Direct evidence of an increasing mutational load in humans

Stéphane Aris-Brosou[1,2,⋆]

[1]Department of Biology, University of Ottawa, Ottawa, ON K1N 6N5, Canada

[2]Department of Mathematics and Statistics, University of Ottawa, Ottawa, ON K1N 6N5, Canada

⋆Correspondence: sarisbro@uottawa.ca

Abstract

The extent to which selection has shaped present-day human populations has attracted intense scrutiny, and examples of local adaptations abound. However, the evolutionary trajectory of alleles that, today, are deleterious has received much less attention. To address this question, the genomes of 2,062 individuals, including 1,179 ancient humans, were reanalyzed to assess how frequencies of risk alleles and their homozygosity changed through space and time in Europe over the past 45,000 years. While the overall deleterious homozygosity has consistently decreased, risk alleles have steadily increased in frequency over that period of time. Those that increased most are associated with diseases such as asthma, Crohn disease, diabetes and obesity, which are highly prevalent in present-day populations. These findings may not run against the existence of local adaptations, but highlight the limitations imposed by drift and population dynamics on the strength of selection in purging deleterious mutations from human populations.

Keywords: ancient DNA, Single Nucleotide Polymorphism (SNP), Genome-Wide Association Study (GWAS), selection, range expansion, Combined Annotation Dependent Depletion (CADD).

Main Text

The role played by natural selection in shaping present-day human populations has received extensive scrutiny [1, 2, 3], especially in the context of local adaptations [4]. However, most studies to date assume, either explicitly or not, that populations have been in their current locations long enough to adapt to local conditions [5], and that they were large enough to allow for the action of selection [6]. If these conditions were satisfied, not only should selection be effective at promoting local adaptations, but deleterious alleles should also be eliminated over time. Surprisingly, this question has only been examined by comparing present-day modern humans from Africa and Europe [7], leading to the consensus that selection was as effective in Europeans as in Africans [8, 9] but without really assessing the effectiveness of selection on a large data set [10]. Here, I examine this question directly, based on the genotype of not only present-day humans, but also of $> 1,000$ ancient humans over the past 45,000 years.

In order to trace the evolutionary trajectory of deleterious alleles in human populations over time, the genotypes of 2,062 individuals, of which 1,179 were ancient humans, were assessed at about 1.2 million Single Nucleotide Polymorphisms (SNPs; the '1240k capture' [11]). The present study focuses on individuals sampled throughout Europe, from the Atlantic to the Ural Mountains, as this is where most ancient DNA has been recovered and genotyped throughout the world. To take into account the history of admixture and replacement events characterizing Europe [12, 11], this region was split into four quadrants along two orthogonal axes roughly cutting through the Alps (Figure 1). These four quadrants represent approximately four distinct areas that played significant roles in the three peopling waves that affected Europe, first from the Fertile Crescent (Q4 in Figure 1) to the rest of Europe during the Paleo-/Mesolithic period, between 45 and 10 thousands

years ago (kya) [13, 14]. These early Europeans were then replaced after the Last Glacial Maximum, 10 kya, by Neolithic farmers [15] who reached the Iberian Peninsula (Q3), and Britain and Scandinavia (Q2) 7-6 kya [16]. A third major migration event swept into Europe, including central Europe (Q1), during the late Neolithic and the early Bronze Age, about 3.5 kyr ago [11, 17]. Under the constraint of analyzing approximately even numbers of individuals across these four quadrants, the timing of these migration events suggested splitting Europe into four different time periods, representing roughly the Mesolithic (t1: 45-10 kya), the Neolithic (t2: 10-3.5 kya), the Bronze Age (t3: 3.5-0 kya), and present-day populations (t4: 0-present) as in Figure 1.

To find which genomic positions among these 1.2 million SNPs are deleterious, a database of markers identified based on genome-wide association studies (DisGeNET) was used [18]. This database contains 83,582 SNPs, and the associated risk alleles were retrieved separately [19] (Supplemental Material and Methods). Strikingly, the average frequency of deleterious mutations significantly increased through each time period in all four quadrants (Figure 2). One possible explanation for this nonadaptive pattern is that most of the SNPs tested are weakly deleterious, and increased in frequency as neutral mutations would have [20].

