Title
Analysis of human accelerated DNA regions using archaic hominin genomes.

Permalink
https://escholarship.org/uc/item/7jf9j36h

Journal
PloS one, 7(3)

ISSN
1932-6203

Authors
Burbano, Hernán A
Green, Richard E
Maricic, Tomislav
et al.

Publication Date
2012

DOI
10.1371/journal.pone.0032877

Peer reviewed
Analysis of Human Accelerated DNA Regions Using Archaic Hominin Genomes

Hernán A. Burbano1*, Richard E. Green2, Tomislav Maricic1, Carles Lalueza-Fox3, Marco de la Rasilla4, Antonio Rosas5, Janet Kelso1, Katherine S. Pollard6,7, Michael Lachmann1, Svante Pääbo1

1 Max Planck Institute for Evolutionary Anthropology, Leipzig, Germany, 2 Department of Biomolecular Engineering, University of California Santa Cruz, Santa Cruz, California, United States of America, 3 Institute of Evolutionary Biology, Consejo Superior de Investigaciones Científicas, University of Pompeu Fabra, Barcelona, Spain, 4 Department of Paleobiology, Museo Nacional de Ciencias Naturales, Madrid, Spain, 5 Department of Prehistory, Departamento de Historia, Universidad de Oviedo, Oviedo, Spain, 6 Gladstone Institutes, University of California San Francisco, San Francisco, California, United States of America, 7 Division of Biostatistics and Institute of Human Genetics, University of California San Francisco, San Francisco, California, United States of America

Abstract

Several previous comparisons of the human genome with other primate and vertebrate genomes identified genomic regions that are highly conserved in vertebrate evolution but fast-evolving on the human lineage. These human accelerated regions (HARs) may be regions of past adaptive evolution in humans. Alternatively, they may be the result of non-adaptive processes, such as biased gene conversion. We captured and sequenced DNA from a collection of previously published HARs using DNA from an Iberian Neandertal. Combining these new data with shotgun sequence from the Neandertal and Denisova draft genomes, we determine at least one archaic hominin allele for 84% of all positions within HARs. We find that substitutions in HARs tend to have come to fixation faster than substitutions elsewhere in the genome and that substitutions in HARs tend to cluster in time, consistent with an episodic rather than a clock-like process underlying HAR evolution. Our catalog of sequence changes in HARs will help prioritize them for functional studies of genomic elements potentially responsible for modern human adaptations.

Introduction

To detect functionally relevant genomic features that underwent positive selection in humans since the separation from their common ancestor with chimpanzees and bonobos, several authors identified genomic regions that are conserved among vertebrates but have accumulated substitutions on the human lineage at an accelerated rate [1,2,3,4]. Here, we refer collectively to these regions as “human accelerated regions” (HARs).

The sequence conservation in HARs suggests that they are subject to functional constraints, while the increased rate of substitutions on the human lineage suggests that their function may have changed in humans. To date, two HARs have been studied in detail with respect to function. One of these (HAR1 in [3]) is part of an RNA gene that is expressed in Cajal-Retzius neurons in the developing human cortex between gestational week 7 and 19, while another (HAR2 in [4]) acts as an enhancer of gene expression in transgenic mice and has limb expression in humans which is not seen in chimpanzees and rhesus macaques [5]. However, it is unclear to what extent all HARs are functionally important. Although some HARs are located near genes that encode transcription factors and other DNA-binding proteins [6], genes involved in neuronal cell adhesion [4], and genes containing polymorphisms correlated with changes in gene expression [1], it has been noted that they are not enriched for cis-regulatory elements [1,2,3,4] although other conserved non-coding sequences are so [7].

