A signature of Neanderthal introgression on molecular mechanisms of environmental responses

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Abstract

Ancient human migrations led to the settlement of population groups in varied environmental contexts worldwide. The extent to which adaptation to local environments has shaped human genetic diversity is a longstanding question in human evolution. Recent studies have suggested that introgression of archaic alleles in the genome of modern humans may have contributed to adaptation to environmental pressures such as pathogen exposure. Functional genomic studies have demonstrated that variation in gene expression across individuals and in response to environmental perturbations is a main mechanism underlying complex trait variation. We considered gene expression response to in vitro treatments as a molecular phenotype to identify genes and regulatory variants that may have played an important role in adaptations to local environments. We investigated if Neanderthal introgression in the human genome may contribute to the transcriptional response to environmental perturbations. To this end we used eQTLs for genes differentially expressed in a panel of 52 cellular environments, resulting from 5 cell types and 26 treatments, including hormones, vitamins, drugs, and environmental contaminants. We found that SNPs with introgressed Neanderthal alleles (N-SNPs) disrupt binding of transcription factors important for environmental responses, including ionizing radiation and hypoxia, and for glucose metabolism. We identified an enrichment for N-SNPs among eQTLs for genes differentially expressed in response to 8 treatments, including glucocorticoids, caffeine, and vitamin D. Using Massively Parallel Reporter Assays (MPRA) data, we validated the regulatory function of 21 introgressed Neanderthal variants in the human genome, corresponding to 8 eQTLs regulating 15 genes that respond to environmental perturbations. These findings expand the set of environments where archaic introgression may have contributed to adaptations to local environments in modern humans and provide experimental validation for the regulatory function of introgressed variants.
Author summary

Humans have populated the entire world thus adapting to live in very different environments. Recent studies have suggested that the presence of Neanderthal DNA sequences in the genomes of modern humans contribute to our ability to respond to pathogens. Here we investigated whether the Neanderthal sequences present in modern human genomes also contribute to our ability to respond to environmental changes. We found that DNA sequences from Neanderthals modify the molecular mechanisms that regulate gene activity in different environments, including in response to stress hormones, caffeine, and vitamin D. We also found an important role of Neanderthal sequences in regulating sugar metabolism. Using experimental data, we provide evidence that Neanderthal sequences modify the activity of several genes in our genomes, including genes important for our ability to respond to a broad set of environmental stimuli.

Introduction

Studies of ancient DNA samples in recent years have discovered that interbreeding occurred between modern humans and our extinct relatives [1,2,3]. Approximately 2% of the genome in modern Europeans and Asians is the result of introgressed Neanderthal sequences [4]. However, Neanderthal ancestry is estimated to be 1.54x the genome average in genomic regions with the lowest density of functional elements. The uneven distribution of these introgressed sequences in the human genome suggests that natural selection may have acted to remove Neanderthal sequences that were deleterious for modern humans [5]. The presence of introgressed archaic sequences in the human genome, instead, raises the question of whether these sequences were maintained in the human genome because they have functional or even beneficial impact in modern humans [6–9]. Accordingly, Neanderthal introgressed haplotypes have been found to occur at higher frequency than expected by drift and to impact genes with important biological functions for human adaptations in different environments [10,11]. These include for example BNC2, a gene associated with skin pigmentation [5,12] and several genes with important immunological functions [13].

Gene regulatory variants in the human genome are associated with complex traits and disease risk, supporting the concept that these variants have important phenotypic consequences [14,15]. Identifying functional non-coding variants that regulate transcriptional processes poses severe challenges because we cannot directly predict function from sequence. Several approaches have been proposed to computationally predict the effect of a nucleotide change on transcription factor binding and gene expression, including methods that are based on sequence content only [16–18] and methods that integrate sequence content information with functional genomic data, like DNase-seq [19–21].

Recent studies have focused on understanding the role of Neanderthal introgression on gene regulation in humans using genetic variants which are associated with gene expression (expression quantitative trait loci, or eQTL) from the multi tissue GTEx consortium [22]. Two independent studies used eQTL mapping and allele-specific expression analysis, respectively, to show that a substantial number of Neanderthal introgressed haplotypes harbor regulatory variants in the human genome [23,24]. Introgressed haplotypes were found to have lower expression compared to modern haplotypes in most tissues [24]. In particular, brain and testis had lower expression of introgressed haplotypes, compared to other tissues. Given the humanspecific and reproductive functions of these tissues, these previous observations support the
hypothesis that Neanderthal introgression may have affected modern human fitness through gene regulation. These observations suggest that it is possible to analyze functional patterns of Neanderthal introgressed sequences to identify potential selection effects on overall pathways or molecular mechanisms.

Studies of genetic regulation of gene expression in cells exposed to pathogens confirmed a role for Neanderthal introgression in immune functions important for the response to bacterial and especially viral infections [25,26]. Adaptation to pathogen exposure is part of a broader set of signals detected in the human genome and interpreted as adaptations to local environments in human populations. In addition to pathogen exposures, human populations adapted to climate, diet and high altitude [27]. One of the limitations in studies of adaptations to local environments is the difficulty to collect and utilize data on past environmental exposures.

More generally, dissecting the complex environment that we are exposed to today and in the past is a difficult task that requires some necessary simplifications. For example, population genetics studies have summarized past environments with latitude or historical climate and subsistence data [28,29]; while epidemiological studies generally focus on current measurable lifestyle environments, such as smoking and drinking. An alternative approach to study genetic variants that modulate the response to environmental changes is through in vitro systems. Though a simplified version of organismal environments, cultured primary cells exposed to controlled treatments have been pivotal in identifying genetic variants that regulate the response to a variety of different exposures including drugs, hormones, pathogens and other chemical stimuli. We recently demonstrated that this approach can be scaled to investigate hundreds of cellular environments, defined as the combination of specific cell types exposed to different treatments [30]. Approximately 50% of the genes under genetic regulation of transcriptional response are also associated with complex traits variation in humans, thus confirming that the molecular phenotypes measured in this experimental setting (gene expression responses) are important mechanisms in human complex traits variation [30].

Here we used this approach to investigate the role of Neanderthal introgression in modern human response to environmental perturbations. We use a functional annotation of regulatory variants that disrupt transcription factor binding, to predict the regulatory function of Neanderthal introgressed alleles and provide an experimental validation of their regulatory function in human cells (Fig 1).

**Results**

**Role of Neanderthal introgressed variants in transcription factor binding and gene regulation**

The availability of large amounts of genetic data from human populations and of a variety of different functional annotations makes it possible to explore several unresolved questions on the role of Neanderthal introgression in human history. To analyze the contribution of Neanderthal introgression to modern human response to environmental perturbations, we first refined the definition of introgressed Neanderthal variants. Specifically, we considered variants in previously defined introgressed regions [2] and also present in 2 of 3 high-quality Neanderthal genomes [31–33], but absent in all African samples from the 1000 Genomes project (S1 Fig). We annotated 177,878 SNPs from the 1000 Genomes project [34] as Neanderthal introgressed variants (N-SNPs).

