Derived *Homo sapiens* cis-eQTL regulation: implications for brain evolution

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Abstract

The high-quality sequence of the genomes of our extinct relatives, Neanderthals and Denisovans, became recently public. At the same time, we have seen the emergence of big databases of modern human genetic variation. However, linking human genetic variation, neuronal phenotypes and, eventually, behaviour, is only possible if we understand how variation and genetic regulation interact. We used two publicly available datasets, the GTEX cis-eQTL database (v7) and a catalog of high-frequency *Homo Sapiens* specific alleles relative to the Neanderthals and Denisovan sequences, to understand how high-frequency Homo Sapiens derived alleles affect gene expression regulation. The resulting dataset shows that genes associated with brain development are affected by *Homo sapiens*-specific eQTL in brain areas key in human evolution such as the cerebellum. We also show that some of these eQTL overlap significantly with putative

regions of positive selection relative to archaic humans [Peyrégne et al., 2017]. Additionally, we tested whether any of the variants are associated with clinical conditions in modern human populations. These findings can inform future experimental work and enrich current venues of research of the Homo Sapiens brain evolution, such as the relationship between clinical and evolutionary research and the recent expansion of the cerebellum in *Homo Sapiens* [Gunz et al., 2010].

Keywords— Human evolution, eQTL, brain evolution

1 Introduction

The high-quality DNA sequencing of two Neanderthal individuals and a Denisovan from Altai and Vindija [Prüfer et al., 2014, 2017, Meyer et al., 2012] has opened numerous research avenues and opportunities for studying the evolution of the *Homo sapiens* brain with unprecedented resolution and precision. Geometric morphometric analysis on endocasts [Bruner et al., 2014, Gunz et al., 2010] have already suggested that the differences between Neanderthal and *Homo sapiens* skulls could be related to changes in neural tissue that might in turn have had consequences for the evolution of human cognition. In particular, the temporal and the parietal lobes, as well as the cerebellum, have been claimed to have expanded during the emergence of *Homo sapiens* evolution [Gunz et al., 2010]. The sequencing of ancient human genomes made it possible perform selective sweep scans to detect areas of the genome that have been significantly affected by natural selection after the split with Neanderthals [Racimo et al., 2014, Peyrégne et al., 2017]. Much of the early efforts relied on determining the function on the few missense mutations that are *Homo sapiens*-specific. However, to characterize the effects of *Homo sapiens*-specific variants we should target not only the variants that affect the structure and function of

the protein, but also those that regulate gene expression. Species-specific regulation of genetic expression levels might also play a big role in determining the modern human brain phenotype [Franchini and Pollard, 2017, Gokhman et al., 2016].

In order to link genotype and brain phenotype, previous work has explored the idea of connecting modern human variation data and brain evolution. A major study [McCoy et al., 2017] explored the effects of Neanderthal and Denisovan introgressed variants in 44 tissues and found reduced differential expression of Neanderthal alleles in the cerebellum and the striatum. In a similar vein, another study [Gunz et al., 2019] examined the effects of archaic introgression on brain skull shape variability in modern human populations to determine which variants are more associated with a globularized skull shape, a feature of *Homo sapiens* skulls.

Expanding on these approaches, we aim to understand the effects of the derived *Homo sapiens*-specific alleles in gene regulation in brain tissue. We took advantage of a recent systematic review of high frequency changes in modern humans [Kuhlwilm and Boeckx, 2019], which offered not only a catalog of *Homo sapiens*-specific fixed missense mutations, but an exhaustive database with all the derived *Homo sapiens* unique alleles in high frequency (90% or more cutoff). We crossed this database with the GTEX version 7 database, which contains information on Expression Quantitative Trait Loci (cis-eQTLs) accross tissues, focusing exclusively on brain tissue. Compared to approaches that have used the GTEx data in light of introgression [McCoy et al., 2017, Gunz et al., 2019], our focus on the effect of the derived allele allows this study to span the whole genome, as opposed to introgressed regions only. Additionally, the data generated, unlike in McCoy et al. [2017], doesn't focus on neanderthal-specific alleles but on the effect of the derived, modern-specific allele (as defined in [Kuhlwilm

and Boeckx, 2019]).

Our results show differential regulation by derived cis-eQTLs in key brain areas and circuits that are claimed to have changed significantly during the emergence of *Homo sapiens*, such as the cerebellum and olfactory signalling pathway [Bastir et al., 2011]. We also found genetic regulation of cellular processes that can potentially impact neurodevelopment and disease, such as folate one-carbon metabolism, aerobic glycolysis regulation, cell-cell adhesion and regulation of cytoskeleton dynamics. Some of these processes, such as aerobic glycolysis, have already been discussed in other studies in the context of human evolution and human-specific diseases such as Alzheimer's Disease [Bufill et al., 2011]. While we limited the scope of our study to brain tissue, we found as well eQTLs associated with neural crest cell development and the craniofacial complex. This is of special interest, as the *Homo sapiens* face has a distinct retracted profile compared to that of archaic humans [Lacruz et al., 2019]. Some of the genes that affect brain development might exert an influence in adjacent tissues [Boeckx, 2017].

