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An ancient view on host pathogen interaction across time and space

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The ancient DNA revolution provided diverse fields with an unprecedented opportunity to look back into the past and shed light on research aspects that were until now subject to speculation and inference from modern data. In particular enrichment methods that allow the targeted retrieval of millions of SNP positions from ancient human genomes, or even complete bacterial and viral genomes have the potential to revolutionize our understanding of host pathogen interactions. Ancient DNA combined with new bioinformatic tools now even allows actual allele calling for immunogenetic systems such as Human Leukocyte Antigen (HLA) across time and space. The coming years will provide us with frequency data of human immunity genes, such as HLA, as well as genome wide data of ancient pathogens from many time periods of human history, and will therefore provide us with a dynamic view on historical human adaptation to pathogen exposure on a population wide scale.

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Introduction

The geographic distribution of allelic variants of the Human Leukocyte Antigen (HLA) immunogenetic system has been extensively studied for the past 50 years. Several alleles exhibit geographic restrictions which has led to several hypothesis in an attempt to explain their distribution. Models such as isolation by distance [1], neutral evolution [2], pathogen-driven selection [3] and natural selection [4] alone have failed to thoroughly explain the patterns uncovered so far. However, present-day data may not be the best to make inferences about the distribution and frequencies of alleles through time and space. Moreover, the ability to look into specific time transects, or to compare the frequencies and distribution of alleles before and after specific biological events (such as epidemics or the arrival of new pathogens into previously unexposed ecosystems) gives an opportunity to study selection *in situ*.

In recent years, rapid technological developments within the field of molecular biology and its application to ancient DNA (aDNA) exponentially enlarged the number of genome-wide data sets from ancient human remains. Next-generation sequencing (NGS) technologies applied to aDNA dramatically expanded the number of past individuals that could be analyzed at a given time and allowed to authenticate ancient DNA and quantify potential contamination therefore massively improving the quality of the obtained data [5]. NGS sequencers have made it possible to increase the number of bases sequenced per run with a concomitant decrease in sequencing costs [6]. From the analyses of short mtDNA fragments [7] the field rapidly shifted to the reconstruction of entire mitogenomes of *Neandertals* [8] and using approaches to discriminate between contamination and authentic endogenous DNA also into the study of early modern humans [9]. It was just a matter of time before the next quantum leap would occur: the study of entire ancient human genomes [10,11] that allowed the characterization of the genetic makeup of modern humans across the Late Pleistocene [12,13], the genomic transitions that occurred during the Neolithic [14–17] and the complexity and genetic history of the formation of the Eurasian human genepool [18–20]. Other major methodological innovations involved the identification of anatomical elements with higher proportion of endogenous DNA content [21], the design of specific protocols to extract and enrich for specific genomic DNA regions [22] and the construction of robust computational pipelines appositely designed for aDNA data processing [23]. In particular targeted enrichment was a game changer: using targeted hybridization capture, those approaches allowed the retrieval of genome wide data even from poorly preserved ancient samples [24], making archaeogenomic studies not only limited to the analyses of a few specimens with outstanding DNA preservation. As a result, thousands of ancient human genomes have been analyzed in the last five years from ancient cultures and populations worldwide. Using the same targeted hybridization capture approaches it is now also possible to enrich and study HLA and related immunogenetic systems across time and space [25].

All this technical and analytical advantages are playing, and will continue to play, a key role on the discoveries related to the recent evolution of our immune system in the years to come. And some research questions that have remained long unanswered may be illuminated by such new types of data. What was the role of archaic introgression in our immunogenetic makeup? How did the Neolithization and the accompanying transition to agriculture shape our immune system, and how did we adapt to the major pandemics that ravaged human populations in our recent past, such as the medieval Black Death or the Old World pathogens that were introduced to the Americas in the 16th century [26]? Answers to those questions will help to draw a better picture on the temporal and spatial selection that our species has gone through.

Testing the pathogen-driven selection

The increase in the amount and quality of the data obtained from ancient human remains boosted the optimism in the field and allowed researchers to venture into new directions. One of the first studies compared pre-Contact Native Americans to a modern group from the same region (the northwest coast of North America) in order to directly explore changes in the frequency of immunity genes that might have been caused by exposure to pathogens introduced after the 15th century. The study found signatures of positive selection in immune-related genes, with the strongest signal found in the HLA gene *HLA-DQA1* [27]. However, this approach pointed only to a genomic region in the HLA cluster, but would not provide information regarding the specific HLA alleles involved in the reported shift. It remains to be shown in the future which HLA alleles and/or haplotypes changed in Native Americans before and after the contact with Europeans, and if these changes are consistently found all over the Americas or if local differences arose as a result of specific pathogen-driven selection. In a second step the identity of the pathogens that caused the above-mentioned shift could be addressed by studying the specific binding and presentation properties of the positively selected alleles.

