

1 **Genetic differences between humans and other hominins contribute to the “human condition”**

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9

10 **Abstract**

11 Throughout the past decade, studying ancient genomes provided unique insights into human  
12 prehistory, and differences between modern humans and other branches like Neanderthals can  
13 enrich our understanding of the molecular basis of the human condition. Modern human variation  
14 and the interactions between different hominin lineages are now well studied, making it reasonable  
15 to explore changes that are observed at high frequency in present-day humans, but do not reach  
16 fixation. Here, we put forward interpretation of putative single nucleotide changes in recent modern  
17 human history, focusing on 571 genes with non-synonymous changes at high frequency. We  
18 suggest that molecular mechanisms in cell division and networks affecting cellular features of  
19 neurons were prominently modified by these changes. Complex phenotypes in brain growth  
20 trajectory and cognitive traits are likely influenced by these networks and other changes presented  
21 here. We propose that at least some of these changes contributed to uniquely human traits.

22

23 *Homo sapiens* appears to be a “very special primate”<sup>1</sup>. Our position among animal species stands  
24 out largely thanks to the composite complexity of our cultures, social structures and communication  
25 systems. It seems very reasonable that this “human condition” is rooted, at least in part, in the  
26 properties of our brain, and that these can be traced to changes in the genome on the modern human  
27 lineage. This phenotype in the population called “anatomically modern humans” emerged in Africa  
28 likely before the deepest divergence less than 100,000-200,000 years ago<sup>2,3</sup>, although complex  
29 population structure may reach back up to 300,000 years ago<sup>4-6</sup>. Except of some early dispersals<sup>7</sup>,  
30 humans most likely peopled other parts of the world than Africa and the Middle East permanently  
31 only after around 65,000 years ago. It has been claimed that the brain of modern humans adopted a  
32 specific, apomorphic growth trajectory early in life that gave rise to the skull shape difference  
33 between modern humans and extinct branches of the genus *Homo*<sup>8</sup>, although the timing of this  
34 change is debated<sup>9</sup>. This ontogenic trajectory, termed the “globularization phase”, might have  
35 contributed to our singular cognitive abilities<sup>8,10,11</sup>.

36 We are now in a favorable position to examine the evolution of human biology with the help of  
37 the fossil record, in particular thanks to breakthroughs in paleogenomics: The recent reconstruction  
38 of the genomes of members of archaic *Homo* populations<sup>12-14</sup> has opened the door to new  
39 comparative genomic approaches and molecular analyses. The split of the lineages leading to  
40 modern humans and other archaic forms (Neanderthals and Denisovans) is estimated to around  
41 600,000 years ago<sup>2</sup>, setting the timeframe for truly modern human-specific changes after this split,  
42 but before the divergence of modern human populations (Fig. 1). Together with efforts to explore  
43 present-day human diversity<sup>15</sup>, this progress has allowed to narrow down the number of candidate  
44 point mutations from ~35 million differences since the split from chimpanzee when comparing only  
45 reference genomes<sup>16</sup> to 31,389 fixed human-specific changes in a previous seminal study<sup>1</sup>.

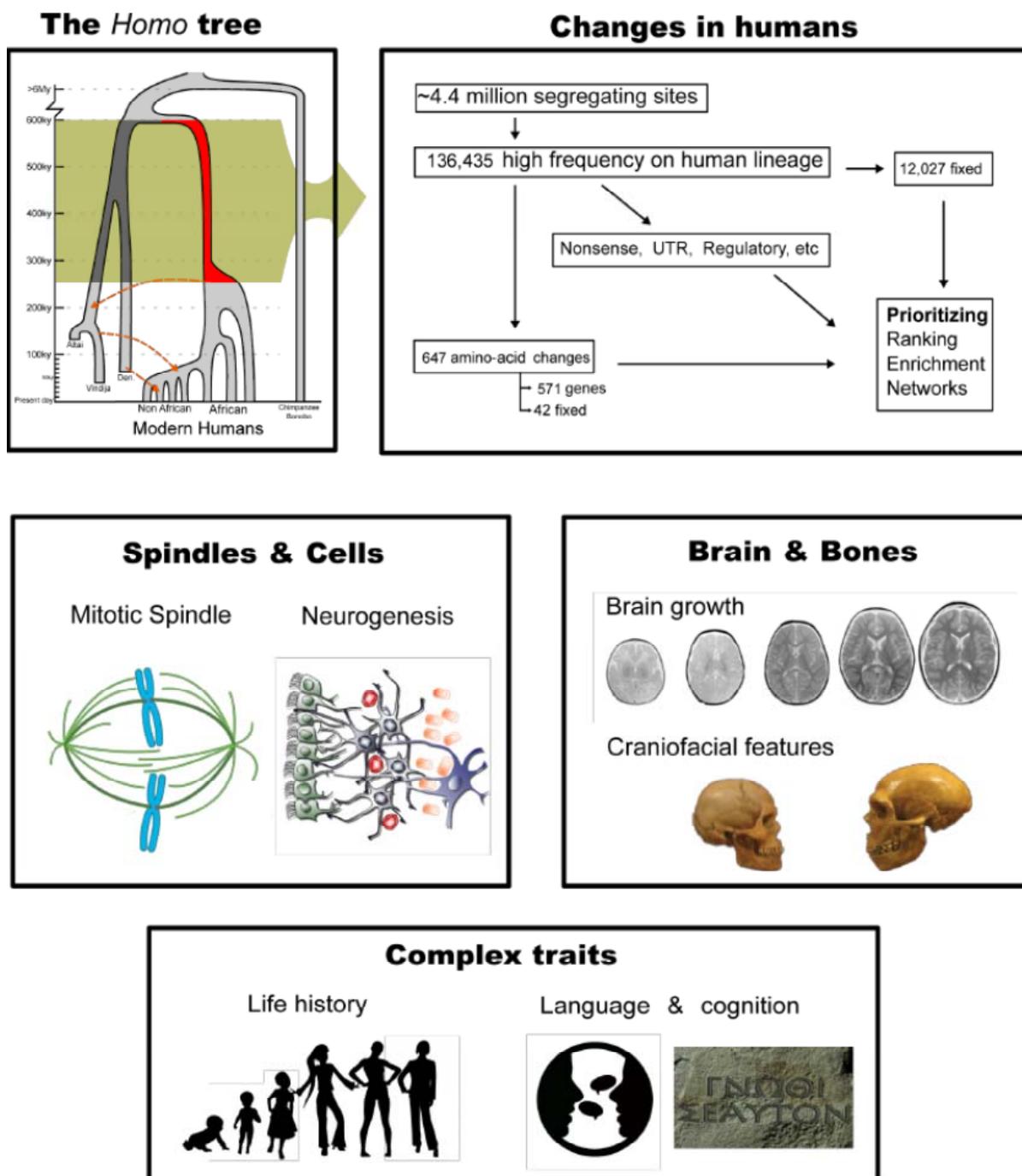
46 Some of these changes have been linked to putative functional consequences<sup>1,13,17</sup>, and evidence  
47 is mounting that several molecular changes affecting gene expression in the brain were subject to  
48 selective pressures<sup>18-22</sup>. Furthermore, the genomic impact of interbreeding events is not evenly  
49 distributed across the genome. Genes expressed in regions of the brain regarded as critical for  
50 certain cognitive functions are depleted in introgressed archaic genetic material<sup>23-26</sup>, and  
51 introgressed alleles are downregulated in some brain regions, suggesting natural selection acting on  
52 tissue-specific gene regulation<sup>27</sup>. Thus, it seems reasonable to conclude that there were differences  
53 between anatomically modern human and Neanderthal brains, and that these underlie at least some  
54 of the characteristics of our lineage<sup>28</sup>. We want to emphasize that such recent differences are likely  
55 to be subtle when compared to those after the split from our closest living relatives on a scale of 6-  
56 10 million years<sup>29</sup>, where fundamental changes arose since the divergence from chimpanzees and  
57 bonobos<sup>30</sup>. The observation of recurrent gene flow between modern human and archaic populations  
58 also implies a broad overall similarity, yet, such subtle differences may still have contributed to the  
59 evolutionary outcome<sup>31</sup>. Obviously, not all human-specific changes are beneficial: While most  
60 mutations may be rather neutral and have little effect on the phenotype, some may have had  
61 deleterious effects or side-effects, possibly increasing the risks for neurodevelopmental or  
62 neurodegenerative disorders in humans<sup>32-34</sup>.

63 The goal of this paper is to provide a set of recent single nucleotide changes in humans since  
64 their split from Neanderthals that could enrich our understanding of the molecular basis of the  
65 recent human condition. The previous focus on fixed alleles was reasonable given limited data<sup>1</sup>, but  
66 having a better grasp of the magnitude of modern human variation and the interaction between  
67 different hominin lineages seems a good reason to cast a wider net, and take into account not only  
68 fixed differences but also high-frequency changes shared by more than 90% of present-day

69 individuals. Here, we present a revised list of 36 genes that carry missense substitutions which are  
70 fixed across 1,000s of human individuals and for which all archaic hominin individuals sequenced  
71 so far carry the ancestral state. In total, 647 protein-altering changes in 571 genes reached a  
72 frequency of at least 90% in the present-day human population. We attempt to interpret this list, as  
73 well as some regulatory changes, since it seems very likely that some of these genes would have  
74 contributed to the human condition.

