13. ÖTZI, THE ICEMAN – LESSONS FROM MODERN MUMMY RESEARCH

Albert Zink[1]*

[1] *Institute for Mummies and the Iceman, EURAC research, Viale Druso 1, 39100 Bolzano, Italy.*
* *albert.zink@eurac.edu*

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ABSTRACT

The Iceman, commonly referred to as Ötzi, is the world's oldest glacier mummy and one of the best studied ancient human remains in the world. Since the discovery of the 5300-year-old Copper Age individual in 1991, at the Tisenjoch in the Eastern Italian Alps, a variety of morphological, radiological, and molecular analyses have been applied that revealed important insights into his ancestry, his life habits and the circumstances surrounding his violent death. In more recent research, the mummy was subjected to modern research methodologies focusing on high-throughput sequence analysis of ancient biomolecules (DNA, proteins, lipids) that are still found to be preserved in his mummified tissues. This application of innovative "-omics" technologies revealed novel insights on the ancestry, disease predisposition, diet and the presence of pathogens in the glacier mummy. In this article the most important and actual results of the molecular studies are presented.

13.1 INTRODUCTION

This review article summarizes the major achievements and recent advances in the study of the Tyrolean Iceman, with a focus on the latest studies using modern sequencing technologies. It is mainly based on two previously published papers, in which general aspects of the Iceman research were already addressed (Zink et al., 2019; Zink and Maixner, 2019).

13.1.1. DISCOVERY AND RESEARCH HISTORY

On the 19th of September 1991, the naturally mummified body of the Iceman, commonly known as "Ötzi", was discovered by the two German hikers Erika and Helmut Simon at the Tisenjoch in the Ötztal Alps at an altitude of 3210 m. The Iceman lived around 3300 B.C. and died at an age of approximately 40-50 years in the high Alps (Gaber and Künzel, 1998). The glacier mummy is currently kept together with

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Figure 1: Finding site of the Iceman in the Tyrolean Alps at an altitude of 3210m.

his well-preserved clothing and equipment at the South Tyrol Museum of Archaeology in Bolzano, Italy. Since his discovery in 1991, the mummy was intensively studied covering a wide range of scientific fields, such as archaeology, prehistory, anthropology and paleopathology (e.g. Barfield et al 1992, zur Nedden and Wicke 1992, Seidler et al., 1992, Spindler 2000). In addition, a variety of methods was applied for the study of the glacier mummy, including radiology and computer tomography (Murphy et al., 2003), histology (Nerlich et al., 2003), isotope analysis (Müller et al. 2003), paleobotany (Oeggl et al., 2007) and the first genetic analysis of his mitochondrial DNA (Ermini et al., 2008).

In more recent years, new technologies were introduced to the study of the Iceman, such as nanotechnology for the study of soft tissue and bone samples (Janko et al., 2010) and spectroscopy of blood remnants on his clothing, Furthermore, a re-evaluation of radiological data (Gostner et al., 2011) and a detailed genetic analysis of his nuclear DNA (Keller et al., 2012) revealed new insights into life and death of the glacier mummy.

Thereby, it was demonstrated that further investigations using new technology, as well as a re-evaluation of existing data can still reveal important findings. As an example, despite previous radiological studies (zur Nedden and Wicke, 1992), 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 scanning technology allowed the researchers to obtain detailed images of the damage caused to the blood vessel by the arrowhead (Pernter et al., 2007). The CT scans further showed that the unnatural position of the left arm is not due to glacier movements. This was demonstrated by the configuration of skin folds and muscles, the intact shoulder joints and the rotated scapula. The haematoma in the soft tissue of the left shoulder can be continually followed through the arrow

Figure 2: The Iceman in his conservation chamber inside the South Tyrolean Museum of Archaeology

Figure 3: Stab wound on the Iceman's right hand.

Figure 4: CT based reconstruction of the upper body of the Iceman showing the arrowhead in the left shoulder.

wound channel into the superficial tissue layers without interruption or tearing. Therefore, any post mortem displacements of these parts can be effectively ruled out. It could be concluded that the Iceman was in this position, in which he should be found more than 5000 years later, within a short time following death (Lippert et al., 2007).