Since the eQTLs affect genes previously identified as under selective sweep in modern humans relative to archaic humans, we tested whether the eQTLs affect genetic regions identified in human positive selection studies. We found *Homo sapiens*-specific eQTLs overlap significantly with regions of positive selection [Peyrégne et al., 2017] in a permutation test (n=10000) (Supp. Fig. 2). We also explored whether the eQTL-associated genes were enriched in transcription factor motifs with a known functionality, as well as their relationship with known clinical conditions.

Overall, the results offer a landscape of *Homo sapiens* specific gene regulation, enriching previously explored venues of research of the field of human evolution studies and opening new possibilities for variant effect testing in the

context of human brain evolution.

2 Results

We used the publicly available GTEx v7 eQTLs lists, which consist of statistically significant allele specific expression changes (as defined by GTEx [The GTEx Consortium et al., 2015]) influencing gene expression dosage in 13 different brain tissues from adult brain samples aged 20-60.

We first selected those eQTLs where the minor allele was also the ancestral one (as defined by Kuhlwilm and Boeckx [2019]). The catalog of derived gene changes in *Homo sapiens* we used for this study [Kuhlwilm and Boeckx, 2019] imposes an arbitrary cutoff of a global 90% that allows some frequencies to be under 90% as long as the overall mean fulfills the requirement. We processed the data so that only those alleles that fulfill the 90% threshold in all metapopulations (as detailed in Kuhlwilm and Boeckx [2019]) are included in order to apply a more stringent filter. Finally, crossing this database with the GTEx allele-specific genetic regulation datasets, we retrieved all the *Homo sapiens* derived alleles that had a statistically significant effect in gene expression in any of the adult human brain tissues and were in high frequency in modern population.

The resulting data includes 1,357 statistically significant unique SNPs associated with regulation of a total of 316 eGenes (i.e., genes affected by cis-regulation by at least one SNP) whose major allele falls into at least 90% frequency in all modern human metapopulations . As expected, we find some degree of functional overlap: 266 of those 1,357 eQTLs regulate various genes in the same tissue or act across different tissues. If we don't account for functional overlap we count a total of 2,507 eQTLs across 13 brain tissues (Supp. Tabl. 1).

Each eQTL has a beta score assigned to it, i.e., a normalized score of the modulatory effect of the SNP in the eGene normalized expression levels, as

rsID	Gene exon	Additional notes
rs17597625	*OR5I1*	Under positive selection in early selective sweep [Moreno-Estrada et al., 2007]
rs2917782	*STARD6*	Cerebellar development [Chang et al., 2012] and Alzheimer's Disease [Yin et al., 2019]
rs2917782	*PCNT*	Associated with Down Syndrome and dwarfism [Rauch et al., 2008]
rs74316182	*PLD4*	Influences myelination [Chiba et al., 2016]

Table 1: A summary of missense variants in high frequency in modern humans where the variant also differentially affects cis-regulation of gene expression.

detailed in the GTEx consortium documentation [The GTEx Consortium et al., 2015]. In this case, the effect of an eQTL is understood as the relative gene expression difference between the minor, ancestral allele and the high-frequency derived allele. As shown in Figure 4 (C), chromosome 13 is underrepresented in terms of eQTL regulation.

In terms of distance to the transcription starting site, eQTLs tend to accumulate near the TSS (Transcription Start Site) of the regulated eGene, with a slight upward tendency (Fig. 4 A). This is in accordance with the GTEx consortium data report [The GTEx Consortium et al., 2015]: the GTEx consortium data exhibits an overall tendency towards transcriptional regulation instead of post-transcriptional regulation. We additionally report a tail of downstream variants far away from the TSS.

When listed by number of occurrences, the gene that accumulates more variability from eQTL regulation is *METTL18* (296 SNPs), followed by *METTL14* (72 SNPs, all affecting gene cis-regulation in the cerebellum), *POLI* (47), a DNA repair gene [Jain et al., 2017], and *KATNAL2* (41), a gene implicated in ciliogenesis and neurodevelopment in *Xenopus* [Willsey et al., 2018]. The full list of occurrences per gene can be found in Supp. Table 1. Overall, close to 45% of SNPs accumulate in introns, consistently with the initial GTEx database reports (Fig. 4 B) [The GTEx Consortium et al., 2015], while the second most abundant subgroup is non-coding transcripts.

We also found 15 missense mutations overlapping with cis-eQTLs, from

Tissue	Up	Down	Total	Tissue	Up	Down	Total
Adrenal gland	78	193	271	Cerebellar hemisphere	101	148	249
Amygdala	7	47	54	Hippocampus	7	44	51
BA9	22	64	86	Hypothalamus	12	74	86
BA24	82	102	184	Nucleus Accumbens	44	84	128
Caudate cortex	33	113	146	Pituitary gland	157	491	648
Cerebellum	115	348	463	Putamen	20	98	118
Substantia Nigra	3	20	23				

Table 2: Summary of up and downregulating high-frequency eQTLs in each brain tissue. BA = Brodmann's Area.

which four affect genes that have appeared in the literature related to neurodevelopment, evolution or disease (Table 1). Some of these missense mutations, such as rs74316182, regulate different eGenes in each tissue (Fig. 4 B). Additionally, there is one stop gained (rs73600054) in a cilium and flagella related gene (*CFAP157*) [Weidemann et al., 2016] and one lost (rs80336323) in *PSPN* (persephin neurothropic factor).

