

ABO genetic variation in Neanderthals and Denisovans

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1 Abstract

Variation at the ABO locus was one of the earliest sources of data in the study of human population identity and history, and to this day remains widely genotyped due to its importance in blood and tissue transfusions. As one of the first genetic markers, variation at the ABO gene has been studied for over 60 years, and yet there are some aspects of its evolution that remain mysterious. Here, we look at ABO blood type variants in our archaic relatives: Neanderthals and Denisovans. Our goal is to understand the genetic landscape of the ABO gene in archaic humans, and how it relates to modern human ABO variation. We analyze coding variation at the ABO locus from next-generation sequences in $\sim 2,500$ individuals from 28 populations, including three Neanderthal and one Denisovan individuals. We use the modern human haplotypes to impute ABO genotypes for the four archaic human genomes. We found that the Siberian Neanderthals, Altai and Chagyrskaya, are both homozygous for a derived Neanderthal variant of the O allele, while the European Neanderthal, Vindija, is a heterozygote for two derived Neanderthal variants, an O variant different from Altai and Chagyrskaya, and a rare cis-AB variant. The Denisovan individual is homozygous for an ancestral variant of the O allele, similar to variants found widely in modern humans. Perhaps more surprisingly, the derived O allele variant found in the Altai Neanderthal can be found at low frequencies in modern European and Southeast Asian individuals, and the derived O allele variant found in the Vindija Neanderthal is also found at very low frequency in East Asian individuals. Our genetic distance analyses suggests both alleles were introgressed through Neanderthal-human gene flow. In summary, our study identifies the genetic variation of the ABO gene in archaic humans, we find that ABO allele diversity in Neanderthals was likely high, and that some of these alleles still survive in modern humans due to inbreeding with Neanderthals.

2 Introduction

ABO was the first blood group discovered in humans, its identity coded by the ABO gene. As ABO blood types can be identified through chemical assays, ABO gene variation became important as a source of genetic information before the advent of sequencing technology. For example, Cavalli-Sforza and Mordant used serological-based determination of ABO blood types in the 1960s to determine if ABO allele frequencies accurately recapitulated historic human migration patterns [Cavalli-Sforza et al., 1964]. Advances in sequencing technology allowed for improved resolution of ABO locus variation, including detection of population-specific rare variants. Rare variants are useful as a tool to understand population migration history. Examples include a rare O variant (O1V542) which is an Ancestry Informative Marker in Indigenous American populations [Estrada-Mena et al., 2010, Villanea et al., 2013], as well as myriad other rare variants private to specific human groups [Yip, 2002, Roubinet et al., 2004]. In addition, Fry et al. [2007] speculates that rare variation is also informative about natural selection due to historical interactions with pathogens such as malaria, norovirus, smallpox, and perhaps others, further highlighting the importance of studying rare ABO variation.

In modern humans, variation at the ABO gene is characterized by the maintenance of higher-than-expected levels of haplotype diversity - a classic signature of balancing selection [Ségurel et al., 2012, Seymour et al., 2004, Villanea et al., 2015]. ABO haplotypes have common functional types (i.e., A, B, and O), which are retained in populations by the effects of balancing selection. At a finer scale, rare variation accumulates through mutation, creating diverging haplotype backgrounds for alleles of identical functional types. For archaic humans - Neanderthals and Denisovans - the recent sequencing of high-coverage genomes opens up the possibility of studying what ABO diversity looks like in these extinct species.

We now know that when anatomically modern humans dispersed out of Africa, they encountered and hybridized with various archaic human groups [Green et al., 2010]. Direct comparisons of archaic and modern human genomes have revealed a complex landscape of admixture between modern humans and both Neanderthals and Denisovans [Browning et al., 2018, Villanea and Schraiber, 2019]. For the majority of genes found in modern humans, negative natural selection has acted on archaic versions removing them from the gene pool [Sankararaman et al., 2016, Petr et al., 2019, Zhang et al., 2020]. However, a handful of archaic versions of genes have risen to high frequency in modern humans through positive natural selection [Huerta-Sánchez et al., 2014, Huerta-Sánchez and Casey, 2015, Racimo et al., 2015, 2016]. It is possible then, that archaic ABO variants could have been inherited by modern humans, and become targets of natural selection in modern human populations.