The whole-genome study indicated that the Iceman had brown eyes, was lactose-intolerant, and had genetic predispositions to several diseases (Keller et al., 2012). The complete genome sequence further provided indications for recent common ancestry between the Iceman and present-day inhabitants of the area near the Tyrrhenian Sea, which was confirmed in later studies (Sikora et al., 2014).

The finding of the well-preserved body of the Iceman did not only increase our understanding of a Neolithic individual's life, but the presence of his clothing and equipment also allowed unique insights into the living circumstances of the people in the early Copper Age. His equipment consisted of several tools that allowed him to prepare fire, repair clothes and other items, he carried an unfinished bow and arrows for hunting, and he had a remarkable axe made out of an elbow-shaped wooden handle and a finely handcrafted copper blade. This allowed the Iceman to spend longer time away from his settlement. His clothing was produced using different types of furs and leather, including a cap, leggings, a loincloth, shoes and a coat (Barfield et al., 1992; Spindler, 2000). In a whole mitochondrial analysis of the Iceman's clothes and quiver the different animal species that were used for the production of his garment and equipment could be further identified. The results showed that he made considered choices of clothing material from both wild and domestic animals, including cattle, sheep, goat, brown bear and roe deer (O'Sullivan et al., 2016). It can be assumed that his clothing was very functional and allowed him to walk up in the high mountains even during cold periods.

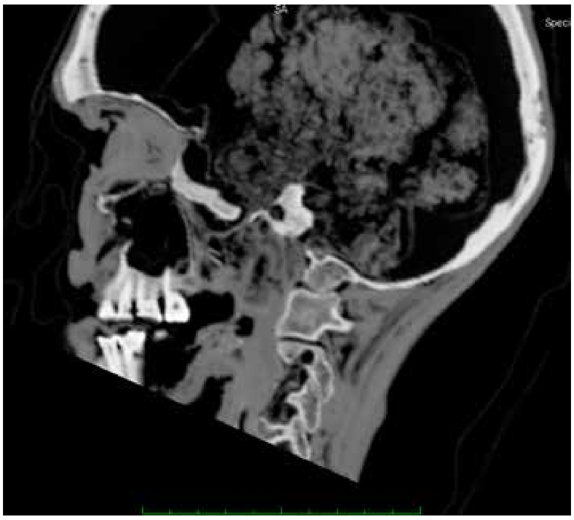

Figure 5: CT image of the skull showing severe dental attrition and mild carious lesions in the teeth.

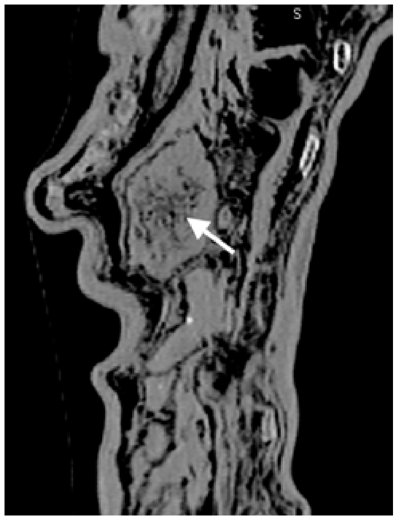

Figure 6: CT image of the abdominal area of the Iceman's body. The stomach (arrow) is filled with material.

13.2 THE ICEMAN'S HEALTH STATUS AND HIS VIOLENT DEATH

The numerous scientific studies that were applied to the glacier mummy revealed detailed insights into his health condition and circumstances of his violent death. It further provided first revelations into how the population of the Copper Age in Northern Italy has dealt with diseases and its consequences, such as illness, pain and stress (Zink et al., 2018).

13.2.1. TRAUMA AND VIOLENT DEATH

In previous studies multiple signs of injuries to the bones and soft tissues at the Iceman's body have been reported (zur Nedden and Wicke, 1992; Murphy et al., 2003, Gostner et al., 2004; Lippert et al., 2006; 2007). Thereby, a major part of the damages has been caused by the glacial environment or during the recovery of the Iceman (Murphy et al., 2002; Gostner et al., 2011). It appears that the Iceman had experienced only one episode of skeletal trauma during his lifetime, that is evidenced by well-healed rib fractures of the posterolateral fifth through ninth ribs on the left side, from which he completely recovered.