We found a splice region variant (rs17801742) in *COL2A1*, linked to Stickler Syndrome (OMIM entry number: #108300) [Guo et al., 2017] and regulating *PIGV*, a gene linked to neural crest cell regulation [Horn et al., 2014] that falls within a region of modern human positive selection [Racimo et al., 2015]. The full list of consequences can be consulted in Supp. Tab. 2.

2.1 Directional regulation of eGenes

To check if there was a significant directionality of regulation in any genetic region or tissue we divided cis-eQTL variants in up and down effects per tissue. (Fig. 4 C , Table 2). We performed a two sample Kolmogorov–Smirnov test under the null hypothesis that the distribution of the high frequency *Homo sapiens* derived eQTLs follows a similar distribution as the GTEx significant eQTLs. The test does not reject that the upregulating eQTLs follow the same distribution as the total GTEx significant variants per tissue, which would be

expected considering that the high-frequency eQTL list is a subset of the total of significant variants in each tissue. However, it reports a significant ($p = 0.04979$, maximum $D^{\hat{}} = 0.5$) difference in the distribution of the downregulating eQTLs relative to the original GTEx v7 lists of significant variants per tissue. Additionally, the data shows a numeric bias towards downregulating eQTLs in all tisues, as visualized in Figure 3 C: in total, 681 alleles are linked to gene upregulation, while 1,826 are linked to gene downregulation across tissues. We tested whether the amount of downregulating to upregulating alleles in the *Homo sapiens* derived data follows the same proportions as the down to upregulating ratios in the unfiltered significant eQTL list with a chi-square goodness-to-fit test. The results show a significant difference ($p < 0.05$) in the down to upregulating ratio compared to the unfiltered GTEx list in all tissues except Brodmann's Area 24 (p-values reported in Supp. Table 3). Note that the allele specific expression measures the effect of the major allele in the GTEx database. Since one of the filters we impose is an equivalency between derived and major allele, in a downregulating eQTL the (ancestral) low frequency allele is decreasing the dosage of the eGene relative to the high-frequency *Homo sapiens*-derived allele. By contrast, upregulation of an eGene by an eQTL means that the ancestral allele is increasing gene dosage relative to the high-frequency derived allele. Given this, the strong bias towards downregulated eQTLs could be reflecting our very limited sample of archaic human genomes. This is consistent with previous results of a study that uses similar resources [McCoy et al., 2017], which reports that Neanderthal alleles reduced genetic expression in the modern human brain. Chromosome Y is depleted of eQTLs in our results for the same reason, as there is no high-coverage genome of a male archaic individual available at the moment (Fig. 4 C).

In terms of the relative proportion of eGenes to eQTLs, cerebellum and pi-

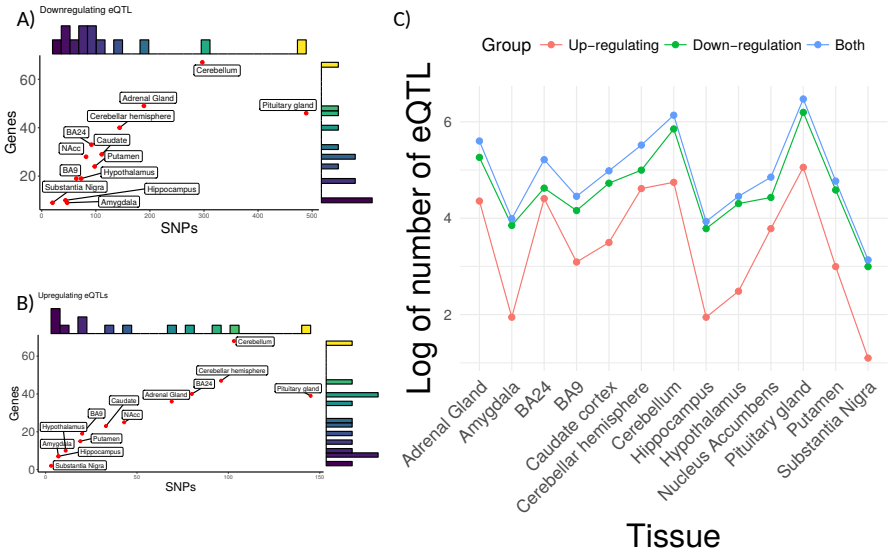

Figure 1: A) and B) Ratio of downregulating (A) and upregulating (B) eQTLs per genes affected in single tissues. C) Log value distribution in upregulating eQTL in human evolution, downregulating and total GTEx original data per tissue.

tuitary gland stand out both in up and downregulated eQTLs. (Figs. 3 A, B). While the cerebellar phenotype has been claimed to be derived in modern humans [Gunz et al., 2019], the pituitary has not been highlighted by any experimental study regarding human evolution to our knowledge. However, a recent account of human evolution has put forward the idea that changes in the hypothalamic–pituitary–adrenal (HPA) axis could be driving the *Homo sapiens* sociocognitive profile (the so-called self-domestication process [Wrangham, 2019]).