75 We will discuss some of their known functions, and how these relate to pathways that might have  
76 been modified during human evolution (Fig. 1), in a bottom-up fashion. Beginning at the molecular  
77 level, changes found in genes associated to the mitotic spindle complex might be relevant, as has  
78 been suggested in previous studies<sup>1,13</sup>. The cellular features of neurons (axons and synapses) have  
79 been considered important in light of their role in traits such as vocal learning<sup>35-37</sup>, and possibly  
80 behavioral phenotypes<sup>38</sup>. Pathways influencing brain organization were modified during hominin  
81 evolution, and we suggest that this might have been extended further since the split from  
82 Neanderthals and other archaics<sup>39</sup>. Finally, we discuss implications for other complex phenotypic  
83 traits, with a focus on cognition and life history trajectory. We restrict our attention to genes where  
84 the literature may allow firm conclusions and predictions about functional effects, since many genes  
85 might have multiple different functions<sup>40</sup>. Obviously, experimental validation will be ultimately  
86 needed to confirm our hypotheses concerning alterations in specific functions.

87



88  
89 **Figure 1:** Conceptual summary of this study.  
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91

92 **Results**  
93

94 **Genetic differences between present-day humans and archaic hominins**

95 Using publicly available data on one Denisovan and two Neanderthal individuals and present-  
96 day human variation (Methods), we calculated the numbers of single nucleotide changes (SNCs)  
97 which most likely arose recently on the respective lineages after their split from each other, and  
98 functional consequences as predicted by VEP (Table 1). Previously, a number of 31,389 sites has  
99 been reported as recently fixed derived in present-day humans, while being ancestral in archaics<sup>1,13</sup>.

100 We find a smaller number of only 12,027 positions in the genome, in part caused by including  
101 another archaic individual and different filters, but mainly by a richer picture of present-day human  
102 variation. The 1,000 Genomes Project as well as other sources contributing to the dbSNP database  
103 now provide data for thousands of individuals, which results in very high allele frequencies for  
104 many loci instead of fixation. Indeed, 29,358 positions show allele frequencies larger than 0.995,  
105 demonstrating that the level of near-fixation is similar to the level of previously presented fixation.  
106 The number of loci with high frequency (HF) changes of more than 90% in present-day humans is  
107 an order of magnitude larger than the number of fixed differences. The three archaic individuals  
108 carry more than twice as many changes than present-day humans; however, we emphasize that  
109 much of this difference is not due to more mutations in archaics, but rather the fact that data for  
110 only three individuals is available, compared to thousands of humans. The variation across the  
111 archaic population is not represented equally well, which makes these numbers not directly  
112 comparable.

113 Present-day humans carry 42 fixed amino acid-changes in 36 genes (Table 2, Fig. 2), while  
114 Neanderthals carry 159 such changes. Additionally, modern humans carry 605 amino acid-changes  
115 at high frequency (human-lineage high-frequency missense changes, referred to as HHMCs),  
116 amounting to a total of 647 such changes in 571 genes (Table S1). Together with 323 SNCs on the  
117 human lineage with low confidence (Methods, Table S2), almost 1,000 putative protein-altering  
118 changes were found across most present-day humans. Generally, synonymous changes are found at  
119 a similar magnitude as missense changes, but only few SNCs altering start and stop codons, and  
120 thousands of changes in putative regulatory and untranslated regions. We admit that some of the  
121 loci presented here are variable across the phylogenetic tree, or less reliable due to low coverage in  
122 the archaics, but we accept this since our intention is retrieve an inclusive picture of possibly  
123 functional recent changes. The 42 protein-altering changes for which the ancestral allele has not  
124 been observed in any present-day human, most of which have been presented before<sup>1</sup>, constitute  
125 without doubt the strongest candidates for understanding the human condition. Only one gene,  
126 *SPAG5*, carries three such SNCs, and four genes (*ADAM18*, *CASC5*, *SSH2* and *ZNHIT2*) carry two  
127 fixed protein-coding changes in all modern humans. We identified 15 SNCs (in *AHR*, *BOD1L1*,  
128 *C1orf159*, *C3*, *DNHD1*, *DNMT3L*, *FRMD8*, *OTUD5*, *PROM2*, *SHROOM4*, *SIX5*, *SSH2*, *TBC1D3*,  
129 *ZNF106*, *ZNHIT2*) that have not been previously described as fixed differences between humans  
130 and archaics. We note that another 12 previously described<sup>1</sup> protein-altering substitutions were not  
131 found among the genotypes analyzed here (in *C21orf62*, *DHX29*, *FAM149B1*, *FRRS1L*, *GPT*, *GSR*,  
132 *HERC5*, *IFI44L*, *KLF14*, *PLAC1L*, *PTCD2*, *SCAF11*). These genotype calls are absent from the  
133 files provided for the three archaic genomes due to different genotype calling and filtering  
134 procedures compared to the original publication of the Altai Neanderthal genome<sup>13,14</sup>. Hence, some  
135 potentially relevant candidate changes were not included here, and future research is necessary to  
136 evaluate these as well. Despite attempting an extended interpretation, our data is thus still not fully  
137 exhaustive.

138 It is noteworthy that the number of fixed SNCs decreased substantially, and it is possible that  
139 single individuals will be found to carry some of the ancestral alleles. Hence, it is important to focus  
140 not only on fixed differences, but also consider variants at high frequency. When analyzing the 647  
141 HHMCs, 68 genes carry more than one amino acid-altering change. Among these, *TSGA10IP*  
142 (Testis Specific 10 Interacting Protein) and *ABCC12* (ATP Binding Cassette Subfamily C Member  
143 12) carry four such changes, and seven more genes (*MUC5B*, *NPAP1*, *OR10AG1*, *OR5M9*, *PIGZ*,  
144 *SLX4*, *VCAN*) carry three HHMCs. 1,542 genes carry at least one HF missense change on the  
145 archaic lineage (archaic-lineage high-frequency missense change, referred to as AHMC, Tables S3,

146 S4). We find an overlap of 122 genes with HHMCs and AHMCs, which is more than expected  
 147 considering that among 1,000 sets of random genes of a similar length distribution, no overlap of  
 148 this extent was observed. The same genes seem to have acquired missense changes on both lineages  
 149 since their divergence more often than expected. We find a high ratio of HHMCs over synonymous  
 150 changes for chromosome 21 (1.75-fold), and a very small ratio (0.18-fold) for chromosome 13. We  
 151 do not find such extreme ratios for AHMCs and corresponding synonymous changes, suggesting  
 152 differences in the distribution of amino acid changes between both lineages (Fig. S1).  
 153

	Fixed human	HF human	Extended human	Fixed archaic	HF archaic	Extended archaic
<b>All</b>	12,027	136,435	83,254	33,498	380,756	983
<b>Non-synonymous</b>	42	647	327	167	1,921	13
<b>Synonymous</b>	41	843	363	193	2,123	14
<b>Start/stop</b>	1	14	10	3	48	2
<b>Splice site</b>	4	23	8	4	54	0
<b>TFBS</b>	28	226	126	87	914	1
<b>Upstream</b>	1,935	19,599	11,235	4,920	55,188	289
<b>5' UTR</b>	180	1,853	1,012	195	2,016	7
<b>3' UTR</b>	77	702	334	506	5,303	19
<b>Downstream</b>	1,922	19,704	11,673	4,956	55,832	281
<b>miRNA</b>	0	1	2	0	4	0
<b>Regulatory element</b>	1,952	20,971	12,320	5,125	59,248	195

154 **Table 1:** Summary of single nucleotide changes. TFBS: Transcription factor binding sites. UTR:  
 155 Untranslated Region. HF: High frequency. Fixed changes are a subset of HF changes.

156

### 157 **Ranking and enrichment**

158 We assessed the impact of mutations for different deleteriousness scores (Table 2), finding 12  
 159 genes with deleterious HHMCs according to SIFT, three according to PolyPhen, and 16 when using  
 160 the Grantham score (>180), measuring the physical properties of amino acid changes. The C-score  
 161 and GWAVA can be used to rank all mutation classes, and we present the top candidates.

162 Then, we attempted a ranking of genes by the density of lineage-specific changes in the dataset.  
 163 As expected, the total number of segregating sites is correlated with gene length (Pearsons'  $R =$   
 164  $0.93$ ). This correlation is weaker for HF human SNCs ( $R = 0.73$ ) and fixed human-specific SNCs ( $R$   
 165  $= 0.25$ ), as well as for fixed ( $R = 0.37$ ) and HF ( $R = 0.82$ ) SNCs in archaics. We conclude that some  
 166 genes with a large number of human-specific changes might carry these large numbers by chance,  
 167 while others are depleted. Indeed, 17,453 (88.9%) of these genes do not carry any fixed human-  
 168 specific change, and 80.5% do not carry fixed archaic-specific changes. Of note, genes that have  
 169 attracted attention in the context of traits related to the "human condition" like *CNTNAP2* and  
 170 *AUTS2* are among the longest genes in the genome, hence changes in these genes should be  
 171 interpreted with caution as they are not unexpected. We ranked the genes by the number of HF  
 172 changes in either modern humans or archaics, divided by their genomic lengths, and categorize the  
 173 top 5% of this distribution as putatively enriched for changes on each lineage (Table S5). We note  
 174 that 191 genes (30.9%) fall within this category for both human HF changes and archaic HF  
 175 changes, as a result of differences in mutation density. In order to distinguish a truly lineage-  
 176 specific enrichment, we calculated the ratios of HF changes for humans and archaics, defining the

177 top 10% of genes in this distribution as putatively enriched (Table S5). Among the genes enriched  
178 for changes on the modern human lineage, 18 carry no HF changes on the archaic lineage, and ten  
179 of these also fall within the 5% of genes carrying many changes considering their length (*ARSL*,  
180 *CLUAP1*, *COL20A1*, *EPPIN*, *KLHL31*, *MKNK1*, *PALMD*, *RIC3*, *TDRD7*, *UBE2H*). These might be  
181 candidates for an accumulation of changes, even though this is not identical to selective sweep  
182 signals. Among these, the collagen *COL20A1* and the Epididymal Peptidase Inhibitor *EPPIN* carry  
183 HHMCs. *ACAD10*, *DST* and *TTC40*, which carry two HHMCs, might be other notable genes with a  
184 human-specific enrichment.

