Please use this PDF proof to check the layout of your article. If you would like any changes to be made to the layout, you can leave instructions in the online proofing interface. First, return to the online proofing interface by clicking "Edit" at the top page, then insert a Comment in the relevant location. Making your changes directly in the online proofing interface is the quickest, easiest way to correct and submit your proof.

Please note that changes made to the article in the online proofing interface will be added to the article before publication, but are not reflected in this PDF proof.

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

Cell
Leading Edge

Review

The genetic changes that shaped Neandertals, Denisovans, and modern humans

Q1 Hugo Zeberg,[1,2,*] Mattias Jakobsson,[3] and Svante Pääbo[1,4,*]
[1]Max Planck Institute for Evolutionary Anthropology, Deutscher Platz 6, 04103 Leipzig, Germany
[2]Department of Neuroscience, Karolinska Institutet, 17165 Stockholm, Sweden
[3]Department of Organismal Biology, Uppsala University, 75236 Uppsala, Sweden
[4]Okinawa Institute of Science and Technology, Onnason 904-0495, Okinawa, Japan
*Correspondence: hugo.zeberg@ki.se (H.Z.), paabo@eva.mpg.de (S.P.)
https://doi.org/10.1016/j.cell.2023.12.029

SUMMARY

Modern human ancestors diverged from the ancestors of Neandertals and Denisovans about 600,000 years ago. Until about 40,000 years ago, these three groups existed in parallel, occasionally met, and exchanged genes. A critical question is why modern humans, and not the other two groups, survived, became numerous, and developed complex cultures. Here, we discuss genetic differences among the groups and some of their functional consequences. As more present-day genome sequences become available from diverse groups, we predict that very few, if any, differences will distinguish all modern humans from all Neandertals and Denisovans. We propose that the genetic basis of what constitutes a modern human is best thought of as a combination of genetic features, where perhaps none of them is present in each and every present-day individual.

Q3 Q2 INTRODUCTION

A group of humans diverged from the ancestors of modern humans some 600,000 years ago in Africa. Members of that group eventually left Africa and become Neandertals in western and Denisovans in eastern Eurasia. Later, modern humans, i.e., the ancestors of all present-day people, emerged in Africa and spread across that continent and beyond, encountering Neandertals and Denisovans as well as other human forms that are likely to have existed at that time outside of Africa. As a result, Neandertals and Denisovans as well as other forms of so-called archaic humans that existed at the time disappeared from the archaeological record by about 40,000 years ago (Figure 1). Modern humans went on to develop culture and technology that changed rapidly and allowed them to become very numerous and colonize all habitable parts of the globe. A central mystery is why modern humans have become very numerous and culturally diverse, whereas Neandertals and Denisovans disappeared.

Although Neandertals, Denisovans, and modern humans share a common ancestry, they were only in limited contact with each other. That means human evolution has essentially played out three times in the last half million years. We can reconstruct aspects of the evolutionary histories of these groups and the limited contacts between them using genome sequences. For modern humans, we have hundreds of thousands of present-day genomes and genome-wide data from thousands of humans who lived during the past 45,000 years.[1–3] For Neandertals, we have three genome sequences of good quality[4–6] and a dozen or so of moderate or low quality,[7,8] allowing us to identify genetic variants that are likely to have been carried by most or even all individuals among them. At present, only one Denisovan genome of good quality is available.[9] Although more archaic genomes will become available, our knowledge of archaic genetic variation will always remain more limited than our knowledge of present-day people.

Comparisons of genomes from the three forms of human show that they exchanged genes several times when they met outside of Africa (Figure 1). Neandertals received gene flow from groups related to the ancestors of modern humans more than 100,000 years ago.[10–12] In addition, Neandertals and Denisovans exchanged genes[5]; for example, about 80,000–90,000 years ago in southern Siberia, an individual who had a Neandertal mother and Denisovan father has been identified.[13] When modern humans started spreading out of Africa and the Near East less than 100,000 years ago, they mixed with Neandertals[14] and Denisovans.[9] As a result, all people who have genetic roots outside of Africa south of the Sahara carry genetic variants that come from Neandertals.[15] Ancestors of people in Asia also mixed with Denisovans,[9,16] and people of Asian ancestry therefore carry Denisovan variants in addition to Neandertal variants. This genetic contribution from Denisovans is particularly large in some populations in Oceania.[16,17]

The field is now moving beyond the description of the mixing between the groups and starting to explore the functional impacts of genetic variants that differ among them. Here, we discuss what we can learn from studying genetic variants of Neandertal and Denisovan origin in present-day people, with an emphasis on physiologically relevant variants. We focus on examples wherein single genetic variants have been linked to certain traits. Other aspects have been reviewed elsewhere.[18–20] Considering that many ancestral variants or variants

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

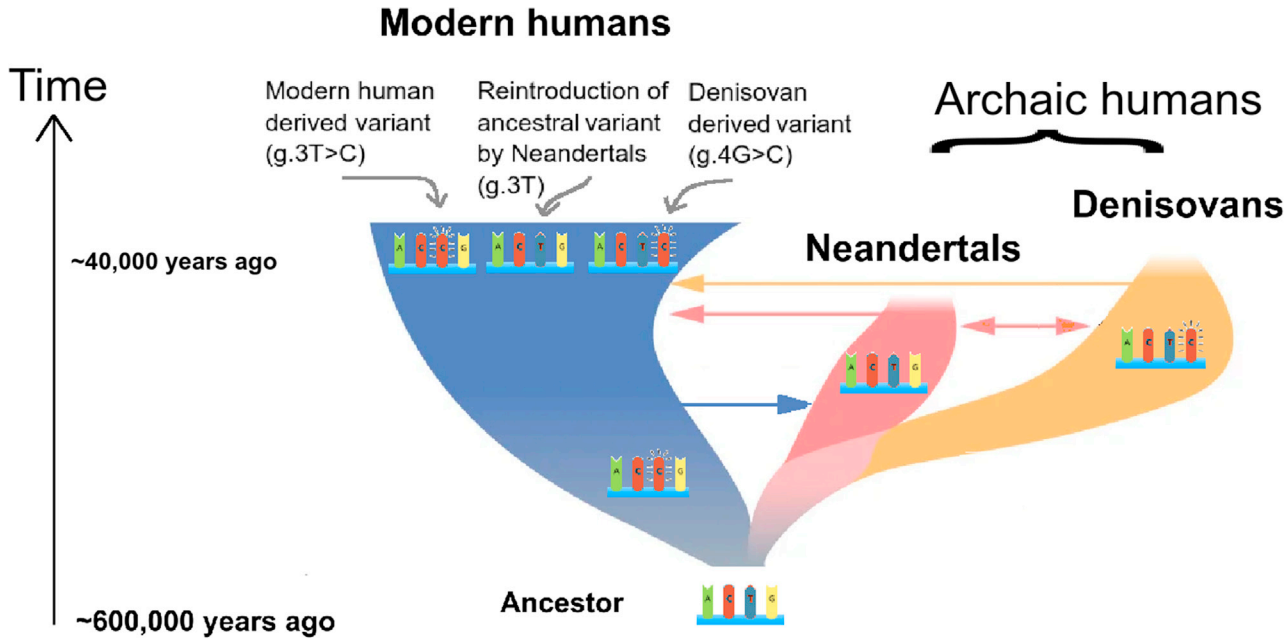


Figure 1. Schematic illustration of the history of archaic and modern humans and DNA sequence evolution
Derived mutations are highlighted. The occurrence of gene flow between groups is illustrated by arrows. The archaic groups contributed both derived and ancestral variants to modern humans. Note that the extent and number of gene flow events and when they occurred are only partially known.

that appeared in the Neandertals or Denisovans are found at low or moderate frequencies in some populations today, we also discuss how we might think about the genetic and biological basis for what sets modern humans apart from Neandertals, Denisovans, and other forms of now-extinct humans.

ARCHAIC VARIANTS

When modern and archaic humans mixed, their first-generation offspring carried one set of modern human chromosomes and one set of archaic chromosomes. As those children lived in modern human populations and in turn had children, the Neandertal or Denisovan chromosomes were broken up and reshuffled in every generation due to the process of recombination. As a result, over the ~2,000 generations that have passed since modern and archaic humans met, archaic DNA segments became shorter and today occur scattered within the genomes of present-day people (Figure 2A). The expected length of an archaic DNA segment can be estimated as $1/(r \times N)$, where r is the recombination rate and N the number of generations. Under the simplifying assumptions of an average recombination rate (~1 centimorgan per mega base pairs) and a single admixture event that happened 2,000 generations ago, the expected typical length for an archaic DNA segment today is approximately 50 kilobases (kb). However, for each genomic region where archaic DNA segments are found, they occur as a distribution of fragments of different lengths, where the ends reflect past recombination events (Figure 2B). However, to be confident that a fragment came over by gene flow some 2,000 generations ago, they must be of substantial length.