Some of the SNPs with the same regulatory directionality (up or down) accumulate in linkage disequilibrium regional clusters that affect specific eGenes, such as in *METTL18* or *METTL14*. (Fig. 4 C). There are also eGenes that are

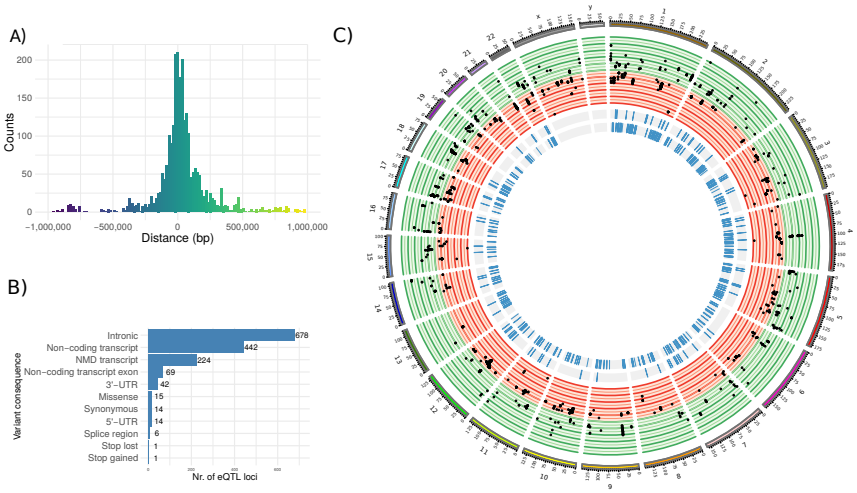

Figure 2: A) A bar plot showing the distribution of eQTL over distance to Transcription Starting Site B) Classification of the genetic consequences in our data C) A Circos plot showing the distribution along the genome of eQTLs. Each line denotes 0.5 steps in beta score (allele specific effects in gene expression), from 3 to -3. Red circles denote downregulation, green circles upregulation of eGenes. Inner rings, in blue: areas showing signals of positive selection relative to archaic humans in [Peyrégne et al., 2017] (innermost) and [Racimo et al., 2014] (outermost).

regulated exclusively in one tissue, both in up and downregulation of the archaic allele. Some cases where the derived allele is increasing the eGene expression in one tissue only are: *METTL14* (cerebellum), *INTS12* (adrenal gland), *STARD6* (Brodmann's Area 24), *BCHE* (caudate) and *ENSG00000254547* (pituitary).

2.2 Overlap with regions of evolutionary significance

To identify whether previous studies could give additional evolutionary significance to any of the genes that are affected by regulation in our data, we have tested whether the eQTLs might regulate genes associated with signals of posi-

tive selection in modern humans versus archaics ([Peyrégne et al., 2017, Racimo et al., 2014]). We ran a permutation test (n=10000) to see if the SNPs accumulate significantly in regions under positive selection relative to archaic humans (Supp. Fig. 2). We found a significant ($p < 0.006$) correlation between eQTLs and regions of positive selection as defined by [Peyrégne et al., 2017]. The same test didn't show a significant overlap with an earlier positive selection study [Racimo et al., 2014].

Overall, some eQTLs lie within regions of selective sweep, but the genes that are affected by them aren't necessarily inside the sweep. It is possible that gene expression modulation of genes outside the selected window is the one driving the statistical significance of the positive selection scan, as it might be targeting mutations that were on their own neutral but that affect translational modification of gene expression levels outside the sweep region. Such a scenario would apply for *PIGV*, *ZNF37A*, *ARID1A*, *GPN2*, *GOLGA4*, *UBR1*, *BAP1*, *TMEM222*, *C3orf35*, *AC097637.2* and *AL117339.5*. An enrichment analysis failed to identify a biological category for this subset of genes. Some of these genes, including *PIGV*, also include other eQTLs that exert cis-regulation over themselves. In order to find out if any of these genes harbouring eQTLs have had a functional role in human evolution, we crossed our data with the results of Zhu et al. [2018], a study that presented transcriptomic data of 16 brain tissues in several developmental stages (see Supp. Tab. 5). We found that 22 of the genes affected by eQTLs are also found in transcriptional heterochronic clusters, ie, blocks of co-expressed genes that have changed their temporal expression profile during the course of evolution relative to the macaque tissue. However, a gene ontology enrichment analysis failed to return any relevant association.

We also tested whether any of the eQTLs fell within deserts of introgression, i.e., genetic windows of at least 1,000 Mb that have resisted genetic introgression

from archaic humans to *Homo sapiens* ([Sankararaman et al., 2016]), as well as within regions of Neanderthal or Denisovan introgression ([McCoy et al., 2017]). The results show that none of our data entries overlap with these regions of special evolutionary interest, and that our data points are not redundant relative to other studies with a similar scope [McCoy et al., 2017]. This is most probably due to the frequency cutoff imposed on the data, as introgression fragments are generally low in frequency (unless adaptative).

2.3 Mechanisms of regulation

We hypothesized that part of the regulation of eGenes could be driven by transcription factor binding site motif variability. We found that there is a number of binding site motif enrichments in genes that are affected by eQTLs in different tissues. We ran a Transcription Factor Binding Site enrichment of each tissue (including both up and down eQTLs regulation) through SNP2TFBS [Kumar et al., 2017]. This tool takes as input a list of rsIDs and maps each SNP to the Transcription Factor Binding Site (TFBS) JASPAR database.