One of the main mechanisms used by human cells to respond to environmental perturbations is through transcriptional changes, which are largely the result of changes in transcription factor binding. To investigate the role of Neanderthal introgression in this cellular response, we focused on N-SNPs that are more likely to have a regulatory function.
Specifically, we considered N-SNPs that are in active footprints for transcription factors. To annotate these N-SNPs, we considered the catalog of 1000 Genomes SNPs in active transcription factor footprints generated by [21] using the DNase-seq data from ENCODE and the RoadMap Epigenomics. This catalog annotates 5.8 million SNPs in active transcription factor footprints across 153 tissues and 1,372 motifs. We identified 58,967 N-SNP-motif pairs, corresponding to 27,349 unique N-SNPs in active footprints for a total of 1,255 unique transcription factor motifs. For each transcription factor, we investigated the predicted effect of N-SNPs on binding across all the binding sites in the genome. When considering all N-SNPs in transcription factor binding sites, we found that 19,924 N-SNPs (73%) were computationally predicted to alter binding (centiSNPs), which was significantly higher than the 66% of all SNPs which are centiSNPs. The Neanderthal allele is predicted to decrease binding in 48% of the cases, increase binding in 44% of cases, and both increase and decrease binding depending on the transcription factor in 8% of cases (Fig 2A). N-SNPs in footprints are more likely to disrupt transcription factor binding (centiSNPs) than non N-SNPs (positive enrichment), with a significant enrichment for 434 transcription factor motifs (BH-FDR < 0.05; range of enrichment odds ratios: [1.5, 67.7]) out of 435 that have at least 10 N-SNPs in footprints. Neanderthal introgressed variants may be absent in binding sites for specific transcription factors that are important for modern human-specific functions. To investigate whether any transcription factor is depleted of N-SNPs in footprints, we considered all TFs with at least 100 SNPs in footprints, regardless of the number of N-SNPs. None of the factors considered showed a significant depletion of N-SNPs in footprints.

Several motifs for transcription factors activated in response to various environmental perturbations were among the most enriched in N-centiSNPs genome-wide. ARNT, which is the most enriched motif, is involved in the metabolism of chemical substances that are not produced by the organism (xenobiotic metabolism) and is also a cofactor for hypoxia-inducible factor 1 and NFAT3 (NFATC4) belongs to a family of factors important for T cell activation [35]. SWI5 is a DNA repair protein involved in cellular response to ionizing radiation. Finally,
AML1, also known as RUNX1 is involved in hair follicle development and epithelial cancer development. We also found enrichment in footprints for activator protein 1 (AP1), which is known to interact with the glucocorticoid receptor in mediating the response to stress, and for multiple transcription factors (c-Jun, ATF2, and Elk1 [36–38]) that are downstream of the JNK signaling pathway, which is often induced by environmental stress.

The top 15 enriched motifs (OR > 38, S1 Table) also include factors associated with glucose metabolism. For example, MAFA activates expression of the insulin gene (INS) [39]. Mutations in this gene result in the autosomal dominant disease Insulinomatosis and Diabetes Mellitus [40]. Furthermore, when we considered 37 transcription factors that mediate the response to glucose and/or insulin [41,42], 16 (43%) were enriched for N-centiSNPs in their footprints genome-wide, suggesting a potential contribution of Neanderthal introgression to modern human metabolism. To further investigate the regulatory function of N-SNPs, we considered a dataset of allele-specific binding for SNPs associated with Type 2 Diabetes risk and assayed through SNP-SELEX [43]. One advantage of this method over other experimental assays is that allele-specific effects are measured in vitro, outside of a cellular context, therefore are independent of any trans-effects and the identity of the transcription factor is known. SNP-SELEX provides a preferential binding score (PBS), which compares the relative binding affinity of two alleles for a specific transcription factor. 15,431 N-SNPs were assayed by

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**Fig 2.** N-SNPs in transcription factor binding sites (A) N-SNPs were annotated in transcription factor binding sites using the centiSNPs annotation, which includes a computational prediction of the SNP’s effect on transcription factor binding. The percentage of N-SNPs in each prediction category is shown in the pie chart. (B) Histogram of enrichments of N-SNPs that disrupt transcription factor binding compared to non N-SNPs for 435 transcription factors. (C) Phenotypic relevance of the top transcription factor motifs enriched for N-centiSNPs in their binding sites and that are involved in environmental responses. {TC "2 N-SNPs in transcription factor binding sites (A) N-SNPs were annotated in transcription factor binding sites using the centiSNPs annotation, which includes a computational prediction of the SNP’s effect on transcription factor binding. The percentage of N-SNPs in each prediction category is shown in the pie chart. (B) Histogram of enrichments of N-SNPs that disrupt transcription factor binding compared to non N-SNPs for 435 transcription factors. (C) Phenotypic relevance of the top transcription factor motifs enriched for N-centiSNPs in their binding sites and that are involved in environmental responses. " ".[43].

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SNP-SELEX across 270 factors. We considered significant PBS values, using a cut-off of \( p < 0.05 \). There were 505 N-SNPs that had significant PBS values. We conducted an enrichment analysis via Fisher’s exact test and found that N-SNPs were more likely to have a significant PBS, thus affecting binding to a transcription factor, when compared to non-N-SNPs in the SNP-SELEX data set (OR = 1.105626, \( p = 0.03861 \)). We investigated if the distribution of PBS was different between the N-SNPs and non-N-SNPs. We used the Kolmogorov-Smirnov test and found that the distribution of PBS for non-N-SNPs was significantly different from the distribution of PBS for N-SNPs (\( D = 0.011458, p = 0.03614 \)). We then repeated the Kolmogorov-Smirnov test for every transcription factor tested both computationally and in SNP-SELEX to find if the directional effects on binding are similar or different between N-SNPs and non-N-SNPs. We filtered our results for an adjusted \( p \)-value less than 0.1 and found 12 factors with significantly different PBS distribution between N-SNPs and non-N-SNPs: for EGR4, ELF2, ETV2, IRF5, POU2F3, ZBTB26, and ZNF740 the Neanderthal allele decreased binding, while for HOXA6, IRF8, PAX2, TGFIF2LX, and ZNF821 the Neanderthal allele increased binding (S3 Fig). We compared our N-centricSNP predictions for the ELF2 transcription factor to the experimental SELEX PBS score and found that both indicated decreased binding for the introgressed variants. Lastly, we conducted a literature search to investigate the potential biological effects of the N-SNPs that alter TF binding in the SNP-SELEX data. Because the variants within the SNP-SELEX data are cis-regulatory variants near type-2 diabetes risk loci, many of the transcription factors were associated with related processes, such as insulin and glucose metabolism or adipogenesis. Some notable examples include IRF5, which when deficient can cause increased insulin sensitivity in obese mice [44]. We found that the Neanderthal allele decreased binding for this transcription factor, which may mean Neanderthals experienced enhanced insulin sensitivity. Another example is PAX2, which may upregulate expression of the glucagon gene [45]. The Neanderthal allele had increased binding for PAX2, indicating Neanderthals may have increased glucagon gene expression.

