Variation and Functional Impact of Neanderthal Ancestry in Western Asia

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Abstract

Neanderthals contributed genetic material to modern humans via multiple admixture events. Initial admixture events presumably occurred in Western Asia shortly after humans migrated out of Africa. Despite being a focal point of admixture, earlier studies indicate lower Neanderthal introgression rates in some Western Asian populations as compared with other Eurasian populations. To better understand the genome-wide and phenotypic impact of Neanderthal introgression in the region, we sequenced whole genomes of nine present-day Europeans, Africans, and the Western Asian Druze at high depth, and analyzed available whole genome data from various other populations, including 16 genomes from present-day Turkey. Our results confirmed previous observations that contemporary Western Asian populations, on an average, have lower levels of Neanderthal-introgressed DNA relative to other Eurasian populations. Modern Western Asians also show comparatively high variability in Neanderthal ancestry, which may be attributed to the complex demographic history of the region. We further replicated the previously described depletion of putatively functional sequences among Neanderthal-introgressed haplotypes. Still, we find dozens of common Neanderthal-introgressed haplotypes in the Turkish sample associated with human phenotypes, including anthropometric and metabolic traits, as well as the immune response. One of these haplotypes is unusually long and harbors variants that affect the expression of members of the \( \text{CCR} \) gene family and are associated with celiac disease. Overall, our results paint a complex first picture of the genomic impact of Neanderthal introgression in the Western Asian populations.

Key words: Anatolia, celiac disease, malaria, metabolism, immunity, genetic anthropology.

Introduction

Recent studies have shown that archaic hominins contributed genetic material to modern humans (Green et al. 2010; Hammer et al. 2011; Reich et al. 2010; Xu et al. 2017). The origin and impact of these introgressions vary geographically and happened through multiple instances. For example, Neanderthals contributed genetic material to all Eurasian peoples, possibly through a single introgression event in the Middle East during the out-of-Africa migrations ~50,000–60,000 years ago (Green et al. 2010). Follow-up analyses have shown evidence for a second, smaller introgression event affecting only Asian groups (Wall et al. 2013). More recent studies have also identified additional geography-specific introgression events from other archaic hominins to ancestors of contemporary human populations (Meyer et al. 2012; Hsieh et al. 2016; Vernot et al. 2016). As such, all modern humans from outside-of-Africa are estimated to carry 1–3% Neanderthal DNA in their genomes (Green et al. 2010; Meyer et al. 2012; Prüfer et al. 2014). Moreover, haplotype level scrutinization of these admixture events revealed a significant depletion of coding sequences among haplotypes admixed from Neanderthals, suggesting strong negative selection acting on these sequences (Sankararaman et al. 2014; Vernot and Akey 2014; Harris and Nielsen 2016; Juric et al. 2016). In contrast, a small but measurable number of introgressed haplotypes have been adaptively maintained in the human population (Huerta-Sánchez et al. 2014; Dannemann et al. 2016; Gittelman et al. 2016; Quach et al. 2016; Racimo...
et al. 2017). In addition, recent studies have now shown that Neanderthal-introgessed haplotypes might affect the expression levels of multiple genes (Dannemann et al. 2017; McCoy et al. 2017).

Based on the current data, it has been suggested that the first and most significant introgression from Neanderthals occurred in Western Asia (Green et al. 2010; Prüfer et al. 2014). Specifically, contemporary East and West Eurasian genomes share more Neanderthal alleles than sub-Saharan African genomes do (Vernot and Akey 2014). This observation is consistent with an introgression event in Western Asia, after the population ancestral to contemporary West and East Eurasians migrated out of Africa, but had not yet split into two isolated populations. However, little is known about the distribution and functional significance of Neanderthal introgression in contemporary Western Asian populations, except for a handful of studies (reviewed in Taskent and Gokcumen 2017). Notably, a recent study found lower proportions of Neanderthal ancestry in ancient genomes from the Middle East (Lazaridis et al. 2016). This study further identified high levels of basal Eurasian ancestry (Lazaridis et al. 2014) in these ancient West Asian genomes, which was negatively correlated with Neanderthal ancestry, suggesting that the hypothetical basal Eurasian lineage carried lower levels of Neanderthal ancestry than other ancestral Eurasian lineages (Lazaridis et al. 2016). The degree of basal Eurasian ancestry could also explain variation in Neanderthal ancestry among present-day West Eurasian genomes. For instance, a high level of basal Eurasian or sub-Saharan African ancestry could underlie the observation that there is a relatively low proportion of Neanderthal ancestry in a present-day Qatari Bedouin population as compared with European and some other Middle Eastern populations (Rodriguez-Flores et al. 2016).