We found a significant enrichment of *RUNX1* TFBS in the cerebellum. *MEIS1* and *RUNX1* regulate Hedgehog signaling through the *ATOH1* transcription factor [Owa et al., 2018]. The Hedgehog signaling pathway lies at the heart of the developmental program of the cerebellum [De Luca et al., 2016]. Gene variants affecting this pathway might have consequences for the development of cerebellar tissue. Within our eQTL list affecting gene expression in the cerebellum we focused on *METTL14*. *METTL14* is of special relevance because it accumulates 72 tissue-specific eQTLs in cerebellar tissue. We hypothesized that we might find that transcription factors binding to sites in *METTL14* are related to cerebellar development, since the cerebellum is known to have expanded recently in human evolution [Gunz et al., 2010]. We found that *METTL14* SNPS

accumulate an enrichment of *MEIS1*, *RUNX1* and retinoic acid (*RXR-RAR*) (Supp. Fig. 3), possibly driving part of the statistical significance in the overall tissue. Additionally, we found other tissues affected by derived eQTLs enriched in transcription factor binding motifs involved in development and disease. The adrenal gland and Brodmann's Area 9 derived eQTLs are enriched in *EGR1* binding sites. *EGR1* is a brain growth factor that plays a key role in stress regulation, schizophrenia and synaptic plasticity [Duclot and Kabbaj, 2017]. We also found that an enrichment in *NFATC2* TFBS in cerebellar hemisphere and hypothalamus. *NFATC2* affects neuronal development and axonal growth [Nguyen and Di Giovanni, 2008]. Finally, we found *STAT6*, a regulator of the neurogenesis in *Xenopus* upon Amyloid-beta42 administration [Bhattarai et al., 2016], in the pituitary.

Additionally, we explored whether derived eQTLs overlapped with any know human miRNA or miRNA seeds (as defined in [Branco et al., 2018]), but found no overlap with our data.

2.4 Clinical data

The ultimate goal of the identification of these derived eQTLs should be bridging genotypical variation with the evolution of phenotypes. By crossing our data with the NCBI Clinvar database [Landrum et al., 2018] — a record of associations of SNPs with a broad range of clinical conditions — we expected to identify the effects of the eQTLs under specific genetic backgrounds to see if they might affect developmental pathways of the brain (full data in Supp. Tab. 4).

Our data shows that a series of conditions associated with changes in the craniofacial structure are linked to some of the modern human eQTLs. Two of the downregulating eQTLs (rs17801742, rs41317939) are known to be related

to Stickler Syndrome (OMIM entry number: #108300), a condition that affects the development of the skull and face, among other effects. Both SNPs are downregulating *PFKM* expression levels in the cerebellum, a gene associated to aerobic glycolysis metabolism usually found in muscles. No known link exists between *PKFM* and Stickler Syndrome.

Another eQTL in our data is related to microcephalic osteodysplastic primordial dwarfism (OMIM: #210720), and epileptic encephalopathy (rs79305633). One of the key characteristics of microcephalic osteodysplastic primordial dwarfism is microcephaly, with the retracted development of the brain starting prenatally [Reference]. Other pathologies linked to the skull-brain co-development found in our data are Noonan Syndrome (OMIM: #613224) and Cowden syndrome (OMIM: #615108). The form of Noonan syndrome linked to the eQTL is characterized by macrocephaly and changes in the craniofacial profile, sometimes accompanied of cognitive consequences. Cowden syndome is characterized by macrocephaly as well, and it is usually linked to variants in the gene coding for PTEN, a key protein in brain development. Interestingly, *PTEN* harbors an excess of modern-specific mutations compared to archaic humans [Kuhlwilm and Boeckx, 2019]. Other variants of interest are one eQTL linked to Charcot-Marie tooth disease (OMIM: #606482), variants associated with Parkinson disease (OMIM: # 260300) and cerebellar ataxia (OMIM: #610743).

Despite these associations, a note of caution should be raised here as we don't fully understand the genetic background that causes these complex disorders. The eQTL-disease relationship is not necessarily causal but could contribute to the epistatic background of tissues of interest.

2.5 Clustered GO analysis

To shed light on whether genes affected by eQTLs affect specific networks of genes we performed a gene ontology (GO) clustered analysis with Metascape [Zhou et al., 2019]. Metascape performs an enrichment analysis that organizes hierarchically GO by establishing a measure of distance between each category and then organizing resulting GO nodes in networks according to the distance between each node. We performed this analysis with a list of all the genes that are affected by differential regulation across all tissues. The results of the analysis (Supp. Fig. 1) reveal a significant number of terms related to the olfactory signalling pathway, cell-cell adhesion and negative regulation of microtubule depolymerization. Geometric morphometrics studies that compared *Homo sapiens* and Neanderthals [Bastir et al., 2011, Kochiyama et al., 2018] had already identified that *Homo sapiens* has significantly larger olfactory bulbs compared to Neanderthals. Cell-cell adhesion also has been found enriched previously in a motif macroanalysis of the Neanderthal and Denisovan genomes [Cserhati et al., 2018]. Cell adhesion is an important process in human-specific cortical folding and apical progenitors cell cycle [Mora-Bermúdez et al., 2016, Wianny et al., 2018]. Microtubule dynamics play an important role in neurodegenerative diseases such as Alzheimer's [Hernández et al., 2013] but also in learning and memory [Dent, 2017].