Moreover, the Iceman was affected by several traumatic injuries that can be related to his violent death. A deep stab wound on Ötzi's right hand is the first evidence that was detected, and subsequent histological analyses revealed that the injury was inflicted a few days before his death (Nerlich et al., 2003).

In 2007, multislice computed tomography (CT) demonstrated that an arrowhead had lacerated the left subclavian artery, leading to a fast, deadly hemorrhagic shock in the Iceman (Pernter et al., 2007). In addition, pre- or perimortem fractures could be identified at the right side of the neuro- and viscerocranium of the skull. This indicated, together with patchy areas of increased radiological transparency in the posterior cerebral regions and the soft tissue swelling in the right facial side, a severe skull and brain injury shortly before death (Lippert et al. 2007). The fact that this can be observed mainly on the right-hand side also indicates a traumatic injury of this side. The diagnosis of a traumatic brain injury was further confirmed by a paleoproteomic and nanotechnological study of the Iceman's brain tissue that showed the presence of clotted blood in the brain (Maixner et al., 2013). The study also demonstrated a significant accumulation of proteins related to stress response and wound healing.

13.2.2. DEGENERATIVE JOINT DISEASES

Evidence for degenerative joint diseases have been found in the Iceman in the cervical and lumbar spine and the joints of the lower limbs. This was demonstrated by the presence of degenerative disk disease at vertebrae C6-7 and apophyseal joint osteoarthritis at the level of C4-5, as well as vertebrae L3-4 and L5-S1. Moreover, the right hip joint and the right sacroiliac joint showed evidence of mild osteoarthritis. The damage observed at the left little toe was most probably caused by frostbite (Murphy et al., 2003). The knee joint of the Iceman shows slight degenerative, osteoarthritic changes in form of calcifications of tendons and their entheses. The enthesopathies in the knee indicated that he was accustomed to strenuous walks in high altitude terrains. These findings support the theory that the Iceman was physically a highly active person who spent a great deal of time on mountain wanderings (Lippert et al. 2006; 2007), despite the assumption that the degenerative changes of the spine and hip were probably painful (Murphy et al., 2003).

13.2.3. DIET AND DENTAL DISEASES

The first stable isotopic analysis (15N/14N) of the Iceman's hair suggested a predominantly vegetarian lifestyle (Macko et al., 1999a; Macko et al., 1999b), which was later changed to a rather omnivorous diet (Dickson et al., 2000). This was further supported by the analysis of a colon sample of the Iceman, that revealed the diet of both animal (roe deer, ibex) and plant material. The plant remains included einkorn wheat bran, possibly eaten in the form of bread and pollen grains of hop-hornbeam (Oeggl, 1999; Dickson et al., 2000; Rollo et al., 2002). A recent multi-omics analysis of the Iceman's stomach content revealed a diet rich in fat, wild animal meat from ibex and red deer, and cereals (Maixner et al., 2018).

As shown in the Iceman, the transition from hunter and gatherer to agriculture resulted in a change of nutritional habits including the use of wild and domestic crops and of domesticated animals. This has, among other things, led to an increased caries rate in Neolithic populations, while Paleolithic people had fairly healthy teeth. The surprisingly high frequency of caries is thought to be mainly due to carbohydrate rich food as observed in Neolithic individuals, including the Iceman. In addition, the teeth of Neolithic individuals are more abraded and pitted owing to hard inclusions from poorly ground up flour (Richards, 2002). Although initial inspections of the Iceman's teeth did not reveal any signs of tooth decay (e.g. Spindler 1995; Murphy et al., 2003), a reassessment of his dental statement revealed a severe degree level of abrasion and several oral pathologies, including dental caries, periodontal tooth loss and dental trauma (Seiler et al., 2013). The authors concluded that despite the multiple observed dental pathologies, the Iceman probably had a functional, yet sometimes painful, dentition.