Overall our results support previous findings that Neanderthal introgression may play a role in the immune response, but importantly also suggest a novel role in regulatory mechanisms for the response to broader environmental stress, and for glucose metabolism.

**Neanderthal introgression regulates the transcriptional response to environmental perturbations**

Previous studies have shown that Neanderthal alleles play an important role in human gene regulation [23,24]. To directly investigate whether Neanderthal alleles regulate the expression of genes that respond to environmental perturbations, we considered expression quantitative trait loci (eQTLs) from the GTEx database and genes differentially expressed in a panel of 52 cellular environments, resulting from 5 cell types and 26 treatments, including hormones, vitamins, drugs, and environmental contaminants [30]. We focused on eQTLs for genes differentially expressed in each cellular environment considered. Individuals carrying a high expression genotype at an eQTL for a gene that responds to a specific environmental stimulus will have an overall greater response compared to individuals with a low expression genotype in the same environment (Fig 3A). Overlapping our N-SNPs catalog with the GTEx database, we found that 30,464 GTEx eQTLs (16,065 unique SNPs) in any tissue are N-SNPs. When we considered the set of differential expressed genes in response to treatments, we found that 27,789 N-SNPs are eQTLs for genes differentially expressed in at least one condition, corresponding to 15,026 unique SNPs and 1,629 unique genes (S2 Table). For 8 cellular environments we identified a significant enrichment for N-SNP eQTLs in genes differentially expressed, compared to genes that do not respond to the treatment considered.
Among these differentially expressed genes regulated by introgressed alleles, we found 239 genes that respond to dexamethasone, a synthetic glucocorticoid that regulates gene expression through activation of the glucocorticoid receptor (GR). The GR is ubiquitously expressed and glucocorticoids regulate different key biological processes depending on the specific body site. For example, in immune cells glucocorticoids act as immunosuppressors to prevent systemic inflammation and sepsis, while in vascular endothelial cells, glucocorticoids regulate angiogenesis and vascular remodeling. Because of the anti-inflammatory function of glucocorticoids, it is likely that the observed enrichment captures the same signature of Neanderthal introgression on transcriptional regulation of immune response, which was reported in other studies [26, 25]. For example, we found a N-eQTL for the gene RAPGEF3, which encodes for a Ras GTPase. This gene is downregulated in response to dexamethasone in PBMCs and was also among the infection eQTLs identified in monocytes treated with the influenza A virus [26].

We observed a 1.2 fold enrichment for N-SNPs that regulate Vitamin D (1,435 genes). Vitamin D synthesis depends on UV exposure, with important trade-offs between UV exposure and skin pigmentation and its effect on Vitamin D production. Our observation suggests a role for Neanderthal introgression in the response to UV exposure however the specific adaptive phenotype remains to be defined. For example, BNC2 is downregulated in response to Vitamin D in melanocytes, but upregulated in PBMCs and vascular...
endothelial cells. In our analysis we found that rs2288634 is a N-eQTL that regulates expression of ATP1B3, a gene down-regulated in response to Vitamin D in PBMCs. This N-eQTL is also associated with hair color in the GWAS catalog. Overall we found a total of 66 N-eQTLs for genes that respond to environmental stimuli that are also significantly associated with a complex trait in the GWAS catalog (S3 Table).

Adaptive introgression of N-SNPs

It is well known that more introgressed segments of Neanderthal DNA have been removed by purifying selection in genomic regions with high selective constraint [46]. Less selective constraint could explain the patterns observed in this study, in terms of excess of N-SNPs predicted to alter TF binding sites as well as N-eQTLs for responding genes. For example, genes with a transcriptional response to environmental perturbations could be less constrained, therefore more likely to harbor introgressed regulatory N-SNPs. However, the set of N-SNPs considered in this study have similar levels of background selection (KS test $p > 0.05$), as measured by the B-statistic from McVicker et al. [47], thus indicating that the observed enrichments are not due to relaxation of selective constraint, but may suggest adaptive introgression in gene regulation (S5 Fig). Selective pressures on introgressed Neanderthal variation can leave a genomic signature indicative of adaptive introgression. To test for possibility of Neanderthal adaptive introgression at the N-eQTLs in Europeans, we computed summary statistics that have been demonstrated to capture the signature of adaptive introgression, including the RD statistic, the Q95 statistic, and the U20 statistic [8]. The RD statistic is defined as the average ratio of the sequence divergence between an individual from the source population and an individual from the admixed population, and the sequence divergence between an individual from the source population and an individual from the non-admixed population. If a region of the genome is adaptively introgressed into a non-African population, the sequence divergence between Neanderthals and non-African populations should be less than the sequence divergence between Neanderthals and African populations, and, as a result, RD would be small. We identified 6,403 N-SNPs in windows that were significant for the RD statistics (lowest 5% of RD values genome-wide), corresponding to 397 genes (S2A Fig). On average, 23.6% of differentially expressed genes in response to all treatments are regulated by an N-SNP in a region with an adaptive introgression signal via RD statistic. For each treatment, the number of genes in regions with evidence of adaptive introgression from the RD statistics is proportional to the number of differentially expressed genes regulated by a N-eQTL (S6 Fig). The U20 statistic is based on allele frequency in the recipient and donor population and identified adaptive introgression variants based on their elevated allele frequency in the recipient population (>20%) compared to the donor population. 6,281 SNPs were in regions of adaptive introgression identified via the U20 statistic, corresponding to 296 genes (S2B Fig). On average, 17.9% of differentially expressed genes in response to all treatments are regulated by an N-SNP in a region with a significant U20 statistic. We also computed the Q95 statistics, that considers “high-frequency” archaic alleles, and represents a summary statistic of the site-frequency spectrum in the recipient population. This statistic should be high when a region contains many alleles at especially high frequencies in the recipient population. 6,917 SNPs had a signal of adaptive introgression (highest 5% of Q95 statistic genome-wide) via the Q95 statistic, corresponding to 408 genes (S2C Fig). On average, 25.4% of differentially expressed genes in response to all treatments are regulated by an N-SNP in a region with an adaptive introgression signal via Q95 statistic.

To identify signatures of adaptive introgression, we considered genomic windows that exceed the critical values on all three adaptive introgression summary statistics (the most
We therefore identified 1000 N-SNPs in windows that exceed the critical values on all three adaptive introgression summary statistics, hence candidates of adaptive introgression. Of these adaptive introgressed N-SNPs, 384 are N-eQTLs and 363 regulate genes that respond to treatments, for a total of 132 genes (Fig 4A). The proportion of genes regulated by N-eQTLs in regions of adaptive introgression is similar across all treatments (7.5% on average, Fig 4B) and it is not significantly different for genes that do not respond to a treatment.

