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News & Views

Excavating signature gene to trace back the extinct hominin Svante Pääbo, DNA hunter was nominated for Nobel Prize winner

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Imprint on extinct

The present human beings are descendants of an ancestral living creature on the earth is known to us, but not sure about the exact timeline of the event. Our earliest ancestors are no longer exist rather extinct. The fossil records of the past 2 million years shows modern humans evolving from earlier prehistoric humans, often referred to as 'archaic humans. During excavation, the evidence of human habitat was ascertained from the evidence of bones, skulls, tools as well as other archaeological artefacts recovered from the site. From the anatomical structure and morphology of the skeleton retrieved from the site of excavation, it has been speculated that it was less than 200,000 years ago when modern humans (skeletons like those of present-day humans) appeared in Africa. All these data are not substantial enough to predict the exact historical path of the origin of modern humans and its divergence from their ancestors. However, at that time and later, when modern human appeared in Europe and western Asia, other 'archaic' hominins were already present in Eurasia (Hublin, 2009). As per scientific data, no consensus exists about which groups were present in eastern Asia (Reich *et al*., 2010). We all are curious to know whether any genetic relationship exists with our ancestors and how much we are related. All these can be derived from DNA analysis of the past with the present. In recent times biologists have shown their interest in retrieving the residual DNA remaining in those archaic specimens. One of the DNA-hunting scientists, Svante Pääbo and his group studied the fossils to explore the history of the evolution of human beings and take us back to our ancestral roots. The moment an organism (man or animal) dies, the long DNA strands of its cells are highly prone to degrade due to DNA clipping enzymes of its own cells. DNA damage includes fragmentation and modification of nucleotides caused by an oxidative process such as deamination of cytosine residue to uracil. The longer the passage of time, the shorter the fragments become; eventually, the short fragments are not enough to compute the exact ancestral history. Further such DNA are highly contaminated with DNA from microbes, other living organisms as well as contemporary humans. To put this miniature debris together to draw a meaningful conclusion is a daunting task. Sequencing of genomic DNA recovered from archaic specimens can provide some clues in this regard. Harvesting human DNA from million years old fossils and sequencing those contaminated DNA is a battle against time.

According to scientific data, an extinct group of human Neanderthals (the 'th' pronounced as 't'), *Homo neanderthalensis* is our closest human relative (Monnier, 2012). Obviously, a question may arise why they are called Neanderthals? In 1830 the first Neanderthal fossil was found in Belgium, but due to a lack of scientific knowledge, scholars failed to recognise those to be extinct human. During 1856, a skeleton was accidentally discovered among cave sediments extracted from the Kleine Feldhofer Cave, Neander Valley,