185 No Gene Ontology (GO) categories are enriched for HHMCs on the human lineage when using  
186 HF synonymous changes as background in a hypergeometric test. This is also true for genes  
187 carrying AHMCs, or HF changes in UTRs or transcription factor binding sites on either lineage. We  
188 applied a test for the ratio of the number of gene-wise HF changes on one lineage over the other  
189 lineage. For changes on the modern human lineage, this yields an enrichment for 12 GO categories  
190 (Table S6), with “soft palate development”, “negative regulation of adenylate cyclase activity”,  
191 “collagen catabolic process” and “cell adhesion” in the biological process category. Among the  
192 cellular components category, the “postsynaptic membrane”, “spermatoproteasome complex”,  
193 “collagen trimer”, “dendrite” and “cell junction” show enrichment, as well as the molecular  
194 functions “calcium ion binding”, “histone methyltransferase activity (H3-K27 specific)” and  
195 “metallopeptidase activity”. We find no GO enrichment for genes with an excess of changes on the  
196 archaic lineage. In order to approach a deeper exploration of genes with associated complex traits in  
197 humans, we explored the NHGRI-EBI GWAS Catalog<sup>41</sup>, containing 2,385 traits. We performed a  
198 systematic enrichment screen, finding 17 unique traits enriched for genes with HHMCs, and 11 for  
199 genes with AHMCs (Table S7). Changes in genes associated to “Cognitive decline (age-related)”,  
200 “Rheumatoid arthritis” or “Major depressive disorder” might point to pathways that could have  
201 been influenced by protein-coding changes on the human lineage. In archaics, genes are enriched,  
202 among others, for associations to traits related to body mass index or cholesterol levels, which  
203 might reflect differences in their physiology. We also find an enrichment of genes associated to  
204 behavioral disorders on the archaic lineage.

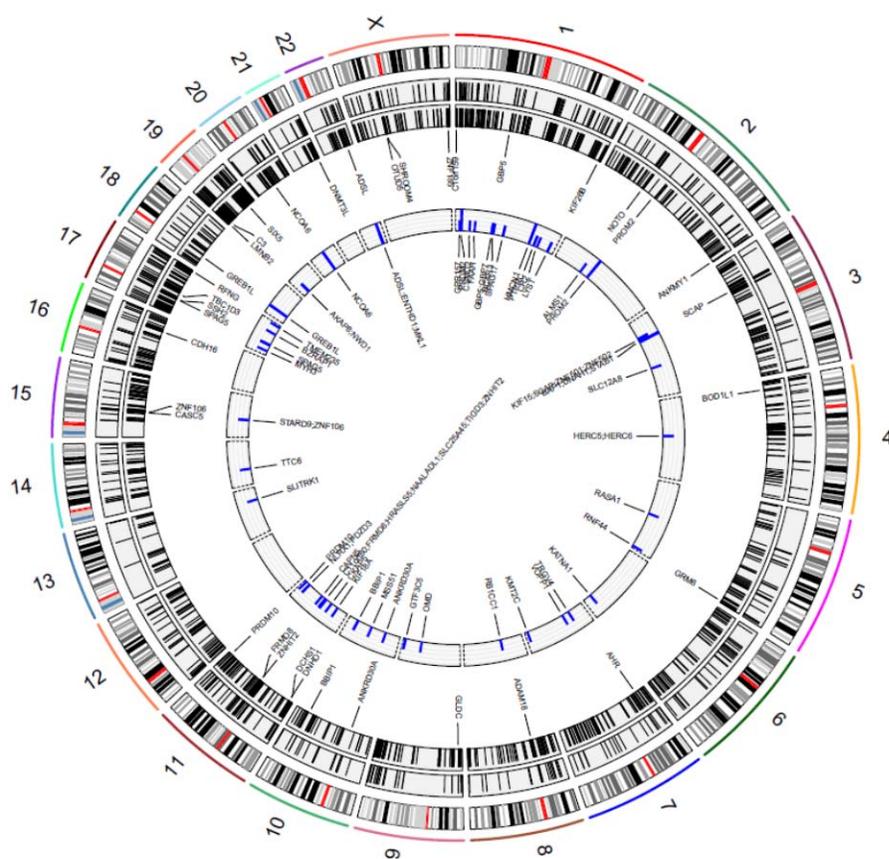
205 We find a significant enrichment of protein-protein interactions ( $P = 0.006$ ) among the gene  
206 products of HHMC genes (Fig. S2), meaning that these proteins interact with each other more than  
207 expected. Functional enrichment is found for the biological process “cellular component assembly  
208 involved in morphogenesis”, most strongly for the cellular components cytoskeleton and  
209 microtubule, as well as the molecular function “cytoskeletal protein binding”. Three proteins have  
210 at least 20 interactions in this network and might be considered important nodes: *TOP2A*, *PRDM10*  
211 and *AVPR2* (Table S8). However, proteins encoded by genes with synonymous changes on the  
212 modern human lineage seem to be enriched for interactions as well ( $P = 0.003$ ), as are proteins  
213 encoded by genes with AHMCs ( $P = 1.68 \times 10^{-14}$ ), with an enrichment in GO categories related to  
214 the extracellular matrix and the cytoskeleton, and the most interacting proteins with more than 40  
215 interactions being *GART*, *LRGUK*, *ARRB1*, *SPTAN1* and *ATM* (Table S8). We caution that these  
216 networks might be biased due to more mutations and possibly more interactions in longer, multi-  
217 domain genes.

218 Regulatory changes might have been important during our evolution<sup>42</sup>, hence we tested for an  
219 overrepresentation of transcription factors (TFs). We find 78 known or putative TFs among the  
220 HHMC genes (Table S9) on the modern human lineage<sup>43</sup>, which is not overrepresented among  
221 genes with HHMCs (with 49.2% of random genes sets containing fewer HHMCs). Despite lack of  
222 enrichment, single TFs on the modern human lineage might have been important, particularly those

223 with an excess of modern human over archaic HF changes (*AHR*, *MACC1*, *PRDM2*, *TCF3*,  
 224 *ZNF420*, *ZNF516*). Others TFs, like *RB1CC1*<sup>13</sup> or *PRDM10* and *NCOA6*<sup>19</sup> have been found in  
 225 selective sweep screens, suggesting contributions of individual TFs, rather than TFs as a class. We  
 226 also tested for an enrichment of gene expression in different brain regions and developmental  
 227 stages<sup>44,45</sup>, using the HF synonymous changes on each lineage as background sets. We find an  
 228 enrichment of gene expression in the orbital frontal cortex at infant age (0-2 years) for genes with  
 229 HHMCs, but no enrichment for genes with AHMCs. Furthermore, when testing the genes with  
 230 HHMCs and using the set of genes with AHMCs as background, “gray matter of forebrain” at  
 231 adolescent age (12-19 years) is enriched, while no enrichment was found for genes with AHMCs.  
 232

<b>Fixed HHMCs</b>	<i>ADAM18, ADSL, AHR, ANKMY1, ANKRD30A, BBIP1, BOD1L1, C1orf159, C3, CASC5, CDH16, DCHS1, DNHD1, DNMT3L, FRMD8, GBP5, GLDC, GREB1L, GRM6, KIF26B, LMNB2, NCOA6, NOTO, OTUD5, PRDM10, PROM2, RFNG, SCAP, SHROOM4, SIX5, SPAG5, SSH2, TBC1D3, ZNF106, ZNF185, ZNHIT2</i>
<b>Selection 2014</b>	<i>C11orf80, CKAP5, GREB1L, HMCN1, NLRX1, PDZD3, PRDM2, RB1CC1</i>
<b>Selection 2015</b>	<i>MSS51, NCOA6, OMD, SPAG17, SPAG5</i>
<b>Selection 2016</b>	<i>ACE, ADSL, ALMS1, ANKRD30A, BZRAP1, DNAH1, GREB1L, KMT2C, NWD1, PROM2, RASA1, STAB1, STARD9, ZNF106</i>
<b>Selection 2017</b>	<i>ADSL, AKAP8, BAP1, BBIP1, BCAR3, CAPN5, CR2, CSMD2, DNAH1, ENTHD1, FAAH, FRMD8, GBP5, GBP7, GPR157, GTF3C5, HERC5, HERC6, HMCN1, HRASLS5, KATNA1, KIF15, KIF18A, LYST, MKL1, MYH3, NAALADL1, NCOA6, PRDM10, PRDM2, PROM2, PTPRC, RNF44, SCAP, SLC12A8, SLC25A45, SLITRK1, TIGD3, TMEM235, TRGV4, TTC6, VOPPI, ZNF501, ZNF502, ZNHIT2</i>
<b>Grantham</b>	<i>ABHD14A-ACY1, ACY1, ABHD14A, CCDC158, CCDC30, DNHD1, EML2, ERI1, GBA3, GREB1, OR1K1, TTC6, UBQLN3, UIMC1, ZBP1, ZNF510, ZNHIT2</i>
<b>SIFT</b>	<i>BEND2, CCT6B, COPA, CUL4B, GBP7, KRTAP10-10, MEPE, NHEJ1, OR1K1, SLC6A15, TPO, ZNF510</i>
<b>PolyPhen-2</b>	<i>FSHR, NLN, TPO</i>
<b>CADD</b>	<i>C11orf80, C5orf66, CCT6B, CDH15, CEP128, CPM, FGF21, FMN2, FUT1, H2AFY, HERC6, KCNK5, KPNA4, KRT33A, KRT8P12, MUM1, NR1H2, OPRM1, PDSS2, ROCK1, RPS15P9, SLC22A31, SUCLG2P4, TMPRSS7, UNC5D</i>
<b>GWAVA</b>	<i>ANK2, COPA, CTRC, CYP2B6, MAPK10, MCTP1, SLC38A6, SYT1, YTHDC1</i>