There are also DNA segments in the human genome that are similar to the Neandertal or Denisovan genomes, not because these segments were contributed from archaic to modern humans but because these segments persisted independently in both archaic and modern humans since the two populations shared a common ancestor about half a million years ago. Since recombination has acted for a much longer time on such fragments, the chance that they are longer than 13 kb is less than 0.05 under the recombination rate assumed above.[22] This gives an approximate idea of the size differences expected between DNA fragments inherited from the common ancestors (unlikely to be more than 13 kb) and those inherited from Neandertals or Denisovans more recently (expected to be about 50 kb). However, when examining any particular segment, it is important to consider that the size also depends upon the local recombination rate in the part of the genome where an archaic DNA segment finds itself. This rate varies not only across the genome but also among populations and over time.[23]

Assuming a mutation rate of $1.61e^{-8}$ per site per generation[24] and a Neandertal branch length of 19,500 generations,[25] a DNA segment inherited from Neandertals with the typical length of 50 kb will carry ~16 variants that result from mutations that happened on the Neandertal lineage, i.e., they are "derived" in Neandertals (Figure 1). In addition, modern humans have accumulated about as many derived changes independently of the Neandertals. Finally, since the Neandertals and modern humans often inherited different versions of DNA segments that carried differences from each other in the ancestral population, the number of differences between modern human and Neandertal DNA segments can be even larger. Thus, archaic DNA segments often

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

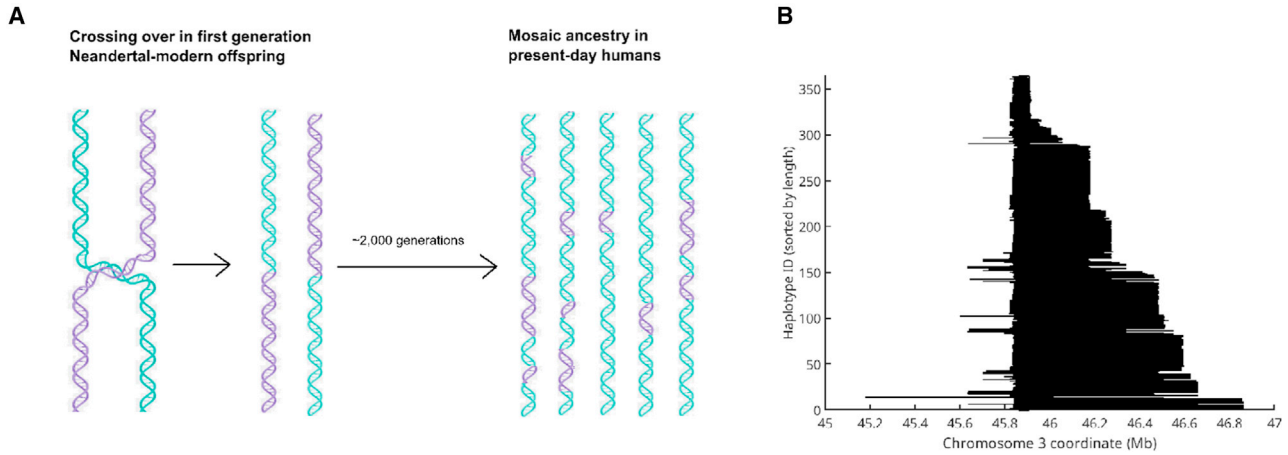


Figure 2. The effect of recombination on archaic DNA sequences in modern humans

(A) In the germ line of the offspring from an archaic and modern human, the two chromosomes recombine. If the descendants in subsequent generations live in modern human communities, further recombination will progressively shorten the archaic DNA segments, resulting varying lengths of DNA segments of archaic ancestry spread across chromosomes in the population.

(B) A region on chromosome 3 that carries Neandertal DNA segments of different lengths that increase the risk for severe COVID-19.[21] The ends of the Neandertal DNA segments reflect past recombination events. Note that numerous ends to the left suggest the existence of a recombination hot spot around chromosomal coordinate 45.85.

stand out in the human genome in that they carry more single-nucleotide variants (SNVs) than surrounding regions.

ARCHAIC GENE FLOW

Although Neandertal variants are found in all populations outside Africa and make up about 2% of the present-day non-African genomes, different individuals in a population often carry different Neandertal variants and the frequencies of individual variants can sometimes differ drastically among populations. A smaller number of Neandertal-derived variants are also found in Africa south of the Sahara as a result of gene flow to Africa from Europe and Western Asia after the Neandertals disappeared.[11]

The fact that the fraction of Neandertal DNA in present-day genomes is relatively consistent at around 2%[15] suggests that the bulk of the Neandertal contributions occurred relatively early, perhaps about 60,000 years ago, in populations that left Africa and became ancestral to all non-Africans. Nevertheless, analyses of modern human genomes derived from specimens older than 40,000 years in Europe have shown that Neandertals also contributed locally to modern human groups. For example, an approximately 40,000-year-old individual from Romania had a Neandertal ancestor 4–6 generations back in her family tree,[26] and three 45,000-year-old individuals found at a site in Bulgaria also had close Neandertal relatives.[27] However, many of these early modern populations did not leave many or even any descendants that contributed genetic variants to present-day populations. Thus, those later contributions by Neandertals may have had less of an impact on present-day populations.[26,27]

Variants coming from Denisovans that are found in mainland Asian and Native American populations contribute to about 0.2% of present-day genomes. In many populations in Oceania, over 5% of the genomes are of Denisovan origin.[17,28] The Denisovan genetic contributions come from at least two distinct Denisovan populations.[29] One of these was quite closely related to the Denisovan genome sequenced from southern Siberia,[9] and its traces can be found in present-day people in Japan, China, and other parts of East Asia. The other Denisovan population contributed to the ancestors of populations over large parts of Asia, including East and South Asia. This Denisovan population was much more distantly related to the Denisovan genome currently available.[29] Contributions from yet other Denisovan populations to the ancestors of people in the Pacific may also have occurred.[28,30,31] A fascinating question is how the genetic changes that accumulated on the archaic and modern human lineages affected the physiology of the three forms of humans, two of which became extinct.

STUDYING THE EFFECTS OF ARCHAIC AND MODERN VARIANTS

The presence of genetic variants (typically SNVs but also insertions and deletions and other changes in the DNA sequences) contributed by Neandertals and Denisovans to present-day people allows us to investigate the effects of such variants by asking whether they are associated with phenotypes in present-day people. These variants can result from mutations that happened on the Neandertal or the Denisovan lineage; i.e., they are derived on those lineages, or—more rarely—from changes occurring on the common lineage that led to the two archaic groups. Notably, archaic DNA segments can also introduce ancestral variants[32] at positions where present-day humans carry derived variants caused by mutations that happened in modern humans (Figure 1).

Variants that occurred on the modern human lineage and are present in almost all individuals today cannot be studied by associating them with phenotypes, as almost no one lacks these variants, and thus, there is no control group. Similarly, variants

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

that emerged in archaic humans and were not contributed to modern humans cannot be studied in present-day people. However, these variants can be studied in model systems. For example, variants can be experimentally changed to the ancestral state or the state seen in archaic humans by genome editing. The effects of these variants can then be studied in cell culture or organoids that partially mimic the physiology of human organs. Another possibility is to introduce modern or archaic human genetic variants into the genomes of mice and study their effects in the organism.[33] However, studying actual carriers of the archaic variants is often very informative. For this to be possible, several requirements must be met.

One requirement is that the archaic variants need to occur at frequencies large enough to be detected in association studies or population cohorts where genetic and phenotypic information are available. Because such studies and cohorts have been mostly generated in populations of European descent, more Neandertal than Denisovan variants have been studied to date. However, large biobanks are increasingly being established in Asia (e.g., BioBank Japan, Tohoku Medical MegaBank, and The Korean Genome and Epidemiology Study), opening the possibilities to also study Denisovan genetic contributions.[34]

Another requirement for studying archaic genetic variants in present-day humans is that they need to have phenotypic effects large enough to be detected. The effect of a single variant is often small and may be impossible to detect in cohorts of a few tens or hundreds of thousands of individuals. Fortunately, some types of genetic variants are relatively likely to have phenotypic effects that can be studied. For example, variants that affect the expression or the amino acid sequences of enzymes or proteins that transport molecules across membranes may have effects that directly affect measurable processes such as catalysis of a chemical reaction or accumulation of molecules in cells. However, many traits of interest are genetically complex, i.e., affected by many genetic variants. In addition, archaic variants involved in complex traits may be hard to detect because their effects may depend on archaic variants elsewhere in the genome that may not exist in present-day humans or be very rare. Below, we discuss both categories. We start with genetic variants that emerged in Neandertals, then discuss the variants that emerged in Denisovans, and finally examine the variants that emerged in modern humans.

GENETIC VARIANTS THAT EMERGED AMONG NEANDERTALS

For Neandertals, three genomes of high quality and a dozen of genomes of lower quality are available. In spite of these small numbers, they vary in age from approximately 130,000 years to approximately 45,000 years and cover much of the Neandertal range from Western Europe to southern Siberia. Thus, any variant that is derived in Neandertals and present in homozygous form in all available Neandertal genomes is very likely to have been present at high frequency among the late Neandertal populations. Most such variants have been studied because they also occur in present-day humans as a result of gene flow. Here, we discuss a few variants that emerged in Neandertals and affect various aspects of human physiology.

Metabolism

One of the first Neandertal DNA segments in present-day humans for which a phenotypic effect was described is on chromosome 17. This DNA segment carries regulatory variants affecting the expression of the pyruvate transporter protein SLC16A11 in the liver and amino acid substitutions that decrease the interactions of SLC16A11 with chaperones needed for its expression at the cell surface.[35] The reduced cell surface expression of SLC16A11 results in changes to fatty acid and lipid metabolism associated with increased risk of diabetes[15] and increased risk for type 2 diabetes.[36] While these variants are located on DNA segments of different lengths (defined here and below as having an association of Neandertal-like alleles in the population of $r^2 > 0.8$ on a scale between 0 and 1), they all share a DNA segment of 73 kb.