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Germany. Then after scientists could figure out what they had recovered earlier and named it according to the name of the valley. Geologist William King suggested the name *Homo neanderthalensis* (Walker *et al.*, 2021). Subsequently, due to matching the Neanderthal DNA against our own, scientists have proved that *Homo neanderthalensis* is a member of the modern human (*Homo sapiens*) neanderthalensis family, much closer kin to us than are chimpanzees. Analysing the DNA sequence of the tip of a finger bone recovered during 2008 from Denisova Cave in the Altai mountains of Russia, another extinct ancestral population has been discovered and named as Denisovans *Homo sapiens denisova* (Krause *et al.*, 2010). It is relevant to mention here that the DNA of Denisovans differed from both extinct Neanderthals and existing modern humans on the earth. When Neanderthal, Denisovans, and modern humans diverged from one another is not yet entirely clear. The use of DNA sequencing tool as transformative technology to trace the human evolution has revolutionized the study of human prehistory but sometimes straining the relationships between archaeologists and geneticists. There is divided opinion among them; half of the archaeologists think ancient DNA can solve everything; the other half thinks ancient DNA is the devil's work. (Callaway, 2018). Svante Pääbo at the Max Planck Institute for Evolutionary Anthropology (EVA) in Leipzig, Germany engaged in ancient DNA research - an entirely new scientific discipline; *paleogenomics* worked extensively to refine the protocols for sequencing and analysing DNA from archaic specimen contaminated with artifacts (Krings *et al.*,1997). Ultimately, he has now illuminated both the distant past and the genetic heritage of people living today by sequencing genomes of extinct hominins. Till date, we do not know what makes us *Homo sapiens* so unique and different from other hominins? In 2009 the scientific group from Paabo`s lab developed an improved gene sequencing technique to decipher the genome of another archaic human, the Denisovan (de-NEE-soh-vens), a closely related species of Neanderthal (Curry, 2022). Findings from the lab of Pääbo has given substantial evidence that a certain amount of genetic information of ancient extinct Neanderthal population has been transferred to modern human (*Homo sapiens*); as of now, the non-Africans modern human being are still retaining near about 2% of Neanderthal ancestry in their genome (Callaway, 2021). Invariably the Nobel Prize in Physiology or Medicine isn't awarded to a single scientist yet. On 3rd October 2022, the Nobel committee decided to honour Svante Pääbo with the Nobel prize in Physiology or Medicine for this outstanding work. Pääbo has narrated the tales of genetic changes of prehistoric human that has revealed who we are, as well as why we are unique and different to be human. It has revolutionised our understanding regarding the evolutionary history of modern humans.

A proficient recipient

In 1955 Estonian descendant Svante Pääbo son of Sune Bergstrom was born in Stockholm. His father had also received the Nobel prize for discovering prosta-glandin in 1982. During a vacation trip to Egypt with his mother at the age of 13, Pääbo developed fascination towards the archaeological wonders of Egypt. From that point, he wanted to become an Egyptologist. In 1975 he joined the faculty of humanities, University of Uppsala University (Uppsala, Sweden) to pursue a degree in Egyptology, but after 2 years, Pääbo discontinued and switched his studies to medicine. In 1980 interrupting medical studies, he was inclined to pursue a PhD in molecular genetics. In 1986 he obtained his PhD degree from the University of Uppsala, Sweden. Subsequently, for two years, he continued as a postdoctoral fellow at the University of Zürich, Switzerland and the University of California at


Svante Pääbo

Berkeley, USA. In 1981, during his PhD carrier, avoiding the displeasure of his professor, he secretly worked on screening DNA samples from biological specimens preserved in his lab. Pääbo tried to extract DNA from a 2400-year-old mummy with repetitive sequence of modern human for his own interest and wrote up the results for the scientific journal 'Nature' which was published in 1985 as a cover story (Pääbo, 1985). From 1990 he occupied the chair as full professor of General Biology, University of Munich, Germany and in 1997, he became Director, Max-Planck-Institute for Evolutionary Anthropology, Leipzig, Germany, and still working on bench (Zagorski, 2006).