In this study, we investigate whether the unique population history of the region affected the distribution of Neanderthal introgression among Western Asian populations, concentrating on the Druze, a small, and ethnically homogeneous closed population of the Levant, and the more diverse population of modern-day Turkey. Western Asia is especially interesting as the region has been central to major demographic events since the early Neolithic (Yunusbayev et al. 2015; Kılınc et al. 2016). Moreover, the distribution of genetic variation in Western Asia may be different than what has repeatedly been observed for European populations, that is, a strong correlation with geography consistent with an isolation-by-distance model (Novembre et al. 2008). Specifically, recent inbreeding, migration, and local isolation may create deviations in the clinal distribution of genetic variation in this region (Gokcumen et al. 2011; Scott et al. 2016), which may give rise to considerable heterogeneity in the levels of Neanderthal introgression among populations.

Materials and Methods
For this study, we used data from 1000 Genomes Project Phase 1 (1000 Genomes Project Consortium 2012), Turkish Genome Research Project (Alkan et al. 2014) and Human Origins data sets (Lazaridis et al. 2014). In addition, we compiled a dataset of ten samples. Specifically, we sequenced 9 genomes: two individuals from Finnish population (with European ancestry), one individual from CEPH population (Utah residents with Northern and Western European ancestry), three individuals from Druze population (from Lebanon and Syria, with Western Asian ancestry), and three individuals from Mbuti Pygmy population (with Central African ancestry), all of which were purchased from Coriell Institute for Medical Research (https://www.coriell.org/). In addition, we also included previously sequenced CEPH sample NA12878 in our variant calling pipeline and compiled a vcf file from all 10 genomes. (https://www.coriell.org/; last accessed November 13, 2017). The samples and the populations used in this study are summarized in supplementary table S1, Supplementary Material online.

The library preparation was conducted by New York Genome Center core facility using standard procedures (TruSeqDNA Nano). Each sample was sequenced to ~30x with 150-bp paired-end sequences using an Illumina HiSeq X platform. Duplicate reads were removed by Picard and BWA was used to map the sequences to Hg19 (Ghr37). The variation calls were done by GATK (McKenna et al. 2010). All mapping and variant calling was done by standard procedures in New York Genome Center. BAM files are available at Short Read Archive and the joint genotyping vcf file can be found at https://gokcumenlab.org/.

The expression quantitative loci information for rs10540 was downloaded from GTEx (The GTEx Consortium 2015). All the bioinformatics analyses were done using publicly available software and custom scripts, which can be found here (https://github.com/taskent/W.Asia-Neanderthal-Introgression/blob/master/Sstar.py; last accessed November 13, 2017). The details of our methodology is described in supplementary methods, Supplementary Material online.

Results
Variation in Neanderthal Ancestry within and among Western Asian Populations
To determine the Neanderthal introgression patterns in Western Asian populations, we first set out to determine relative Neanderthal ancestry levels in two distinct populations: the Druze and the population of present-day Turkey, as compared with other Eurasian populations. To do this, we first analyzed the Human Origins (HO) data set (Lazaridis et al. 2014) and calculated D-statistics of the form D(HO Druze or Turkish, HO Eurasian or
African; Neanderthal, Chimpanzee) for various population combinations.