Some other Neandertal gene variants with metabolic consequences of medical importance have also been described, for example, one that increases the risk for protein-caloric malnutrition[37] and others that affect the metabolism of commonly used drugs.[38]

Sensory organs

Another Neandertal DNA segment in present-day humans with phenotypic effects is located on chromosome 2 and encodes the sodium channel SCN9A (Nav1.7), which initiates the sensation of pain in peripheral nerve endings. In Neandertals, this protein carries three amino acid changes, and the combination of two of these amino acid changes shortens the time that the channel remains refractory after having been activated, causing it to be open longer after being stimulated. This may make nerve cells more sensitive. About 0.4% of people in the United Kingdom carry a 23-kb-DNA ($r^2 > 0.8$) fragment with the Neandertal version of *SCN9A*. These carriers report experiencing more pain than non-carriers in questionnaires. This increase corresponds approximately to the increase in pain experienced by people with each additional 8 or 9 years of life.[39] Since people who express the Neandertal version of SCN9A in heterozygous form experience more pain, it is tempting to suggest that Neandertals, who carried this version of the protein in homozygous form, were more sensitive to pain than present-day people. If so, this may even have been a selective advantage given that biallelic loss-of-function mutations in *SCN9A*, which causes congenital insensitivity to pain, reduces life expectancy.[40]

Gestation

A 56-kb-DNA fragment of Neandertal origin on chromosome 11 encodes the progesterone receptor, which—activated by the steroid hormone progesterone—functions as a transcription factor that regulates gene expression. It carries several differences to other versions of this fragment, including an amino acid variant in the encoded protein and the insertion of an *Alu* element, a transposable element active in humans and other primates. In some populations, the Neandertal version of the gene occurs at a carrier frequency of up to 21%. Since it is associated with an increased risk of premature births in present-day humans,[41] it has been suggested to represent an evolutionary disadvantage to Neandertals as premature babies represent a risk, especially in the absence of modern medical care.[42]

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

However, the Neandertal variants are also associated with an approximately 15% decreased risk for bleeding and miscarriages early in pregnancy as well as with having increased numbers of siblings.[43] It is therefore tempting to speculate that it represents an evolutionary trade-off where the Neandertal variants rescues pregnancies that would otherwise have resulted in miscarriages, but the price paid is that some of these pregnancies result in premature births. Notably, two different versions of the Neandertal progesterone receptor gene have been contributed to modern humans, and both have risen in frequency. This has been shown by an increase in their occurrence in skeletal remains of individuals over the past 10,000 years.[43] Both Neandertal versions result in higher expression of the progesterone receptor and may thus mediate a higher progesterone effect during pregnancies. This is compatible with the finding that progesterone administration lowers miscarriage rates in women who previously experienced miscarriages[44] and suggests that increased progesterone effects mediated either by higher hormone levels or by higher receptor levels may protect at-risk pregnancies.

Immune system

Infectious diseases are a major selective factor and therefore influenced the fate of many archaic gene variants. For example, Neandertal DNA segments carrying genes encoding proteins that interact with viruses are particularly likely to have risen to high frequencies and are therefore likely to have been advantageous in the ancestors of present-day Europeans.[45] Similarly, Neandertal variants that influence the transcription of genes involved in the responses, especially viruses, have been frequently introduced into present-day populations.[46,47] Furthermore, genomic regions that contain innate immunity genes seem to harbor more Neandertal variants than the rest of the coding genome.[48] Some Neandertal gene variants that influence the immune response to pathogens may also increase the risk of autoimmune disease.[15,34,49]

One example of an immunological archaic contribution is a 143-kb-long DNA segment on chromosome 4. It contains three genes encoding Toll-like receptors, which are expressed on dendritic cells and macrophages and recognize conserved features of microbes and activate innate immune responses. Two different Neandertal variants and one Denisovan variant of this region occur in modern humans.[49] Since three different archaic variants have been contributed to modern humans and persisted there, it seems that they have been advantageous. The archaic variants increase the expression of the receptors and are associated with increased resistance to *Helicobacter pylori* infections.[50] As is the case for other variants that affect immune responses, these variants differ in frequency among present-day populations, suggesting that local selection from pathogens may have affected them.

A striking example of archaic variants affecting the response to infections is a Neandertal DNA segment of 49 kb on chromosome 3. It carries 13 nucleotide substitutions[21] and confers an almost 2-fold higher risk of needing mechanical ventilation or dying from infection with severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2).[51] The underlying mechanism may involve not only the expression of *LZTFL1*, one of the genes encoded on the fragment,[52] but also the expression of other genes.[47]

The Neandertal variants on chromosome 3 also influence the expression of other genes in the region, notably *CCR5*, which encodes a chemokine receptor that serves as a co-receptor for the human immunodeficiency virus (HIV). It is less expressed in individuals carrying the Neandertal DNA segment, leading to an approximately 25% reduction of the risk of becoming infected with HIV. Thus, although this Neandertal segment is negatively selected in the SARS-CoV-2 pandemic, it has positive effects in other contexts.[53] This seems to also have been the case in the past. Whereas the Neandertal variant has reached carrier frequencies approaching 60% in South Asia, it is almost absent in East Asia,[21] perhaps suggesting that other infectious diseases in the past have caused the frequency of the Neandertal DNA fragment to increase in South Asia and decrease in East Asia.

Association studies of COVID-19 disease severity have also revealed that a 75-kb-long DNA segment on chromosome 12, previously shown to derive from Neandertals,[54] is protective against severe disease. Unfortunately, its effect size is almost five times smaller than that of the Neandertal risk variant on chromosome 3.[51,55] The chromosome 12 segment encodes three genes that synthesize molecules involved in the activity of an RNase that degrades double-stranded RNA. The Neandertal variant encodes the ancestral splice form of one of the genes,[56] which becomes localized to membrane structures where the SARS-CoV-2 virus replicates and is therefore presumably more efficient in eliminating cells infected by this and other RNA viruses.[57] Tracking this variant over time by using 1,641 ancient modern human genomes has shown that it has risen in frequency twice in the past in European populations,[55] probably in response to infectious diseases.

The fact that many Neandertal gene variants affect phenotypes related to infectious diseases may not be coincidental. Infections are a major factor affecting human populations and one that changes particularly rapidly over time. Archaic human populations in Eurasia likely encountered distinct pathogenic challenges, necessitating adaptations in their immune systems. Such local adaptations to pathogens are a well-documented phenomenon in modern humans, a prominent example being the relationship between sickle cell anemia and malaria resistance. There is little reason to doubt that archaic humans also underwent similar evolutionary pressures. The differences in immune systems between archaic and modern humans might be a particularly clear manifestation of their prolonged geographic separation.

Complex traits

Most human traits like height or cognitive abilities vary continuously in the population and are influenced by many genetic variants in many parts of the genome, as well as by the environment. Studying how gene flow from archaic humans has affected such complex traits is demanding, most notably because the effect size of each genetic variant is generally small. Current analyses therefore do not rely on direct associations between individual variants and complex phenotypes. Instead, it is investigated whether all Neandertal or Denisovan variants in a population, when combined, tend to influence a complex trait in a particular

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

direction or account for a portion of variance in the risk for a complex disease. Such studies have shown, for example, that Neandertal alleles explain a significant fraction of the variation in risk for depression and sun-induced skin lesions.[37]

Some studies have also asked whether there is an over- or underrepresentation of archaic variants that influence a certain trait. Generally, complex traits were found to be less influenced by Neandertal ancestry than expected. Of 405 complex traits, dermatological traits were the most influenced, while cognitive traits were the least influenced by Neandertal DNA.[58] The latter observation is compatible with the observation that the expression of genes in the brain are less often influenced by Neandertal variants than the expression of genes in other organs.[59]

Gene expression

Many complex traits may be influenced by gene expression levels. One study has shown that in the individuals who carry one Neandertal and one modern variant of a gene,[60] the Neandertal variants tend to be expressed less than the modern human variants. This is particularly pronounced in testis and the brain, especially the cerebellum and basal ganglia, suggesting that selection against Neandertal regulatory sequences is particularly strong in those tissues. This is compatible with the relatively low influence of Neandertal variants on cognitive traits.[58]

Recent studies have shown that older archaic variants were more tolerated among modern humans than more recent variants. Variants shared between a Siberian Neandertal[5] and a Neandertal from Croatia[6] are likely to be older than variants found in just one of these individuals, while variants shared between Neandertals and Denisovans are likely to be even older. In the case of gene regulation, it has been shown that archaic variants upregulating the expression of genes are underrepresented in present-day populations, except for variants shared between Neandertals and Denisovans.[59] Thus, variants that persisted in archaic groups for a long time may have more benign effects on modern humans than variants that originated more recently in Neandertals. This could be because the archaic groups had more time to eliminate deleterious variants through purifying selection. It is also possible that older archaic variants were more compatible with ancestral traits that are more often shared with the ancestors of modern humans. In addition, deleterious genetic variants may have accumulated particularly readily in late Neandertals because their population size was small,[61] which allowed slightly deleterious variants to become frequent more easily.[62]

GENETIC VARIANTS THAT EMERGED AMONG DENISOVANS

As only a single high-quality genome of a Denisovan is currently available, little is known about how frequent any particular variant was among them. In addition, relatively few examples of Denisovan genetic contributions with phenotypic consequences are known. The primary reason is that only a few association studies and biobanks are available in Asia, a situation that is fortunately now rapidly changing.