Experimental validation of Neanderthal introgressed alleles

Association studies, including eQTLs, are limited in their ability to identify true causal variants in the region of association with the trait of interest. Even considering the lead eQTL for each eGene does not ensure that the true causal variant is being considered. Massively parallel reporter assays (MPRAs) can be used to test the gene regulatory function of DNA sequences carrying individual variants by transfecting them in human cells as part of reporter gene plasmids. To validate N-SNPs that regulate gene expression response, we used BiT-STARR-seq, an MPRA we recently developed to test allele-specific regulatory function for tens of thousands of centiSNPs. We identified 21 N-SNPs (9.3%) with allele-specific regulatory function (BH-FDR < 0.1) when considering the 226 N-SNPs included in the library of variants used in [48] (Fig 5A, Table 1 and S4 Table). This validation rate was expected and was not significantly different than the validation rate in the original study, which did not distinguish between N-SNPs and modern SNPs (2,720 out of 43,500, 6.2%). Interestingly, the Neanderthal allele led to increased gene expression for 20 of the 21 N-SNPs, which was a significantly different
pattern compared to modern human alleles (binomial test). This result could not be explained by differences in background selection between genomic regions harboring experimentally validated compared to non-validated N-SNPs (S4 Fig). Of these 21 N-SNPs, 13 are centiSNPs, 8 are eQTLs in GTEx and 5 are infection eQTLs in a study of macrophages exposed to listeria and salmonella [25]. The N-centiSNP rs4784812 is in a region with significant evidence for adaptive introgression based on both the RD and the Q95 statistics (RD p-value = 0.046, Q95 p-value = 0.040). The Neanderthal allele at rs4784812 is predicted to alter binding for six transcription factors (NF-Y, CCAAT box, ELK4, or with an ETS DNA binding domain).

We then investigated whether the 8 N-SNPs GTEx eQTLs regulate genes that respond to environmental perturbations. We found that these genes respond to treatments and each of them is differentially expressed in response to several different treatments, as shown in Fig 5B and 5C. While this set of 8 eQTLs is not a random sample of regulatory N-SNPs, nevertheless,
each of the genes they regulate is likely to be central to the cellular response to multiple environmental perturbations, rather than to specific stimuli.

Three notable examples are rs72773986, rs28514987 and rs4362387. rs72773986 is an infection eQTL and a GTEx eQTL for ERAP2, and is predicted to alter binding of EPAS1, a key transcription factor for response to hypoxia. ERAP2 encodes a zinc metalloaminopeptidase involved in antigenic response and polymorphisms at this locus are responsible for differential response to bacterial infection and influenza [49]. ERAP2 was also associated with susceptibility to Crohn’s disease [50]. rs28514987 is in a region with significant signals of adaptive introgression by U20 and Q95 statistics (U20 p-value = 0.022, Q95 p-value = 0.031). It is also a GTEx eQTL for BRSK2 and is in a bZIP911 binding site. BRSK2 is expressed in pancreatic islets and negatively regulates insulin secretion [51]. rs4362387 is located within an AP1 binding site and is a GTEx eQTL for SPIRE2 and 6 additional genes in the region. This genomic region also contains the MC1R gene, which is implicated in skin and hair pigmentation and rs4362387 is also an eQTL also for MC1R. The expression of both SPIRE2 and MC1R is highly upregulated by Vitamin D (fold change = 2.6–2.7, FDR = 0) and likely share regulatory elements. In GTEx, a strong eQTL effect (both in the single tissue and multi-tissue analysis) is observed in the skin tissues, where the T allele (Neanderthal allele) is associated with higher expression of both genes (Skin sun exposed; SPIRE2: normalized effect size: 0.52; MC1R:

Table 1. N-SNPs validated by MPRA. Columns 1–12 are: 1) rsID; 2) Chromosome; 3) Position; 4) Modern human allele; 5) Neanderthal allele; 6) Allele frequency among all 1000 Genomes populations; 7) RD p-value; 8) U20 p-value; 9) Q95 p-value; 10) transcription factor motif name; 11) GTEx gene name; 12) Infection eQTL gene name.

| rsID   | chr | pos  | MH     | Nean | AF     | RD    | U20 | Q95 | TF Name           | GTEx gene name | iEQTL gene name |
|--------|-----|------|--------|------|--------|-------|-----|-----|--------------------|----------------|-----------------|
| rs79244900 | 1   | 234041317 | C     | T    | 0.059 | 0.363 | 1   | 0.040 | ATF5,ATF2c-Jun,CREB,DREB1B,CREM | NA             | NA              |
| rs146121273 | 15  | 52436165 | A     | C    | 0.017 | 0.548 | 1   | 0.418 | Elk-1,Ets,NUR77,PPARA | NA             | NA              |
| rs79097783 | 21  | 44229777 | T     | G    | 0.027 | 0.128 | 1   | 0.100 | NA                 | NA             | NA              |
| rs17178611 | 21  | 44229685 | A     | G    | 0.027 | 0.128 | 1   | 0.100 | NA                 | NA             | NA              |
| rs75522770 | 21  | 44268431 | G     | A    | 0.026 | 0.096 | 1   | 0.132 | NA                 | NA             | NA              |
| rs72785456 | 12  | 11309644 | A     | G    | 0.016 | 0.088 | 1   | 0.123 | NA                 | NA             | NA              |
| rs17431446 | 12  | 120884291 | C     | T    | 0.041 | 0.450 | 1   | 0.123 | StuAp,Pax-3,AML1   | NA             | NA              |
| rs4362387  | 16  | 90075226 | C     | T    | 0.057 | NA    | NA  | NA  | STB4,AP1           | DEF8,SPIRE2,ZNF276, DBNDD1,AFL3L1P,TCF25 | NA              |
| rs41390949 | 16  | 57447951 | G     | A    | 0.050 | 0.190 | 1   | 0.164 | NA                 | NA             | NA              |
| rs74813334 | 16  | 57298926 | G     | T    | 0.110 | 0.270 | 1   | 0.123 | NA                 | PLLP,RSPRY1    | NA              |
| rs60403102 | 16  | 57297312 | G     | A    | 0.114 | 0.270 | 1   | 0.123 | NA                 | RSPRY1,PLLPP   | NA              |
| rs78951230 | 9   | 85198625 | G     | A    | 0.028 | 0.894 | 1   | 0.152 | NA                 | NA             | NA              |
| rs28514987 | 11  | 1487024  | C     | G    | 0.049 | 0.146 | 0.022 | 0.031 | bZIP911           | MOB2,BRSK2     | MOB2            |
| rs72773986 | 5   | 96159717 | G     | A    | 0.070 | 0.207 | 1   | 0.062 | HIF-2alpha         | AC008906,1,ERAP1,CAST, ERAP2 | ERAP2          |
| rs72786879 | 16  | 57410998 | G     | A    | 0.064 | 0.640 | 1   | 0.164 | NA                 | NA             | NA              |
| rs76730157 | 8   | 123793459 | G    | A    | 0.021 | 0.196 | 1   | 0.272 | StuAp,ZF5,MBP1     | NA             | NA              |
| rs55931338 | 1   | 3321941  | A     | G    | 0.018 | 0.738 | 1   | 0.272 | GBP,TAF-1,GFB,E4F1,CPRF-3,TGA1b, GBF1 | NA         | NA              |
| rs4784812 | 16  | 57536204 | A     | C    | 0.081 | 0.040 | 1   | 0.046 | NF-Y,CCTAT box,c-ets-1 (ets1),ELK4, /EMBO10/Fhl1, /EMBO10/Gm4881 | NA         | NA              |
| rs77060867 | 16  | 57298163 | G     | A    | 0.110 | 0.270 | 1   | 0.123 | NA                 | PLLP,RSPRY1    | NA              |
| rs187433595 | 11  | 119205315 | T     | C    | 0.005 | 0.710 | 1   | 0.418 | GABP-alpha         | NA             | NA              |
| rs77417541 | 9   | 93660685 | C     | T    | 0.044 | 0.406 | 1   | 0.176 | LBP-1              | NA             | NA              |