13.2.4. INTESTINAL DISEASES

The excellent preservation of the Iceman has allowed to study his intestines and its contents and, thereby, obtain important information on the composition of his diet and the presence of intestinal diseases. In samples taken from the ileum and colon, a high number of eggs of the whipworm (Trichuris trichiura) were detected (Aspöck et al., 1996; Aspöck, 2000). Thereby, it was assumed that he may have had various intestinal problems from time to time, such as abdominal pains and diarrhea, even if it remained difficult to estimate the intensity of the whipworm infestation (Aspöck, 2000). Another finding during the systematic reevaluation of the radiological examinations carried out on the Iceman, was the presence of three gallbladder stones (Gostner et al., 2011). The evidence of the gallstones further underlined the omnivorous diet of the Iceman (Dickson et al., 2000) and contradicted initial assumptions of a more vegetarian diet. Although gallstones may remain asymptomatic in many people, it cannot be excluded that the Iceman experienced some pain in the upper right abdomen. Finally, a metagenomic analysis of the Iceman's stomach contents revealed the presence of the stomach pathogen Helicobacter pylori in his guts (Maixner et al., 2016). By using next-generation sequencing and targeted enrichment, interesting insights into the ancestry and evolution of the pathogen could be revealed, that showed that the ancient Helicobacter pylori strain was a potentially virulent strain that is today strongly associated with gastric disease (Jones et al., 2010). Due to the absence of the stomach mucosa in the Iceman's stomach, it remains difficult to obtain a definite diagnosis and reveal whether the Iceman could have suffered from gastritis, a stomach ulcer, or even gastric carcinoma. Nevertheless, it seems to be well possible that the Iceman could have had at least some periods of stomach problems and pain caused by the H. pylori infection.

13.3 ANCIENT DNA STUDIES OF THE ICEMAN

A breakthrough in the research on the Iceman was achieved by the application of modern sequencing technologies. Ancient DNA or paleogenetic studies of the Iceman revealed new and important information on his ancestry, disease predisposition and the presence of pathogens. In the following, a short overview of the development of this relatively new field will be given and the latest findings in the Iceman will be summarized.

13.3.1. PALEOGENETICS

About 30 years ago, the field of paleogenetics emerged, when the first studies on the retrieval of DNA from ancient specimens have been published (Higuchi et al., 1984; Pääbo 1985). Since then, ancient DNA analysis significantly changed from the detection of small DNA fragments from single specimens to large-scale genome wide studies of past populations (Skoglund et al., 2012). By using modern sequencing technologies, it has become possible to perform detailed research in human evolution (Prüfer et al., 2014), population dynamics and past migration patterns (Brandt et al., 2013; Sankararaman et al., 2014), and gain insights into the phenotypes of our ancestors, such as skin and eye color (Rasmussen et al., 2010). Paleogenetic analysis further enhanced the detection of bacteria, viruses and parasites, indicating the presence of infectious diseases, such as tuberculosis, leprosy and malaria in ancient human populations (Zink et al., 2005; Hawass et al., 2010). Moreover, advances in the application of DNA array capture and next-generation sequencing (NGS) technologies allowed full genome reconstructions of ancient pathogens that lead to new insights into disease evolution. As an example, the combination of DNA array capture and next-generation sequencing (NGS) technologies allowed the reconstruction of complete bacterial genomes of Yersinia pestis and Mycobacterium leprae strains from Medieval Europe, showing a remarkable genetic conservation of both pathogens throughout the last 1000 years (Schuenemann et al., 2013; Bos et al., 2011; Wagner et al., 2014).

Although the detection of ancient DNA is still limited by factors that lead to the degradation of DNA, such as the activity of enzymes and microorganisms, hydrolytic and oxidative processes, high temperature and humidity, soil DNA may survive up to 800,000 years in the case of Pleistocene fauna (Orlando et al., 2013) or 400,000 years for hominin fossils from the Iberian Peninsula (Meyer et al., 2014). The risk of amplifying exogenous contaminants and the highly fragmented endogenous DNA used to be a major limitation and challenge in the era of PCR-based approaches (Pääbo et al. 2004). This was mainly overcome by the application of high-throughput sequencing technologies and targeted enrichment strategies. This approach can be used to identify endogenous ancient sequences by looking for DNA degradation patterns that accumulate over time and which are typical of ancient DNA (Krause et al. 2010).

The nuclear sequencing of the 5300-year-old Tyrolean Iceman represented the first whole genome study of an ancient mummy (Keller et al., 2012). In the following years, the application of next-generation sequencing technologies has led to an increasing number of genomic information on mummies (Gomez-Carballa et al., 2015; Schuenemann et al., 2017) and the reconstruction of pathogen genomes, such as those of M. tuberculosis, hepatitis B virus and H. pylori (Kay et al., 2015; Kahila Bar-Gal et al., 2012; Patterson Ross et al., 2018; Maixner et al., 2016).