Here, we investigate population-specific rare variation of the ABO gene by combining data from the 1,000 Genomes Project, the Neanderthal genome project [Prüfer et al., 2014], the Chagyrskaya Neandertal genome project [Mafessoni et al., 2020], and the Denisovan

Genome Project [Meyer et al., 2012]. Our naive expectation for ABO archaic alleles is to find common functional types with analog function to the modern human A, B, and O alleles, as well as for mutation to give rise to rare haplotype variants. We find that the Denisovan individual presents variants that are similar to modern ABO haplotypes found in African and non-African living individuals, possibly retained in both lineages since before the Denisovan-human split. The Altai, Chagyrskaya, and Vindija individuals all present unique Neanderthal ABO haplotypes (Figure 1). Furthermore, these Neanderthal variants are found in select human non-African populations as a result of human-Neanderthal admixture. Finally, we find that the high sequence divergence observed in the introgressed Neanderthal ABO haplotypes is consistent with the effects of balancing selection.

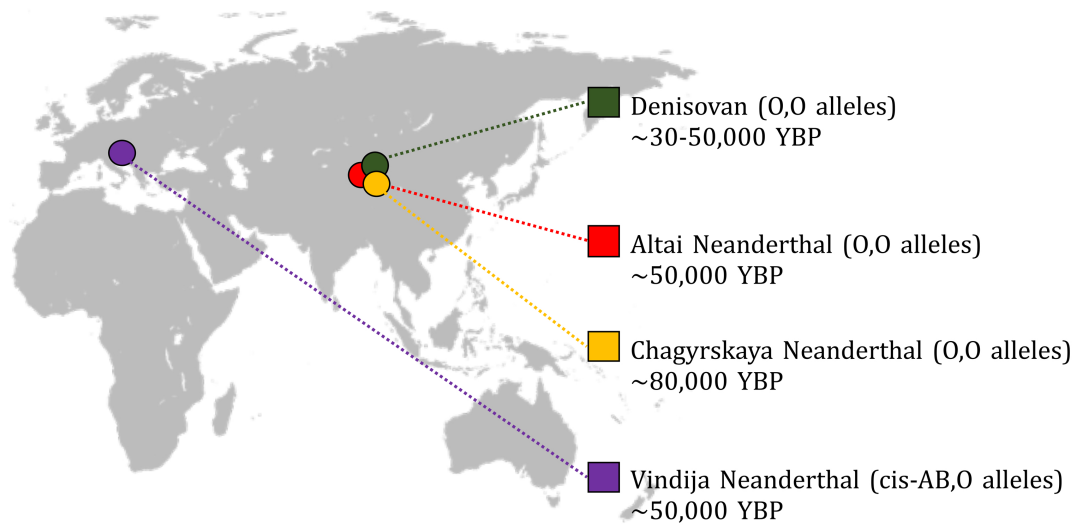


Figure 1: Geographic location of the archaic individuals studied here, including ABO functional allele types.

3 Results

3.1 ABO variation in the 1000 Genomes Project

In modern humans, common allele variation occurs in exons 6 and 7 of the ABO gene, where four common SNPs determine the common functional allele types. In addition, a loss-of-function (LOS) mutation in exon 6 is responsible for the O haplotype [Yamamoto et al., 1992]. This LOS mutation is commonly a deletion (denoted here as a "deletional O allele"), but it can also be the result of an insertion, or a base pair mutation producing a