It has been noted that nucleotide substitutions in HARs show an excess of A/T to G/C substitutions [6,8]. This is reminiscent of GC-biased gene conversion (BGC), a non-adaptive process associated with recombination in eukaryotes. BGC is the non-reciprocal copying of a stretch of DNA from one chromosome into the other [9] and favors fixation of GC alleles over AT alleles in yeast [10] and presumably in primates [11]. Since recombination, and therefore BGC, tends to be localized to recombination hotspots [12], and since these often shift their locations over short evolutionary times in primates [13,14], an increase in the rate of substitutions in a genomic region in a certain evolutionary lineage may be due to a recombination hotspot that has appeared in that region and lineage. Thus, it has been hypothesized that repeated events of BGC are the source of human-specific substitutions in many HARs [8,9].

One limitation in investigating HARs and their potential role in recent human evolution is that it is unknown when during the 5–7
millions of years since the divergence of the human lineage from the chimpanzee lineage substitutions in HARs occurred. The genomes of extinct close relatives of present-day humans offer the possibility to determine when such substitutions took place. Recently, two draft genome sequences of archaic hominins have been determined: Neandertals [15] and Denisovans [16]. Neandertals and Denisovans were sister groups whose DNA sequences diverged from those of modern humans on average about 800,000 years ago. These two draft genomes, of about 1.3- and 1.9-fold genomic coverage, respectively, provide a means to determine if nucleotide substitutions observed in human DNA sequences occurred before or after the divergence of the common ancestor of Neandertals and Denisovans from the lineage leading to modern humans. However, of all nucleotide substitutions assigned to the human lineage since the divergence of humans from chimpanzees, a total of only 30 and 40% are covered by at least one sequenced DNA fragment in the Neandertal and Denisovan genomes, respectively [15,16]. This is below the theoretical expectation from random fragmentation and sampling of DNA (65 and 85%, respectively) [17] and is likely to be due to the fact that preservation, recovery, and mapping of ancient DNA fragments are not random.

To provide more complete coverage of particular regions of interest, several approaches that are able to recover specific genomic regions have been developed or applied to ancient DNA. Several approaches that are able to recover specific genomic regions have been developed or applied to ancient DNA. For example, several approaches that are able to recover specific genomic regions have been developed or applied to ancient DNA. For example, several approaches that are able to recover specific genomic regions have been developed or applied to ancient DNA. One such approach used hybridization capture on microarrays to recover specific genomic regions in ancient DNA. We designed an Agilent oligonucleotide capture array covering 2,615 HARs (total of ~1 Mb) identified in four studies [1,2,3,4] and used this array to capture Neandertal DNA sequences from 40 DNA libraries from a Neandertal from El Sidrón Cave, Asturias, Spain (Sidrón 1253) [22,23]. The captured DNA fragments were sequenced on the Illumina GAIIx platform and mapped [24] to the human reference genome. From the capture of these Sidrón libraries we obtained at least one sequencing read covering 5,711 (63%) of the human lineage-specific substitutions within HARs. From the Neandertal [15] and Denisovan [16] draft genomes, we retrieved 3,779 (42%) and 3,468 (38%) such positions, respectively. Combining the three data sets produced a total coverage of 7,566 (84%) positions with human lineage-specific changes within HARs (Fig. 1a–b).

To determine the archaic state at each position, we filtered the Sdrón Neandertal data for PCR duplicates and used a maximum likelihood approach to generate consensus sequences from overlapping, independent fragments and join them in “mini-contigs”, as described previously [15,16]. In order to avoid biases induced by sequencing errors, we considered only positions where the Neandertal carried the derived (human-like) or the ancestral (chimpanzee-like) nucleotide. We refer to positions where at least one archaic hominin carries the nucleotide seen in the human reference genome as “old alleles” since they are likely to have been present in the common ancestors of present-day humans and Neandertals and Denisovans. We refer to positions where neither the chimpanzees nor any available archaic hominin genome carries the human nucleotide as “recent alleles” in the sense that they had not come to fixation in the common ancestor of the archaic hominins and modern humans. Therefore, they represent substitutions that occurred recently along the human evolutionary lineage. In all further analyses we use positions recovered from one or more of the three data sets: the Sidrón Neandertal, the Neandertal genome and the Denisova genome. For positions that overlapped among datasets the agreement in terms of whether old or recent alleles were seen was 96–99% (Fig. 1c). We therefore restricted the analyses to the positions where all datasets agreed.