To test if weakly deleterious mutations are driving the increase in risk allele frequency, their relative pathogenicity was assessed by the Combined Annotation Dependent Depletion (CADD) score [21], which take values from -6 (the least deleterious) to 100. The values observed here range from about -5 to 15, are sharply distributed around 0, and are skewed to the right (Figure S1). To cover the observed CADD scores, their range was split into five bins. In all four quadrants, the mutations that are expected to be the most weakly deleterious (up to CADD = 2.5) significantly increased in frequency (Figure 2A). As these mutations are the most numerous (Figure S1), they are most likely responsible

for the overall increase in frequency. However, the CADD analysis also reveals that the most deleterious mutations, with CADD > 10, did not significantly decrease in frequency (Figure 2A). If these mutations were recessive, the deleterious alleles would mostly be present in the heterozygous genotypes, and their homozygosity would be low.

To assess the effect of dominance on the general pattern of increasing frequency of risk alleles through time, the homozygosity of the risk alleles was calculated (Figure 2B). Overall, homozygosity decreased significantly through time, but again, this pattern is mostly driven by the least deleterious alleles (up to CADD $= 2.5$). The most deleterious ones (CADD > 10), on the other hand, saw their heterozygosity decrease recently (in t4) only in southwestern Europe (Q3). In all the three other quadrants, the heterozygosity of the most deleterious alleles did not change significantly (Figure 2B), suggesting that these alleles are only partially recessive.

To better understand which diseases increase in frequency through time, robust linear models were fitted to each SNP, irrespective of its CADD score. From the corresponding volcano plot (Figure 3A), two groups of SNPs can be defined: those whose frequency significantly decrease (top left area of volcano plot) or increase (top right area) through time. In the first group, quadrant Q1 has both the most negative slopes and the smallest CADD scores (Figure 3B; Dunn test, FDR < 0.05), and the most affected phenotypes, with a CADD score greater than 1, include "age at menarche" and "drug dependence" (Table S1). In the second set, quadrant Q2 has the most positive slopes even if all quadrants has similar CADD scores (Figure 3C; Dunn test, FDR < 0.05), and the most affected phenotypes, with a CADD score greater than 1, include "asthma," "Crohn disease," "Diabetes Mellitus" and "obesity" (Table S1), diseases that tend to be frequent in present-day European populations. Enrichment analyses confirmed that SNPs involved in addictive behavior are overrepresented in the set of SNPs with decreasing frequency,

while common diseases are overrepresented in the group with increasing frequencies. Note that in both sets of SNPs, the CADD scores are never greater that 10, suggesting that the most deleterious alleles either do not change in frequency, and / or do so at a very slow rate – which again is consistent with their not being eliminated through time.

The efficacy of selection at removing deleterious mutations depends on fitness effects, with the most deleterious variants being eliminated with the greatest ease. However, this efficacy can be affected by a number of factors such as the effective population size, which is arguably the most critical [22]. Indeed, ancient human demographics is marked during the Last Glacial Maximum (LGM) by a severe bottleneck, which left traces in present-day individuals of European ancestry [23] as well as in other worldwide populations [24]. The results presented here are therefore consistent with the population genetics context, which permitted the increase in frequency over time of deleterious variants.

Although the variance of deleterious allele frequencies in the oldest time period (t1: the Mesolithic) is very large, their median is also quite high (~ 0.25), begging the question of the origin of these variants. Previous work showed that some of these deleterious variants found in modern humans could have a Neanderthal origin, and it was quickly suggested that selection could be efficient at removing these variants [25], especially in East Asians, as selection is mostly acting on weakly deleterious mutations located in protein-coding genes [26]. However, recent work showed that the decline of Neanderthal ancestry in modern humans was likely due to demographic events such as migrations between West Eurasian and African populations, rather than widespread and systematic selection against these alleles [27]. This is entirely consistent with the results presented here, where frequencies of even the most weakly deleterious variants do not significantly change over time. This is potentially due to most of the SNPs used here being located outside of protein-coding genes – which again is consistent with the depletion of Neanderthal alleles

in regulatory and conserved noncoding region [27].