233 **Table 2:** Genes with fixed non-synonymous changes on the human lineage, genes under positive  
 234 selection with HHMCs, and deleterious candidate HHMCs. Selection 2014: Prüfer *et al.*, 2014.  
 235 Selection 2015: Zhou *et al.*, 2015. Selection 2016: Racimo, 2016. Selection 2017: Peyrégne *et al.*,  
 236 2017.  
 237



238  
 239 **Figure 2:** Features discussed in this study. From inside to outside: Genes with HHMCs and  
 240 signatures of positive selection (compare Table 2), genes with fixed non-synonymous SNPs on the  
 241 human lineage, HHMCs, AHMCs, karyogram of human chromosomes.

242  
 243

## 244 Discussion

245  
 246

### 247 The kinetochore and spindle complex

248 It has been proposed previously that protein-coding changes in cell cycle-related genes are highly  
 249 relevant candidates for human-specific traits<sup>1,13</sup>. Indeed, three genes (*CASC5*, *SPAG5*, and *KIF18A*)  
 250 have been singled out as involved in spindle pole assembly during mitosis<sup>1</sup>. Other genes with  
 251 protein-coding SNPs (*NEK6* and *STARD9/KIF16A*) turn out to be implicated in the regulation of  
 252 spindle pole assembly as well<sup>46,47</sup>. Furthermore, it has been claimed<sup>13</sup> that genes with fixed non-  
 253 synonymous changes in humans are also more often expressed in the ventricular zone of the  
 254 developing neocortex, compared to fixed synonymous changes. Since the kinetochore-associated  
 255 genes *CASC5*, *KIF18A* and *SPAG5* are among these genes, it has been emphasized that this “may be  
 256 relevant phenotypically as the orientation of the mitotic cleavage plane in neural precursor cells  
 257 during cortex development is thought to influence the fate of the daughter cells and the number of  
 258 neurons generated<sup>48,13</sup>. Several fixed SNPs on the modern human lineage are observed for *CASC5*  
 (two changes) and *SPAG5* (three changes), which is also among genes with a relatively high

259 proportion of HF changes (Table S5). The changes in *KIF18A*, *KIF16A* and *NEK6* can no longer be  
260 considered as fixed, but occur at very high frequencies (>99.9%) in present-day humans.

261 We attempted to determine whether an enrichment of genes with HHMCs on the human lineage  
262 can be observed in the ventricular zone for the same data<sup>45</sup>, but instead find an enrichment in the  
263 intermediate zone, where less than 5% of random gene sets of the same size are expressed.  
264 However, synonymous HF changes also show an enrichment in this layer, as well as genes with  
265 AHMCs (Table S10), suggesting an overrepresentation of genes that carry mutations in the coding  
266 regions rather than lineage-specific effects. However, we were able to broadly recapitulate the  
267 observation of an enrichment of expression in the ventricular zone if restricting the test to genes  
268 with non-synonymous changes at a frequency greater than 99.9% in present-day humans, which is  
269 not observed for corresponding synonymous and archaic non-synonymous changes (Table S10).  
270 Expression of genes with AHMCs is enriched in the intermediate zone. Among the 28 genes  
271 expressed in the ventricular zone that carry almost fixed HHMCs, four might be enriched for HF  
272 changes in humans (*HERC5*, *LMNB2*, *SPAG5*, *VCAMI*), and one shows an excess of HF changes  
273 on the human compared to the archaic lineage (*AMKMY1*). Other notable genes discussed in this  
274 study include *ADSL*, *FAM178A*, *KIF26B*, *SLC38A10*, and *SPAG17*. We find 126 genes (Table S9)  
275 with 143 HHMCs that putatively interact with proteins at the centrosome-cilium interface<sup>49</sup>, which  
276 is more than expected using 1,000 random gene sets of a similar length distribution, for which  
277 98.9% contain fewer genes with HHMCs. However, 99.9% of random sets also contain fewer genes  
278 with AHMCs, suggesting that differences between humans and archaics might lie in the particular  
279 genes rather than their numbers. The centrosome-cilium interface is known to be critical for early  
280 brain development, and centrosome-related proteins are overrepresented in studies on the  
281 microcephaly phenotype in humans<sup>50</sup>, which we will discuss below. Some of the genes listed here  
282 and discussed elsewhere in this study, such as *FMRI*, *KIF15*, *LMNB2*, *NCOA6*, *RB1CC1*, *SPAG5*  
283 and *TEX2*, harbor not only HHMCs, but an overall high proportion of HF changes on the human  
284 lineage.

285 Among the 15 fixed protein-coding changes identified here but absent from previous analyses<sup>1,13</sup>,  
286 some might also contribute to complex modifications of pathways in cell division: The AHR  
287 protein is involved in cell cycle regulation<sup>51</sup> and shows an excess of HF changes on the human  
288 lineage, the dynein DNHD1 might be recruited to the kinetochore<sup>52</sup> and is overexpressed in fetal  
289 brain<sup>53</sup>, and the SSH2 protein (two fixed changes, one of which is first described here; and one on  
290 the archaic lineage) might interact with spindle assembly checkpoint proteins<sup>54</sup>. SHROOM4, which  
291 is associated to a mental retardation syndrome with delayed speech and aggressive behavior<sup>55</sup>, may  
292 also be relevant<sup>56</sup>. Other proteins that carry two HHMCs are involved in mitosis, for example the  
293 spindle checkpoint regulator CHEK1<sup>57</sup>, the Dynein Axonemal Heavy Chain 1 (encoded by  
294 *DNAH1*), the mitotic regulator AZI1 (*CEP131*)<sup>58</sup>, the Cyclin D2 (*CCND2*) and the Protein Tyrosine  
295 Phosphatase Receptor Type C (*PTPRC*)<sup>59</sup>. Other genes with HHMCs that could be part of the same  
296 functional network are *FOXMI*<sup>60</sup> and *FMRI*<sup>61</sup>, which carry a putative enrichment of HF changes,  
297 and *TOP2A*<sup>62</sup>. The TOP2A protein shows the largest number of interactions (53) with other HHMC-  
298 carrying proteins, while CHEK1, KIF18A, KIF15 and PTPRC are among highly-interacting  
299 proteins with more than ten interactions, which suggests that these proteins might function as  
300 interaction hubs in modifications of the cell division complex. Furthermore, enrichment in cell-  
301 cycle related GO categories has been found for candidate regions for ancient positive selection<sup>20</sup>,  
302 and *ANAPC10* has been highlighted, containing two potentially disruptive intronic changes that are  
303 fixed derived in modern humans and ancestral in both Neanderthals and Denisovans. This gene

304 carries a total of 39 HF changes (11 of them fixed) specific to modern humans, but none for  
305 archaics.

306 All of this suggests that the cell cycle machinery might have been modified in a specific way in  
307 humans compared to other hominins. One particular example of specific consequences for a  
308 relevant SNC on the human lineage is *SPAG5*: One of the three fixed non-synonymous changes in  
309 the *SPAG5* protein is a Proline-to-Serine substitution at position 43. This position is phosphorylated  
310 in humans<sup>63</sup> during the mitotic phase of the cell cycle, directly through the protein phosphatase 6  
311 (*PPP6C*) at the Serine at this position<sup>64</sup>, with the effect of a modification of the duration of the  
312 metaphase. *PPP6C* regulates the mitotic spindle formation<sup>65</sup>, and the *PPP6C* gene itself carries five  
313 HF SNCs on the modern human lineage, one of which is a TF binding site (for HNF4A/HNF4G),  
314 and only one SNC on the archaic lineage. This specific substitution in *SPAG5* seems likely to  
315 influence the duration of the metaphase through phosphorylation, as a molecular consequence of  
316 this HHMC.

317 On the archaic lineage, we find an AHMC in the *ASPM* gene, along with 24 other HF changes,  
318 but none in modern humans, resulting in an excess of archaic SNCs. The proteins *ASPM* and *CIT*,  
319 which carries an AHMC that is listed among the most disruptive non-synonymous derived SNCs in  
320 archaics<sup>17</sup> (Table S31), are known to co-localize to the midbody ring during cytokinesis and  
321 regulate spindle orientation by affecting the dynamics of astral microtubules<sup>66</sup>. These proteins  
322 regulate astral microtubules and thus the orientation of cell division in archaics, whereas in modern  
323 humans we find proteins regulating kinetochore microtubules, thus the timing of cell division. This  
324 difference could indicate two alternative ways of modulating cell division on the different lineages.  
325

### 326 **Cellular features of neurons: Axons, the myelin sheath and synapses**

327 Moving on from differences on the molecular level to cellular features of neurons, it is clear that  
328 wiring is key to cognitive ability, and in this context, axon guidance is relevant: To form critical  
329 networks during the early development of the brain, axonal extensions of the neurons in the cortical  
330 region must be sent and guided to eventually reach their synaptic targets. Studies conducted on  
331 avian vocal learners<sup>37,67</sup> have shown a convergent differential regulation of axon guidance genes of  
332 the *SLIT-ROBO* families in the pallial motor nucleus of the learning species, allowing for the  
333 formation of connections virtually absent in the brains of vocal non-learners. In modern humans,  
334 genes with axon-guidance-related functions such as *FOXP2*, *SLIT2* and *ROBO2* have been found to  
335 lie within deserts of archaic introgression<sup>25,26,68</sup>, suggesting incompatibilities between modern  
336 humans and archaics for these regions. *SLITRK1* might have been under positive selection<sup>19</sup>, and  
337 carries one HHMC, and *SLIT3* carries an excess of HF changes in modern humans. Several genes  
338 involved in wiring carry HHMCs, which we want to delineate here.