**Estimates of human DNA contamination**

One of the major challenges when working with ancient hominin DNA is the contamination of extracts with contemporary human DNA. Although this specimen has been excavated using procedures that minimize contamination of human DNA [25] and processed using laboratory procedures designed limit and detect contamination [26], it is nevertheless essential to estimate the level of human DNA contamination directly from the sequencing data. Here, we used two approaches to estimate modern human contamination in the Sidrón dataset based on mitochondrial DNA (mtDNA) and autosomal positions.

The first approach used mtDNA. The mtDNA sequence from the Sidrón 1253 bone has been previously determined [18] and differed from almost all (99%) present-day human mtDNAs at 130 positions. We estimated the level of modern human mtDNA contamination by classifying each Sidrón mtDNA fragment carrying at least one of the 130 positions as modern-human-like or Neandertal-like [27]. From a total of 127,610 informative fragments, 473 were modern-human-like, indicating an mtDNA contamination level of 0.36%, with a 95% binomial confidence interval between 0.33–0.4%.

The second test estimated modern human contamination using autosomal positions, where present-day humans are fixed for the derived allele as judged from the present-day human polymorphism database dbSNP (v. 131). Briefly, for every such position, the Neandertal individual is expected to be homozygous (for the ancestral or the derived allele) or heterozygous, yielding an expectation of seeing only one allele (if the Sidrón individual was homozygous) or a draw of the two alleles with equal probabilities (if he was heterozygous). Contamination or sequencing errors will skew these expectations. We applied a maximum likelihood model that exploits this aspect of the data [20] and estimated an upper bound of contamination of <1% (Fig. S1). Thus, both a mtDNA, which is not model dependent, and an autosomal-based estimates, which is directly relevant for the data analyzed, suggest that the contamination is low.

**HAR substitutions along the human lineage**

We found that 8.3% of substitutions in HARs are recent (Fig. 2a), i.e., not shared with the Sidrón Neandertal or the Neandertal and Denisova genomes. By contrast, 12.4% of substitutions genome-wide are recent. Hence, the fraction of recent alleles in HARs is about 30% smaller than expected from substitutions genome-wide ($P_{\text{bootstrap}}<0.001$). For A/T to G/C (weak to strong, W2S) and G/C to A/T (strong to weak, S2W) substitutions, the fractions of recent substitutions in HARs are 6.4% and 11.4%, respectively (Fig. 2a). Thus, recent W2S substitutions are 50% fewer than expected from substitutions genome-wide ($P_{\text{bootstrap}}<0.001$), whereas recent S2W substitutions are only 8% fewer and not statistically different from the genome-wide expectation ($P_{\text{bootstrap}}=0.09$). When W2S and S2W substitutions in HARs are compared to W2S and S2W substitutions genome-wide, we found that the fractions of recent substitutions are in both cases smaller than for substitutions genome-wide ($P_{\text{bootstrap}}<0.001$) (Fig. 2a). When positions recov-
In the Sidron Neandertal and the two ancient hominin draft genomes were analyzed separately the same trends are found. However, in the Neandertal genome the difference in the percentage of recent alleles for W2S and S2W changes is larger (Fig. S2). This is likely an artifact resulting from the treatment of the Neandertal libraries with restriction enzymes in order to increase the amount of endogenous DNA [15] (Fig. S3), resulting in a lower recovery of GC-rich HARs in the Neandertal genome. When the published sets of HARs were analyzed separately, the same trends were also seen (Fig. S4 and Table S1, S2) despite the fact that these were ascertained in different ways in each of the publications. We conclude that W2S HAR substitutions are significantly older than S2W substitutions (Mann-Whitney U test (MWU) $P < 10^{-15}$). They are also older than substitutions outside HARs and older than substitutions in various functional classes in protein-coding genes (synonymous, non-synonymous, 5’-UTR and 3’-UTR changes) as determined from the Neandertal and Denisova draft genomes ($P_{\text{bootstrap}} < 0.001$ for all comparisons) (Fig. 2b).