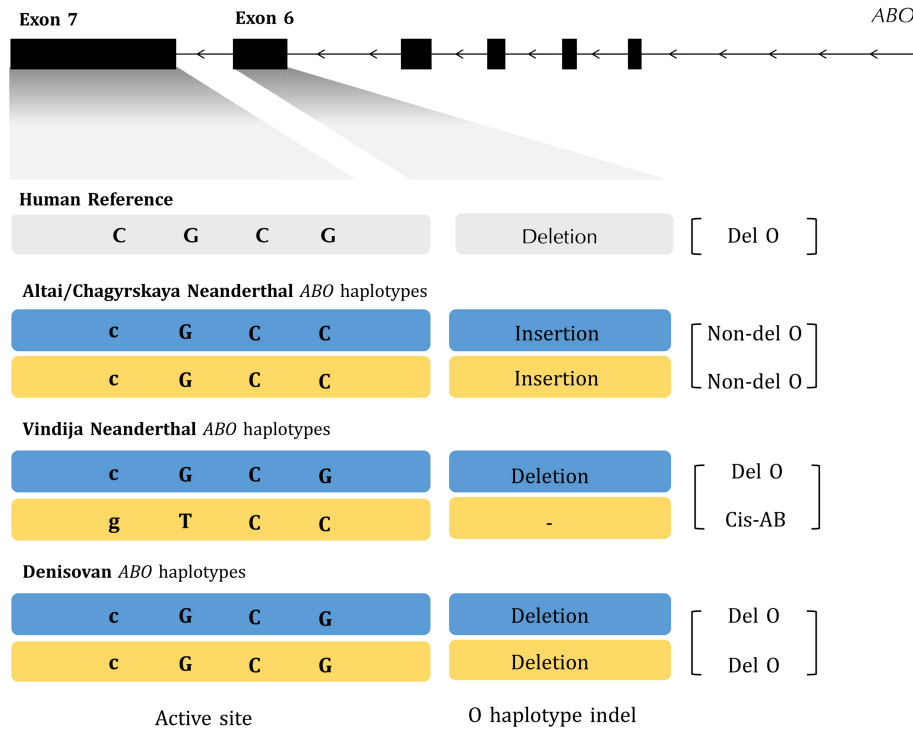


Figure 2: Functional SNPs of interest along the ABO exons. SNP variants are shown for one Denisovan and three Neanderthal individuals. Nucleotides in lower-case font represent missing data in the archaic genomes that was imputed from modern data (see methods).

premature stop codon (denoted here as a "non-deletional O allele", Figure 2). We extracted those positions for all archaic haplotypes, as summarized in Table 1.

Then, to understand archaic ABO functional variation at the rare haplotype level, we summarized the ABO variation in 2,504 diploid individuals in the 1,000 Genomes Project panel (the coding sequence of 5,008 chromosomes), identifying 69 variable sites, and resulting in a total of 108 unique haplotypes: 20 unique A types (945 chromosomes), 9 unique B types (720 chromosomes), a single cis-AB type (1 chromosome), 72 deletional O types (3280 chromosomes), and 6 non-deletional O types (17 chromosomes, Supp. Figure 1).

3.2 Archaic human ABO haplotype structure

Using the coding variation at the ABO locus in the 1000 Genomes Project panel as a reference, we identified ABO blood types for four archaic individuals (Table 1, Supp. Figure 1). The single Denisovan individual presents two unique forms of deletional O haplotypes,

Position	136131315	136131322	136131415	136131592	136132908	Imputed ABO type
Human Common A	C	G	C	G	TC	
Human Common B	G	T	T	C	TC	
Human Common Deletional O	C	G	C	G	T	
Human Non-deletional O	C	G	C	C	TC	
Vindija hap. 1	c	G	C	G	T	Deletional O type
Vindija hap. 2	g	T	C	C	TC	Cis-AB type
Altai hap. 1	c	G	C	C	TC	Non-deletional O type
Altai hap. 2	c	G	C	C	TC	Non-deletional O type
Chagyrskaya hap. 1	c	G	C	C	TC	Non-deletional O type
Chagyrskaya hap. 2	c	G	C	C	TC	Non-deletional O type
Denisovan hap. 1	c	G	C	G	T	Deletional O type
Denisovan hap. 2	c	G	C	G	T	Deletional O type

Table 1: Archaic SNPS for the common ABO variants, including position in the human genome (hg19, build37). Imputed ABO allele types for the four archaic individuals are shown on the right-most column. Bases in lower-case represent missing data in the archaic genomes that was imputed from modern data (see methods).

with no exact matches in the 1000 Genomes Project individuals. The Altai Neanderthal individual is homozygous for a rare non-deletional O haplotype found in modern humans [Yip, 2002]. The Chagyrskaya Neanderthal individual was also homozygous for the same non-deletional O haplotype, although it differed from the Altai Neanderthal version at three positions (136131539 A→G, 136133506 A→G, and 136137547 A→C). The Vindija Neanderthal individual is heterozygous for an O allele, which is deletional and found in modern humans, and a functional allele similar to a rare cis-AB modern human allele found in a single individual in the 1000 Genomes Panel (HG02537). Cis-AB alleles are exceedingly rare recombinant alleles which code for a hybrid A and B antigen ([Chun et al., 2019]). Finding a deletional O allele in the Vindija individual is consistent with a previous study, which found the deletion responsible for the common O genotype in two other European Neanderthals [Lalueza-Fox et al., 2008].