13.3.2. THE ICEMAN'S GENOME

The first paleogenetic studies of the Iceman targeted the mitochondrial DNA, starting with the analysis of the hypervariable region (HVS1) (Handt et al., 1994). In a later study the entire mitochondrial genome was successfully analyzed (Ermini et al., 2008, Rollo et al., 2006).

Several years later, a whole-genome sequencing study of the Iceman's genomic DNA was initiated. The improvements in modern sequencing technologies together with the excellent preservation of his biomolecules as demonstrated in other studies (e.g. Janko et al., 2010), appeared to be a good precondition for a successful study. DNA was extracted from a small bone sample taken from the Iceman's left ilium under sterile conditions in the Iceman's preservation cell using established protocols, Subsequently, a sequencing library was generated and high-throughput sequencing was performed on a SOLiD 4 platform (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, an overall coverage of the human genome of 96% was retrieved. A comparison with the previously published mitochondrial DNA showed a full concordance and thereby confirmed the authenticity of the ancient Iceman DNA.

The sequencing results revealed many interesting insights into physiological parameters, diseases and the ancestry of the Iceman. Thereby, it could be shown that he had likely brown eyes and that the Iceman was lactose intolerant. The latter represents one of the most significant genetic traits connected with the beginning 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).

The Iceman genome underwent a further detailed analysis of genetic risk factors, specifically for DNA sequence variations, so called SNPs (single nucleotide polymorphisms) that are linked with diseases. The most intriguing finding was that the Iceman showed a strong genetic pre-disposition for increased risk for coronary heart disease (CHD). This was of particular interest as the CT scans of the Iceman revealed major calcification in carotid arteries, distal aorta, right iliac artery and coronary arteries, as strong signs for a generalized atherosclerotic disease (Murphy et al., 2003, Gostner et al., 2018). The genetic pre-disposition could have significantly contributed to the development of the arterial calcifications.

Other traditional cardiac risk factors, such as being overweight, tobacco use, lack of physical activity and a high fat diet, can generally be ruled out in the glacier mummy. Based on the previous studies mentioned above, the Iceman was walking intensively in the mountain area, he had a slim and well-trained body, and his nutrition was well-balanced with low amounts of proteins and saturated fats. Tobacco was not available in that time period, although dark staining in his lungs indicate that he inhaled smoke during his life, most likely from open fires.

13.3.3. ANCESTRY AND GENETIC HISTORY

The genome analysis of the Iceman allowed a detailed analysis of the Y-chromosome for a better understanding of his ancestry and proximity to early European populations. The results have shown that the Iceman belonged to a rare Y-haplogroup (G2a2b), which is today encountered at a low frequency in Europe. Only on Corsica and Sardinia this Y-haplogroup is still represented relatively frequently. From this it could be concluded that the Iceman and the population on Sardinia and Corsica had common ancestors who immigrated to Europe during the Neolithic period. In wide parts of Europe, the representatives of this group were superseded over the course of time and only in remote regions such as the Mediterranean islands could they survive in greater numbers until today.

In contrast, studies of the Iceman have shown that his mitochondrial DNA (mtDNA) belongs to a novel lineage of haplogroup K1 (K1f) not found in extant populations. In a recent analysis, haplogroup K DNA samples were analyzed from more than 800 individuals (Coia et al., 2012; Capocasa et al., 2013; Pichler et al., 2006) and collected new modern samples from South Tyrol. The results were compared to 1077 complete K1 mtDNA sequences from modern populations. The study revealed that the K1f haplogroup is most probably absent in present-day populations and therefore, we suggested that mtDNA Iceman´s lineage could have disappeared during demographic events starting in Europe from c. 5,000 years BP (Coia et al., 2016).