In the HARs, 96% of the old alleles and 16% of the recent alleles are fixed among present-day humans as judged from dbSNP (v. 131). When we sampled 1,000 times ~2,000 HAR-sized regions from the two archaic genomes 94% of the old alleles are fixed in present-day humans, while only 8% of the recent alleles are fixed. Recent alleles in HARs are thus about twice as likely to be fixed than is expected from genome-wide rates ($P_{\text{bootstrap}} < 0.001$), indicating that substitutions in HARs tend to fix faster than substitutions elsewhere in the genome (Fig. 3). This applies to both recent W2S and recent S2W alleles in HARs, where 16% and 14%, respectively are fixed and significantly higher than the genome-wide averages ($P_{\text{bootstrap}} < 0.001$ for both W2S and S2W alleles). The percentages of recent fixations of W2S and S2W alleles were not significantly different from each other (Fisher exact test (FET) $p = 0.31$).

**Temporal clustering of HAR substitutions**

To test whether the substitutions within individual HARs occurred clustered in time, we randomly sampled one substitution in each HAR and classified it as old or recent. The HARs were thus divided into two groups. For each group we calculated the percentage of recent alleles using all other positions in the HARs. The percentages of recent alleles in each group were divided by

**Figure 1. Substitutions in HARs recovered from the array capture experiment (Sidron) and two published hominin genomes (Neandertal and Denisova).**

(A) Number of substitutions on the human lineage recovered in each dataset. (B) Fraction of substitutions recovered in each dataset and all datasets together. (C) Pie charts of substitutions from all possible overlaps among datasets (agree: datasets have same state; disagree: at least two datasets differ).

doi:10.1371/journal.pone.0032877.g001
8.3%, the percentage of recent alleles observed across all HARs. If substitutions in HARs occurred gradually along the human lineage, this ratio will not be different from one, whereas if the changes in HARs clustered in time the ratio will be different from one. The ratios were 2.26 and 0.87 for recent and old alleles, respectively (Fig. 4), indicating that if a HAR carries a recent allele, it is about 2.3 times more likely than expected to carry a second recent allele ($P_{\text{bootstrap}} < 0.001$). In contrast, if it carries an old allele, it is about 15% less likely than expected to carry a second substitution which is recent ($P_{\text{bootstrap}} < 0.001$). We conclude that substitutions in individual HARs tend to be clustered in time. The same patterns were found when each of different published HAR datasets were analyzed separately (Fig. S5). When we apply the same approach to protein-coding parts of genes using the ancient hominin draft genomes [15,16] substitutions in HARs were found to be significantly more clustered than both synonymous (MWU at $P < 10^{-15}$ for both recent and old alleles) and non-synonymous substitutions (MWU at $P < 10^{-15}$ for both recent and old alleles) (Fig. 4). To test if the differences in clustering ratios between HARs and genes was caused by the length difference between the regions studied (short HARs and long genes), we also applied the method to protein-coding exons (median length = 171 bp). Again, the clustering ratios of HARs for both old and new substitutions were significantly more clustered than non-synonymous substitutions within exons (MWU at $P < 10^{-15}$ and $P < 10^{-17}$, respectively) (Fig. 4).