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In the MPRA data, the Neanderthal allele increases expression of the reporter gene, thus showing the same direction of effect as the one observed in the eQTL data, and is also predicted to alter API binding.

Discussion

In vitro studies allow researchers to analyze molecular phenotypes in specific environments. While these environments are a simplified version of organismal exposures, they have two key advantages: they are tightly controlled and can simulate environments that are difficult to measure in vivo, including ancient environments. To directly investigate whether Neanderthal introgression may contribute to modern humans’ ability to respond to a wider range of stressors, beyond pathogens, we used data on transcriptional response in 35 cellular environments that reflect common individual exposures, including dietary components, environmental contaminants, metal ions, over the counter drugs, and hormonal signaling. While some of these compounds clearly represent very recent exposures in contemporary human history, the underlying hypothesis is that these compounds target existing response mechanisms. For example, endocrine disruptors, such as BPA, which are commonly found in plastics, target the estrogen response pathway [52]. Our approach combines functional genomics and paleogenomics, identifying Neanderthal introgressed variants which may have gene regulatory function. We hypothesized that Neanderthal introgression may have contributed to adaptations to environmental changes by introducing regulatory variants that modify binding of specific transcription factors to a large number of downstream targets. When considering human transcription factors, variation in the binding sites is more common than non-synonymous variants in the transcription factor genes themselves. This is in line with the idea that changes in the function of master regulators would have large pleiotropic effects [53]. Variation in transcription factor binding sites, instead, introduces specific functional consequences but preserves unaltered the overall function of the transcription factor [54,55]. Using computational predictions and experimental data on eQTLs and transcriptional response, we assigned a putative function to 33,248 variants which are likely to contribute to modern human response to the environment. For 21 N-eQTLs we validated their ability to modify gene expression in massively parallel reporter gene assays.

While most studies of archaic introgression in the genome of modern humans have focused on non-African populations, recent results from Chen et al [56] indicated the presence of Neanderthal haplotypes in sub-Saharan African populations due to back migration from non-Africans. Our set of N-SNPs excludes variants where the Neanderthal allele is present in African populations, potentially missing Neanderthal alleles re-introduced in sub-Saharan African genomes. It is thus possible that functionally important N-SNPs have been missed in the present study due to our inclusion criteria. Indeed, when considering N-SNPs that are polymorphic in African populations, we identified 1919 (out of 6,884) that are eQTLs and regulate a total of 1158 genes. Of these genes, 654 are regulated by N-SNPs found in sub-Saharan African populations and would be missed if considering only N-SNPs found in non-Africans. The BiT-STARR assay included 61 of the N-SNPs potentially introgressed in sub-Saharan African populations, 7 of which showed allele-specific regulatory function (S5 Table). While further studies are needed to fully understand the impact of archaic introgression introduced by back-migration to Africa, our results suggest a regulatory function for some of these N-SNPs present in African genomes.

While several studies have highlighted a potential contribution of archaic introgression to human phenotypes, the molecular evidence in most cases is suggestive and does not provide experimental data in support of a putative molecular mechanism linking archaic alleles to...
specific functions. Assigning a function to non-coding variants is a daunting task, which is further complicated by the limited availability of archaic genomes and the inability to directly measure archaic molecular phenotypes [57]. By focusing directly on introgressed alleles and considering fine scale computational predictions, here we have provided an annotation of Neanderthal introgressed alleles at single nucleotide resolution. One advantage of this annotation is that it pinpoints transcription factors whose function is likely to be affected by these archaic variants. For 434 transcription factor motifs Neanderthal introgressed alleles are predicted to modify DNA binding. These putative molecular mechanisms represent ideal candidates for follow-up studies focused on specific transcription factors and/or variants.

Another advantage of focusing on the specific introgressed nucleotides within a full haplotype is the ability to directly test and validate the gene regulatory function of these non-coding variants. Massively parallel reporter assays have been successful in identifying new regulatory variants and validating computational predictions of regulatory elements and variants [58–63]. One key advantage of this approach is the ability to test tens of thousands of variants in parallel because the assays use a library of plasmid that can be designed to investigate a catalog of variants of interest. At the same time, these assays cannot test genetic variants within their native chromatin context and by design are unable to test long distance interactions. As a consequence, additional introgressed variants that we were not able to validate in this study may indeed regulate gene expression in other environmental contexts. Nevertheless, we provide a proof of concept that applying computational and experimental functional genomics approaches enables researchers to assay the gene regulatory function of archaic alleles in modern human cells. Recently, MPRA data have been used to show that ancestral alleles reintroduced by Neanderthal introgression have activity levels similar to non-introgressed variants, while Neanderthal derived alleles are depleted for regulatory activity compared to reintroduced ancestral alleles [64]. Interestingly, in the dataset we considered, 95% of the functionally validated Neanderthal alleles led to increased gene expression. If future studies show this to be the case for other introgressed non-coding variants, it would suggest that variants that disrupt function at the gene regulatory level were not tolerated by modern humans and therefore are less likely to be introgressed in our genomes.

One key result of our study is the finding that Neanderthal introgression may contribute not only to modern human’s ability to respond to pathogens, as previously reported, but likely contributes to our ability to adapt and respond to a broader set of environmental challenges. We discovered that introgressed alleles are enriched in binding sites for transcription factors that regulate response to environmental stressors, including hypoxia and ionizing radiation, and also for transcription factors involved in glucose metabolism. These do not seem to be incidental findings, as they find support in the results from our complementary approaches. For example, For example, we found an enrichment for Neanderthal alleles that disrupt binding for 16 transcription factors involved in the response to glucose and insulin. Additionally, using SNP-SELEX data targeting SNPs involved in glucose metabolism and T2D risk, we identified sets of transcription factors where Neanderthal introgression increases or decreases binding with likely consequences for glucose and insulin metabolism. The transcription factors include IRF5 and PAX2, which are involved in insulin sensitivity and glucagon gene expression respectively. One of the N-eQTLs functionally validated in the BiT-STARR-seq assay regulates the expression of BRSK2, a gene that plays a role in the regulation of insulin secretion in response to elevated glucose levels [51]. This N-eQTL is in a region of adaptive introgression, characterized by significant U20 and Q95 statistics.