3.3 Evidence of archaic introgression in modern human ABO types

To identify if the archaic ABO haplotypes are related to the modern versions found in the 1000 Genomes Project individuals, we calculated sequence divergence between ABO haplotypes (hg19 coordinates, 9:136130563-136150630) using Haplostrips. We then visualized these distances grouped by modern human population (Figure 3, Figure 4). Note that admixed populations were excluded from this analysis, the justification can be found in the supplement, and Supp. Table 3. Our results indicate that all archaic haplotypes have close equivalents in modern humans. In the case of Denisovans, their unique O alleles are close to other O alleles common in modern populations, in particular O alleles found in African individuals. This suggests that the Denisovan O allele version is ancestrally shared between Denisovans and modern humans. This is not unlikely, considering balancing selection has maintained the same ABO functional alleles for millions of years among primate species

[Ségurel et al., 2012].

Conversely, for Neanderthals, both the Altai/Chagyrskaya-like and Vindija-like O allele types have almost identical haplotype equivalents in modern humans in non-African populations. The geographic pattern of these alleles is a compelling case for introgression, the result of admixture between Neanderthals and modern humans in Europe, Southeast Asia, and East Asia.

To confirm if the Neanderthal-like ABO alleles found in Europe, Southeast Asia, and East Asia are the result of introgression, we searched in the list of introgressed genome fragments reported in Browning et al. [2018]. We found introgressed genome fragments that overlap with the genome coordinates of the ABO gene in seven populations in the 1000 Genomes Project panel (Supp. Table 1), supporting introgression as the likely cause of shared ABO haplotypes between Neanderthals and modern humans.

3.4 Introgressed Neanderthal haplotypes are highly divergent relative to the rest of the genome

We found two different introgressed Neanderthal O haplotypes in modern humans, an Altai/Chagyrskaya-like O allele found in modern Europeans and Southeast Asians, and a Vindija-like O allele found in modern East Asians. Given that this pattern runs opposite to the geographic location of the Vindija Neanderthal (Croatia) and Altai and Chagyrskaya Neanderthals (Siberia), we wanted to compare the affinity of ABO introgressed haplotypes to the Vindija (y-axis) and Altai (x-axis) Neanderthals, relative to the affinity of all other introgressed fragments in the genomes of a European (IBS) and East Asian (JPT) population.

Figure 5 shows the distribution of affinities to the two Neanderthals on a 2D contour density plot. We plotted the introgressed fragments detected by Sprime to visualize them on an x-y axis. If all alleles in an introgressed fragment are identical to the version in either Neanderthal chromosome, the match score is 1. If none of the alleles match, the score is 0. Scores in between reflect the proportion of alleles that match over all alleles. As observed in the figure, a large number of fragments match both Neanderthals highly, noted by the high density of fragments at the top right corner. Conversely, fragments that have very low affinities to the Neanderthals, plotted in the bottom left corner, represent either false positive fragment calls, or introgressed fragments that match to a different archaic species.

Our plot confirms that the European/Southeast Asian introgressed non-deletional O haplotype is closer in affinity to the Siberian Neanderthal, and the East Asian introgressed deletional O haplotype is closer in affinity to the Croatian Neanderthal, however, neither introgressed haplotype was identical to the Neanderthal version. Our plot also indicates that the introgressed ABO haplotypes fall outside the variation of other introgressed genome fragments in Europe, and on the margins of other introgressed genome fragments in East Asia. This pattern suggests that introgressed ABO haplotypes are more divergent than expected relative to other introgressed genome fragments.