13.4 THE ICEMAN'S STOMACH PATHOGEN

The application of next-generation sequencing and targeted enrichment to samples of the Iceman's stomach has led to the detection and full genome reconstruction of the stomach pathogen Helicobacter pylori (Maixner et al., 2016). The bacterium is of particular interest for clinicians as well as evolutionary biologists, as it is found in approximately half the world's human population and about 10% of the carriers develop stomach diseases, such as gastritis, stomach ulcers or gastric carcinoma. The bioinformatics sequence analysis showed that the ancient H. pylori of the Iceman can be classified as virulent strain that is today associated with inflammation of the gastric mucosa. Moreover, using a paleo-proteomics approach, proteins in the Iceman's stomach were observed that are known to be involved in the inflammatory host response. These results supported the presence of a possible stomach disease in the Iceman, but as the stomach mucosa was not preserved, it remained impossible to obtain a definite diagnosis and reveal whether the Iceman could have suffered from gastritis, stomach ulcer, or even gastric carcinoma.

The phylogeographic assignment of the ancient H. pylori strain revealed a surprising result, as the 5,300-year-old bacterium matched to the modern population hpAsia2, commonly found in Central and South Asia and not to hpEurope, the modern H. pylori strain found in most Europeans. As previous analyses have clearly shown that the Iceman is not of Asian origin, but grew up and lived at the southern side of the Alps, the results have to be interpreted differently. An answer could have been obtained by considering the evolution history of the stomach pathogen that is closely linked to its human host. The H. pylori strains found in most Europeans today (hpEurope) have putatively originated from recombination of the two ancestral populations Ancestral Europe 1 and 2 (AE1 and AE2). In previous work, it was assumed that the admixture of the two ancestral strains has occurred in the Middle East or Western Asia between 10,000 and 52,000 years ago and hpEurope was introduced into Europe with the first Early Neolithic farmers (Moodley 2012). However, the study has shown that the Iceman's H. pylori is a nearly pure representative of the bacterial population of Asian origin (AE1) that existed in Europe before hybridization (Maixner et al., 2016). This suggested that the second ancestral population (AE2), which originated in North East Africa, arrived in

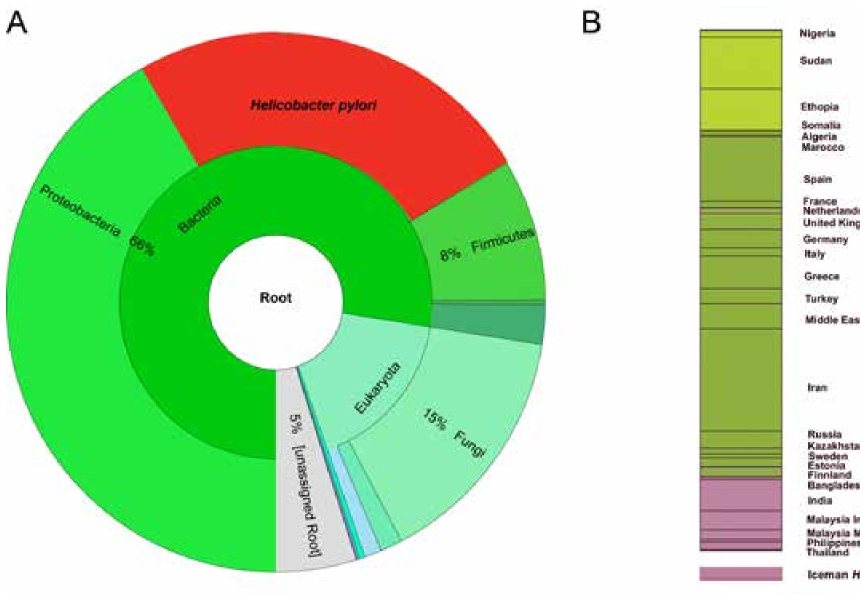

Figure 7: Sequencing and comparative analysis of the ancient H. pylori genome detected in the Iceman stomach content. a) Taxonomic overview of the sequence reads with enrichment for H. pylori. b) Bioinformatic analysis using a multilocus sequence typing database displays the population partitioning of hpEurope, hpAsia2, and hp-NEAfrica and the Iceman's H. pylori.

Europe within the past few thousand years, which is much more recent than previously hypothesized. Taken together, the metagenomic diagnostic approach and genome reconstruction revealed not only that the ancient Helicobacter pylori strain was a potentially virulent strain that is today strongly associated with gastric disease, but it provided interesting insights into the ancestry and evolution of the pathogen and underlined the high complexity of ancient European population history.