3 Discussion

Previous work such as [McCoy et al., 2017, Gunz et al., 2019] had already highlighted molecular mechanisms of evolutionary change in highly-derived tissues in *Homo sapiens*. Here we propose that such changes also rely on regulatory changes in human evolution in several tissues. Highly derived structures in-

dentified in previous literature, such as the cerebellum [Gunz et al., 2010] and the olfactory bulbs [Kochiyama et al., 2018] are prominent in our data. The GO clustered analysis revealed a significant enrichment in olfactory signalling pathway related genes in our data. An early study on the subject [Bastir et al., 2011] suggests that the olfactory signalling pathway overlaps significantly with the memory and learning processing circuitry. Differential regulation of the olfactory circuitry might have consequences for derived aspects of *Homo sapiens* cognition.

We also found that the cerebellum accumulates more differential regulation than the rest of tissues (Figs. 3 A, B). In terms of molecular mechanisms, our results highlight *METTL14* as a central node of regulation of cerebellar development. *METTL14* is the second gene in terms of number of eQTLs. However, in this case the differential regulation is tissue specific and accumulates in the cerebellum, making it a strong candidate for one of the genes underlying the cerebellar growth phenotype. *METTL14*, along with *METTL3*, is part of a complex that regulates expression of N6-methyladenosine (m6a), a epitranslational RNA modificator that has an important role in cerebellar development in mice [Ma et al., 2018].

Supporting the idea of a brain-face coregulatory genetic network, the data shows other genes related to the craniofacial complex expressed in the brain. *PIGV* and *WHSC1* are involved in hyperphosphatasia-mental retardation syndrome (OMIM: #239300) [Horn et al., 2014] and Wolf-Hirschhorn syndrome (OMIM: #194190) [Yu et al., 2017], respectively. These two disorders are characterized by distinctive craniofacial profiles. Both *PIGV* and *WHSC1* are upregulated in *Homo sapiens* in our data. Additionally, *PIGV* is associated with a signal of positive selection relative to the archaic human genomes [Racimo et al., 2015]. The way the brain and the craniofacial complex share some of the

genetic regulatory networks is reflected in the amount of *Homo sapiens* derived EQTLs in the brain linked with disorders that affect the fusion of the skull bones or typical deviations of the craniofacial disorder such as cleft palate (see section 2.4). These findings open up the possibility of using modern population clinical data to establish genetic regulatory networks that can inform about the molecular evolution of the *Homo sapiens* species, and viceversa.

Other aspects of our results can be also found at the crossroads between clinical data and evolutionary studies. Some of the eQTLs affect differential regulation of both metabolic programming and microtubule dynamics. Our data shows distinct regulation of genes such *GAPDH* and *PFKM*. Glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) is one of the enzymes of the aerobic glycolysis metabolic chain. The idea that aerobic glycolysis may have played a role in human evolution was proposed before in [Bufill et al., 2011]. Modern humans have a protracted neuronal development [Somel et al., 2009], a phenotype that has been suggested [Bufill et al., 2011, Bauernfeind et al., 2014] to be associated with a species-specific metabolic program involving an upregulation of aerobic glycolysis. Aerobic glycolysis correlates with key stages of the development of the human brain, such as myelination, synaptogenesis and axonal elongation [Bauernfeind et al., 2014]. Changes in brain metabolism could also be underlying human specific neurodegenerative diseases correlated with metabolic failures, such as Alzheimer's disease [Bufill et al., 2013]. *GAPDH* is know to provide glycolytic energy in fast axonal transport during vesicle trafficking [Zala et al., 2013] and is implicated in Alzheimer's disease, among other neurodegenerative diseases [El Kadmiri et al., 2014].

Our data also shows that genes affected by eQTLs are enriched in the regulation of microtubule dynamics. Microtubule depolymerization plays a crucial role in neuronal development and synaptic plasticity [Dent, 2017], as well as in

the pathogenesis of Alzheimer's Disease [Hernández et al., 2013]. Among the genes affecting microtubule dynamics we found one that is associated to a cellular phenotype in the same tissue where the human eQTL is affecting expression levels: *FMN1* in the hippocampus. Overexpression of *FMN1* promotes primary dendritic development in the hippocampus [Simon-Areces et al., 2011]. Whether *FMN1* overexpression would have the same effect in human hippocampus is currently unknown, but this example shows how the present study can open new testable hypotheses for human evolution studies concerning understudied subcortical structures.

All in all, our work shows the potential of using human variation databases as a valuable point of entry to bridge genotype and phenotype in brain evolution studies.

4 Methods

We accessed the *Homo sapiens* high-frequency variant annotation data from [Kuhlwilm and Boeckx, 2019]. The data is publicly available in https://doi.org/10.6084/m9.figshare.8184038. We then applied more stringent criteria to the high-frequency criteria [Kuhlwilm and Boeckx, 2019]. The study in Kuhlwilm and Boeckx [2019] defines an arbitrary cutoff point of *Homo sapiens* derived 90% frequency based on previous work, but the cutoff is global instead of relative to metapopulation allele frequency, i.e., it is required that the global frequency of an allele be more than or equal to 90%, but specific populations can have lower frequencies. Since the data itself already included metapopulation frequency, we applied a more rigurous filter and removed any alleles that where below 90% in any of the considered metapopulations. A full description of the methods to create these annotations can be obtained from the original paper [Kuhlwilm and Boeckx, 2019]. We also obtained the publicly avail-