Maternal string in offspring

Precise selection of a suitable segment of DNA that carries sufficient information to be a distinguishing marker for species differentiation and is tiny enough to complete the sequencing job in a minimal time period is a challenging task. Such DNA segment must exhibit slow mutation with as few insertions and deletions as possible. Beyond nucleus, a minute quantity of DNA is also available inside the mitochondria. In man and animals, mitochondrial DNA (mtDNA) occurs as a double helical, circular molecule and several copies of such molecules are present in each mitochondrion. Mitochondrial DNA offers several advantages over chromosomal DNA, such as slow mutation rate, limited exposure to recombination, haploid mode of maternal inheritance and high copy number per cell. While only 2 copies of nuclear DNA are present in each cell, the same cell harbours 10^2-10^4 copies of circular mitochondrial DNA, and therefore success rate of mitochondrial DNA recovery is much higher. Mitochondrial DNA of 16569 base pairs codes for 37 genes in human, whereas genomic DNA is too big, having billion base pairs for 30,00 or more genes. The replication of mtDNA is independent of the cell cycle governed by the nuclear DNA (nucDNA) encoded polymerase γ. The mtDNA of a mother is transferred to offspring entirely except for minor random changes due to mutation. Therefore, screening the mtDNA in biological sample provide some clue to trace half of the ancestral parent. Due to the above, sequencing of mtDNA is mainly preferred for DNA barcoding of extinct animal species (Goswami *et al.*, 2014). Sequencing mtDNA of an extinct member of the horse family with the head of zebra and the rump of donkey, the quagga (*Equus quagga quagga*) that died in 1883, was successfully traced after a century gap (Higuchi *et al.*, 1984). Due to invention of PCR, the retrieval of ancient DNA sequences became possible. However, empirical studies with theoretical knowledge indicated DNA in fossil remains dirty with damaged-DNA. The storing conditions greatly influence the decay of DNA. Lower the temperature, less is damage. Sequencing the complete genome of organisms from archaeological samples is quite possible due to advancements in sequencing technologies. Ludovic Orlando, an evolutionary biologist at the University of Copenhagen, was able to sequence DNA from near about a 560,000-to-780,000-year-old horse leg bone buried in the permafrost of the Canadian Yukon (Orlando *et al.*, 2013). Restriction enzyme analysis of mtDNA collected from African and non-African population indicated all the population are of common origin (Cann *et al.*, 1987). Once complete sequence data of the human genome (haploid nuclear DNA) was published (Venter *et al.*, 2001), followed by Simon's genome diversity project data (Mallick *et al.*, 2016), more and more groups are inclined to explore the evolutionary past of human.

Clean gene approach

Within the stretch of mtDNA, Pääbo was looking for a specific gene segment, the inconspicuous region that does not code for any protein but is an essential tracking element for gathering evolutionary relationships among species and population. During cleaning the dirty fossil to amplify the desired segment, there is every possibility of contaminating it with a DNA smear of its own. The earlier effort to amplify the mtDNA from archaic sample resulted enough contaminant ended in culling (Green *et al.*, 2008), afterwards Pääbo along with postdoc

student Richard Edward Green (biomolecular engineering) made clean room approach for sample preparation to minimize the modern human DNA contaminants. In their technical advancement, 4-base DNA insert was added (DNA tag) in the Neanderthal sample as a refinement step so that it could easily identify the modern DNA contaminant. The product lacking the tag DNA indicates a contaminated sample - an alarming signal to discard the sample and not to analyse. Two sets of primer of 105 base pair representing the hypervariable region of the human mtDNA (control region) were selected for PCR amplification. Finally, 123 clones of 379 nucleotides representing 13 fragments were sequenced. While comparing it with the mtDNA sequence of 2051 human collection and 59 collections of chimpanzees, the sequence did not match with any of the existing human (*Homo sapiens*). The mismatching sequence data provided an indication that the collected archaic samples were from the different lineage of human population known to be Neanderthals, the extinct species. (Krings *et al*., 1997). Fiurther Pääbo's lab switched to next generation sequencing to accumulate substantial amount of information in the shortest time. Interestingly the gene sequence analysis from the Neanderthal bone indicated to be of a female species. Further sequencing data supplemented with bone thickness indicated the bone fragment was derived from a female of nearly 13 years of age (Warren, 2018). Using candidate gene approach targeting *mc1r* gene for pigmentation, responsible for pale skin and red hair was spotted in Neanderthal and modern human, given an indication that at least some Neanderthals had pale skin and red hair, like that of *Homo sapiens* of Europe. As mutation of *foxp2* gene impaired speech (Culotta, 2007), Pääbo picked up *foxp2* gene linked to ability for speech in modern human and confirmed its presence in Neanderthal.