13.5 THE ICEMAN'S LAST MEAL

The detection of the completely filled stomach of the Iceman allowed the reconstruction of his last meal (Maixner et al., 2018). Thereby, a combined approach, including classical microscopy as well as modern molecular analyses using the whole spectrum of available biomolecules (ancient DNA, proteins, metabolites, lipids), has been applied to samples of the stomach. The first macro- and microscopic analysis showed an extraordinarily well preservation of the specimens with compact pieces of plant and animal food remains that display a hydrophobic "fatty-like" character. The analysis showed that the animal macro fossil remains consist primarily of adipose tissue and muscle fibers that were unambiguously assigned to the wild mountain goat ibex by using a multi-omics approach (metagenomics, proteomics and lipidomics). Additionally, traces of red deer DNA in the Iceman's last meal were detected.

A microscopic and spectroscopic analysis of the ibex meat fibers provided insights into how the Copper Age meat has been prepared. The well-preserved meat fibers still show striated fiber structures that disappear as soon as meat gets cooked or fried and therefore, indicated that the Iceman consumed smoked or air-dried meat. A lipid analysis of the remarkably high proportion of fat in the Iceman's stomach did show that the triglycerides distribution patterns are consistent with the consumption of ibex muscle fat and subcutaneous adipose tissues. Thereby, the consumption of dairy products in his last meal could be excluded and, instead, ibex was the food source for both the meat and the fat for the Iceman.

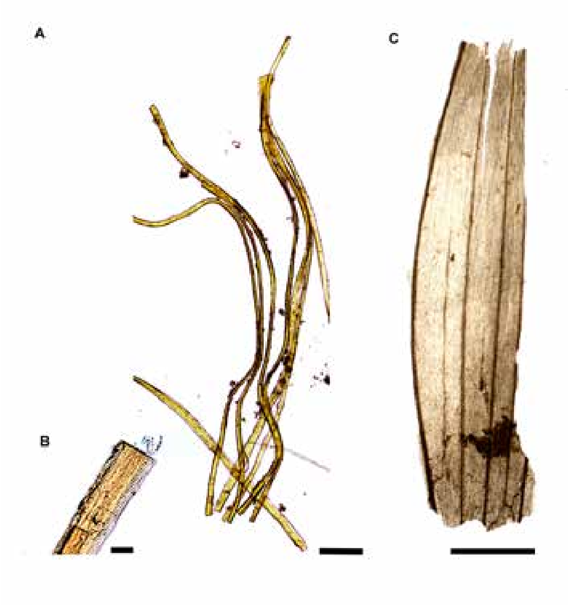

Figure 8: Animal and plant macro remains detected microscopically in the Iceman stomach content. a) muscle fibers. b) zoomed-in view of one muscle fiber showing the striated fiber structures. c) Part of a wheat grain spikelet.

The majority of plant macro remains in the Iceman's stomach content could be attributed to cereal bran. The microscopic and molecular analysis indicated that the bran derives from the diploid einkorn wheat (Triticum monococcum). Beside this early domesticated wheat species, a continuous presence of the toxic bracken fern (Pteridium aquilinum) was detected in the analysed content material.

13.6 OUTLOOK

Despite 28 years of intense research on the Iceman, there are still open questions and important discoveries to be made. In particular, the continuous development of molecular methods will allow more sensitive analyses of all different biomolecules (DNA, RNA, proteins, lipids, glycans), elements and metabolites preserved in the Iceman. The combination of medical imaging with cutting-edge molecular research will provide more important insights on disease predispositions and evolution of pathogens, as shown in the studies on arteriosclerosis (Keller et al., 2012) and H. pylori (Maixner et al., 2016).

The analysis of the Iceman microbiome holds an enormous potential for future studies. In a recent study, we have contributed to the investigation of Prevotella copri, an important member of the human gut microbiome (Tett et al., 2019). The comprehensive analysis of modern and ancient gut microbiomes provided a deep insight into the genetic diversity and evolutionary history of P. copri, suggesting a loss of strain diversity due to Westernization and changes in diet. This work underlined the potential in comparing ancient and modern gut microbiomes and provided first evidences for a decline in gut microbiota diversity within the last millennia which is a possible underlying factor linked to the rise of modern diseases such as obesity, asthma, or food allergies (Tett et al., 2019).

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