Another study focused on a time transect of the last 2000 years in the region of modern-day Poland. They found that SNPs within innate immune response genes (SLC11A1 and IL10) could have been the subject of non-stochastic evolutionary forces, although the effect of inbreeding and reduced gene flow may have influenced changes in allele frequencies over time [28]. Well-controlled, geographically restricted studies are important to analyze frequency fluctuations of immunity genes variants and the effect of specific pathogens and ecological conditions on past and present human populations.

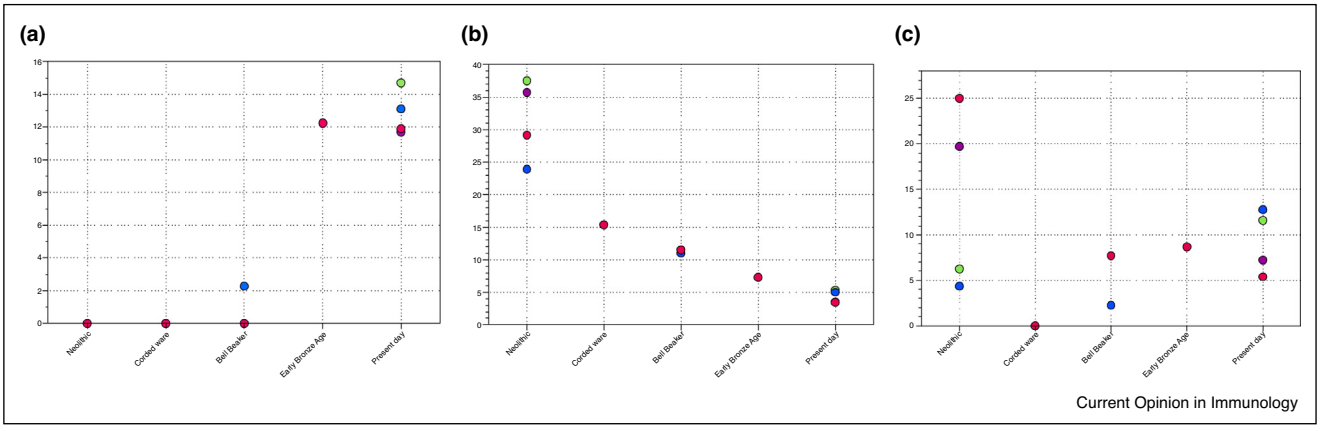
Changes across time and space

Probably one of the most exciting questions to be answered with these new approaches is how are these changes associated with the arrival of newly introduced pathogens and whether variation through time responds to local adaptation or to genetic drift, or an interplay between the two forces. In Eurasia, some immune-related genes have been suggested to be under selection since the Neolithic period [19]. It is predictable, thus, that immunogenetic diversity would vary over long time periods, but it is very difficult to infer how this variation occurred from modern data alone. Genome wide data from central European Neolithic individuals show that there is a significant change in the frequency and diversity of HLA alleles [25]. Those findings are further supported by HLA capture data recovered from Central European archaeological sites that span from the Mesolithic period to the early Bronze Age (Barquera *et al.* unpublished). Our results suggest that there are three types of changes found among HLA alleles through the last 10 000 years in Europe (Figure 1): alleles that were absent for most of the time until recently (after Bronze Age; such as HLA-B*07), alleles that were once common and gradually decreased in frequency (like HLA-B*27:05) and alleles that have been present throughout the entire time transect varying through time and space (like HLA-B*44:02). What we acknowledge today as alleles commonly found in the European gene pool poorly resembles the diversity observed in this region over the course of the last 10 000 years. But, is this variation due to adaptation and pathogen-host co-evolution, or due to specific epidemic or pandemic events that shaped this diversity or simply a product of genetic drift? To answer this question, we must look for the variation of HLA alleles under selection by relevant pathogens. One of those alleles is HLA-DRB1*15:01, which has been previously associated with the susceptibility to infection by *Mycobacterium leprae* [29], and was recently reported to be associated with the susceptibility to lepromatous leprosy in infected individuals from medieval northern Europe [30].