339 Some of the aforementioned microtubule-related genes, specifically those associated with axonal  
340 transport and known to play a role in post-mitotic neural wiring and plasticity<sup>69</sup>, are associated with  
341 signals of positive selection, such as *KIF18A*<sup>70</sup> or *KATNAI1*<sup>71,72</sup>. Furthermore, an interactor of  
342 *KIF18A*, *KIF15*<sup>73</sup>, might have been under positive selection in modern humans<sup>19</sup>, and contains two  
343 HHMCs (but also five AHMCs). Versican (*VCAN*), which promotes neurite outgrowth<sup>74</sup>, carries  
344 three HHMCs, and *SSH2* (two HHMCs) might be involved in neurite outgrowth<sup>75</sup>. *PIEZO1*, which  
345 carries a non-synonymous change that is almost fixed in modern humans, is another factor in axon  
346 guidance<sup>76</sup>, as well as *NOVA1*<sup>77</sup>, which is an interactor of *ELAVL4*<sup>78</sup>, a gene that codes for a  
347 neuronal-specific RNA-binding protein and might have been under positive selection in  
348 humans<sup>19,22</sup>. Furthermore, among genes with the most deleterious regulatory SNCs, we find the  
349 Netrin receptor *UNC5D*, which is critical for axon guidance<sup>79</sup>.

350 The establishment of new connections requires protection, particularly as some of these  
351 connections reach long distance and are associated with enhanced activity following rewiring  
352 events, like for vocal motor neurons in songbirds<sup>67</sup>. The gene *MAL*, which is implicated in myelin  
353 biogenesis and function, shows up in selective sweep regions<sup>19,20</sup> and is enriched for HF changes on  
354 the human lineage, while its orthologue *MAL2* carries a HHMC. A gene with HHMCs that is  
355 associated with the organization of the axon initial segment and nodes of Ranvier during early  
356 development is *NFASC*<sup>80</sup>. The protein encoded by this gene is a L1 family immunoglobulin cell  
357 adhesion molecule, and we find that also the *LICAM* gene carries an AHMC<sup>81</sup>. *NFASC* is also an  
358 interactor of *DCX*<sup>82</sup>, which might have been under positive selection in humans<sup>19</sup> and is enriched  
359 for HF SNCs on the human lineage, but carries an AHMC as well. At least two genes associated  
360 with the process and timing of myelination, *PTEN*<sup>83</sup>, and *NCMAP*<sup>84</sup> are among genes with an excess  
361 of HF SNCs in modern humans. Other genes carrying HHMCs in our dataset associated with  
362 myelination include *SCAP*<sup>85</sup>, *RBICCI*<sup>86</sup>, *TENM4*<sup>87</sup>, *CDKLI*<sup>88</sup> and *ADSL*<sup>89</sup>, and genes with an  
363 excess of changes on the human lineage with similar functions include *FBXW7*<sup>90</sup>, *KIFAP3*<sup>91</sup>, and  
364 *AMPH*<sup>92</sup>. The *AMPH* protein interacts closely with the huntingtin protein *HTT* (which also carries a  
365 HHMC) and is involved in myelination processes<sup>93</sup>.

366 Another interesting class that emerges from the set of genes is related to synaptic vesicle  
367 endocytosis, critical to sustain a high rate of synaptic transmission. We find a formal enrichment of  
368 genes with an excess of HF changes on the human compared to the archaic lineage with gene  
369 products located in the postsynaptic membrane and dendrites. *PACSINI*<sup>94</sup> carries a HHMC, is  
370 among genes with an excess of HF changes, and has been highlighted as putatively under positive  
371 selection on the human lineage, along with other synaptic plasticity related genes such as  
372 *SIPA1L1*<sup>19,20,22</sup>, *SH3GL2*<sup>95</sup> and *STXIA*<sup>96</sup>. Among genes harboring HHMCs and related to synaptic  
373 vesicle endocytosis, we find *LMNB2*<sup>97</sup> and *SV2C*<sup>98</sup>. Finally, *SYTI*, which is critical for synaptic  
374 vesicle formation<sup>99</sup>, carries a deleterious HHMC (Table 2). Synaptic properties have been  
375 mentioned before in the context of human specific traits, for instance in postnatal brain  
376 development in humans, chimpanzees and macaques<sup>100</sup>, with a focus on synaptogenesis and  
377 synaptic elimination in the prefrontal cortex. A period of high synaptic plasticity in humans has  
378 been related to a cluster of genes around a transcription factor encoded by the *MEF2A* gene. Even  
379 though this gene neither carries a protein-altering change nor shows a particular pattern in our  
380 analysis, any of the 26 HF SNCs it harbors on the modern human lineage could have had a  
381 functional impact not captured here. Apart from that, several of the genes with an excess of HF  
382 changes in modern humans do belong to this cluster: *CLSTN1*, *FBXW7*, *GABBR2*, *NRXN3*, *PTPRJ*,  
383 *PTPRN2*, *SLIT3*, and *STXIA*, three of which (*CLSTN1*, *FBXW7* and *STXIA*) are associated with  
384 signals of positive selection<sup>19</sup>. In addition, the above-mentioned *AMPH* interacts via *CDKL5*<sup>101</sup>  
385 with *HDAC4*<sup>102</sup>. The latter exhibits an excess of HF changes in modern humans, and is known to  
386 repress the transcriptional activation of *MEF2A*<sup>103</sup>. A putative signature of positive selection  
387 upstream of *MEF2A*<sup>104</sup> suggests that this may be part of a broader network which might be  
388 supported by our analysis. Finally, *ENTHDI/CACNAII*, which contains a HHMC that can no longer  
389 be considered as fixed, but occurs at a very high frequency (>99.9%), lies in a selective sweep  
390 region<sup>19</sup>. The protein encoded by this gene is involved in synaptic vesicle endocytosis at nerve  
391 terminals<sup>105</sup> and is regulated by the *MEF2* gene family<sup>106</sup>.

392

### 393 **The brain growth trajectory**

394 The number of neurons in the brain might be influenced by some of the changes in kinetochore-  
395 associated genes<sup>13</sup>, and their organization and neuronal wiring clearly impose structural demands on

396 the organization of the brain. We have presented candidate genes and networks for these features  
397 above, but brain organization might involve broader networks. Brain growth factors identified by  
398 disease phenotypes in modern humans such as micro- and macrocephaly have been highlighted  
399 previously as potentially relevant for physiological differences between humans and archaics<sup>18</sup>.  
400 Although an early analysis suggested several candidate genes associated to microcephaly, not all of  
401 these could be confirmed by high-coverage data. Among eleven candidate genes<sup>18</sup>, only two  
402 (*PCNT*, *UCP1*) are among the HHMC gene list presented here, while most of the other changes are  
403 not human-specific, and only *PCNT* has been related to microcephaly<sup>107</sup>. Nevertheless, more such  
404 changes are found on both lineages: For example, our data reveals that in archaics there are AHMCs  
405 in the microcephaly candidate genes *ASPM*<sup>108</sup> and *CIT*<sup>109</sup>. The ASPM-katanin complex controls  
406 microtubule disassembly at spindle poles and misregulation of this process can lead to  
407 microcephaly<sup>110</sup>, which is of interest given the presence of a HHMC in *KATNA1* and a fixed non-  
408 coding change in *KATNB1*, while no such changes were observed in archaics<sup>111</sup>. Other genes  
409 associated with microcephaly that harbor non-synonymous SNCs are *CASC5* (two in humans, one  
410 in archaics)<sup>112</sup>, *CDK5RAP2* (in humans), *MCPHI* (in archaics)<sup>113</sup>, *ATRX* (one in humans and  
411 archaics each)<sup>114</sup>, and *NHEJ1*<sup>115</sup> (a deleterious one in humans, and one in archaics). The SPAG5  
412 protein, which carries three fixed HHMCs, has been claimed to interact with CDK5RAP2<sup>116</sup>, is a  
413 direct target of PAX6<sup>117</sup>, via which it affects cell division orientation, and therefore is critical in the  
414 course of brain development. Disease mutations in *SCAP* or *ADSL* have also been associated with  
415 microcephaly phenotypes as well<sup>118,119</sup>, and Formin-2 (*FMN2*), which carries a deleterious  
416 regulatory change in modern humans, influences the development of the brain causing  
417 microcephaly in mice<sup>120</sup>.