High altitude adaptation

One striking example of Denisovan influence on present-day populations is a 33-kb Denisovan DNA segment on chromosome 2 that occurs at an allele frequency of over 80% among Tibetans, while being absent or very rare in other Asian populations.[22,63] It encodes EPAS1, a transcription factor induced by hypoxia that is involved in adaptation to low oxygen levels. Denisovans were present on the Tibetan high plateau[64,65]; some of them may thus have been adapted to life at high altitudes and presumably contributed this genetic predisposition to modern humans as they arrived in the region.

Cold adaptation and facial morphology

Another example of a Denisovan genetic contribution is a 28-kb segment on chromosome 1, carrying the genes *WARS* and *TBX15*. It is present in almost 100% of Greenlandic Inuit and several other populations.[66] The Denisovan variants affect the expression of genes that may influence adaptation to low temperatures, possibly by inducing brown fat.[67] Curiously, this segment has also been associated with the thickness of the upper lip and its protrusion.[68] However, the genetic variant in the 28 kb segment most strongly associated with lip morphology (rs3790553) is not present in the Denisovan genome sequenced to date.[9] This does not rule out that another Denisovan variant may be associated with the trait or that this variant might be present in a Denisovan genome not sequenced. However, we caution that in this and other cases, an archaic DNA segment could have recombined with a modern human variant associated with the trait early after its introduction into the modern human population. It is also possible that a genetic variant has emerged on the archaic DNA segment after it was introduced into modern humans.

GENETIC VARIANTS THAT EMERGED AMONG MODERN HUMANS

Genetic changes that occurred on the modern human lineage and are present in almost all people today (i.e., are "fixed") set modern humans apart from archaic humans.[69] These changes are interesting because they could underlie biological differences that may be important for the modern human phenotype. However, the extent to which any single such change might cause a phenotype is often unclear. Nevertheless, it seems that many changes that has occurred on the modern human lineage and affected, for example, gene expression may have been more beneficial for modern humans than changes that occurred in the archaic lineages. This is suggested by the observation that archaic DNA segments, which affect gene expression and other traits, tend to be enriched for ancestral variants rather than for variants derived in archaic humans.[32]

Another complicating factor is that many traits of interest may be complex and therefore influenced by genetic changes that have not reached fixation. For instance, body height is influenced by variation at many positions in the genome. If we assume that there is selection pressure favoring tall individuals in a population, it is then possible that the height of individuals will increase by height-increasing alleles at many different positions in the

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

genome becoming more frequent in the population without any of them becoming very frequent or present in all individuals.

Still, some individual variants that emerged in modern humans may have effects that can be identified and studied. For example, nucleotide substitutions that occurred in transcription factor binding sites in modern humans may change the expression of genes.[70] Other examples are changes that affect the structure of enzymes, some of which we discuss below.

Purine biosynthesis

Changes that cause amino acid substitutions in enzymes are particularly amenable to investigation because the function of the enzyme can be studied *in vitro* and the concentrations of substrates and products can be determined in cells and tissues. An example is adenylosuccinate lyase (ADSL), an enzyme that catalyzes two steps in the synthesis of purines, molecules that are building blocks of DNA and RNA and have many other important functions in the cell. ADSL carries an amino acid substitution that occurred in modern humans and is found in almost all people today. It reduces the stability of the enzyme.[33,71] The modern version of the enzyme causes lower levels of purines to be synthesized in the cells. This was shown by experimentally introducing the ancestral version of the enzyme that Neandertals and Denisovans carried into human cells in tissue culture and the modern human version of the enzyme into mice.[33] Relative to apes, human tissues display lower purine levels, and this difference is largely explained by the amino acid substitution in ADSL. However, it is unclear what consequences this has at the organismal level. One approach to address this is to study mice or other model animals into which the derived, modern human-like substitution has been introduced. However, an obvious caveat is that the genetic background of the mouse is different from that of humans. Another approach is to find rare people who carry the ancestral version of ADSL today.

Oxidative stress

The enzyme glutathione reductase is important for maintaining a reduced intracellular environment and preventing oxidative stress. In most present-day humans, glutathione reductase carries an amino acid substitution that is not seen in archaic humans and other primates. When expressed *in vitro*, the ancestral enzyme produces more oxygen radicals in the absence of its substrate oxidized glutathione, suggesting that it may be less efficient in preventing oxidative stress when cells are not exposed to oxidative stress.[72] The ancestral version of the enzyme is still present in some people today due to gene flow from Neandertals, and its effect can be studied using the UK BioBank data, where 0.06% of the participants carry this variant. Indeed, the ancestral version is associated with increased risk of atherosclerosis and inflammatory bowel disease—diseases that have an inflammatory component that can be exacerbated by oxidative stress.[72] This supports the hypothesis that the modern human version of the enzyme provides better protection against oxidative stress.

Splicing

The protein NOVA1 is involved in splicing and processing of 3′ ends of transcripts. It carries an amino acid substitution in modern humans. Brain organoids generated from human stem cells that have been genetically modified to carry the ancestral variant showed differences in morphology, synaptic protein expression, and electrophysiology.[73] While this experiment is a valuable approach to study the physiological relevance of the genetic difference, the observed effects may be due to deletions of the target gene that often occur as a side effect of genome editing.[74,75] Interestingly, the ancestral version of NOVA1 occurs in at least four individuals in current databases, including in TOPMed where other data and phenotypes are available. Thus, the effect of the ancestral variant could be studied in human carriers.

Neurogenesis

Three proteins involved in chromosome segregation during cell division, KIF18A, KNL1, and SPAG5, carry one, two, and three modern human-specific amino acid substitutions, respectively. Introducing these six changes into mice (alone and in combinations)[76] revealed that the modern human changes in the *Knl1* and *Kif18A* prolong a part of mitosis, the metaphase, when the chromosomes are lined up before being pulled apart to the two daughter cells. This prolongation is observed in apical progenitors that generate neurons during brain development. When the three ancestral variants were introduced into *KNL1* and *KIF18A* in human stem cells that were then used to generate brain organoids, a shortening of metaphase was observed. Notably, the shortened metaphase in the organoids with ancestral variants is similar to the difference in metaphase length that is observed between chimpanzee and human organoids.[76] The prolongation of metaphase in modern humans seems to correlate with a reduction in the number of mis-segregating chromosomes. This suggests that these changes may increase the accuracy of chromosomal segregation in early neurogenesis. Interestingly, the modern human version of *KNL1* is found in some late Neandertals as the result of gene flow from modern human ancestors, adding to the evidence that contacts between the three forms of humans occurred on numerous occasions.[12]

In modern humans, the enzyme transketolase-like 1 (TKTL1) in the pentose phosphate pathway carries an amino acid substitution. When the archaic and modern human versions of the proteins are overexpressed in the developing brains of either mice or ferrets, the modern human version results in the generation of more basal radial glia cells.[77] Conversely, human brain organoids generated from stem cells carrying the ancestral form of TKTL1 generate fewer basal radial glia cells and fewer neurons. This suggests that this amino acid substitution causes metabolic changes that affects neurogenesis early in development.

Both the changes in KNL1 and KIF18A that affect chromosomal segregation and the change in TKTL1 that affects the generation of neuronal precursors have effects during early brain development. Further work is needed to elucidate if these effects have any consequences later during development when other mechanisms may compensate for early effects or for the adult brain.

ANCESTRAL VARIANTS IN PRESENT-DAY PEOPLE

Historically, people of European descent have been the primary subjects of genomic studies. Therefore, the initial lists of genetic

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

changes that were thought to exist in all present-day humans were incomplete, as they only considered some of the World's populations. As more human genomes from more parts of the world are sequenced, ancestral genetic variants that were thought to be absent in present-day humans are found in some populations. Conversely, populations of European descent are found to carry ancestral variants that do not exist almost anywhere else.

There are two reasons why ancestral variants may occur today. First, some ancestral variants have persisted since the common ancestors of modern and archaic humans lived 600,000 years ago. This is especially frequent in populations in Africa, where the genetic diversity is greater than outside of Africa. Second, ancestral variants have been contributed to present-day people by Neandertals and Denisovans. Populations with higher archaic ancestry, for example, in Oceania,[9,28] are likely to have particularly high levels of reintroduced ancestral variants. Figure 3 illustrates these two scenarios using genomes of 25 Khoe-San[78] (a diverse group of indigenous people in southern Africa) and 25 Ayta[28] (an indigenous group in the Philippines) individuals. Of the 113 variants that were considered to be nearly fixed among present-day people (see https://bioinf.eva.mpg.de/catalogbrowser), we find that 42 are found in their ancestral form in Khoisan, Ayta, or both (Figure 3). A striking example of this is TKTL1. The ancestral version of TKTL1 exists in present-day humans at an over-all variant frequency of about 0.03%. However, in the Khoe-San, its frequency is around 32%. The high frequency of the ancestral TKTL1 variant is likely an example of a genetic variant that has been lost in most other groups.