To investigate whether both substitutions that are likely to be due to BGC, i.e. W2S substitutions, and those that are not, i.e. S2W substitutions, cluster in time, we performed the analysis above for HARs where 0–50% of substitutions were of the W2S type (n = 680) and for those where more than 50% were W2S (n = 849). For recent substitutions, the clustering ratios in these two groups were 1.71 and 2.16, respectively. Both ratios were significantly higher than one ($P_{\text{bootstrap}} < 0.001$), and different from each other (MWU at $P < 10^{-15}$) (Fig. 5). For old substitutions, the clustering ratio of the over 50% W2S group was significantly lower than one ($P_{\text{bootstrap}} = 0.01$), whereas the 0–50% group was not ($P_{\text{bootstrap}} = 0.07$). Thus, temporal clustering exists for HARs that are potentially affected by BGC and for those that are not, although clustering is stronger amongst HARs with more W2S substitutions. These findings suggest that substitutions in all HARs, and especially those with some evidence of BGC, fixed surprisingly quickly in the human lineage.

### Discussion

Since Neandertals and Denisovans are sister groups [16] their genomes can be used in concert to gauge when substitutions occurred along the human evolutionary lineage. Here, we complement the analysis of these two published low-coverage
Human Accelerated Regions in Archaic Hominins

Figure 4. Temporal clustering analysis of HAR substitutions and protein-coding genes. (A) Clustering ratios for recent substitutions. HAR ratios computed using all datasets, and changes in genes computed from Neandertal and Denisova genomes. Non-syn gene = non-synonymous substitutions in full-length protein sequences; syn gene = synonymous substitutions in full-length protein sequences; syn exon = synonymous substitutions in individual exon sequences. (B) Clustering ratios for old substitutions. In both panels, the red line shows ratio = 1, which indicates an absence of clustering. Error bars are 95% confidence intervals calculated from an empirical distribution after repeating the sample procedure 1,000 times.

doi:10.1371/journal.pone.0032877.g004

Figure 5. Temporal clustering analysis of HARs. HARs were split in two bins according to their percentage of W2S substitutions: 0 to 50% and >50%. (A) Clustering ratios for recent substitutions of each type. (B) Clustering ratios for old substitutions of each type. In both panels, the red lines and confidence intervals are calculated as in Figure 4.

doi:10.1371/journal.pone.0032877.g005

Neandertals and Denisovans, which is estimated to be 270,000 to 440,000 years ago [15]. Those substitutions that we do not observe in the currently available archaic genomes are “recent”, i.e. they existed as polymorphisms in the common ancestor of the archaic hominins and modern humans or occurred in modern humans at a later time. Thus, even a single observation of the ancestral allele allows us to say that an allele is recent. This information can be used to identify potentially interesting HARs that have changed recently in human evolution.

By analyzing evolutionary and sequence characteristics of substitutions in HARs and comparing them to genome-wide
Materials and Methods

Neandertal DNA extraction and library preparation

We extracted DNA from a Neandertal bone (Sidrón 1253) in our clean room facility as previously described [29]. The bone was excavated in El Sidrón, Spain [23]. During excavation precau-

tionary measures were taken to avoid contaminating the bone with present-day human DNA [25]. In ancient DNA cytosines deaminate to uracils, which can then lead to misidentification of some cytosines for thymines in retrieved sequences [26,30]. To remove uracils from ancient DNA we treated the extracts with uracil-DNA glycosylase and endonuclease VIII [31]. The extracts then were turned into 454 sequencing libraries as described in [20]. The libraries carry a Neandertal library-specific "key" sequence (TGAC) that only Neandertal libraries produced in our clean room carry [26]. We used 1.4 grams of the Neandertal bone and 475 µl of the extracts for the production of 10 libraries.

Array design

To capture Neandertal libraries we used Agilent custom 244 K capture arrays. We designed overlapping microarray probes of 60 bases targeting 2613 HARs that were identified in 4 different studies [1,2,3,4]. Probes were tiled every 9 bases across the target regions. Probes containing repetitive elements were discarded [32]. We used the human reference sequence NCBI Build 36.1 (hg18) to design the probes. In addition to probes targeting the HARs, we include probes that target human mitochondrial DNA (mtDNA) sequence in order to estimate the level of present-day human contamination.

