



ARCHEOLOGY, FORENSIC DENTISTRY AND DENTAL PULP DNA: SOME HISTORICAL CASES

Dental Science

Xavier Riaud*

Docteur En Chirurgie Dentaire, Docteur En Épistémologie, Histoire Des Sciences Et Des Techniques, Lauréat Et Membre Associé National De L'académie Nationale De Chirurgie Dentaire. *Corresponding Author

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Today, dental pulp DNA, free from any dental pathology, constitutes a remarkable means of investigation in odontology but also in archeology. Indeed, numerous historical researches in forensic dentistry use this scientific device in order to specify different elements such as the origin of the body, its identification, the cause of its death, its eventual filiations or even its place in the long chain of the human evolution. Here are few historical examples where dental pulp DNA gave extraordinary results.

Origin

In Segudet, Andorra, human remains coming from a newly-discovered prehistoric tomb were found in the parish of Ordino, at 1324m of altitude. This body belonged to the ancient Neolithic Age (in the Epicardial period, from 4300 and 4050 BC). These remains were submitted for analysis at the Paleoanthropology Laboratory of the "Unitat d'Antropologia, Dept. Biologia Animal, Vegetal i Ecologia, Universitat Autònoma de Barcelona". They were identified under the S-2001 acronym. The skeleton was diagnosed as a female of between 30-35 years of age at death. A mitochondrial DNA analysis of the upper right canine (I3) was performed and it helped classifying this individual within the European haplogroup K, characterized by an age group from 12,900 to 18,000 years. Therefore, this haplogroup was present in Neolithic populations of the Basque Country and was mainly linked to the indigenous people of Andorra (Díaz N., Solórzano E., Montiel, R., García, C., Yañez C., Malgosa, A., 2004).

Ötzi is the name given to a frozen and dehydrated body which was found in September 1991 in the Ötztal Alps, on the border between Austria and Italy. Aged of 46 years, this man is said to have lived between 3,350 and 3,100 BC. The analysis of its dental DNA helped determining that the body belonged to the K1 subcluster of the European haplogroup K which was highly met in the south of the Alps and in the region of Ötztal (Aquaron, 2005 & 2008).

Forensic identification

On 15th April 1912, at 2:20, the Titanic sank after striking an iceberg. Out of 1,496 passengers, 2,208 of them died. Few bodies were recovered. Among them, an unknown child was discovered on April 21 and it was buried in the Fairview Lawn Cemetery of Halifax, Canada. At the end of summer 1998, with the injunction of families who wished to identify bodies that could possibly be identified as relatives, the body of this child was exhumed. A Swedish family, the Pålssons, requested the exhumation of this body. They thought that this child could be Gösta Leonard Pålsson, who was two years and three months old when the disaster occurred. During the exhumation, only three teeth (55, 73, and 84) from the child's mouth were found. A mitochondrial DNA analysis and comparison of the family and of the remains were performed and helped to determine that there was no filiation. Later on, it was acknowledged that the three teeth were temporary teeth.

An appropriate analysis of dental anatomy proved that these teeth were those of a baby whose age was between 9 and 15 months, no more. Then, a microscopic and histological study confirmed the presence of dentin in lacteal tooth 84 but not in the other two.

In the old DNA testing laboratory of the Brigham Young University in Utah, mitochondrial DNA was isolated from this dentin. This mitochondrial DNA comes from our genital mothers. Genealogists succeeded in finding and getting a DNA sample of descendants of the mothers' line of descent of all the children who were less than 3 and

who died in the shipwreck. Two boys corresponded to the predetermined genetic profile: one was 19 months and the other, 13 months. As a matter of fact, they discovered that the second boy matched the estimated age criteria (from 9 to 15 months). His name was Eino Viljam Panula. His remains were once again buried (Titley, Pynn, Chernecky, Mayhall, Kulkarni & Ruffman, 2004).

Copernicus (1473-1543), the famous astronomer, notably proved that such as planet Earth, the group of planets rotated around the sun, the axis of the Earth oscillating like a whirligig. Therefore, he identified that the Sun, not the Earth, was at the centre of the solar system. He was buried in the lofty cathedral of Frombork, on the banks of the Vistula but no one knew exactly where.

In 2005, new archaeological research was carried out and this time, archaeologists explored the St. Cross Altar, one of the sixteen altars leaning against the pillars of the cathedral. Beneath the marble tiling, they found the remains of 13 bodies, among which were the skull and the teeth of a man around seventy years old. A team of specialists from the Institute of Forensic Research, Cracow and from the University of Uppsala in Sweden were able to identify the last remains as Copernicus's. The research was carried out with all the most modern procedures of the judicial police.