As ongoing and future genome-sequencing projects include more geographically diverse populations, even more ancestral versions of genomic changes that are currently only known in their derived forms are likely to be found. Ultimately, when biobanks that contain not only individual information about genomic variation but also phenotypic information will include more diverse and previously understudied groups, they will enable the investigation of many more archaic variants and their effects on physiologically relevant phenotypes.

A COMBINATORIAL VIEW OF HUMAN MODERNITY

The occurrence of rare ancestral variants in cases where most people carry derived variants raises questions about how to define modern humans from a genetic perspective. To gain a perspective on this, it may be useful to consider how paleoanthropologists define Neandertals and modern humans based on skeletal morphology using features such as the robustness of their skeleton, the presence of prominent brow ridges, and elongated crania with occipital "buns." Most of these features are derived in Neandertals.[79] However, these features can also be found in modern humans. For example, some people today have as robust skeletons or as prominent brow ridges as Neandertals. Nevertheless, only Neandertals carry a combination of all or most of these features.

Similarly, the genetic basis of the modern human phenotype should be seen as a combination of derived genetic features, where not every feature is present in every modern human. Rather, some derived modern features may exist in their ancestral forms in some people today either because they have persisted from the common ancestor of archaic and modern humans or because of archaic gene flow. Thus, from a genetic perspective, modern humans can be defined as carrying combinations of derived variants, which are common but not present in all of us today (Figure 4). Conversely, some derived genetic variants typical of modern humans are present in some archaic humans who lived late enough to carry variants that were introduced into archaic population from modern humans, as illustrated by the derived form of *KNL1* seen in some Neandertals.[12]

Of course, modern humans may also exhibit some changes that are almost completely fixed (if very rare back-mutations are disregarded). One example is a substitution in the *AHR* gene that changes an amino acid in the aryl hydrocarbon receptor and reduces its ability to induce the expression of enzymes that metabolize aromatic hydrocarbons.[80] This variant has, to our knowledge, not been seen in any present-day human.

There are some genomic regions in present-day humans that are devoid of variants contributed by Neandertals and Denisovans. They are particularly enriched in variants fixed among modern humans because they contain hardly any archaic genetic contributions. These genomic regions may be of particular interest to understand modern human uniqueness.[15,17,81]

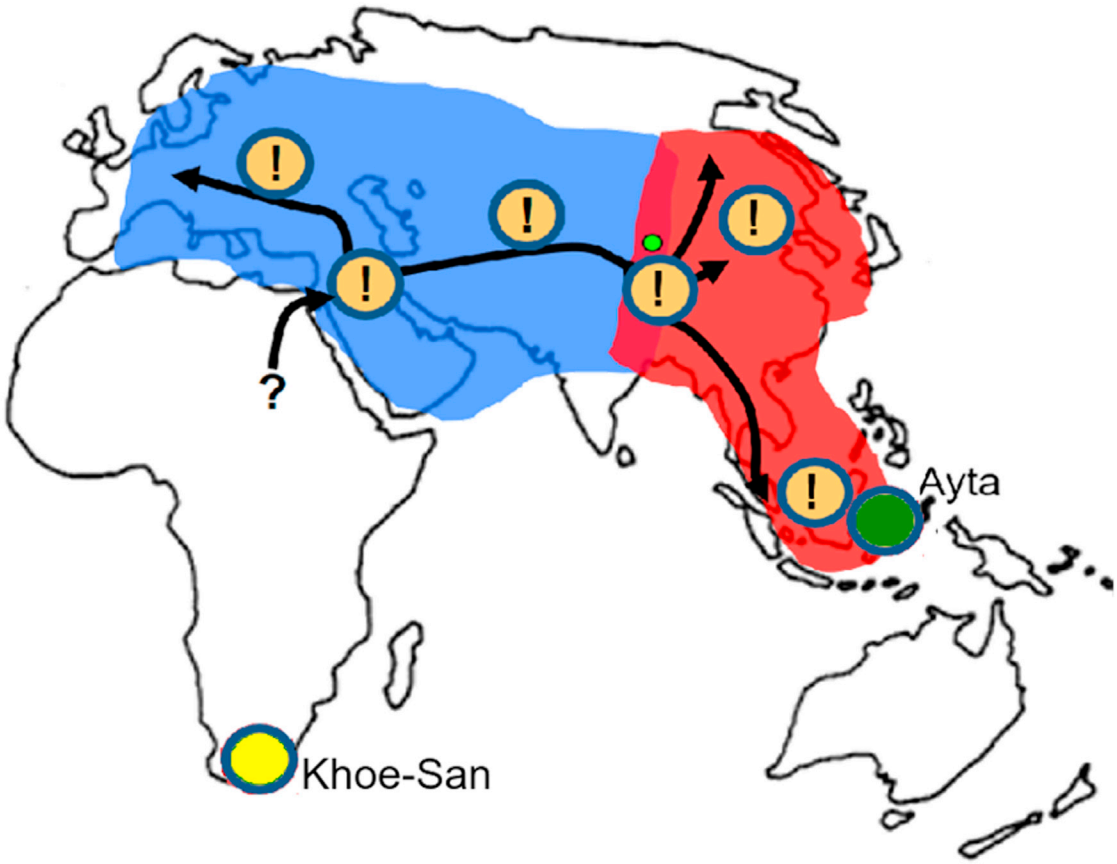
CONCLUSIONS AND OUTLOOK

The genomes of Neandertals and Denisovans have shown that they had common ancestors with modern humans about 600,000 years ago. In contrast, the ancestor of all present-day humans existed perhaps about 300,000 years ago. As a result, only 1.5%–7% of the genome are made up of regions where archaic genomes fall outside the variation of present-day people.[82] These regions harbor genetic variants the frequency of which differs drastically between modern and archaic humans. However, variants in other regions of the genome may also differ between these groups. Differences in both types of regions in the genome are likely to be important for the modern human phenotype. From a genetic perspective, modern humans are best defined as a combination of genetic features wherein each individual carries most but not necessarily all of these genetic features. This "combinatorial view" of what constitutes a modern human does not negate the fact that individual genetic changes may be more functionally important than others. In fact, it is likely that an "explosive combination" of several or many important changes came together and became frequent in the population that became the ancestor of modern humans. These changes were the genetic basis that allowed modern humans to embark on a historical trajectory radically different from other human forms that existed both before and contemporaneously with modern humans. A grand challenge for the future is to identify these genomic changes.

Gene flow between archaic and modern humans offers a way to study the physiological consequences of many of the variants typical of both modern and archaic humans. In the case of variants that changed in modern humans, mixing between the groups often introduced the ancestral versions into present-day people. However, variants introduced by archaic gene flow

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

A



B

Gene	AA	AAF Khoe-San	AAF Ayta
APOF	A178G	0.36	0.16
COX7B2	Q16R	0.02	.
CSGALNACT1	I240V	.	0.04
EDEM3	R20S	.	0.08
EFCAB4B	V474I	.	0.02
ENTHD1	R292T	0.26	.
EVC2	G488S	.	0.04
FAAH	A476G	.	0.04
GPR132	E328Q	0.02	.
HMGXB3	G1214S	0.02	.
ITGB4	R1748H	0.02	.
KATNA1	T343A	0.04	0.12
KLLN	N131S	0.26	.
LHFPL4	splice	.	0.40
MFSD12	L326F	0.02	.
MIIP	H280Q	.	0.04
MPL	T374A	.	0.06
NEK6	H325D	0.02	.
NOP14	T493R	.	0.12
NOTO	I237N	.	0.02
OSBP2	M760V	0.06	.

Gene	AA	AAF Khoe-San	AAF Ayta
PIEZO1	G307S	.	0.10
PIGZ	T275M	.	0.02
PNLIP	M414K	0.10	.
RB1CC1	R1216K	0.10	0.08
RPTN	H246L	.	0.16
SH2D4A	E284K	0.12	.
SLC12A1	S24N	0.06	.
SLC8A1	T22I	0.04	.
SPAG17	T1415A	0.04	0.02
SPAG17	Y431D	0.02	0.02
SPAG5	H410D	0.02	.
SPAG5	G162E	0.02	.
SPAG5	S43P	0.02	.
STARD9	T3925A	.	0.10
TCHH	K1209E	.	0.12
TKTL1	R317K	0.32	0.00
TMPRSS2	V70A	.	0.10
USH2A	K5026E	.	0.22
VCAN	D3042N	0.02	0.02
ZNF106	A697T	.	0.10
ZNF292	I2574T	0.06	.

Figure 3. "Unique" modern human genetic variants occur in their ancestral form in some present-day people

(A) Map illustrating how modern humans originated in Africa and spread across Africa and Eurasia, mixing (exclamation marks) with Neandertals (blue) in western Eurasia and Denisovans (red) in eastern Eurasia.

(B) The ancestral versions of 42 out of 113 protein-changing variants that were considered to be nearly fixed (see https://bioinf.eva.mpg.de/catalogbrowser) can be found in 25 individuals from the Khoe-San in southern Africa (yellow)[78] and 25 individuals from the Ayta in Malaysia (green).[28] aa, amino acid substitution; AAF, ancestral allele frequency.