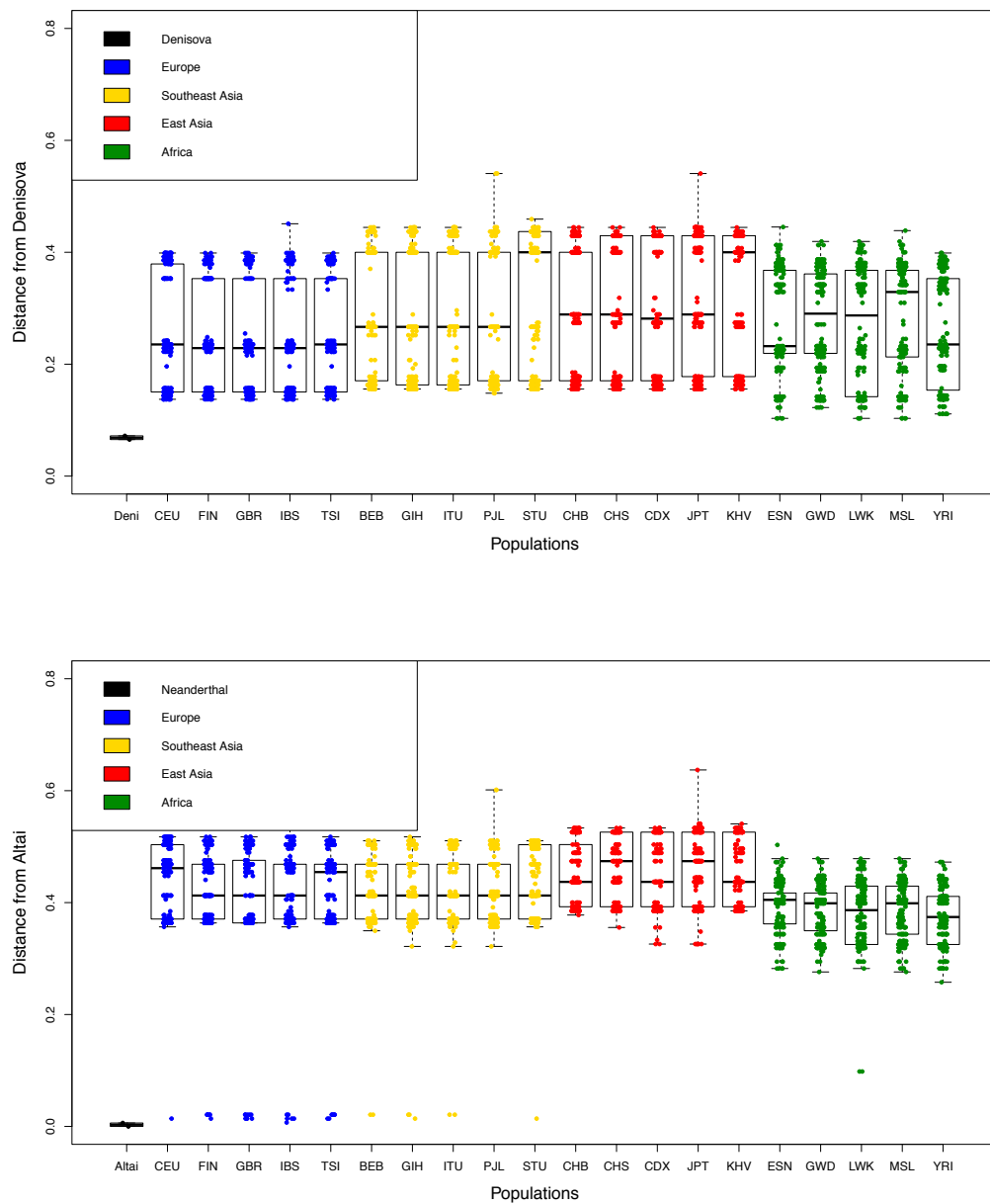


Figure 3: Haplotype distances calculated in Haplostrips for the 1000 Genomes Project populations, polarized relative to the archaic allele: top) Denisova, bottom) Altai Neanderthal (and Chagyrskaya Neanderthal).