Several studies in the last decade have identified genetic variants that modify how cells respond to environmental perturbations. These molecular gene-environment interactions have shown that genetic variants have varying effects on gene expression depending on the
specific context considered and that these genetic effects are also important for complex trait variation in humans [25,26,65–76]. The majority of these studies have focused on the response to pathogens thus there is limited availability of eQTL datasets to investigate the role of Neanderthal introgression on GxE in modern humans. However, the response to environmental changes depends not only on interaction effects but also on additive effects between environmental variables and genotypes. In other words, an individual carrying a high expression genotype at an eQTL for a gene that responds to a specific environmental stimulus, will have an overall greater response than an individual with a low expression genotype in the same environment. This is because the gene is induced and consequently the genetic effect is amplified [77]. The observed enrichment of N-eQTLs for responding genes suggests a contribution of Neanderthal introgression to modern humans’ transcriptional response to treatments that represent the in vitro counterpart for common exposures, some of which may have represented a selective pressure during human history. Some examples include Vitamin D (a proxy for solar radiation), aldosterone (the main hormone regulating fluid balance), tunicamycin (endoplasmic reticulum stress agent), and glucocorticoids, which exert an anti-inflammatory function in the human body. Glucocorticoids are regulated by the hypothalamus-pituitary-adrenal axis in response to stress, thus representing a molecular connection between stress and immune function.

We also found that genes responding to vitamin D, which is produced in response to solar radiation [78], are enriched for Neanderthal eQTLs (OR = 1.2, FDR). Previous studies suggested that Neanderthal introgressed haplotypes contribute some of the genetic variation underlying the red hair phenotype in modern humans [23,79]. A non-synonymous variant in the MC1R gene was identified in a Neanderthal genome but was not seen in subsequently sequenced individuals, thus suggesting that it may represent a polymorphic site in Neanderthal populations. The N-eQTL we functionally validated is significantly associated with red hair in the UK Biobank data () and is also an eQTL for MC1R and SPIRE, two genes that are differentially expressed in response to Vitamin D. In transcriptome wide association studies, both genes are associated with skin and hair pigmentation phenotypes, including red hair color and childhood sunburn [80] (Fig 5D). While it is difficult to pinpoint a specific phenotypic effect for each of the introgressed sequences, it is likely that the most relevant environmental stressor is solar radiation. Overall, our results raise the possibility that multiple alleles and mechanisms underlie the red hair phenotype not only in humans but also in Neanderthals. As more response eQTL datasets become available, they will enable further analysis of the contribution of Neanderthal and other archaic humans’ introgression to gene expression response in modern humans, including for example estimating the portion of gene expression response heritability that can be attributed to archaic introgression.

With increasing availability of ancient DNA sequences, combining paleogenomic and functional genomic tools will allow researchers to explore in greatest depth the functional differences and similarities of modern humans from our extinct relatives. Together with other recent studies, we have demonstrated the value of taking these complementary approaches. Our results uncovered a putative role of Neanderthal introgression to modern humans’ ability to adapt and respond to environmental changes, with potential consequences for glucose metabolism and response to solar radiation.

**Methods**

**Identification of introgressed Neanderthal variants**

We determined a list of putative Neanderthal introgressed SNPs by screening the biallelic SNPs from the 1000 genomes project (1KGP) [34] based on three criteria. The conservative
criteria address the notions that Neanderthal introgression occurred outside of Africa and that the Neanderthal-introgressed haplotypes are not observed at certain regions of the human genome. Briefly, the criteria for a Neanderthal-introgressed SNP are: 1) the SNP is within the genomic regions reported to harbor the Neanderthal introgressed haplotypes [2], we used the combined regions from all the populations tested including Europeans, East Asians, South Asians, and Melanisians. We intended to be more generous when defining the Neanderthal introgressed regions to establish a more general input for samples with different ancestry, and compensate this relaxed stringency with conservative screening steps described below; 2) the SNP is confidently genotyped in at least two of the three high coverage Neanderthal genomes available [31–33] and is fixed among the genotyped alleles; 3) the SNP has all of the 504 sub-Saharan Africans (ESN, GWD, LWK, MSL, YRI) in 1KGP fixed for an allele alternative to the Neanderthal one, and is polymorphic among the non-Africans (present in at least one individual). The rationale of these criteria is to address the notion that much of the Neanderthal introgression occurred outside of Africa. We found a total of 186,871 SNPs that pass these criteria for further analysis. We further removed structural variants and multi-allelic SNPs to end up with 177,578 N-SNPs.

As illustrated by Chen et al [56], sub-Saharan African populations contain Neanderthal introgressed sequences likely due to migration from ancestral Europeans back to Africa. Our requirement that the modern human allele be fixed in sub-Saharan African populations filtered out 6,884 SNPs which could potentially be Neanderthal in origin. As a result, we analyzed these SNPs separately to determine the number which regulate differentially expressed genes and which were validated by BiT-STARR-seq, as described below.

N-SNPs in transcription factor binding sites

We annotated N-SNPs present in transcription factor binding sites using the centiSNP annotation from Moyerbrailean et al [21]. Specifically, we used the centiSNPs Compendium (http://genome.grid.wayne.edu/centisnps/compendium/) which summarizes all 1000 Genomes Project SNPs in 1,372 transcription factor motifs across 153 tissues and estimates the likelihood that a SNP disrupts transcription factor binding. N-SNPs were located within 1,255 transcription factor motifs. N-centiSNPs are defined as those that are predicted to alter binding of at least one transcription factor. For each of the 435 transcription factor motifs with at least 10 N-SNPs within their binding sites, we performed Fisher’s exact test to determine whether N-SNPs in transcription factor binding sites were more likely to disrupt transcription factor binding than non-introgressed SNPs within transcription factor binding sites. Multiple-test correction was performed using the Benjamini-Hochberg procedure [81]. Input data and results from the Fisher’s exact tests can be found in S1 Table.