418 Genes associated with brain growth trajectory changes lead not necessarily to a decrease but also  
419 an increase of brain size<sup>121</sup>, suggesting that the disease phenotype of macrocephaly might point to  
420 genes relevant in the context of brain growth as well. One of the few genes with several HHMCs,  
421 *CASC5*, has been found to be associated with gray matter volume differences<sup>122</sup>. It has been claimed  
422 that mutations in *PTEN* alter the brain growth trajectory and allocation of cell types through  
423 elevated Beta-Catenin signaling<sup>123</sup>. This gene is also present among differentially expressed genes  
424 in human neurons compared to chimpanzee neural progenitor cells during cerebral cortex  
425 development, which may relate to a lengthening of the prometaphase-metaphase in humans  
426 compared to chimpanzees that is specific to proliferating progenitors and not observed in non-  
427 neural cells<sup>124</sup>. We find that *PTEN* falls among the genes with the highest number of HF SNCs on  
428 the human lineage per length, and also among the genes with an excess on the modern human over  
429 the archaic lineage, suggesting that regulatory changes in this gene might have contributed to  
430 human-specific traits. This is also the case for the HHMC-carrying transcription factor TCF3, which  
431 is known to repress Wnt-Beta-Catenin signaling and maintain the neural stem cell population during  
432 neocortical development<sup>125</sup>. Among other macrocephaly-related genes with HHMCs in *RNF135*<sup>126</sup>,  
433 *CULAB*<sup>127</sup> and *CCND2*<sup>128</sup>, the latter also shows a large number of HF changes on the human  
434 lineage, and the HHMC in *CULAB* is inferred to be deleterious (Table 2). Other macrocephaly  
435 candidates such as *NFIX*<sup>129</sup>, *NSD1*<sup>130</sup> and *GLI3*<sup>131</sup> have been claimed to have played an important  
436 role in shaping the distinctly modern human head<sup>132</sup> and show numerous SNCs in non-coding  
437 regions. *GLI3* might have been under positive selection<sup>19</sup> and carries 20 HF SNCs on the human,  
438 but only one on the archaic lineage. Two of the very few genes hypothesized to regulate expansion  
439 and folding of the mammalian cerebral cortex by controlling radial glial cell number and fate,  
440 *TRNP1*<sup>133</sup> and *TMEM14B*<sup>134</sup>, exhibit HF 3'-UTR changes in modern humans, and *TRNP1* shows an  
441 excess of changes on the modern human lineage. The expression of these two genes in the outer

442 subventricular zone might be important<sup>135</sup>, since this is an important region for complexification of  
443 neocortical growth in primates<sup>136</sup>, and for which an enriched activation of mTOR signaling has been  
444 reported<sup>137</sup>. In addition to other genes in the mTOR-pathway, such as *PTEN*<sup>138</sup> or *CCND2*, two  
445 possibly interacting modulators<sup>139</sup> of the mTOR signaling pathway stand out in our dataset: *ZNHIT2*  
446 with one deleterious SNC (Table 2) might have been under positive selection<sup>19</sup>, and *CCT6B* carries  
447 a deleterious change according to both SIFT and C-score. The TF encoded by *RBICCI* is essential  
448 for maintaining adult neuronal stem cells in the subventricular zone of the cerebral cortex<sup>140</sup>. This  
449 gene carries a HHMC, a regulatory SNC that has been suggested to modify transcriptional  
450 activity<sup>141</sup>, and a signature of positive selection<sup>13</sup>.

451 Changes in the genes mentioned here could have contributed to the brain growth trajectory  
452 changes hypothesized to give rise to the modern human-specific globular braincase shape during the  
453 past several 100,000 years<sup>8,10,39</sup>. On the archaic side, an enrichment of genes with AHMCs  
454 associated to “Corneal structure” may relate to archaic-specific changes in brain growth-trajectories  
455 since the size and position of the frontal and temporal lobes might affect eye and orbit  
456 morphology<sup>142</sup>, and the macrocephaly-associated gene *RIN2*<sup>143</sup> carries an AHMC. Finally, changes  
457 that might have affected the size of the cerebellum can be found in our dataset, such as the HF  
458 regulatory SNCs found in *ZIC1* and *ZIC4*<sup>144</sup>, and the deleterious HHMC in *ABHD14A*, which is a  
459 target of *ZIC1*<sup>145</sup>.

460

#### 461 **The craniofacial phenotype**

462 Differences other than brain-related properties are likely to have emerged after the split from  
463 archaic humans, some of which may have had an impact on cognition more indirectly<sup>146,147</sup>. Among  
464 the genes harboring HHMCs and found in selective sweep regions, the gene encoding the TF  
465 *PRDM10* stands out, since this is the second-most interacting protein within the HHMC dataset.  
466 Although little is known about *PRDM10*, it may be related dendrite growth<sup>148</sup> and to neural crest  
467 related changes that contributed to the formation of our distinct modern face<sup>149</sup>. Changes in genes  
468 related to craniofacial morphology would complement previous observations<sup>17,132</sup>, and we find an  
469 enrichment of genes with an excess of HF SNCs on the modern human lineage for the GO term  
470 “soft palate development”, which might relate to craniofacial properties that are relevant for  
471 language<sup>150</sup>. Among genes harboring an excess of HF SNCs associated with specific facial features,  
472 we find *RUNX2*, *EDAR*, and *GLI3*<sup>151</sup>, *NFATC1*<sup>152</sup>, *SPOP*<sup>153</sup>, *DDR2*<sup>154</sup> and *NELL1*<sup>155</sup>, possibly due  
473 to changes in regulatory regions, while mutations in the HHMC-carrying gene encoding for the TF  
474 *ATRX* cause facial dysmorphism<sup>156</sup>. In addition, genes with HHMCs such as *PLXNA2*<sup>157</sup>, *EVC2*<sup>158</sup>,  
475 *MEPE*<sup>159</sup> and *SPAG17*<sup>160</sup> are known to affect craniofacial bone and tooth morphologies. These  
476 genes appear to be important in determining bone density, mineralization and remodeling, hence  
477 they may underlie differences between archaic and modern human facial growth<sup>161</sup>. Some of these  
478 facial properties may have been present in the earliest fossils attributed to *H. sapiens*, like the Jebel  
479 Irhoud fossils<sup>4</sup>, deviating from craniofacial features which emerged in earlier forms of *Homo*<sup>162</sup>, and  
480 may have become established before some brain-related changes discussed here<sup>39,163</sup>.

481 Other craniofacial morphology-related genes, such as *DCHS2*<sup>151</sup>, *HIVEP2*<sup>164</sup>, *HIVEP3*<sup>165</sup>,  
482 *FREMI*<sup>166</sup>, and *FRASI*<sup>167</sup> harbor AHMCs, while another bone-related gene, *MEF2C*<sup>168</sup>, shows an  
483 excess of HF changes on the archaic lineage. These changes may underlie some of the derived  
484 facial traits of Neanderthals<sup>169</sup>.

485

486

487

## 488        **The impact on cognition and language**

489        It has long been hypothesized that language and its neurological foundation were important for  
490 the evolution of humans and uniquely human traits, closely related to hypotheses on the evolution  
491 of cognition and behavior. It is noteworthy that among traits associated with cognitive functions  
492 such as language or theory of mind, the timing of myelination appears to be a good predictor of  
493 computational abilities<sup>170,171</sup>. Computational processing might have been facilitated by some of the  
494 changes presented here, at least in some of the circuits that have expanded in our lineage<sup>172</sup>, since  
495 subtle maturational differences early in development<sup>173</sup> may have had a considerable impact on the  
496 phenotype. This might be linked to the specific brain growth trajectory in modern humans<sup>11</sup>, and  
497 reflected in the morphology of the parietal and temporal lobes<sup>174,175</sup>, as well as in the size of the  
498 cerebellum<sup>8</sup>. Archaic hominins likely had certain language-like abilities<sup>176,177</sup>, and hybrids of  
499 modern and archaic humans must have survived in their communities<sup>178</sup>. However, another  
500 important hint for human-specific features is that genes associated with axon guidance functions,  
501 which are important for the refinement of neural circuits including those relevant for speech and  
502 language, are found in introgression deserts<sup>36,179</sup>, and especially the *FOXP2* region is depleted for  
503 archaic introgression, which seems to be a unidirectional and human-specific pattern<sup>68</sup>. This,  
504 together with putative positive selection after the split from Neanderthals<sup>180</sup> and regulatory changes  
505 affecting *FOXP2* expression<sup>181</sup>, could indicate modifications of a complex network in cognition or  
506 learning, possibly related to other brain-related, vocal tract<sup>132</sup> or neural changes<sup>182</sup>. We suggest that  
507 some other genes with changes on the human lineage might have contributed more specifically to  
508 cognition-related changes, although we admit that the contribution of single SNCs to these  
509 functions is less straightforward than their contribution to molecular mechanisms, since disease  
510 mutations in many genes may have disruptive effects on cognitive abilities.