Q8

Please cite this article in press as: Zeberg et al., The genetic changes that shaped Neandertals, Denisovans, and modern humans, Cell (2024), https://doi.org/10.1016/j.cell.2023.12.029

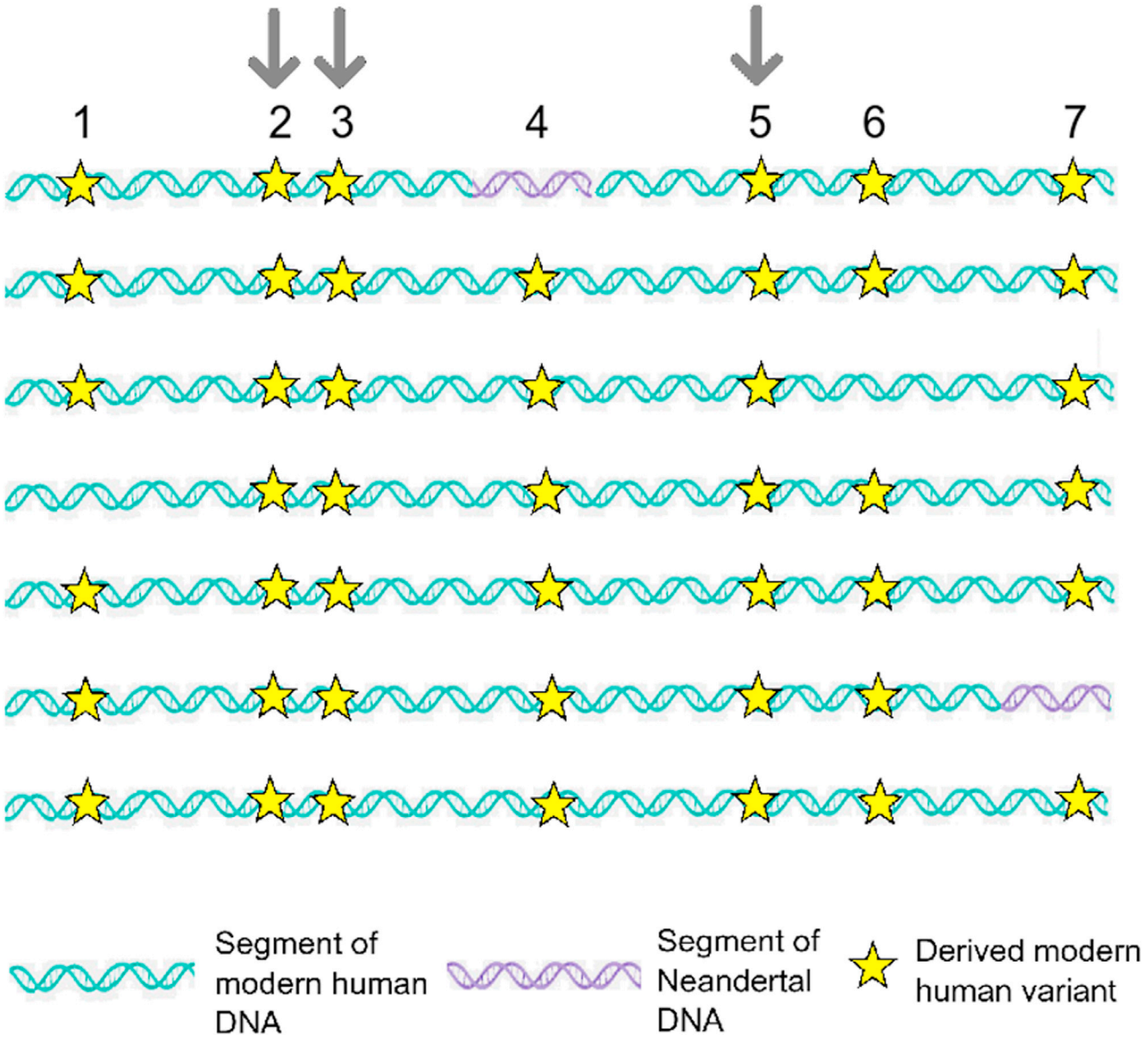


Figure 4. Schematic illustration of the present-day chromosomes and the "combinatorial view" of human genetic modernity

In this example, seven chromosomes carried by present-day people carry seven genetic variants that are frequent among modern humans. Three of them (variants 2, 3, and 5, arrows) occur on all chromosomes. The others occur on many but not all chromosomes, either because the ancestral variants have persisted in the modern human population (variants 1 and 6) or because Neandertals contributed ancestral variants to modern humans (variants 4 and 7). However, all chromosomes (and thus all individuals) carry almost all of the variants that together define modern humans from a genetic perspective.

are often rare, which limits our ability to study them. A promising prospect is that biobanks become not only bigger but also more diverse in terms of the ancestry of their participants. This is important, as variants that are rare in large populations will sometime have reached appreciable frequencies in smaller populations wherein random genetic drift plays a larger role than in larger populations (Figure 3). The phenotypic effects of some ancestral variants can therefore potentially be studied in small populations.

Finally, it is important to keep in mind that when ancestral variants are associated with effects during development or in adult individuals, such ancestral variants are not in any sense "primitive" or "pathological." They functioned well for hundreds of thousands of years in healthy archaic humans that were closely related to modern humans and most or all of them presumably function well in people today.

ACKNOWLEDGMENTS

This work was supported by the Max Planck Society and the NOMIS foundation (S.P.) and the Swedish Research Council (H.Z., grant number: 2021-03050; M.J., grant numbers: 2018-05537 and 2022-04642). We thank Dr. Linda Vigilant for valuable input for the manuscript and Dr. Carolina Bernhardsson and Dr. Maximilian Larena for technical support.

DECLARATION OF INTERESTS

The authors declare no competing interests.