To further investigate the regulatory function of N-SNPs in glucose metabolism, we used a data set that analyzed allele-specific binding of SNPs with a minor allele frequency >1% in regulatory sequences within 500kb of type 2 diabetes lead SNPs from GWAS or SNPs in linkage disequilibrium with them () using SNP-SELEX [43]. SNP-SELEX is an in vitro assay that evaluates binding of specific transcription factors to DNA oligos outside of a cellular context. Briefly, to perform SNP-SELEX, oligos matching 40 bp sequences including the SNP of interest are synthesized. To examine binding, in each experiment the oligos are combined with a single transcription factor. Unbound oligos are washed from the plate such that only oligos bound to the transcription factor remain and can be identified through sequencing. The enrichment of each oligo present in the final sample is used to determine binding affinity and to calculate binding effect for each SNP. This relative enrichment is used to calculate the preferential binding score (PBS), which is defined as the difference in relative enrichment between two alleles.
PBS were obtained from supplemental data 2 [43]. We intersected the SNPs tested in this data set with our library of N-SNPs. We performed Fisher’s Exact Test for enrichment analyses. We then performed the Kolmogorov-Smirnov test on the PBS values to compare the distribution of PBS between N-SNPs and non-N-SNPs. We also repeated this analysis for each individual transcription factor.

**N-SNP regulation of differentially expressed genes**

To link N-SNPs to genes they regulate, we annotated N-SNPs which were significant eQTLs in any of 48 tissues in GTEx v7 (https://storage.googleapis.com/gtex_analysis_v7/single_tissue_eqtl_data/GTEx_Analysis_v7_eQTL.tar.gz). 16,065 N-SNPs were GTEx eQTLs in any tissue, regulating 1,939 genes. We then annotated N-eQTLs which regulated differentially expressed genes in response to environmental perturbation from Moyerbrailean et al [30]. We chose all treatment-cell type combinations with at least 1,000 differentially expressed genes and downloaded the gene expression data from http://genome.grid.wayne.edu/gxebrowser/Tables/Supplemental_Table_S6.tar.gz. In total, we considered five cell types (melanocytes, peripheral blood mononuclear cells, lymphoblastoid cell lines (LCLs), smooth muscle cells, and human umbilical vein endothelial cells) and the 26 treatments shown in Fig 3B for a total of 52 treatment-cell type combinations. These experiments were performed in cell lines from European (N = 3), African (N = 3) and African American (N = 9) individuals. The cell types were chosen by [30] because: they are relevant for the biology of complex traits, they are readily available primary cells (with normal karyotype, as opposed to cancer cell lines), can be passaged and cultured for multiple experiments and finally they can be collected non-invasively, therefore representing potentially ideal cell types for future larger eQTL mapping studies. We identified treatments in which differentially expressed genes were enriched for Neanderthal regulation using Fisher’s exact test. We limited our analysis to genes with an eQTL and tested for an enrichment of N-eQTL genes in genes differentially expressed for each treatment-cell type combination separately. For treatments which were tested in multiple cell types, we meta-analyzed the enrichment odds ratios for each treatment using a fixed effects model with inverse variance weights to obtain a single enrichment per treatment. Multiple-test correction was performed using the Benjamini-Hochberg procedure [81].

**Experimental validation of N-SNPs from BiT-STARR-seq**

To validate N-SNPs which cause changes in gene expression, we used data from a massively parallel reporter assay (MPRA) known as BiT-STARR-seq performed in LCLs [48]. Allele-specific cis-regulatory effects in MPRA are independent of the ancestry of the cell line used. Potential ancestry-specific trans effects (e.g. expression of a specific TF) would equally affect both alleles and should not create a bias. Results from BiT-STARR-seq were downloaded from https://genome.cshlp.org/content/suppl/2018/10/17/gr.237354.118.DC1/Supplemental_Table_S1_.txt. We also included an annotation for Neanderthal SNPs which were eQTLs in macrophages infected with *Listeria* or *Salmonella* from Nedelec et al [25] (downloaded from https://genome.cshlp.org/content/suppl/2018/10/17/gr.237354.118.DC1/Supplemental_Table_S4.txt). 226 N-SNPs were tested by BiT-STARR-seq for allele-specific regulatory differences, and 21 were significant after multiple test correction (Benjamini-Hochberg adjusted p value < 0.1). BiT-STARR-seq results for all 226 N-SNPs can be found in S4 Table.
Adaptive Introgression summary statistics

To test for adaptive introgression, we chose a set of statistics developed by Racimo et al [8] and shown to robustly identify adaptive introgression. A description of each statistic is provided below. Because each individual statistic may yield significant results for other demographic or non-adaptive processes independently of adaptive introgression [8,82], here we decided to take a conservative approach and define as adaptive introgressed the N-SNPs in windows that exceed the critical values on all three adaptive introgression summary statistics.

RD statistic is defined as the average ratio of sequence divergence between an individual from the recipient and an individual from the donor population, and the divergence between an individual from the outgroup and an individual from the donor population. The U20 statistic is defined as the number of uniquely shared alleles between the recipient and donor population that are of frequency <1% in the outgroup, 100% in the donor, and >20% in the recipient population. The Q95 statistic is defined as the 95% quantile of the distribution of derived allele frequencies in the recipient population, that are of frequency <1% in the outgroup and 100% in the donor population [8].

In this analysis, we computed the above statistics in non-overlapping 50-kb windows across the autosomes using modern human genome data from Phase 3 of the 1000 Genomes Project [34] as in Zhang et al [82]. We used individuals from the CEU population as the recipient population, individuals from YRI population as the non-introgressed outgroup, and the unphased, high-quality whole genome sequence from the Altai Neanderthal individual [31] as the donor population. We define the statistical values for each eQTL as the AI summary statistic values of each 50kb window that contains the eQTL SNP. The CEU was chosen as the recipient population to match the ancestry of the functional genomics data from Moyerbrailean et al [30], which were collected in cells from donors with European and African ancestries.

To identify signatures of AI, we adopted an “outlier approach” where we define the critical value for each statistic as the most extreme 5% quantile value from its genome-wide distribution [8,82] (S6 Table). We plotted the genome-wide distribution of the AI statistics as Manhattan plots in S2 Fig, with windows containing N-eQTLs highlighted in red. The solid line shows the critical value of each statistic.

Supporting information

S1 Table. Enrichment of N-centiSNPs to disrupt transcription factor binding. Each row represents a transcription factor. Columns 1–12 are: 1) Motif; 2) Odds ratio; 3) Lower confidence interval; 4) Upper confidence interval; 5) Enrichment p-value; 6) Number of N-SNPs which are predicted disrupt transcription factor binding; 7) Number of non-Neanderthal SNPs which are predicted to disrupt transcription factor binding, 8) Number of N-SNPs in transcription factor binding site which are not predicted to disrupt binding, 9) Number of non-Neanderthal SNPs in transcription factor binding site which are not predicted to disrupt binding, 10) FDR of enrichment, 11) transcription factor motif name {TC "1 Enrichment of N-centiSNPs to disrupt transcription factor binding. Each row represents a transcription factor. Columns 1–12 are: 1) Motif; 2) Odds ratio; 3) Lower confidence interval; 4) Upper confidence interval; 5) Enrichment p-value; 6) Number of N-SNPs which are predicted disrupt transcription factor binding; 7) Number of non-Neanderthal SNPs which are predicted to disrupt transcription factor binding, 8) Number of N-SNPs in transcription factor binding site which are not predicted to disrupt binding, 9) Number of non-Neanderthal SNPs in transcription factor binding site which are not predicted to disrupt binding, 10) FDR of enrichment, 11) transcription factor motif name" \f t}.