511        The basal ganglia are a brain region where *FOXP2* expression is critical for the establishment  
512 and maintenance of language-related functions<sup>183,184</sup>. Several genes carrying HHMCs have been  
513 described previously as important for basal ganglia functions related to language and cognition. The  
514 HTT protein has long been implicated in the development of Huntington's disease, which is  
515 associated with corticostriatal dysfunction, and is known to interact with *FOXP2*<sup>185</sup>. Mutations in  
516 *SLITRK1* have been linked to Tourette's syndrome, a disorder characterized by vocal and motor tics,  
517 resulting from a dysfunction in the corticostriatal-thalamocortical circuits<sup>186</sup>. *NOVA1* regulates  
518 RNA splicing and metabolism in a specific subset of developing neurons, particularly in the  
519 striatum<sup>187</sup>. As pointed out above, *NOVA1* is an interactor of *ELAVL4*, which belongs to a family of  
520 genes known to promote the production of deep layer *FOXP2*-expressing neurons<sup>188-190</sup>, and part of  
521 a neural network-related cluster that has been highlighted as putatively under positive selection in  
522 humans<sup>22</sup>. Within this network,  $\alpha$ -synuclein (encoded by *SCNA*) might serve as a hub and is  
523 specifically expressed in brain regions important for vocal learning regions in songbirds<sup>67</sup>. *SCNA*  
524 and *SV2C*, which carries a HHMC, are involved in the regulation of dopamine release, with *SV2C*  
525 expression being disrupted in *SCNA*-deficient mice and in humans with Parkinson's disease<sup>191</sup>.  
526 Genes in the cluster of selection signals<sup>22</sup> are implicated in the pathogenesis of Alzheimer's disease,  
527 which (together with Huntington's and Parkinson's diseases) is linked to a *FOXP2*-driven  
528 network<sup>192</sup>. Some introgressed archaic alleles are downregulated in specific brain regions<sup>27</sup>,  
529 especially pronounced in the cerebellum and basal ganglia. One notable example is *NTRK2*, which  
530 shows an excess of HF changes on the human lineage and a signature of positive selection<sup>19</sup>, and is  
531 also a *FOXP2* target<sup>35</sup>, a connection which has been highlighted for the vocal learning circuit in  
532 birds<sup>193</sup>. Other genes harboring HHMCs such as *ENTHD1*<sup>106</sup> and *STARD9*<sup>194</sup>, as well as genes in  
533 introgression deserts<sup>25</sup>, have been associated with language deficits. It may indeed have taken a

534 complex composite of changes to make our brain fully language-ready<sup>195</sup>, where not all changes  
535 needed to reach fixation.

536 In the broader context of cognition, we find an enrichment of HHMCs in genes associated to  
537 “Alzheimer's disease (cognitive decline)” and “Cognitive decline (age-related)”, with seven  
538 associated genes (*COX7B2*, *BCAS3*, *DMXLI*, *LIPC*, *PLEKHG1*, *TLL2* and *VIT*). Two other genes  
539 linked to Alzheimer's are *PTEN*<sup>196</sup>, and *RBICCI*<sup>197</sup>. Among genes with deleterious HHMCs,  
540 *SLC6A15* has been associated to emotional processing in the brain<sup>198</sup>, and may be part of  
541 modifications in glutamatergic transmission<sup>199</sup>, a category found in selective sweep regions<sup>147</sup>.  
542 *GPR153*, which carries one HHMC and two AHMCs, influences behavioral traits like decision  
543 making in rats, and is associated with various neuropsychiatric disorders in humans<sup>200</sup>. Another  
544 interesting candidate change in the context of cognitive abilities might affect the Adenylosuccinate  
545 Lyase (*ADSL*), for which the ancestral Neanderthal-like allele has not been observed in 1,000s of  
546 modern human genomes. This gene has been associated to autism<sup>201</sup>, is part of behavioral traits like  
547 “aggressive behavior” which have been found to be enriched on the human lineage<sup>17</sup>, and several  
548 studies detected a signal of positive selection in modern humans<sup>19,20,202</sup>. These observations make  
549 *ADSL* a strong candidate for human-specific features, particularly in light of the fact that the  
550 relevant HHMC is located in a region that is highly conserved and lies close to the most common  
551 disease mutation leading to severe adenylosuccinase deficiency<sup>20</sup>. Other relevant genes, similar to  
552 *ADSL* in carrying a fixed HHMC and being frequently found in selective sweep screens, are  
553 *NCOA6*, which might be related to autism as well<sup>203</sup>, and *SCAP*. Downregulation of the cholesterol  
554 sensor encoded by this gene has been shown to cause microcephaly, impaired synaptic transmission  
555 and altered cognitive function in mice<sup>119</sup>. We want to emphasize that the networks presented in the  
556 previous sections influencing brain growth and neural wiring are likely to impact cognitive  
557 functions, since disruptions in these networks would impair the healthy human brain. Furthermore,  
558 we find an enrichment of AHMCs in genes associated to Parkinson's disease and “Attention deficit  
559 hyperactivity disorder and conduct disorder”, suggesting that changes may have taken place in  
560 related networks on the archaic lineage as well.

561

### 562 **Life history and other phenotypic traits**

563 Apart from their consequences for cognitive functions, it has been suggested that changes  
564 involved in synaptic plasticity might be interpreted in a context of neoteny<sup>19,100,204,205</sup>, with the  
565 implication of delayed maturation in humans<sup>206</sup> and a longer timeframe for brain development.  
566 However, given their similar brain sizes<sup>207</sup>, humans and Neanderthals might both have needed a  
567 long overall maturation time<sup>208,209</sup>. Accordingly, notions like neoteny and heterochrony are unlikely  
568 to be fine-grained enough to capture differences between these populations, but early differences in  
569 infant brain growth between humans and Neanderthals<sup>8,210</sup> could have rendered our maturational  
570 profile distinct during limited developmental periods and within specific brain regions, imposing  
571 different metabolic requirements<sup>211</sup>. One of the brain regions where such differences are found is  
572 the orbitofrontal cortex (OFC)<sup>174</sup>, and we find that the OFC at infant age (0-2 years) is enriched for  
573 the expression of genes that carry HHMCs compared to synonymous SNCs. We suggest that the  
574 development of the OFC in infants might have been subject to subtle changes since the split from  
575 Neanderthals rather than a general developmental delay, which is particularly interesting given that  
576 this brain region has been implied in social cognition<sup>212</sup> and learning<sup>213</sup>.

577 Genes carrying HHMCs are enriched for expression in the gray matter of the forebrain at the  
578 adolescent age compared to AHMC-carrying genes, hence additional human-specific modifications  
579 during this period might have taken place, possibly linked to changes in myelination described

580 above. It has been suggested that differences in childhood adolescence time existed between  
581 humans and Neanderthals, after a general developmental delay in the hominin lineage<sup>214,215</sup>. Dental  
582 evidence suggests an earlier maturation in Neanderthals than modern humans<sup>216</sup>, and it has been  
583 claimed that Neanderthals might have reached adulthood earlier<sup>217</sup>. Furthermore, an introgressed  
584 indel from Neanderthals causes an earlier onset of menarche in present-day humans<sup>218</sup>, supporting  
585 at least the existence of alleles for earlier maturation in the Neanderthal population. Among the  
586 genes carrying fixed HHMCs, *NCOA6* has also been linked to age at menarche and onset of  
587 puberty<sup>219</sup>, as well as placental function<sup>220</sup>. This putative TF is enriched in HF changes and has been  
588 suggested to have been under positive selection on the modern human lineage<sup>19,202</sup>. The HHMC is  
589 located nearby and three 5'-UTR variants within a putatively selected region<sup>22</sup>, with an estimated  
590 time of selection at around 150 kya (assuming a slow mutation rate). Even though this gene carries  
591 an AHMC as well, it remains possible that modern humans acquired subtle differences in their  
592 reproductive system through lineage-specific changes in this gene. A delay in reproductive age may  
593 influence overall longevity, another trait for which our data set yields an enrichment of genes with  
594 HHMCs (*SLC38A10*, *TBC1D22A* and *ZNF516*).

595 The male reproductive system might have been subject to changes as well, since we find that  
596 several proteins in spermatogenesis seem to carry two HHMCs: Sperm Specific Antigen 2 (*SSFA2*),  
597 Sperm Associated Antigen 17 (*SPAG17*), *ADAM18*<sup>221</sup> and *WDR52*<sup>222</sup>, out of which *ADAM18* and  
598 *SPAG17* also carry AHMCs. Lineage-specific differences in genes related to sperm function or  
599 spermatogenesis might have been relevant for the genetic compatibility between humans and  
600 Neanderthals. Another gene harboring a HHMC with similar functions is *EPPIN*<sup>223</sup>, which shows  
601 no HF changes on the archaic, but 27 such SNCs on the modern human lineage. The gene encoding  
602 for the Testis Expressed 2 protein (*TEX2*) is enriched for HF changes in both humans and archaics,  
603 with one HHMC and five AHMCs, but its function is not yet known. Another possible SNC that  
604 might be relevant in this context is a splice site change in *IZUMO4*, since proteins encoded by the  
605 *IZUMO* family form complexes on mammalian sperm<sup>224</sup>. The adjacent exon is not present in all  
606 transcripts of this gene, suggesting a functional role of this splice site SNC. Finally, genes in the  
607 GO category “spermatoproteasome complex” are enriched for an excess of HF changes on the  
608 human compared to the archaic lineage.

609 It has been found that Neanderthal alleles contribute to addiction and, possibly, pain sensitivity  
610 in modern humans<sup>225,226</sup>. In this context, an interesting protein-truncating SNC at high frequency in  
611 humans is the loss of a stop codon in the opioid receptor *OPRM1* (6:154360569), potentially  
612 changing the structure of the protein encoded by this gene in some transcripts. Other mutations in  
613 this gene are associated to heroin addiction<sup>227</sup>, and pain perception<sup>228</sup>, but also sociality traits<sup>229</sup>.  
614 Interestingly, a recent study found a pain insensitivity disorder caused by a mutation in *ZFHX2*<sup>230</sup>,  
615 which carries an AHMC, and three HHMCs are observed in *NPAPI*, which might be associated  
616 with the Prader-Willi syndrome, involving behavioral problems and a high pain threshold<sup>231</sup>. Such  
617 changes may point to differences in levels of resilience to pain between Neanderthals and modern  
618 humans.