Capture and sequencing of Neandertal libraries

Before capture, the 10 libraries were amplified with PCR as previously described [20] and pooled into one library for a total of 16.8 µg required for the capture. Two serial captures were performed on 244 k Agilent arrays as described in [20]. To sequence the captured library on the Illumina platform we converted the 454 DNA libraries to Solexa sequencing libraries as described in [20]. The converted libraries were sequenced on two lanes of the Illumina GAII sequencer together with PhiX 174 variant spiked in. Manufacturer’s sequencing protocol for a paired-end run with 2×76 cycles and v4 chemistry was used except that special sequencing primers were used [20]; the primers were designed so that in the first read the Neandertal-libraries specific “key” sequence was read [20]. DNA sequences are deposited in the European Bioinformatics Institute Sequence Read Archive, with study accession number ERP000837.

Processing and mapping of Neandertal reads

The sequencing run was processed and base calling was performed as described in [20]. Neandertal reads were aligned to the human reference sequence NCBI Build 36.1 (hg18) using BWA [24]. Reads that aligned to hg18 mtDNA were used to assemble Sidrón mtDNA. The mtDNA was assembled using an iterative mapping assembly program as described in [20]. The identification of the changes that happened in the human lineage was done using whole genome alignments as described in [15,16].

Using Neandertal overlapping reads, a consensus Neandertal sequence was generated using “mini-contigs” as described in [15,16].

Authenticity estimates

El Sidrón 1253 mtDNA differs from almost all (99%) modern human’s mtDNA at 130 positions [18]. We estimated the level of modern human mtDNA contamination after microarray capture by classifying each Sidrón mtDNA fragment carrying an informative site as human-like (polluting) or Neandertal-like (clean) [27]. The autosomal contamination estimate was calculated using a likelihood framework [20].
Statistical analysis
Statistical tests were performed in the R environment (http://www.r-project.org). $P_{\text{bootstraps}}$ were calculated using 1000 non-parametric bootstrap iterations.

Supporting Information
Figure S1 Autosomal authenticity estimate. (A) Likelihood surface for contamination and heterozygosity as variables. Likelihood ratio computed vs. the maximum likelihood, with colors corresponding to rejection cutoffs using the $\chi^2$ distribution. (B) Constrained likelihood surface, where heterozygosity is held constant – the horizontal axis represents this constant. Confidence intervals plotted using the $\chi^2$ distribution with one degree of freedom.

(TIF)

Figure S2 Percentage of recent substitutions in the human lineage for different datasets. The red line shows the genome-wide average percentage of recent substitutions (12%). Recent substitutions are defined as those found in modern humans but not in the ancient hominins. “Total” refers to the total number of positions recovered in each archaic hominin. “Private” refers to positions only recovered in a given archaic hominin. Error rates for HARs are 95% confidence intervals calculated from an empirical distribution after 1,000 bootstraps. Color code explained in inset.

(TIF)

Figure S3 Relation between the presence of restriction enzyme motifs used in Neandertal genome enrichment (horizontal axis) and the fraction of human lineage substitutions recovered in HARs (vertical axis). The horizontal axis shows the minimum number of restriction enzyme motifs present in a HAR. The black points show the median percentage of human lineage substitutions recovered for all HARs. The red discontinuous line shows the median percentage of human lineage substitutions recovered for all HARs independent of the presence of restriction enzyme motifs. (A) Sidron. (B) Neandertal. (C) Denisova.

(TIF)

Figure S4 Percentage of recent substitutions for different groups of HARs. The red line shows the genome-wide average percentage of recent substitutions (12%). Recent substitutions are defined as those found in modern humans but not found in the ancient hominins. Error rates for HARs are 95% confidence intervals calculated from an empirical distribution after 1,000 bootstraps. Color code explained in inset. The lower overall percentage of recent substitutions from the Prabhakar dataset is due to the fact that one of the criteria used to identify these HARs required that substitutions be fixed in humans.