REFERENCES

1. 1000 Genomes Project Consortium, Auton, A., Brooks, L.D., Durbin, R.M., Garrison, E.P., Kang, H.M., Korbel, J.O., Marchini, J.L., McCarthy, S., McVean, G.A., et al. (2015). A global reference for human genetic variation. Nature *526*, 68–74.
2. Karczewski, K.J., Francioli, L.C., Tiao, G., Cummings, B.B., Alföldi, J., Wang, Q., Collins, R.L., Laricchia, K.M., Ganna, A., Birnbaum, D.P., et al. (2020). The mutational constraint spectrum quantified from variation in 141,456 humans. Nature *581*, 434–443.
3. Mallick, S., Micco, A., Mah, M., Ringbauer, H., Lazaridis, I., Olalde, I., Patterson, N., and Reich, D. (2023). The Allen Ancient DNA Resource (AADR): a curated compendium of ancient human genomes. Preprint at bioRxiv.
4. Mafessoni, F., Grote, S., Filippo, C. de, Slon, V., Kolobova, K.A., Viola, B., Markin, S.V., Chintalapati, M., Peyrégne, S., Skov, L., et al. (2020). A high-coverage Neandertal genome from Chagyrskaya Cave. Proc. Natl. Acad. Sci. USA *117*, 15132–15136.
5. Prüfer, K., Racimo, F., Patterson, N., Jay, F., Sankararaman, S., Sawyer, S., Heinze, A., Renaud, G., Sudmant, P.H., Filippo, C. de, et al. (2014). The complete genome sequence of a Neanderthal from the Altai Mountains. Nature *505*, 43–49.
6. Prüfer, K., Filippo, C. de, Grote, S., Mafessoni, F., Korlević, P., Hajdinjak, M., Vernot, B., Skov, L., Hsieh, P., Peyrégne, S., et al. (2017). A high-coverage Neandertal genome from Vindija Cave in Croatia. Science *358*, 655–658.
7. Castellano, S., Parra, G., Sánchez-Quinto, F.A., Racimo, F., Kuhlwilm, M., Kircher, M., Sawyer, S., Fu, Q., Heinze, A., Nickel, B., et al. (2014). Patterns of coding variation in the complete exomes of three Neandertals. Proc. Natl. Acad. Sci. USA *111*, 6666–6671.
8. Hajdinjak, M., Fu, Q., Hübner, A., Petr, M., Mafessoni, F., Grote, S., Skoglund, P., Narasimham, V., Rougier, H., Crevecoeur, I., et al. (2018). Reconstructing the genetic history of late Neanderthals. Nature *555*, 652–656.
9. Meyer, M., Kircher, M., Gansauge, M.T., Li, H., Racimo, F., Mallick, S., Schraiber, J.G., Jay, F., Prüfer, K., Filippo, C. de, et al. (2012). A high-coverage genome sequence from an Archaic Denisovan individual. Science *338*, 222–226.
10. Kuhlwilm, M., Gronau, I., Hubisz, M.J., de Filippo, C., Prado-Martinez, J., Kircher, M., Fu, Q., Burbano, H.A., Lalueza-Fox, C., de la Rasilla, M., et al. (2016). Ancient gene flow from early modern humans into Eastern Neanderthals. Nature *530*, 429–433.
11. Chen, L., Wolf, A.B., Fu, W., Li, L., and Akey, J.M. (2020). Identifying and interpreting apparent Neanderthal ancestry in African individuals. Cell *180*, 677–687.e16.
12. Peyrégne, S., Kelso, J., Peter, B.M., and Pääbo, S. (2022). The evolutionary history of human spindle genes includes back-and-forth gene flow with Neandertals. eLife *11*, e75464.
13. Slon, V., Mafessoni, F., Vernot, B., Filippo, C. de, Grote, S., Viola, B., Hajdinjak, M., Peyrégne, S., Nagel, S., Brown, S., et al. (2018). The genome of the offspring of a Neanderthal mother and a Denisovan father. Nature *561*, 113–116.
14. Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W., Fritz, M.H.-Y., et al. (2010). A draft sequence of the neandertal genome. Science *328*, 710–722.
15. Sankararaman, S., Mallick, S., Dannemann, M., Prüfer, K., Kelso, J., Pääbo, S., Patterson, N., and Reich, D. (2014). The genomic landscape of Neanderthal ancestry in present-day humans. Nature *507*, 354–357.
16. Reich, D., Green, R.E., Kircher, M., Krause, J., Patterson, N., Durand, E.Y., Viola, B., Briggs, A.W., Stenzel, U., Johnson, P.L.F., et al. (2010). Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature *468*, 1053–1060.
17. Sankararaman, S., Mallick, S., Patterson, N., and Reich, D. (2016). The combined landscape of denisovan and Neanderthal ancestry in present-day humans. Curr. Biol. *26*, 1241–1247.
18. Brand, C.M., Colbran, L.L., and Capra, J.A. (2022). Predicting archaic hominin phenotypes from genomic data. Annu. Rev. Genomics Hum. Genet. *23*, 591–612.
19. Irving-Pease, E.K., Muktupavela, R., Dannemann, M., and Racimo, F. (2021). Quantitative human paleogenetics: what can ancient DNA tell us about complex trait evolution? Front. Genet. *12*, 703541.
20. Kerner, G., Choin, J., and Quintana-Murci, L. (2023). Ancient DNA as a tool for medical research. Nat. Med. *29*, 1048–1051.
21. Zeberg, H., and Pääbo, S. (2020). The major genetic risk factor for severe COVID-19 is inherited from Neanderthals. Nature *587*, 610–612. Q9
22. Huerta-Sánchez, E., Jin, X., Asan, Bianba, Z., Peter, B.M., Vinckenbosch, N., Liang, Y., Yi, X., He, M., Somel, M., et al. (2014). Altitude adaptation in Tibetans caused by introgression of Denisovan-like DNA. Nature *512*, 194–197.
23. Peñalba, J.V., and Wolf, J.B.W. (2020). From molecules to populations: appreciating and estimating recombination rate variation. Nat. Rev. Genet. *21*, 476–492.
24. Lipson, M., Loh, P.R., Sankararaman, S., Patterson, N., Berger, B., and Reich, D. (2015). Calibrating the human mutation rate via ancestral recombination density in diploid genomes. PLoS Genet. *11*, e1005550.
25. Ågren, R., Patil, S., Zhou, X., FinnGen., Sahlholm, K., Pääbo, S., and Zeberg, H. (2023). Major genetic risk factors for Dupuytren's disease are inherited from neandertals. Mol. Biol. Evol. *40*, msad130.
26. Fu, Q., Hajdinjak, M., Moldovan, O.T., Constantin, S., Mallick, S., Skoglund, P., Patterson, N., Rohland, N., Lazaridis, I., Nickel, B., et al. (2015). An early modern human from Romania with a recent Neanderthal ancestor. Nature *524*, 216–219.
27. Hajdinjak, M., Mafessoni, F., Skov, L., Vernot, B., Hübner, A., Fu, Q., Essel, E., Nagel, S., Nickel, B., Richter, J., et al. (2021). Initial Upper Palaeolithic humans in Europe had recent Neanderthal ancestry. Nature *592*, 253–257.
28. Larena, M., McKenna, J., Sanchez-Quinto, F., Bernhardsson, C., Ebeo, C., Reyes, R., Casel, O., Huang, J.Y., Hagada, K.P., Guilay, D., et al. (2021). Philippine Ayta possess the highest level of Denisovan ancestry in the world. Curr. Biol. *31*, 4219–4230.e10.
29. Browning, S.R., Browning, B.L., Zhou, Y., Tucci, S., and Akey, J.M. (2018). Analysis of human sequence data reveals two pulses of archaic denisovan admixture. Cell *173*, 53–61.e9.
30. Jacobs, G.S., Hudjashov, G., Saag, L., Kusuma, P., Darusallam, C.C., Lawson, D.J., Mondal, M., Pagani, L., Ricaut, F.X., Stoneking, M., et al. (2019). Multiple deeply divergent denisovan ancestries in papuans. Cell *177*, 1010–1021.e32.
31. Choin, J., Mendoza-Revilla, J., Arauna, L.R., Cuadros-Espinoza, S., Cassar, O., Larena, M., Ko, A.M.-S., Harmant, C., Laurent, R., Verdu, P., et al. (2021). Genomic insights into population history and biological adaptation in Oceania. Nature *592*, 583–589.
32. Rinker, D.C., Simonti, C.N., McArthur, E., Shaw, D., Hodges, E., and Capra, J.A. (2020). Neanderthal introgression reintroduced functional ancestral alleles lost in Eurasian populations. Nat. Ecol. Evol. *4*, 1332–1341.
33. Stepanova, V., Moczulska, K.E., Vacano, G.N., Kurochkin, I., Ju, X., Riesenberg, S., Macak, D., Maricic, T., Dombrowski, L., Schörnig, M., et al. (2021). Reduced purine biosynthesis in humans after their divergence from Neandertals. eLife *10*, e58741.
34. Dannemann, M. (2021). The population-specific impact of neandertal introgression on human disease. Genome Biol. Evol. *13*, evaa250.
35. Rusu, V., Hoch, E., Mercader, J.M., Tenen, D.E., Gymrek, M., Hartigan, C.R., DeRan, M., Grotthuss, M. von, Fontanillas, P., Spooner, A., et al.

(2017). Type 2 diabetes variants disrupt function of SLC16A11 through two distinct mechanisms. Cell *170*, 199–212.e20.