able single-tissue cis-eQTLs eGene and significant variant-gene associations (v7) (https://gtexportal.org/home/datasets). We matched eQTL ID to rs IDs with a lookup table provided by GTEx. The chi-square goodness-to-fit and the Kolmogorov–Smirnov test were performed in R. To investigate the allelic consequences and the clinvar data we used the Biomart tool [Zerbino et al., 2018]. We performed the gene ontology cluster enrichment analysis with Metascape [Zhou et al., 2019] and the transcription factor enrichment with SNP2TFBS [Kumar et al., 2017]. We generated all the main figures with the ggplot2 R package [Wickham, 2009] and Circos [Krzywinski et al., 2009]. Supplementary Figure 1, 2 and 3 where generated by Metascape [Zhou et al., 2019], RegioneR [Gel et al., 2015] and SNP2TFBS [Kumar et al., 2017] respectively. The miRNA data was extracted from the supplementary tables 6 and 7 of Branco et al. [2018]. We used the supplementary table S5 from Racimo et al. [2014], the supplementary table S2 from Peyrégne et al. [2017] for the human selective sweep data, and the data from Sankararaman et al. [2016] for the deserts of introgression. The evolutionary clusters data comes from the supplementary table S20 of Zhu et al. [2018]. We performed the permutation test (n=1000) with the R package RegioneR [Gel et al., 2015].

Data acces

All raw data is publicly available as described in Methods. Processed single-tissue data and clinical data can be found here: https://github.com/AGMAndirko/GTEx_project and in the supplementary materials.

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Author Contributions

Conceptualization: CB & AA; Data Curation: AA; Formal Analysis: AA; Funding Acquisition: CB; Investigation: CB & AA; Methodology: CB & AA; Software: AA; Supervision: CB; Visualization: CB & AA; Writing — Original Draft Preparation: CB & AA; Writing — Review & Editing: CB & AA.

Disclosure declaration

The authors declare no conflict of interest.

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Competing interest

Authors declare NO competing financial or non-financial interest.

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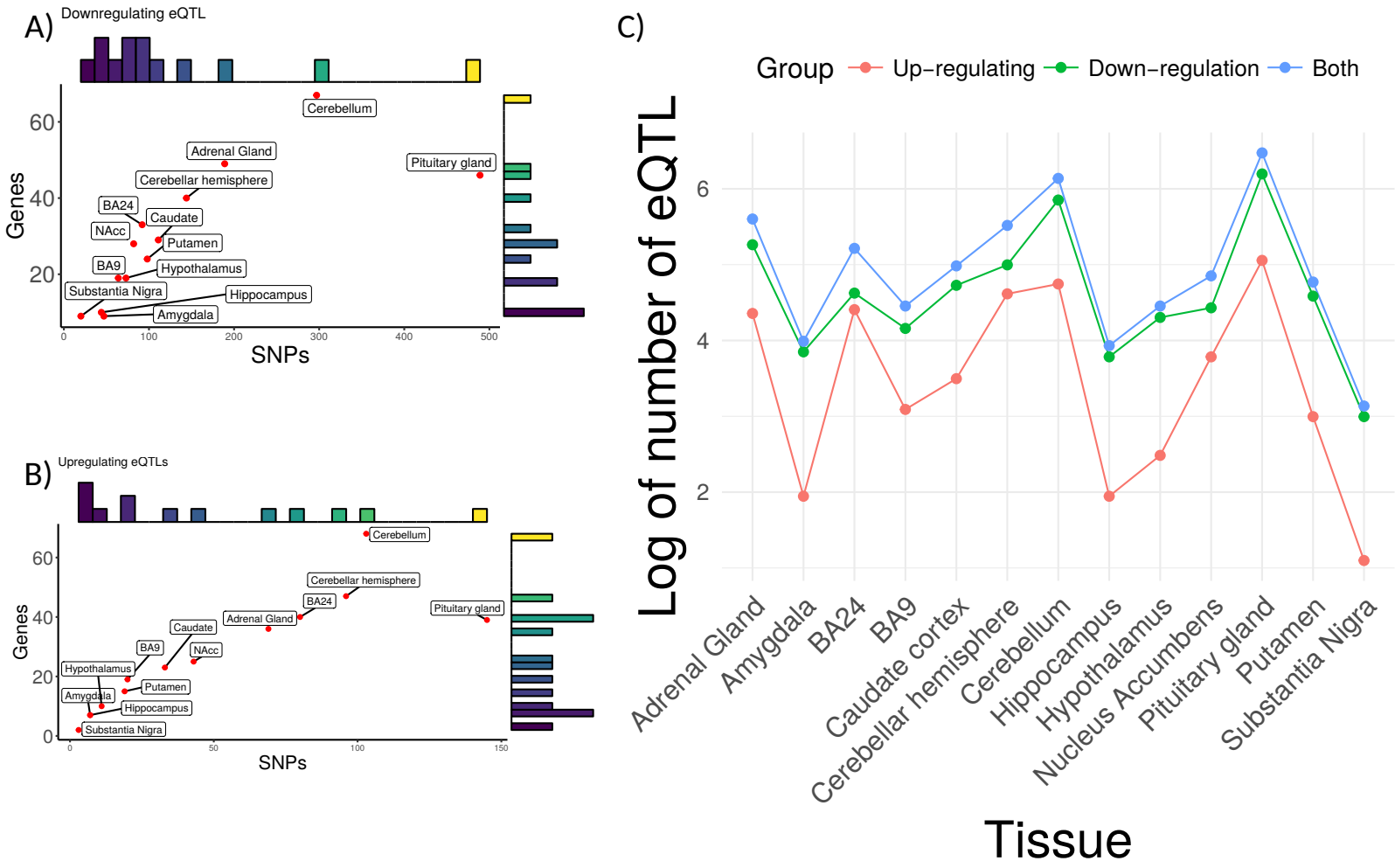