(TIF)

Figure S5 Temporal clustering analysis of HARs from all datasets. The clustering ratios were calculated independently for each group of HARs. (A) Clustering ratios for recent substitutions. (B) Clustering ratios for old substitutions. In both panels, the red line shows ratio = 1, which indicates an absence of clustering. Error bars are 95% confidence intervals calculated from an empirical distribution after repeating the sample procedure 1,000 times.

(TIF)

Table S1 Comparisons between HARs and genome-wide estimate (12%) of new alleles for different HARs’ datasets.

(DOC)

Table S2 Comparisons between W2S and S2W percentage of new changes for different HARs’ datasets.

(DOC)

Table S3 HARs with recent human lineage changes fixed in modern humans according to dbSNP 131. Total refers to the total number of human lineage changes in each HAR. The total number is split in W to S, S to W, and other type of substitutions. The Ensembl gene ID appears alone if the HAR overlap with the gene at least partially. The number after the ‘@’ character shows the distance in base pairs to the nearest gene, when the HAR does not overlap any gene.

(DOC)

Acknowledgments
We thank Aída M. Andrés, Claudia S. Burbano, Michael Dannemann and Cesare de Filippo for helpful comments on the manuscript; Michael Dannemann, Martin Kircher, Kay Prüfer, Michael Siebauer and Udo Stenzel for bioinformatics and statistical advice; and Tony Capra and Dennis Kostka for input on data analysis.

Author Contributions
Conceived and designed the experiments: HAB REG SP. Performed the experiments: TM. Analyzed the data: HAB ML. Contributed reagents/materials/analysis tools: HAB REG TM CL-F MöllR AR JK KSP ML SP. Wrote the paper: HAB KSP SP.

References
1. Bird CP, Stranger BE, Liu M, Thomas DJ, Ingle CE, et al. (2007) Fast-evolving noncoding sequences in the human genome. Genome Biol 8: R118.
2. Bush EC, Lahn BT (2008) A genome-wide screen for noncoding elements important in primate evolution. BMC Evol Biol 8: 17.
3. Polliard KS, Salama SR, Lambert N, Lambert MA, Coppena S, et al. (2006) An RNA gene expressed during cortical development evolved rapidly in humans. Nature 443: 167–172.
4. Prabhakar S, Noonan JP, Paabo S, Rubin EM (2006) Accelerated evolution of conserved noncoding sequences in humans. Science 314: 786.
5. Prabhakar S, Visel A, Akiyama JA, Shoukry M, Lewis KD, et al. (2008) Human-specific gain of function in a developmental enhancer. Science 321: 1346–1350.
6. Pollard KS, Salama SR, King B, Kern AD, Dreszer T, et al. (2006) Forces shaping the fastest evolving regions in the human genome. PLoS Genet 2: e160.
7. Pennacchio LA, Altuv N, Moses AM, Prabhakar S, Nohre GA, et al. (2006) In vivo enhancer analysis of human conserved non-coding sequences. Nature 444: 499–502.
8. Galtier N, Durif L (2007) Adaptation or biased gene conversion? Extending the null hypothesis of molecular evolution. Trends Genet 23: 273–277.
9. Duret L, Galtier N (2009) Biased gene conversion and the evolution of mammalian genomic landscapes. Annu Rev Genomics Hum Genet 10: 283–311.
10. Mancera E, Bourgon R, Broozi A, Huber W, Steinmetz LM (2008) High-resolution mapping of meiotic crossovers and non-crossovers in yeast. Nature 454: 479–485.
11. Durif L, Arndt PF (2008) The impact of recombination on nucleotide substitutions in the human genome. PLoS Genet 4: e1000071.
12. Paigen K, Pelcov P (2010) Mammalian recombination hot spots: properties, control and evolution. Nat Rev Genet 11: 221–233.
13. Puk SE, Hinds DA, Koehler K, Nickel B, Patil N, et al. (2005) Fine-scale recombination patterns differ between chimpanzees and humans. Nat Genet 37: 429–434.
14. Winkler W, Myers SR, Richter DJ, Onofrio RC, McDonald GJ, et al. (2005) Comparison of fine-scale recombination rates in humans and chimpanzees. Science 308: 107–111.
15. Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, et al. (2010) A draft sequence of the Neandertal genome. Science 320: 710–722.
16. Reich D, Green RE, Kircher M, Krause J, Patterson N, et al. (2010) Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature 468: 1053–1060.