Genetic tests were carried out on the dental material but they never proved that it was Copernicus's DNA.

Finally, the precious material to help identifying Copernicus was found in Sweden. Indeed, a hair was found in a book of 1518, entitled *Calendarium Romanum Magnum* and written by Johannes Stoeffler. Copernicus had used this manual all his life. The Swedish had taken the book during the Polish-Swedish wars of the 17th century. The comparison between the dental DNA and that of the hair led to the identification of the famous astronomer's remains. Finally, on May 22, 2010, Nicolaus Copernicus was solemnly buried in the cathedral of Frombork, 467 years after his death (Fabre, 2010).

Poisoning

The case of Diane de Poitiers (around 1499/1500-1566), the favourite mistress of Henry II, Francis I's son and King of France cannot be ignored.

The Duchess of Etampes (around 1508-after 1575), Francis I's official mistress and Diane de Poitiers's rival "*had a devoted poet put into verses a caricatural portrayal of her enemy. In this poem, nothing had been left out: her wrinkles, tainted hair and fake teeth* (Bordonove, 2007), ..."

The Duchess of Etampes nicknamed her rival "*the toothless old lady*", relating to her fake teeth. Indeed, she asked a satirical poet, Jean Voûté, to mock Diane, then aged 38 years old. Author of a calumnious publication, in his hendecasyllables printed in Paris in 1537, he criticized her artificial teeth (Desgardins, 1909). Ambroise Paré also described her prosthetic dental device in his book « *Dix livres de chirurgie, avec le Magasin des instruments nécessaires à icelle* » published in Jean Le Royer, en 1564, à Paris (Charlier, Georges, Huynh-Charlier, Carlier & Poupon, 2009).

In 2008, close to the church of Anet, the remains of Diane de Poitiers were exhumed. There, an intact toothless jaw, a left hemimaxillary and a tooth were collected. The remains were displayed with her last

portrayal painted by Clouet in 1562 preserved in the Museum of Chantilly. They corresponded to the face features (Charlier, Huynh-Charlier & Carlier, 2009). As for the tooth, DNA testing was carried out on it which revealed a considerably more important gold rate than average. Diane often drank drinkable gold which was used as an elixir of life and beauty at the time. It is thought that this gold slowly poisoned and killed her (Hofstein, 2009).

Moreover, for information, “(...) *the jaw presented alveolar positions for the teeth #31, 32 and 42 which were missing. All the other teeth had been lost ante-mortem for such a long time that the bone had perfectly healed and that the horizontal branches had gotten thinner to the extent of giving them a senile aspect. The left hemimaxillary still carried tooth # 24 which showed a marked occlusal usury and a definite parodontopathy*” (Charlier, Georges, Huynh-Charlier, Carlier & Poupon, 2009).

Bacteraemia following an epidemic

In December 1812, the Great Army beat a retreat in Vilnius. In autumn 2001, a communal grave containing the remains of hundreds of French soldiers was exhumed. The research was given to the National Centre for Scientific Research (CNRS). This team began the excavations of the site, the anthropological study and the analysis of the uniforms. The soil, tissue and teeth samples were given to the “Unité des Rickettsies” and the emerging pathogens to the National Centre for Scientific Research. The scientists succeeded in separating the lice from the samples with a technique that they invented. Hence five types of lice were located. In three of them, the bacterium *Bartonella quintana*, the typhus carrier, was isolated. Then the scientists studied the dental pulp extracted from the teeth of thirty-five soldiers. This pulp, which was ground to powder after the DNA testing, allowed determining the existence of bacteria which had contaminated their hosts: *Bartonella quintana* in seven bodies and *Rickettsia prowazekii* in three others. In other words, after several examinations, thirty percent of the buried soldiers of Vilnius suffered and died from epidemics caused by lice. Subsequently, those insects which carried Typhus played a role of paramount importance in the Russian retreat (Raoult, 2001-2010). This pathology was the first cause of death in the campaign. It was responsible for eighty percent of the death caused by diseases. According to Oleg Sokolov, 300,000 men died during the Russian campaign with five men dying following a disease for one man during the battles (Riaud, 2010).

The 2005 excavation of the 15th century protestant graveyard of Saint-Maurice was conducted by the “Institut national de recherches archéologiques préventives” in collaboration with the local laboratory of Archeology of Val-de-Marne. It highlighted the archeological remains of the first temple and 165 burials. The first paleopathologic analyses showed the symptoms of Vitamin D resistant rickets on the major part of the population buried as well as the presence of plague in three human beings.