An allelic frequency however represents a snapshot of a dynamic process, where variants are generated by mutations, and eliminated by selection (or lost by drift). The initial increase, between the Mesolithic and the Neolithic (t1 and t2, respectively), could be due to drift, but also to an increased mutational pressure. Little evidence however seems to support this latter possibility, except maybe for recent times, where humans tend to expose themselves to mutational agents, leading to a relaxation of selection against mildly deleterious mutations [28]. As a result, nonadaptive factors following the initial LGM bottleneck remain the most plausible explanation for the monotonic increase in frequency of deleterious alleles in the European human population.

Again, this is entirely consistent with the demographic context, as this geographic region has been affected by at least three migration waves, associated with rapid demographic expansion(-s) [23]. Indeed, range expansions have been shown to enable new deleterious mutations to behave almost like neutral variants, hereby facilitating their increase in frequency [29]. This would suggest that (i) the deleterious mutations observed post-LGM are novel, *i.e.* do not have a Neanderthal origin, and (ii) increase in frequency because of a nonadaptive factor (repeated range expansions). One signature that is missing from typical range expansions is a frequency gradient along the expansion axis (from Q4 to the other quadrants; [30]); this absence of gradient may however be caused by multiple expansions, whose individual directions are not necessarily overlapping. This may also be the reason why an increase in the homozygosity of deleterious variants was not observed here. As a result, the existence of geographic regions of high fitness in which the effects of deleterious mutations would be partially masked, as predicted by range expansion models [31], seems unlikely in European humans.

The assessment of the phenotypic effects of variants was based on CADD scores, which

correlate with a number of experimental data related to pathogenicity, disease severity, *etc.* [21], but that also show a number of discrepancies in select cases [32], or in a study of 12,391 cancer-related SNPs [33]. While this number represents 12% of the SNPs analyzed here, it is unlikely that the results presented here are affected by the predictive power of CADD scores. Indeed, previous studies used a different score, the Genomic Evolutionary Rate Profiling (GERP) Rejected Substitution (RS) score, to predict the phenotypic impact of mutations [34], and showed either the existence of a spatial increase of deleterious mutations from Africa to the rest of the world, consistent with the expansion axes out of Africa [24], or an increase of deleterious alleles at the front of a range expansion compared to the inner core of the source population [35]. The data presented here add a temporal dimension to these previous spatial results, which were found at a broader scale. It is therefore possible that the results presented here, limited to Europe, be generalizable to the rest of the non-Subsaharan human populations. Simulations can be useful by shedding light on some of the processes affecting selection [8, 9], but are usually limited to simple scenarios [27] that are no substitute for actual data from the past. As more ancient DNA from the rest of the world is accumulating [36, 37, 38], it will soon be possible to test the effectiveness of selection at purging deleterious mutations from non-Subsaharan human populations.

Declaration of Interests

The authors declare no competing interests.

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Supplemental Data

Document S1. Figure S1, Table S1, Supplemental Material and Methods, Supplemental References, and Supplemental Data.