36. SIGMA Type 2 Diabetes Consortium, Williams, A.L., Jacobs, S.B., Moreno-Macías, H., Huerta-Chagoya, A., Churchhouse, C., Márquez-Luna, C., García-Ortíz, H., Gómez-Vázquez, M.J., Burtt, N.P., et al. (2014). Sequence variants in SLC16A11 are a common risk factor for type 2 diabetes in Mexico. Nature *506*, 97–101.
37. Simonti, C.N., Vernot, B., Bastarache, L., Bottinger, E., Carrell, D.S., Chisholm, R.L., Crosslin, D.R., Hebbring, S.J., Jarvik, G.P., Kullo, I.J., et al. (2016). The phenotypic legacy of admixture between modern humans and Neandertals. Science *351*, 737–741.
38. Haeggström, S., Ingelman-Sundberg, M., Pääbo, S., and Zeberg, H. (2022). The clinically relevant CYP2C8*3 and CYP2C9*2 haplotype is inherited from Neandertals. Pharmacogenomics J. *22*, 247–249.
39. Zeberg, H., Dannemann, M., Sahlholm, K., Tsuo, K., Maricic, T., Wiebe, V., Hevers, W., Robinson, H.P.C., Kelso, J., and Pääbo, S. (2020). A Neanderthal sodium channel increases pain sensitivity in present-day humans. Curr. Biol. *30*, 3465–3469.e4.
40. Weisman, A., Quintner, J., and Masharawi, Y. (2019). Congenital insensitivity to pain: a misnomer. J. Pain *20*, 1011–1014.
41. Tiwari, D., Bose, P.D., Das, S., Das, C.R., Datta, R., and Bose, S. (2015). MTHFR (C677T) polymorphism and PR (PROGINS) mutation as genetic factors for preterm delivery, fetal death and low birth weight: a Northeast Indian population based study. Meta Gene *3*, 31–42.
42. Li, J., Hong, X., Mesiano, S., Muglia, L.J., Wang, X., Snyder, M., Stevenson, D.K., and Shaw, G.M. (2018). Natural selection has differentiated the progesterone receptor among human populations. Am. J. Hum. Genet. *103*, 45–57.
43. Zeberg, H., Kelso, J., and Pääbo, S. (2020). The Neandertal progesterone receptor. Mol. Biol. Evol. *37*, 2655–2660.
44. Coomarasamy, A., Devall, A.J., Brosens, J.J., Quenby, S., Stephenson, M.D., Sierra, S., Christiansen, O.B., Small, R., Brewin, J., Roberts, T.E., et al. (2020). Micronized vaginal progesterone to prevent miscarriage: a critical evaluation of randomized evidence. Am. J. Obstet. Gynecol. *223*, 167–176.
45. Enard, D., and Petrov, D.A. (2018). Evidence that RNA viruses drove adaptive introgression between neanderthals and modern humans. Cell *175*, 360–371.e13.
46. Quach, H., Rotival, M., Pothlichet, J., Loh, Y.-H.E., Dannemann, M., Zidane, N., Laval, G., Patin, E., Harmant, C., Lopez, M., et al. (2016). Genetic adaptation and neandertal admixture shaped the immune system of human populations. Cell *167*, 643–656.e17.
47. Jagoda, E., Xue, J.R., Reilly, S.K., Dannemann, M., Racimo, F., Huerta-Sanchez, E., Sankararaman, S., Kelso, J., Pagani, L., Sabeti, P.C., et al. (2022). Detection of Neanderthal adaptively introgressed genetic variants that modulate reporter gene expression in human immune cells. Mol. Biol. Evol. *39*, msab304.
48. Deschamps, M., Laval, G., Fagny, M., Itan, Y., Abel, L., Casanova, J.L., Patin, E., and Quintana-Murci, L. (2016). Genomic signatures of selective pressures and introgression from archaic hominins at human innate immunity genes. Am. J. Hum. Genet. *98*, 5–21.
49. Dannemann, M., Andrés, A.M., and Kelso, J. (2016). Introgression of Neandertal- and Denisovan-like haplotypes contributes to adaptive variation in human Toll-like Receptors. Am. J. Hum. Genet. *98*, 22–33.
50. Mayerle, J., Hoed, C.M. den, Schurmann, C., Stolk, L., Homuth, G., Peters, M.J., Capelle, L.G., Zimmermann, K., Rivadeneira, F., Gruska, S., et al. (2013). Identification of genetic loci associated with Helicobacter pylori serologic status. JAMA *309*, 1912–1920.
51. Pairo-Castineira, E., Clohisey, S., S, Klaric, L., Bretherick, A.D., Rawlik, K., Pasko, D., Walker, S., Parkinson, N., Fourman, M.H., et al. (2021). Genetic mechanisms of critical illness in COVID-19. Nature *591*, 92–98.
52. Downes, D.J., Cross, A.R., Hua, P., Roberts, N., Schwessinger, R., Cutler, A.J., Munis, A.M., Brown, J., Mielczarek, O., Andrea, C.E. de, et al. (2021). Identification of LZTFL1 as a candidate effector gene at a COVID-19 risk locus. Nat. Genet. *53*, 1606–1615.
53. Zeberg, H. (2022). The major genetic risk factor for severe COVID-19 is associated with protection against HIV. Proc. Natl. Acad. Sci. USA. *119*, e2116435119.
54. Mendez, F.L., Watkins, J.C., and Hammer, M.F. (2013). Neandertal origin of genetic variation at the cluster of OAS immunity genes. Mol. Biol. Evol. *30*, 798–801.
55. Zeberg, H., and Pääbo, S. (2021). A genomic region associated with protection against severe COVID-19 is inherited from Neandertals. Proc. Natl. Acad. Sci. USA *118*, e2026309118.
56. Sams, A.J., Dumaine, A., Nédélec, Y., Yotova, V., Alfieri, C., Tanner, J.E., Messer, P.W., and Barreiro, L.B. (2016). Adaptively introgressed Neandertal haplotype at the OAS locus functionally impacts innate immune responses in humans. Genome Biol. *17*, 246.
57. Wickenhagen, A., Sugrue, E., Lytras, S., Kuchi, S., Noerenberg, M., Turnbull, M.L., Loney, C., Herder, V., Allan, J., Jarmson, I., et al. (2021). A prenylated dsRNA sensor protects against severe COVID-19. Science *374*, eabj3624.
58. McArthur, E., Rinker, D.C., and Capra, J.A. (2021). Quantifying the contribution of Neanderthal introgression to the heritability of complex traits. Nat. Commun. *12*, 4481.
59. Telis, N., Aguilar, R., and Harris, K. (2020). Selection against archaic hominin genetic variation in regulatory regions. Nat. Ecol. Evol. *4*, 1558–1566.
60. McCoy, R.C., Wakefield, J., and Akey, J.M. (2017). Impacts of Neanderthal-introgressed sequences on the landscape of human gene expression. Cell *168*, 916–927.e12.
61. Mafessoni, F., and Prüfer, K. (2017). Better support for a small effective population size of Neandertals and a long shared history of Neandertals and Denisovans. Proc. Natl. Acad. Sci. USA *114*, E10256–E10257.
62. Harris, K., and Nielsen, R. (2016). The genetic cost of Neanderthal introgression. Genetics *203*, 881–891.
63. Zhang, X., Witt, K.E., Bañuelos, M.M., Ko, A., Yuan, K., Xu, S., Nielsen, R., and Huerta-Sanchez, E. (2021). The history and evolution of the Denisovan-EPAS1 haplotype in Tibetans. Proc. Natl. Acad. Sci. USA *118*, e2020803118.
64. Chen, F., Welker, F., Shen, C.C., Bailey, S.E., Bergmann, I., Davis, S., Xia, H., Wang, H., Fischer, R., Freidline, S.E., et al. (2019). A late Middle Pleistocene Denisovan mandible from the Tibetan Plateau. Nature *569*, 409–412.
65. Zhang, D., Xia, H., Chen, F., Li, B., Slon, V., Cheng, T., Yang, R., Jacobs, Z., Dai, Q., Massilani, D., et al. (2020). Denisovan DNA in Late Pleistocene sediments from Baishiya Karst Cave on the Tibetan Plateau. Science *370*, 584–587.
66. Racimo, F., Gokhman, D., Fumagalli, M., Ko, A., Hansen, T., Moltke, I., Albrechtsen, A., Carmel, L., Huerta-Sánchez, E., and Nielsen, R. (2017). Archaic adaptive introgression in TBX15/WARS2. Mol. Biol. Evol. *34*, 509–524.
67. Fumagalli, M., Moltke, I., Grarup, N., Racimo, F., Bjerregaard, P., Jørgensen, M.E., Korneliussen, T.S., Gerbault, P., Skotte, L., Linneberg, A., et al. (2015). Greenlandic Inuit show genetic signatures of diet and climate adaptation. Science *349*, 1343–1347.
68. Bonfante, B., Faux, P., Navarro, N., Mendoza-Revilla, J., Dubied, M., Montillot, C., Wentworth, E., Poloni, L., Varón-González, C., Jones, P., et al. (2021). A GWAS in Latin Americans identifies novel face shape loci, implicating VPS13B and a Denisovan introgressed region in facial variation. Sci. Adv. *7*, eabc6160.
69. Pääbo, S. (2014). The human condition—A molecular approach. Cell *157*, 216–226.
70. Weyer, S., and Pääbo, S. (2016). Functional analyses of transcription factor binding sites that differ between present-day and archaic humans. Mol. Biol. Evol. *33*, 316–322.

71. Van Laer, B., Kapp, U., Soler-Lopez, M., Moczulska, K., Pääbo, S., Leonard, G., and Mueller-Dieckmann, C. (2018). Molecular comparison of Neanderthal and Modern Human adenylosuccinate lyase. Sci. Rep. *8*, 18008.

72. Coppo, L., Mishra, P., Siefert, N., Holmgren, A., Pääbo, S., and Zeberg, H. (2022). A substitution in the glutathione reductase lowers electron leakage and inflammation in modern humans. Sci. Adv. *8*, eabm1148.

73. Trujillo, C.A., Rice, E.S., Schaefer, N.K., Chaim, I.A., Wheeler, E.C., Madrigal, A.A., Buchanan, J., Preissl, S., Wang, A., Negraes, P.D., et al. (2021). Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment. Science *371*, eaax2537.

74. Maricic, T., Helmbrecht, N., Riesenberg, S., Macak, D., Kanis, P., Lackner, M., Pugach-Matveeva, A.D., and Pääbo, S. (2021). Comment on "Reintroduction of the archaic variant of NOVA1 in cortical organoids alters neurodevelopment". Science *374*, eabi6060.

75. Riesenberg, S., Kanis, P., Macak, D., Wollny, D., Düsterhöft, D., Kowalewski, J., Helmbrecht, N., Maricic, T., and Pääbo, S. (2023). Efficient high-precision homology-directed repair-dependent genome editing by HDRobust. Nat. Methods *20*, 1388–1399.

76. Mora-Bermúdez, F., Kanis, P., Macak, D., Peters, J., Naumann, R., Xing, L., Sarov, M., Winkler, S., Oegema, C.E., Haffner, C., et al. (2022). Longer metaphase and fewer chromosome segregation errors in modern human than Neanderthal brain development. Sci. Adv. *8*, eabn7702.

77. Pinson, A., Xing, L., Namba, T., Kalebic, N., Peters, J., Oegema, C.E., Traikov, S., Reppe, K., Riesenberg, S., Maricic, T., et al. (2022). Human TKTL1 implies greater neurogenesis in frontal neocortex of modern humans than Neanderthals. Science *377*, eabl6422.

78. Schlebusch, C.M., Sjödin, P., Breton, G., Günther, T., Naidoo, T., Hollfelder, N., Sjöstrand, A.E., Xu, J., Gattepaille, L.M., Vicente, M., et al. (2020). Khoe-San genomes reveal unique variation and confirm the deepest population divergence in Homo sapiens. Mol. Biol. Evol. *37*, 2944–2954.

79. Hublin, J.J. (2009). Out of Africa: modern human origins special feature: the origin of Neandertals. Proc. Natl. Acad. Sci. USA *106*, 16022–16027.

80. Hubbard, T.D., Murray, I.A., Bisson, W.H., Sullivan, A.P., Sebastian, A., Perry, G.H., Jablonski, N.G., and Perdew, G.H. (2016). Divergent Ah Receptor Ligand Selectivity during Hominin Evolution. Mol. Biol. Evol. *33*, 2648–2658.

81. Vernot, B., Tucci, S., Kelso, J., Schraiber, J.G., Wolf, A.B., Gittelman, R.M., Dannemann, M., Grote, S., McCoy, R.C., Norton, H., et al. (2016). Excavating Neandertal and Denisovan DNA from the genomes of Melanesian individuals. Science *352*, 235–239.

82. Schaefer, N.K., Shapiro, B., and Green, R.E. (2021). An ancestral recombination graph of human, Neanderthal, and Denisovan genomes. Sci. Adv. *7*, eabc0776.