Going further back in time: the immunogenetic make-up of Pleistocene hominins

Another set of questions that can now be addressed is related to the early spread of modern humans outside Africa. Is it possible that archaic hominin introgression, that was shown to be on the order of 2%–5% in all modern humans outside Africa [10,31], would have played a major role in the ability of our species to successfully overcome the immune challenges present in the new continents that humans encountered after leaving Africa? Do we owe our success in colonizing the whole world to immunogenetic variants acquired by admixture with other hominins? First attempts to address this subject have been made by analyzing the available genomic data of one of our closest relatives, the Denisovans that were recently discovered [32]. Abi-Rached *et al.* [33] claimed that HLA-B*73:01 was introgressed into early modern humans in Asia based on the reconstruction of two HLA class I

Figure 1



Changes in the frequency of selected *HLA-B* alleles through time spanning from the Late Mesolithic/Neolithic period through present day. Frequencies from Russia are indicated by a green dot, from France in purple, from Germany in blue, and from Czech Republic in pink. **(a)** Frequencies through time for HLA-B*07:02. **(b)** Frequencies through time for HLA-B*27:05. C. Frequencies through time for HLA-B*44:02. The individuals analyzed (R Barquera *et al.*, abstract 755, Society for Molecular Biology and Evolution, Manchester, UK, June 2019) were collected from archaeological sites in France (Obernai, Gougenheim, Gurgy, Pendimoun, Les Bréguières, Fleury-sur-Orne; N = 84), Germany (Niedertiefenbach, N = 23 [25]; Profen, N = 22), Czech Republic (Brandy's, Prague West District, Hostivice-Palouky, Kněževes; N = 93), and the Republic of Tatarstan, Volga region (Murzihinskiy II tomb; N = 8), and span in time from the Late Mesolithic to the Early Bronze Age (~7000–3600 BP). Present-day data (France, N = 1406; Germany, N = 39,689; Czech Republic, N = 5099; Russia, N = 207) were extracted from published data compiled in the Allele Frequencies Data Net website [42].

haplotypes from the first Denisovan genome published [31] (HLA-A*11:01~B*73:01~C*12:02 or HLA-A*11:01~B*73:01~C*15:05). However, the available shotgun data of this extinct hominin was rather low in coverage hampering attempts to correctly call HLA alleles. In that study, the authors reported the presence of HLA-B*73:01 based on inferred linkage disequilibrium between the assigned *HLA-A* and *HLA-C* alleles and HLA-B*73:01 in modern human populations. The *HLA-B* typing could not be directly carried out due to the low quality of the data. Further analysis on the high coverage Denisova genome [34] that reanalyzed the HLA locus called the B*73:01 introgression hypothesis into question [35]. Higher coverage genomes or targeted enrichment with capture approaches may be helpful to perform HLA genotyping of archaic humans in the future and, aided by local genome ancestry analyses, answer whether or not HLA alleles were introgressed from Neanderthals or Denisovans into the early modern human gene pool. Currently it remains largely an open question, which HLA alleles or haplotypes (if any) were introduced from Neandertals and Denisovans and what role they might have played in our adaptation to pathogens for the past ~50 000 years. If the archaic hominins carried variants that were passed on into the later Pleistocene and Holocene modern human populations that could point to local adaptation in Eurasia for tens of thousands of years. If they did not contribute HLA variants, it might tell us that, while we share a common genetic pool with our closest genetic relatives, our immunity genes were selected multiple times independently by the same environment. If the immunogenetic diversity found among Denisovans, Neandertals and modern humans is completely different, it might tell us that hominin groups have responded quite differently to the same environment or that evolution has selected other alleles to deal with the same immune challenges during the late Pleistocene [36]. Recent studies suggested that there is no observable decrease in the genetic diversity of immunity genes in later Neandertals, although the authors claim that this needs confirmation with a larger sample size [37].

Other immune-related genes involved in resistance to pathogen infections and the immune response were found enriched within archaic introgressed regions in modern human genomes [the 2'-5' oligoadenylate synthetase (OAS) locus (OAS1/2/3), TNFAIP3 and the Toll-like receptor (TLR) TLR6~TLR1~TLR10 genetic cluster] [38–41], so there is no reason to think that the fitness of HLA loci would have not benefited from archaic introgression.

Conclusions

The newly emerging data from the field of archaeogenomics will bring answers to long-debated questions related to the recent evolution and selection forces on human immunity genes by studying host pathogen interactions through time and space. Increasing the sample size of immunity genes from ancient human populations will help in the near future to achieve the statistical significance typically required by association studies dealing with HLA and KIR alleles and haplotypes. The

replication of the already observed events related to archaic introgression of immunity genes or local adaptation to recently introduced pathogens in other regions and larger population sizes will confirm whether those findings are representative or rather a single spatial and temporal event. Such data will provide a better understanding on how human populations have dealt with pathogens and adaptation to new environments in the past and might help to reveal selective forces on immunity genes by emerging pathogens in the future.

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Conflict of interest statement

Nothing declared.

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