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Figures

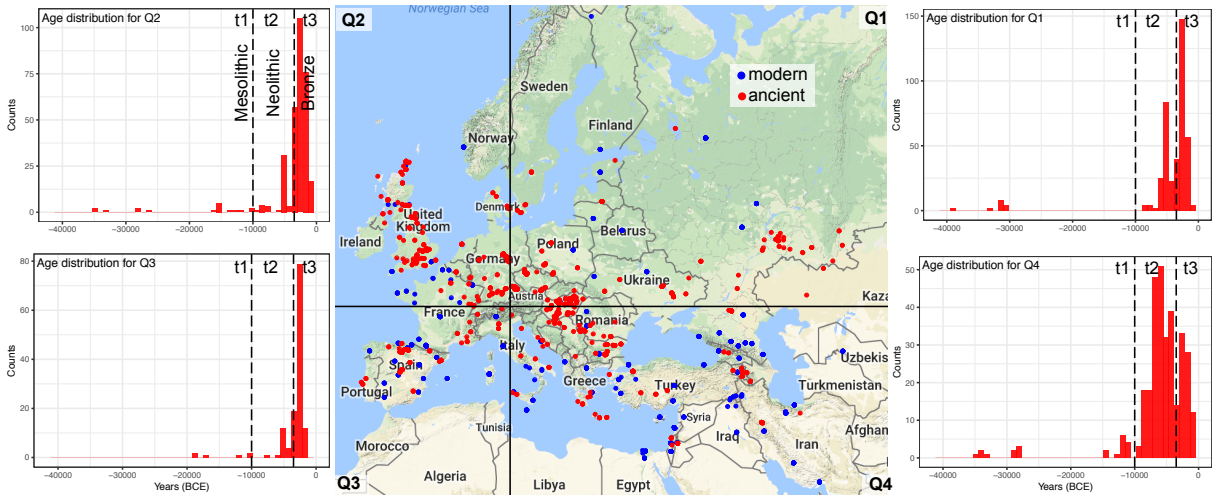

Figure 1. Spatiotemporal distribution of the human genomes analyzed in this study. Center: Sampling location of modern (blue) and ancient (red) individuals throughout Europe. The maps is divided into four quadrants representing northeastern (Q1), northwestern (Q2), southwestern (Q3), and southeastern (Q4) Europe. By each quadrant, the age distributions of the ancient humans are shown; the four time periods considered correspond roughly to the mesolithic (t1: 45,000–10,000 BCE), the neolithic (t2: 10,000–3,500 BCE), the bronze age (t3: 3,500–0 BCE), and modern humans (t4: the 2000 CE). Map background: Google (2018).

