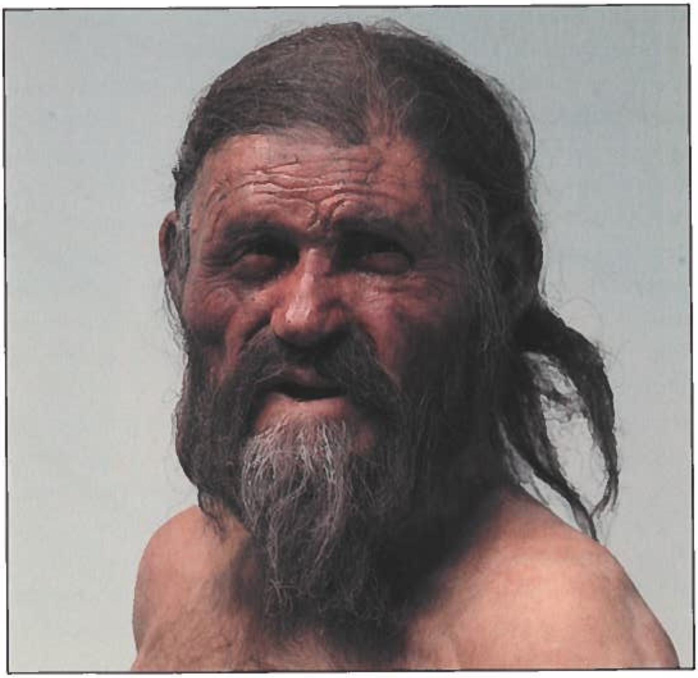


Figure 17: Facial reconstruction of the Iceman showing the brown eyes based on the genetic analysis.

ing previously published protocols, the DNA was extracted at the Institute of Human Genetics in Tübingen, Germany, and subsequently a sequencing library was generated at febit GmbH in Heidelberg, Germany. Finally, the high-throughput sequencing was performed on a SOLiD 4 platform at Life Technologies facilities, Foster City, CA, USA. The next-generation sequencing approach revealed about 40% reads that mapped unambiguously to the human reference genome. Thereby, we were able to retrieve an overall coverage of the human genome of 96%. A comparison with the previously published mitochondrial DNA showed a full concordance and thereby confirmed the authenticity of the ancient Iceman DNA. A first analysis of the sequencing data further revealed that the Iceman most probably had brown eyes, in contrast to the previous assumption that he had blue eyes. In the ongoing study we are currently testing the ability of the Iceman to digest milk. This is of particular interest, as this represents one of the most significant genetic traits connected with the beginnings of agriculture in Europe. The advent of farming in Northern Italy is thought to have occurred between 7000-6900 BP (Itan et al., 2009). Lactase persistence is associated with a polymorphism in a certain genetic region, the MCM6 gene (Enattah et al., 2002). In previous ancient DNA analyses it was assumed that the derived allele was rare in the Neolithic and gained in frequency over the next millennia and was widespread in Central Europe by the Middle Ages (Burger et al., 2007).
Furthermore, the Iceman genome is analysed for genetic risk factors, specifically for DNA sequence variations, so called SNPs (single nucleotide polymorphisms) that are linked with different diseases, such as cardio-vascular diseases, tumors, diabetes, etc. The genetic results will then be compared to other investigations, in particular the radiological studies. For example, computer tomography scans of the Iceman revealed major calcification in carotid arteries, distal aorta and right iliac artery as strong signs for a generalized atherosclerotic disease (Murphy et al., 2003). Since his lifestyle as inferred by radiological and stable isotope

data did not entail major environmental cardiovascular risk factors, a genetic predisposition might be conceivable.

Finally, a detailed analysis of the Y chromosome of the Iceman could allow a better understanding of his ancestry and the proximity to early European populations.

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