Bone beneath the stone

Denisova Cave is located near the Anui River in the Altai region of Russian Siberia that borders with Mongolia and China, and is a key site from which large numbers of hominin fossil bones lacking traditional morphological identification were excavated. The Altai region, and the cave specifically, were the habitat of Neanderthals. Analysing the DNA sequencing data derived from the tip of the little finger of the right hand (separated from the rest of the finger bone) recovered during excavation from Denisova Cave, Viviane Slon and Svante Pääbo

Fig. 1. Virtual restoration of finger bone, missing fragment information is lacking: Callaway, 2019

established another lineage of extinct hominin species named Denisovans (Krause *et al*., 2010). As the rest of the part of the finger bone was missing, digital reconstruction of the complete finger bone was attempted; it appeared to be a structurally slim bone with much similarity of the fingers of modern human than the digits of Neanderthal which is comparatively stout enough (Fig. 1). Further they sequenced the nuclear DNA of a molar tooth found in Denisova Cave carrying a mitochondrial genome extremely like that of the finger bone. The morphological feature of the tooth did not match with Neanderthal or modern human indicative of a different lineage of human species (Reich *et al*., 2010). Employing collagen peptide fingerprinting technique supported through in-depth peptide sequencing analysis, nearly 2315 unidentifiable bone fragments from Denisova Cave were screened that revealed the presence of mtDNA from Neanderthal lineage. The radiocarbon dating indicated its origin to be more than 500,000 years old (Brown *et al*., 2016). A direct descendant of two different groups of early humans has been found in Russia. Genome

analysis of a bone recovered in a Siberian cave indicated the bone was of a 13 years old female who died around 90,000 years ago and was a hybrid having half of its genome from a Neanderthal mother and another half from a Denisovan father (Slon *et al*., 2018). It has been estimated that Neanderthals and Denisovans were contemporary. Genetic variation in ancient and modern humans has given indication that Denisovans and Neanderthals must have bred with each other and with *Homo sapiens.* Analysing the wide-ranging genome sequencing data of several archaic human specimens retrieved from different geographical locations and screening the genome of the modern ethnic population across the world suggests that Neanderthal and *Homo sapiens* might have interbred for which the present-day humans of European or Asian descent harbouring nearly 1 - 4% Neanderthal genome. Pääbo and his group while analysing the DNA sequence of Melanesian individuals has shown that all these individuals harbour on average 104 MB archaic sequence in their genome, out of which 48.9 MB of Neanderthal origin and 42.9 MB of Denisovan, whereas 12.2 Mb is an ambiguous sequence. It indicates that Melanesian individuals have both Neanderthal and Denisovan ancestry (Vernot *et al*., 2016).

Prospect of health aspect

As per recent report genetic inheritance of Neanderthal carried over to modern human is the major risk factor for severe COVID-19. The frequency of Neanderthal risk haplotype in south Asia is near about 30 %, whereas it is almost absent in east Asia. It indicates the tragic effect of gene flow (Zeberg *et al*., 2020). European and African descent differ in their immune response behaviour towards infection due to differential gene expression of the Neanderthal gene which they harbour. Neanderthal genome is responsible for the differential behaviour of African descent responding more strongly to infection and being more prone to autoimmune diseases. This ethnic difference towards infection is due to Neanderthal gene inheritance. (Reardon *et al*., 2016). Neanderthal gene sequence has been found to be associated with lipid catabolism, and the frequency of these genes is three times more in the European population with its beneficial effect (Khrameeva *et al*., 2014). Ancient genetic inheritance from the Neanderthal gene is linked with type-2 diabetes. Similarly, adaptation of human beings at high altitudes on the Tibetan plateau with low oxygen concentration is regulated by the hypoxia pathway EPAS1 gene found in Denisovans and in Tibetans (Huerta-Sánchez *et al*., 2014). The Neanderthal gene also contribute in schizophrenia (Gregory *et al*., 2021). Therefore, these ancestral gene have several health implications in our modern life. Simply we can say that we have received some gift that we cannot shift.

In brief

Mapping Neanderthal genome, researchers have gleaned enough genetic information about our ancient relatives and cousins, yet few relevant questions remain unanswered, such as what they looked like, were they smart, could they talk, did they have sex with modern human, why did they go extinct (Balter, 2009). Excavation of human genome ancestry may rewrite the evolutionary history.

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