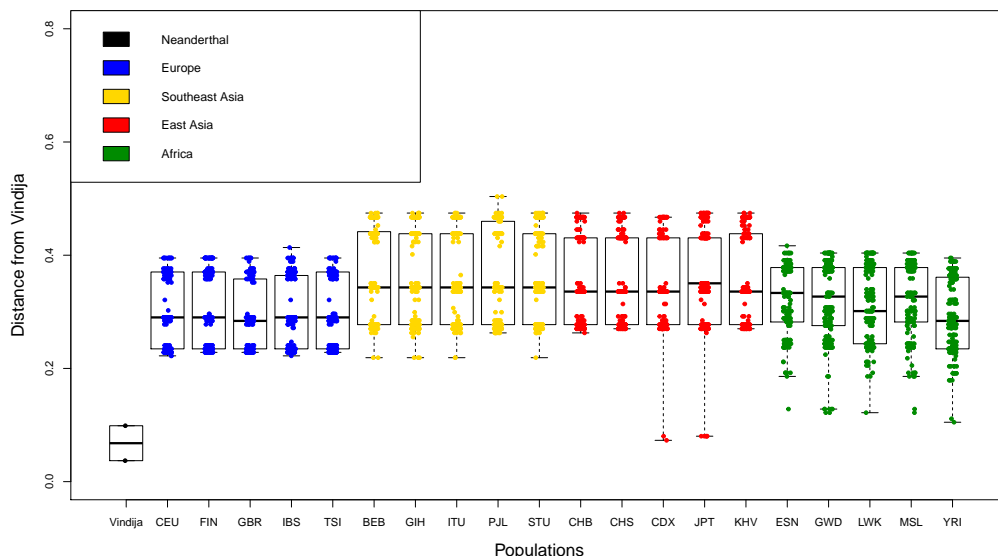


Figure 4: Haplotype distances calculated in Haplostrips for the 1000 Genomes Project populations, polarized relative to the Vindija Neanderthal archaic alleles. Note that the two Vindija haplotypes are unphased, and heterozygous alleles are randomly assigned between the two chromosomes.

4 Discussion

We found that archaic humans possessed unique ABO haplotypes, which are structurally similar to modern human ABO alleles, and in some cases are still found in modern humans through introgression. The similarities found in the coding region suggest those alleles are functionally identical to modern ABO alleles. While it is difficult to speculate on the selective background for archaic specific ABO haplotypes, it is interesting to find Neanderthal ABO alleles at a moderate frequencies in modern humans, as there is compelling evidence for strong selection against Neanderthal versions of functional genes [Sankararaman et al., 2016, Harris and Nielsen, 2016, Petr et al., 2019, Juric et al., 2016]. The most likely explanation is that both Neanderthal O alleles identified here are selectively neutral relative to modern human O alleles, and thus its frequency in modern humans is just a consequence of neutral demographic effects.

The geographic distribution of Neanderthal O alleles in modern human populations; where the Altai/Chagyrskaya O type is found in Europe and Southeast Asia, and the Vindija O type is found exclusively in East Asia, is consistent with independent Neanderthal-modern human introgression events. This result is consistent with findings in other genomic

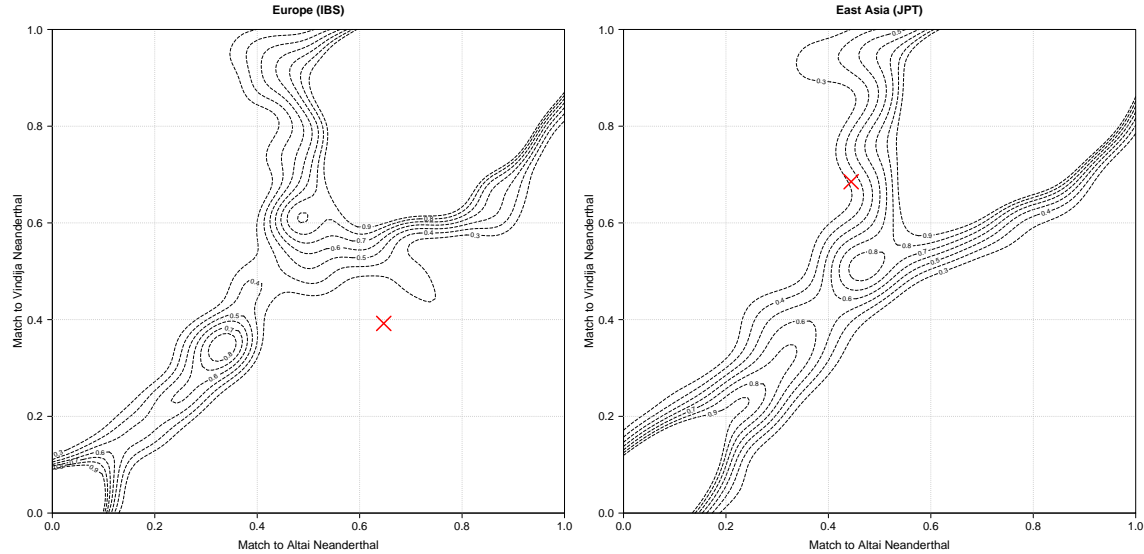


Figure 5: Contour density plots of match proportion of introgressed segments to the Vindija and Altai Neanderthal genomes. The plot visualizes the affinity of all Sprime inferred fragments to the Altai and Vindija Neanderthals (0=completely different, 1=identical to Neanderthal). The red x marks the introgressed ABO alleles we found in a) European and b) Asian populations.

regions, where at least two Neanderthal variants are found in modern humans with different geographic distributions [Taskent et al., 2020, Zeberg et al., 2020]. However, because the pattern is inverted - the European Neanderthal type is found in East Asian modern humans and vice-versa - it's possible that both Neanderthal O alleles were maintained in the same Neanderthal population or populations that admixed with modern humans.