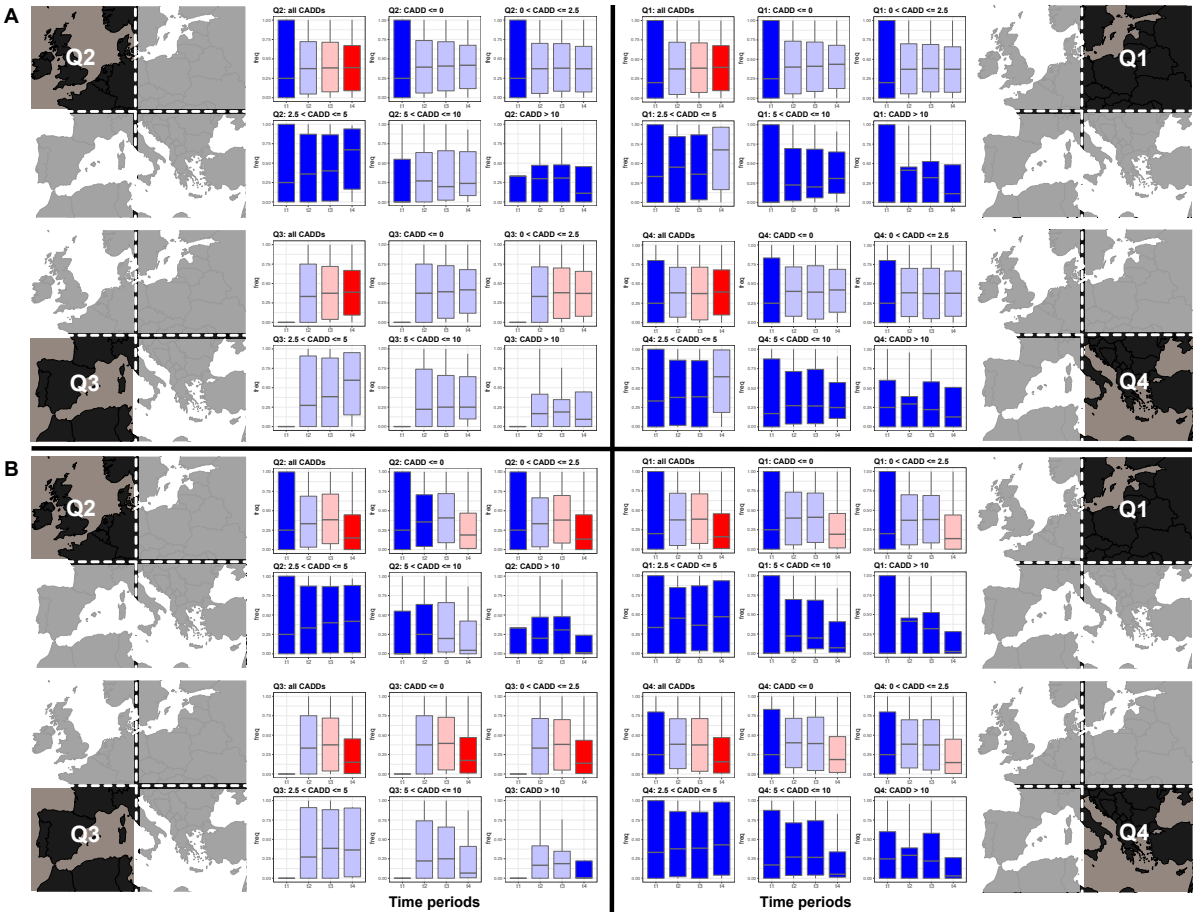

Figure 2. Evolution of SNP frequencies through space and time. (**A**), Frequencies of risk alleles. (**B**), Homozygousity at risk alleles. The top left panel in each quadrant show the frequency distributions at all SNPs over the four time periods, while the next five panels show the frequency distributions at SNPs in each severity category, ranging from benign (CADD < 0) to severe (CADD ≥ 10) effects. Color differences in each panel indicate significance of the Dunn test at the 1% level with Benjamini-Hochberg correction for multiple tests – colors are ordered from significantly lower (cooler) to significantly higher (warmer) frequencies of the risk alleles.

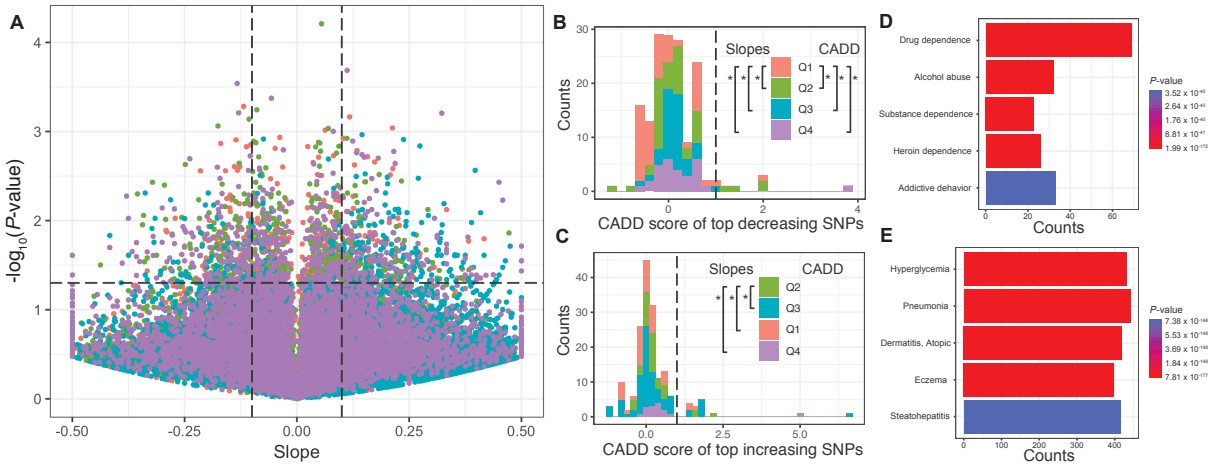

Figure 3. Volcano plot of the regressions of SNP frequencies through time. (**A**), The plot shows the P-values, transformed on a $-\log_{10}$ scale, as a function of the slope estimates of the robust regressions, with the four quadrants color coded (Q1: salmon, Q2: green, Q3: blue, Q4: magenta). (**B**), Distribution of the CADD scores for the SNPs whose frequency decreases through time ($P \leq 0.05$, slope ≥ 0.1). The Dunn test was used to assess differences between slopes (left of inset legend) and CADD scores (right of inset legend) at an FDR ≤ 0.05. (**C**), Same as in (**B**) for the SNPs whose frequency increases through time. Top five enriched gene names among the top decreasing (**D**), and top increasing (**E**) genes identified from the volcano plot with CADD scores ≥ 1.