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## 621 **Conclusion**

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623 The long-term evolutionary processes that led to the human condition<sup>1</sup> is still subject to debate  
624 and investigation, and the high-quality genomes from archaic humans provide opportunities to  
625 explore the recent evolution of our species. We want to contribute to an attempt to unveil the

626 genetic basis of specific molecular events in the time-window after the split from these archaic  
627 populations and before the emergence of most of the present-day diversity. We sought to combine  
628 different sources of information, from genome-wide enrichment analyses to functional information  
629 available for specific genes, to identify threads linking molecular needles in this expanded haystack.  
630 In doing so, we have mainly built on existing proposals concerning brain-related changes, but we  
631 have divided the observations into different biological levels, from cellular changes through brain  
632 organization differences to complex phenotypic traits. Only future experimental work will  
633 determine which of the changes highlighted here contributed significantly to making us “fully  
634 human”. We hope that our characterization and presentation of some new candidate genes will help  
635 prioritize inquiry in this area.

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### 649 **Author contributions**

650 M.K. and C.B. analyzed data and wrote the manuscript.

651

### 652 **Competing interests statement**

653 The authors declare no competing interests.

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### 656 **Supplementary Table & Figure legends**

657

658 **Table S1:** List of HHMCs and genomic features

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660 **Table S2:** List of low-confidence HHMCs and genomic features

661

662 **Table S3:** List of AHMCs and genomic features

663

664 **Table S4:** List of low-confidence AHMCs and genomic features

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666 **Table S5:** Top 5% of genes by HF SNC density on the modern human and archaic lineages, and  
667 top 10% of genes by relative excess of HF SNCs on one lineage over the other.

668

669 **Table S6:** GO enrichment for genes with relative excess of HF SNCs on the human over the  
670 archaic lineage

671

672 **Table S7:** GWAS enrichment for genes with HHMCs or AHMCs

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674 **Table S8:** Number of interactions among genes with HHMCs or AHMCs

675

676 **Table S9:** Genes with HHMCs that are TFs, or at the centrosome interface

677

678 **Table S10:** Enrichment in developing brain zones for genes with HHMCs or AHMCs,  
679 proportion of random gene sets with larger overlap (Methods).

680

681 **Figure S1:** Distribution of missense and non-synonymous HF SNCs across chromosomes.

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683 **Figure S2:** STRING graph of interactions among genes with HHMCs.

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## Methods

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689 We used the publicly available high-coverage genotypes for three archaic individuals: One  
690 Denisovan<sup>12</sup>, one Neanderthal from the Denisova cave in Altai mountains<sup>13</sup>, and another  
691 Neanderthal from Vindija cave, Croatia<sup>14</sup>. The data is publicly available under  
692 <http://cdna.eva.mpg.de/neandertal/Vindija/VCF/>, with the human genome version *hg19* as  
693 reference. We applied further filtering to remove sites with less than 5-fold coverage and more than  
694 105-fold coverage in the Altai Neanderthal or 75-fold coverage in the other archaic individuals, if  
695 such cases occurred. We also removed sites with genotype quality smaller than 20, and  
696 heterozygous sites with strong allele imbalance (<0.2 minor allele frequency). Although these  
697 permissive filters increase power compared to previous studies, we caution that in some cases  
698 genotypes might be incorrect. We added the genotype and coverage for the exome and chromosome  
699 21 sequences of the Vindija and El Sidrón Neanderthals from previous studies<sup>2,17</sup>, with 75-fold and  
700 50-fold coverage cutoffs, respectively. These studies provided data for the same Vindija  
701 individual<sup>14</sup>.

702 We applied the Ensembl Variant Effect Predictor<sup>232</sup> in order to obtain inferences for protein-  
703 coding and regulatory mutations, scores for SIFT<sup>233</sup>, PolyPhen<sup>234</sup>, CADD<sup>235</sup> and GWAVA<sup>236</sup>, and  
704 allele frequencies in the 1000 Genomes and ExAC human variation databases<sup>15,237</sup>. We used the  
705 inferred ancestral allele from<sup>238</sup>, and at positions where this information was not available, the  
706 macaque reference allele, *rheMac3*<sup>239</sup>. We determined the allele frequencies in present-day humans  
707 using the dbSNP database build 147<sup>240</sup>. We retrieved the counts for each allele type, and  
708 summarized the counts of non-reference alleles at each position. Grantham scores<sup>241</sup> were  
709 calculated for missense mutations.

710 Data processing and database retrieval was performed using bcftools/samtools v1.0<sup>242</sup>, bedtools  
711 v2.16.2<sup>243</sup>, and R/Bioconductor<sup>244</sup>, rtracklayer<sup>245</sup> and biomaRt<sup>246</sup>, and plotting with RCircos<sup>247</sup>. We  
712 analyzed all positions where at least two alleles (human reference and alternative allele) were  
713 observed among the human reference and at least one out of three of the high-coverage archaic  
714 individuals in at least one chromosome. The 22 autosomal chromosomes and the X chromosome  
715 were analyzed, in the absence of Y chromosome data for the three female archaic individuals. The  
716 data for 4,409,518 segregating sites is available under [<http://tdb.database>]. The following subsets  
717 were created:

718 Fixed differences: Positions where all present-day humans carry a derived allele, while at least  
719 two out of three archaics carry the ancestral allele, accounting for potential human gene flow into  
720 Neanderthals.

721 High-frequency (HF) differences: Positions where more than 90% of present-day humans carry a  
722 derived allele, while at least the Denisovan and one Neanderthal carry the ancestral allele,  
723 accounting for different types of errors and bi-directional gene flow.

724 Extended high-frequency differences: Positions where more than 90% of present-day humans  
725 carry a derived allele, while one of the following conditions is true: a) Not all archaics have reliable  
726 genotypes, but those that have carry the ancestral allele. b) Some archaics carry an alternative  
727 genotype that is not identical to either the human or the ancestral allele. c) The Denisovan carries  
728 the ancestral allele, while one Neanderthal carries a derived allele, which allows for gene flow from  
729 humans into Neanderthals. d) The ancestral allele is missing in the EPO alignment, but the macaque  
730 reference sequence is identical to the allele in all three archaics.

731 We also created corresponding lists of archaic-specific changes. Fixed changes were defined as  
732 sites where the three archaics carry the derived allele, while humans carry the ancestral allele at  
733 more than 99.999%. High-frequency changes occur to less than 1% in present-day humans, while at  
734 least two archaic individuals carry the derived allele. An extended list presents high-frequency  
735 changes where the ancestral allele is unknown, but the macaque allele is identical to the present-day  
736 human allele.

737 A ranking of mutation density was performed for genes with protein-coding sequences and their  
738 genomic regions as retrieved from Ensembl. For each gene, unique associated changes as predicted  
739 by VEP were counted. A ranking on the number of HF changes per gene length was performed for  
740 all genes that span at least 5,000 bp in the genome and carry at least 25 segregating sites in the  
741 dataset (at any frequency in humans or in archaics), in order to remove genes which are very short  
742 or poor in mutations. The top 5% of the empirical distribution was defined as putatively enriched  
743 for changes on each lineage. The ratio of lineage-specific HF changes was calculated for the subset  
744 of genes where at least 20 lineage-specific HF changes were observed on the human and the archaic  
745 lineages combined. The top 10% of the empirical distribution was defined as putatively enriched for  
746 lineage-specific changes.

747 We performed enrichment tests using the R packages ABAEnrichment<sup>44</sup> and DescTools<sup>248</sup>. We  
748 used the NHGRI-EBI GWAS Catalog<sup>41</sup>, and overlapped the associated genes with protein-coding  
749 changes on the human and archaic lineages, respectively. We counted the number of HF missense  
750 changes on each lineage and the subset of those associated to each trait (“Disease trait”), and  
751 performed a significance test (G-test) against the number of genes associated to each trait, and all  
752 genes in the genome, with a P value cutoff at 0.1. This suggests a genome-wide enrichment of  
753 changes for each trait. We then performed a G-test between the numbers of HF missense changes  
754 on each lineage, and the subset of each associated to each trait (P-value cutoff at 0.1), to determine  
755 a difference between the two lineages. We then performed an empirical test by creating 1,000  
756 random sets of genes with similar length as the genes associated to each trait, and counting the  
757 overlap to the lineage-specific missense changes. At least 90% of these 1,000 random sets were  
758 required to contain fewer missense changes than the real set of associated genes. Only traits were  
759 considered for which at least 10 associated loci were annotated.

760 Gene Ontology (GO) enrichment was performed using the software FUNC<sup>249</sup>, with a significance  
761 cutoff of the adjusted p-value < 0.05 and a family-wise error rate < 0.05. When testing missense  
762 changes, a background set of synonymous changes on the same lineage was used for the  
763 hypergeometric test. When testing genes with relative mutation enrichment, the Wilcoxon rank test

764 was applied. Enrichment for sequence-specific DNA-binding RNA polymerase II transcription  
765 factors (TFs) and TF candidate genes from<sup>43</sup>, and genes interacting at the centrosome-cilium  
766 interface<sup>49</sup> was tested with an empirical test in which 1,000 random sets of genes were created that  
767 matched the length distributions of the genes in the test list. The same strategy was applied for  
768 genes expressed in the developing brain (Table S10)<sup>45</sup>. Protein-protein interactions were analyzed  
769 using the STRING online interface v10.5<sup>250</sup> with standard settings (medium confidence, all sources,  
770 query proteins only) as of January 2018. The overlap with selective sweep screens considers  
771 HHMCs within 50,000 bp of the selected regions<sup>13,19,22</sup>.  
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