17. Lander ES, Waterman MS (1988) Genomic mapping by fingerprinting random clones: a mathematical analysis. Genomics 2: 251–239.

18. Briggs AW, Good JM, Green RE, Krause J, Maricic T, et al. (2009) Targeted retrieval and analysis of five Neandertal mtDNA genomes. Science 325: 318–321.

19. Stiller M, Knapp M, Stenzel U, Hofreiter M, Meyer M (2009) Direct multiplex sequencing (DMPS)—a novel method for targeted high-throughput sequencing of ancient and highly degraded DNA. Genome Res 19: 1843–1848.

20. Burbano HA, Hodges E, Green RE, Briggs AW, Krause J, et al. (2010) Targeted investigation of the Neandertal genome by array-based sequence capture. Science 328: 723–725.

21. Hodges E, Rooks M, Xuan Z, Bhattacharjee A, Benjamin Gordon D, et al. (2009) Hybrid selection of discrete genomic intervals on custom-designed microarrays for massively parallel sequencing. Nat Protoc 4: 960–974.

22. De Torres T, Ortiz JE, Eggins H, Valladas H, et al. (2009) Dating of the Hominoid (Homo Neanderthalensis) remains accumulation from el Sidron cave (Piloña, Asturias, North Spain): an example of a multi-methodological approach to the dating of upper Pleistocene sites. Archaeometry.

23. Rosas A, Martinez-Maza C, Bastir M, Garcia-Tahernero A, Lalueza-Fox C, et al. (2006) Paleobiology and comparative morphology of a late Neandertal sample from El Sidron, Asturias, Spain. Proc Natl Acad Sci U S A 103: 19266–19271.

24. Li H, Durbin R (2009) Fast and accurate short read alignment with Burrows-Wheeler transform. Bioinformatics 25: 1754–1760.

25. Fortea J, de la Rasilla M, García-Tahernero A, Gigís E, Rosas A, et al. (2008) Excavation protocol of bone remains for Neandertal DNA analysis in El Sidron Cave (Asturias, Spain). J Hum Evol 55: 353–357.

26. Briggs AW, Stenzel U, Johnson PL, Green RE, Kelso J, et al. (2007) Patterns of damage in genomic DNA sequences from a Neandertal. Proc Natl Acad Sci U S A 104: 14616–14621.

27. Green RE, Malaspina AS, Krause J, Briggs AW, Johnson PL, et al. (2008) A complete Neandertal mitochondrial genome sequence determined by high-throughput sequencing. Cell 134: 416–426.

28. Dittrich S, Kowacs Z, Yuan SH, Stauder G, Kogl M, et al. (2011) TOX3 is a neuronal survival factor that induces transcription depending on the presence of CITED1 or phosphorylated CREB in the transcriptionally active complex. J Cell Sci 124: 232–260.

29. Rohland N, Hofreiter M (2007) Ancient DNA extraction from bones and teeth. Nat Protoc 2: 1756–1762.

30. Hofreiter M, Jaenicke V, Serre D, Haeseler AV, Paabo S (2001) DNA sequences from multiple amplifications reveal artifacts induced by cytosine deamination in ancient DNA. Nucleic Acids Res 29: 4793–4799.

31. Briggs AW, Stenzel U, Meyer M, Krause J, Kircher M, et al. (2009) Removal of deaminated cytosines and detection of in vivo methylation in ancient DNA. Nucleic Acids Res 38: e87.

32. Hodges E, Xuan Z, Balija V, Kramer M, Moïla MN, et al. (2007) Genome-wide in situ exon capture for selective resequencing. Nat Genet 39: 1522–1527.