Figure 3: A) and B) Ratio of downregulating (A) and upregulating (B) eQTLs per genes affected in single tissues. C) Log value distribution in upregulating eQTL in human evolution, downregulating and total GTEx original data per tissue.

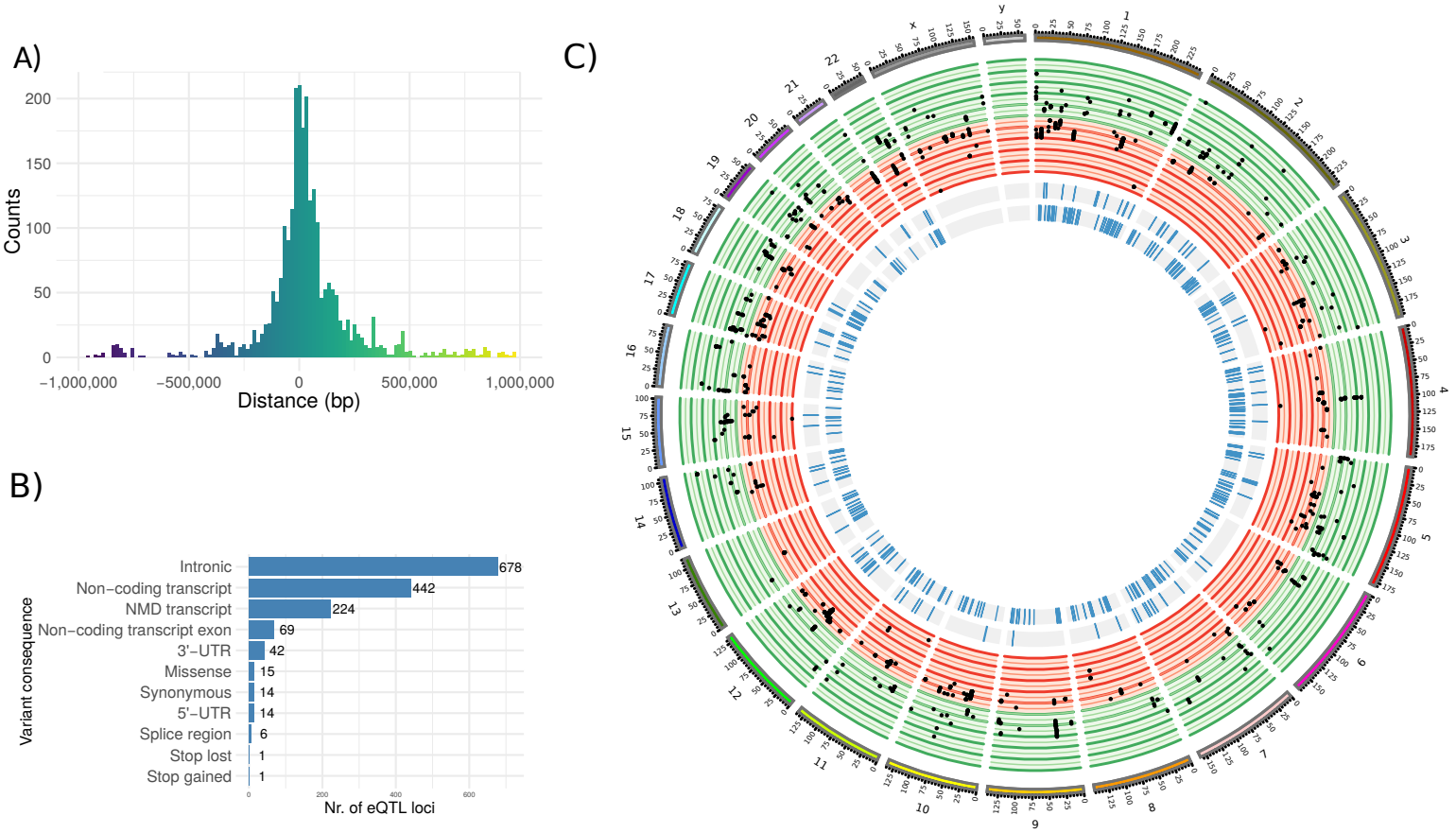

Figure 4: A) A bar plot showing the distribution of eQTL over distance to Transcription Starting Site B) Classification of the genetic consequences in our data C) A Circos plot showing the distribution along the genome of eQTLs. Each line denotes 0.5 steps in beta score (allele specific effects in gene expression), from 3 to -3. Red circles denote downregulation, green circles upregulation of eGenes. Inner rings, in blue: areas showing signals of positive selection relative to archaic humans in [Peyrégne et al., 2017] (innermost) and [Racimo et al., 2014] (outermost).