(TXT)
S2 Table. N-eQTLs. For each N-SNP, we annotated the gene it regulates in GTEx and whether that gene is differentially expressed in Moyerbrailean et al [30]. Allele frequencies are from the 1000 Genomes project. Columns 1–47 are: 1) Chromosome; 2) Position; 3) rsID; 4) GTEx SNP ID. "NA" indicates the SNP is not an eQTL in GTEx; 5) Modern human allele; 6) Neanderthal allele; 7) Global allele frequency; 8) East Asian allele frequency; 9) American allele frequency, 10) European allele frequency, 11) South Asian allele frequency, 12) African allele frequency, 13) Ensembl gene id, 14) Gene symbol, 15–46) Treatments from Moyerbrailean et al [30]. "1" indicates the gene is differentially expressed in that condition in at least one cell type, 47) "1" indicates the gene is not differentially expressed in response to any treatment.

S3 Table. GWAS catalog overlap with N-eQTLs which regulate genes which respond to the environment. 66 N-eQTLs which regulate DE genes were found in the GWAS catalog. Columns 1–10 are: 1) Chromosome; 2) 0-based coordinate; 3) 1-based coordinate; 4) rsID; 5) Trait; 6) Risk allele; 7) P-value; 8) Odds ratio or beta; 9) 95% confidence interval; 10) PubMed ID.

S4 Table. BiT-STARR results for N-SNPs. Results for the 226 N-SNPs tested by BiT-STARR-seq by Kalita et al [48]. The table was downloaded from S1 Table from Kalita et al [48]. We added a final column indicating the FDR calculated using the 226 N-SNPs only.

S5 Table. N-eQTLs. For each of the N-SNPs filtered out due to it being polymorphic in Africans, we annotated the gene it regulates in GTEx, whether that gene is differentially expressed in Moyerbrailean et al [30], and if the SNP was validated by BiT-STARR-seq. Columns 1–43 are: 1) Chromosome; 2) Position; 3) rsID; 4) GTEx SNP ID. "NA" indicates the SNP is not an eQTL in GTEx; 5) Modern human allele; 6) Neanderthal allele; 7) Global allele frequency, 8) Ensembl gene id, 9) Gene symbol, 10–41) Treatments from Moyerbrailean et al [30]. "1" indicates the gene is differentially expressed in that condition in at least one cell type, 42) "1" indicates the gene is not differentially expressed in response to any treatment 43) BiT-STARR-seq results. NA indicates SNP was not tested, 0 indicates it was not validated, and 1 indicates validation.
Africans, we annotated the gene it regulates in GTEx, whether that gene is differentially expressed in Moyerbrailean et al [30], and if the SNP was validated by Bit-STARR-seq. Columns 1–43 are: 1) Chromosome; 2) Position; 3) rsID; 4) GTEx SNP ID. "NA" indicates the SNP is not an eQTL in GTEx; 5) Modern human allele; 6) Neanderthal allele; 7) Global allele frequency, 8) Ensembl gene id, 9) Gene symbol, 10–41) Treatments from Moyerbrailean et al [30]. "1" indicates the gene is differentially expressed in that condition in at least one cell type, 42) "1" indicates the gene is not differentially expressed in response to any treatment 43) BiT-STARR-seq results. NA indicates SNP was not tested, 0 indicates it was not validated, and 1 indicates validation" [f t].

(S6 Table. Adaptive introgression outliers. 1000 SNPs had outlier values for all three introgression statistics: RD, Q95, and U20. Columns 1–13 are: 1) Chromosome; 2) Position; 3) rsID; 4) GTEx SNP ID. "NA" indicates the SNP is not an eQTL in GTEx; 5) Modern human allele; 6) Neanderthal allele; 7) Neanderthal allele frequency, 8) Ensembl gene id, 9) Gene symbol, 10) 50 kb genomic window where SNP resides, 11) RD statistic, 12) Q95 statistic, 13) U20 statistic. [f t].

(TXT)

(S1 Fig. Identification of N-SNPs Overview of how N-SNPs were selected. [f f].)

(TIF)

(S2 Fig. Adaptive introgression statistics (A) RD, (B) U20, and (C) Q95 statistics for 50kb windows genome-wide. The horizontal line indicates the 5th percentile, and windows with p<0.05 with a N-eQTL are colored red. [f f].

(TIF)

(S3 Fig. SNP-SELEX analysis of N-SNPs that alter transcription factor binding. Density plots for significant Kolmogorov-Smirnov test results comparing the distribution of PBS values for N-SNPs and non-N-SNPs for individual transcription factors. The blue color represents N-SNPs and the red represents non-N-SNPs. [f f].

(TIF)

(S4 Fig. B-statistics for N-SNPs validated by BiT-STARR-seq vs N-SNPs not validated by BiT-STARR seq [f f].

(TIF)

(S5 Fig. B-statistic comparisons (A) Distribution of B statistics for centiSNPs which are N-SNPs and centiSNPs which are not N-SNPs. (B) Distribution of B statistics forSNPs in TF
footprints with $> = 10$ N-SNPs in footprint and for SNPs in TF footprints with $< 10$ N-SNPs in footprint. (C) Distribution of B statistics for centiSNPs in 16 TF footprints relevant for glucose metabolism that are enriched for N-centiSNPs and for a control set of centiSNPs in 21 TF footprints relevant for glucose metabolism but that are not enriched for N-centiSNPs. (D) Distribution of B statistics for N-centiSNPs and non-centi N-SNPs in footprints for the most enriched TFs (ARNT, SWI5, AML1, AP1, c-Jun, ATF2, Elk1).

(A) Distribution of B statistics for centiSNPs which are N-SNPs and centiSNPs which are not N-SNPs. (B) Distribution of B statistics for SNPs in TF footprints with $> = 10$ N-SNPs in footprint and for SNPs in TF footprints with $< 10$ N-SNPs in footprint. (C) Distribution of B statistics for centiSNPs in 16 TF footprints relevant for glucose metabolism that are enriched for N-centiSNPs and for a control set of centiSNPs in 21 TF footprints relevant for glucose metabolism but that are not enriched for N-centiSNPs. (D) Distribution of B statistics for N-centiSNPs and non-centi N-SNPs in footprints for the most enriched TFs (ARNT, SWI5, AML1, AP1, c-Jun, ATF2, Elk1).

S6 Fig. Relationship between number of DEGs and significant AI per treatment. The x-axis represents the number of DEGs per treatment, and the y-axis represents the number of genes in regions with evidence of adaptive introgression from the RD statistics. Each dot represents a different treatment.

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