To contextualize the diversity of the introgressed Neanderthal O alleles relative to all introgressed genome fragments in modern humans, we generated affinity contour density plots (Figure 5). All introgressed genome fragments found in the European and East Asian individuals present similar affinity to either Neanderthal genome (following a diagonal line in the affinity map), with a skew towards the Vindija Neanderthal, as this individual is more closely related to the population that interbred with modern humans than the Altai Neanderthal [Prüfer et al., 2017]. The Altai-like O allele found in Europeans has a higher affinity for the Altai individual, yet appears as an outlier in the relative affinity of all other genome fragments (Figure 5a). Conversely, The Vindija-like introgressed O allele found in East Asians has a higher affinity for the Vindija individual, and its affinity falls on the edge of the relative affinity of all other genome fragments (Figure 5b).

Based on these results, it appears both O haplotypes are extremely divergent relative to other genome fragments introgressed into modern human genomes, which are more

shared between the Vindija and Altai individuals. Given that neither the Vindija or Altai individuals were part of the population that directly interbred with modern humans, the most consistent explanation for this pattern is that these two very divergent alleles were maintained in the Neanderthal population that interbred with modern humans, retaining a larger haplotype diversity relative to other genome elements. If this was the case, these various haplotypes were likely retained in the population by balancing selection, which is expected to maintain ancestral diversity much longer than neutral regions of the genome, just as balancing selection maintains ABO variation in modern humans.

4.1 Conclusion

Human genetic variation in the ABO gene is a classic marker for genetic diversity in humans [Cavalli-Sforza et al., 1964]. Here, we provide an in-depth description of the genetic diversity of the ABO gene in four archaic humans, based on published ancient genomes. We found that archaic ABO haplotypes are polymorphic at the same positions which define modern human ABO function, and have posited that these archaic alleles must function similarly to modern human alleles. Furthermore, we found Denisovan-specific O alleles, which are genetically similar to modern O alleles, while Neanderthal-specific O alleles are derived relative to human alleles, but found today at low frequencies due to past human-Neanderthal admixture.

Finding four different Neanderthal variants in late-era Neanderthals is unexpected. A common perception, based on long runs of homozygosity seen across Neanderthal genomes, is that late-era Neanderthals were extremely inbred and thus had reduced genetic diversity. The high allele diversity found in these Neanderthals was possibly maintained through balancing selection at the ABO locus. This notion dovetails with our contour map results, showing introgressed Neanderthal O haplotypes falling outside of the genome divergence of all other fragments, and suggests that balancing selection operated in Neanderthals similarly to modern humans.

5 Acknowledgements

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6 Methods

6.1 Haplotype construction and determination of ABO subtype from NGS datasets

Coding sequence data for the ABO locus (5,008 ABO chromosomes) were obtained from a publicly available global reference panel, the 1,000 Genomes Project (Phase III), which contains a diverse set of individuals from multiple populations [Sudmant et al., 2010]. We obtained genotype calls for the ABO locus from each NGS dataset included in our analysis in variant call format (VCF) file using the Genome Analysis Tool Kit (GATK) to call single nucleotide variants (SNVs) as well as insertions and deletions (indels) [McKenna et al., 2010]. Both indels and SNVs are important for blood group calling because the primary differences between the A and B haplotypes are SNVs while the common cause of the O blood type is a single base deletion that causes nonsense mediated decay of the RNA transcript resulting in absence of protein [Yamamoto et al., 2012]. In order to resolve ABO haplotypes from these NGS datasets, we employed PHASE 2.1.2 for haplotype construction of the different chromosomal alleles for each individual [Scheet and Stephens, 2006]. A comprehensive summary of methods used to call blood group variants from NGS data can be found in Wheeler and Johnsen [2018].