The plague bacterium was identified on two male and female bodies coming from the necropolis; the third was recognized on a mummified burial found in 1986 in a lead sarcophagus. The buried man was a protestant English nobleman called Thomas Craven and who was a member of the reformed Parisian church. He died in 1636 and was 18 years of age.

The biomolecular analysis was carried out by the Rickettsies laboratory of the School of Medicine in Marseille. The dental samples were taken from seven sepulchers including the mummy of the English nobleman Thomas Craven and from six random sepulchers. Two genes among which the sequence was specific to the Plague bacterium, *Yersinia pestis*, were amplified by the Polymerase Chain Reaction technique in front of negative witnesses. In total, three people were diagnosed as being infected by the plague whereas the other was diagnosed as potentially being infected. Therefore, Thomas Craven's mummy was the first identified plague-stricken individual (Hadjouis, Vu, Aboudharam, Drancourt & Andrieux, 2006).

Filiation

The son of the crown prince Frederic III and of his wife Victoria, the United Kingdom's royal princess, Wilhelm II (1859-1941) claimed his Prussian origin and questioned the United Kingdom's maritime supremacy regardless of his titles. He became emperor in 1888. His reign was known for his exacerbated militarism. He dismissed Chancellor Bismarck in 1890 and did not renew the agreement of

mutual assistance between Germany and Russia. His aggressive foreign policy clashed with that of the United Kingdom and isolated him diplomatically. He did not cause the First World War alone but he did nothing to prevent it. During the war, he was commander in chief of the army but soon, he lost his authority and credibility. Ousted from power, Wilhelm's abdication both as German Emperor and King of Prussia was abruptly announced on 9 November 1918. He went into exile in the Netherlands. He died in Doorn in his property during the German occupation of the Netherlands and was buried in this town (<http://fr.wikipedia.org>, 2010).

The castle of Doorn now opens its doors to visitors. When the guide passes by the Emperor's bedroom where he actually died, he usually takes a jewel case containing a tooth from the bedside table. Showing it to the visitors, he explains that it is thanks to it that the existence of an illegitimate daughter could be dispelled (Lamendin, 2002).

Indeed, a female painter whose canvases were displayed in an art gallery claimed her so-called imperial ancestry. In 1996, DNA testing was carried out from the tooth that the guide now displays to the visitors' eyes. The conclusion was unequivocal. There was no filiation and the deception was over (Lamendin, 2002; Riaud, 2007).

Chronology

In August 2008, Richard Green of the Max Planck Institute for Evolutionary Anthropology succeeded in doing the first sequencing of the whole mitochondrial DNA of a Homo neanderthalensis of 38,000 years old. The results confirmed that the last common ancestor of the Neanderthal Men and Homo sapiens is supposed to have lived 660,000 years ago (but for 140,000 years). As for Svante Pääbo, also coming from the Max Planck Institute, he started in 1997 his work of deciphering sequences with the first mitochondrial DNA testing. The international scientific team conducted by Svante Pääbo deciphered a sequence of around a million of nucleotides of the Neanderthal thanks to a new technique called *pyrosequencing* which allows analyzing DNA sequences without amplifying it. The team of Edward Rubin who worked with the same equipment in partnership with Pääbo, used another method and identified 65,000 DNA base pairs. The two analyses drew two close conclusions relating to chronology. For Pääbo's team, Homo sapiens and Homo neanderthalensis diverged 500,000 years ago whereas Rubin's team concluded that the two species had shared a common ancestor 700,000 years ago and that they had parted 370,000 years ago. The difference between the two genomes was lower to 0, 5%. Rubin and his colleagues did not find traces of crossbreeding of the modern man with the Neanderthal (Defrance, 2008). In 2010, Pääbo's team published results relating to the analysis of 4 billions of nuclear DNA base pairs coming from the fossil bones of three Neanderthals. They showed that those three Neanderthals shared more genetic features with the modern Eurasian Homo sapiens than with those from Sub-Saharan Africa. Currently, researchers envisaged the hypothesis according to which the Neanderthals had contributed to the genome of the non-African modern human populations (Green, 2010).

Conclusion

Today DNA testing as a means used in a scientific research following a historical or archeological investigation became an essential procedure. Apart from the age estimation where results are not convincing and where many questions are still raised, these fields of investigation are quasi limitless, provided that the DNA remains intact and readable given the former vestige discovered on the sights. However, even if it is remarkable, this procedure remains expensive.

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