6.2 Inclusion of high coverage paleogenomic datasets

In addition to analyzing ABO haplotype structure in the 1,000 Genomes Project panel, we extracted coding variation from four archaic human genomes; pertaining to a Neanderthal individual from Croatia ($\sim 42X$ coverage for the coding region of ABO) [Prüfer et al., 2017], two Neanderthal individuals from the Altai Mountains: one from the Denisova cave ($\sim 30X$ coverage for the coding region of ABO) [Prüfer et al., 2014], and one from the Chagyrskaya cave ($\sim 28x$ coverage for the coding region of ABO) [Mafessoni et al., 2020]. Finally, a single Denisovan individual from the Denisova cave in the Altai Mountains ($\sim 21X$ coverage for the coding region of ABO) [Meyer et al., 2012]. These individuals are estimated to be at least 50,000 years old. To impute function from these sequences, we extracted all 69 variable loci identified in our initial haplotype construction of the 1,000 Genomes Project and characterized them in all archaic humans. It should be noted that all archaic genome assemblies are missing data between positions 9:136131272-136131321 (hg19), so nucleotide data at those positions was imputed based on the closest haplotype match in modern humans (see the supplement), and is reported in lower-case font in all figures and tables.

Understanding the function of any archaic gene is limited by our ability to phase archaic genome sequences, as population-based phasing methods cannot be applied directly to a single individual from an extinct population [Browning and Browning, 2011]. In our study, the impact of incorrect phasing is mostly absent, because the Denisovan, Altai and Chagyrskaya Neanderthals are all homozygous for ABO sequence identity. However, we

recognize phasing the Vindija heterozygous cis-AB and O alleles could be subject to errors. To lessen this bias, we used the introgressed Vindija-like O sequence from modern humans as a template to manually resolve heterozygous positions to either chromosome (Supp. Table 2).

6.3 Haplotype distances

We used Haplostrips [Marnetto and Huerta-Sánchez, 2017] to quantify the relatedness of modern ABO haplotypes with the archaic haplotypes from the Neanderthal and Denisovan individuals. Each haplostrip is polarized to one the archaic genome (reference haplotype), and each subsequent haplotype is ordered by genetic similarity, from most related to least related. The archaic haplotypes are unphased (only coding regions were phased in the previous section), but because they are highly homozygous, they behave as phased haplotypes. We used the genetic distances calculated by Haplostrips to rank the proximity of modern human haplotypes to archaic haplotypes. We used R [R Development Core Team, 2008] to visualize the genetic distances between individuals in the 1000 genomes populations to the archaic ABO haplotypes. The distance information we used to generate these figures can be found in the supplement.

Identifying the origin of archaic introgression in admixed populations is complex. For example, American populations can trace portions of their genomes to European and African ancestry, as a consequence of European colonization of Native Americans, and the African slave trade. Because of these historical events, archaic introgression present in modern American individuals can be inherited from any of these sources. For the American populations in the 1000 Genomes Project panel, including two African populations sampled in the Southwest United States and Barbados, we used ancestry calls from Martin et al. [2017] to distinguish if purported archaic ABO alleles sit in European-ancestry genome tracks. This allowed us to properly track archaic introgression to Europe, rather than being retained ancestrally in African populations, or introgression with the Asian ancestral populations from which Native Americans descend. For our genetic distance results, we thus excluded six populations sampled in America (MXL, PUR, PEL, CLM), including two African populations (ASW,ACB), after determining that all introgressed ABO haplotypes are exclusively located in European ancestry tracks, thus providing a confusing look at archaic introgression (see Supp. Table 4).

6.4 Contour density plots of match proportion of introgressed segments to the Neanderthal genomes

In order to look more closely at the Neanderthal ABO alleles in present-day humans, we plotted two-way densities of match rate to the Altai Neanderthal and Vindija Neanderthal genomes, using the method described in Browning et al. [2018]. We used the archaic SNP calls generated in Browning et al. [2018] using Sprime statistics. We also used Browning

et al. [2018]’s match-mismatch calls to check if the purported introgressed SNPs also match the Vindija, and Altai archaic genomes. We used R [R Development Core Team, 2008] to visualize the affinity data. We plotted a contour density figure using the Neanderthal match/non-match scores in the Iberian (IBS) and Japanese (JPT) 1000 Genomes Panel populations, reported in Browning et al. [2018]. We mapped the affinity of all introgressed genome fragments to either the Altai and Vindija genomes in these two representative European and East Asian populations. We then matched the variable sites in the sequence of the Altai-like and Vindija-like introgressed ABO haplotypes to the Altai and Vindija genomes to visualize their affinity relative to other introgressed genome fragments. The resulting contour density plots is interpreted as a topological map, showing the affinity of all introgressed fragments in a modern population relative to the two Neanderthal genomes. Then, we highlight the position of the introgressed ABO haplotypes relative to all other introgressed genome element, in order to quantify their relative sequence divergence.

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