Our results showed that these two present-day Western Asian populations consistently carry lower proportions of Neanderthal introgression relative to Europeans, Central Asians, and East Asians (fig. 1A). This observation is concordant with the trends documented globally in present-day populations (Mallick et al. 2016) as well as using ancient genomes (Lazaridis et al. 2016). We found the same trend comparing individual Neanderthal ancestry estimates of the form $D_{\text{Test}, \text{Yoruba}} - D_{\text{Neanderthal}, \text{Chimp}}$ among West Asian and other Eurasian groups. The difference in estimated admixture proportions between Western Asians and other Eurasians is systematic and significantly lower for Western Asians ($P < 0.01$, Wilcoxon Rank Sum Test, fig. 1B).

Even though lower Neanderthal ancestry is a consistent trend for Western Asian populations, it also shows noticeable variation. To determine whether this variation originates from within- or between-population differences, or both, we calculated $D$-statistics for each individual within a given population $D_{\text{Individual}, \text{Test}}$, Yoruba; Neanderthal, Chimpanzee (supplementary fig. S1, Supplementary Material online). Indeed, we observed that both within- and between-population variation of $D$-statistics were higher among Western Asian populations as compared with other continental groups. For example, the Druze has lower levels of
Neanderthal ancestry as compared with European, Central Asian, and East Asian populations. However, this population also shows a higher level of within-population variation in Neanderthal ancestry. We further noticed two distinct groups of Western Asian populations with respect to Neanderthal ancestry that they carry, populations associated with the Arabian peninsula showed conspicuously low Neanderthal ancestry as compared with other Eurasian populations, whereas those from northern and eastern regions of Western Asia showed only slightly lower levels of Neanderthal ancestry as compared with other Eurasians (supplementary fig. S1, Supplementary Material online). This is concordant with the recent observation that the Qatari populations of Bedouin ancestry, but not all Middle Easterners, showed lower levels of Neanderthal ancestry as compared with European, Central Asian, and East Asian populations (Rodriguez-Flores et al. 2016). Overall, our results showed that D-statistics between pairs of Western Asian populations are more variable than D-statistics between pairs of European or Asian populations (supplementary table S1, Supplementary Material online). We further quantified these observations and calculated an ~42% increase in the standard deviation of D-statistic values among Western Asian populations, compared with the same values calculated among European populations, a significant difference as assessed by random permutations (P < 0.001, supplementary fig. S2, Supplementary Material online).

To validate our findings with higher power, we used two whole genome sequencing data sets (1000 Genomes Project Consortium 2012; Alkan et al. 2014). First, we calculated similar D-statistics with published Turkish genomes of high coverage, which we merged with the 1000 Genomes Phase I data set. This indicated lower Neanderthal ancestry in the Turkish population relative to other Eurasian populations (fig. 1C), as we had observed earlier in Turkish genomes in Human Origins data set. Second, we sequenced three Druze, three European, and three Pygmy genomes all with high coverage (>30×) using (~150 bp) paired-end reads. The library preparation, sequencing platform, mapping, and calling algorithms for these genomes are identical to avoid any technical biases (see supplementary methods, Supplementary Material online). Unexpectedly, the sequenced Druze individuals were found to have similar levels of Neanderthal ancestry as European individuals (fig. 1D), in contrast to lower levels found using the 39 independent Druze individuals of the Human Origins data set.

To rule out sample mix up and further investigate the source of this variation in Druze, we merged the data sets using common variants genotyped in our data set and the Human Origins data set. ADMIXTURE analysis on this merged data set confirmed that the three Druze individuals sequenced had similar ancestry components as the Druze in the Human Origins data set (fig. 1E). Using the same merged data set, we also conducted individual Neanderthal ancestry estimate comparisons (supplementary fig. S3A, Supplementary Material online). This analysis showed that the D-statistics from three Druze individuals that we sequenced in this study actually falls within the distribution of the variation of D-statistics observed in Druze from the Human Origins data set (supplementary fig. S3B, Supplementary Material online). Based on these, we conclude that sampling bias is the main source of the observed disparity between data sets, which marks the higher level of variation in Neanderthal ancestry within and among Western Asian populations.

It is plausible that immigration, isolation, and inbreeding (Scott et al. 2016) have created genetic structure and heterogeneity within and across Western Asian populations. Heterogeneity especially with regards to varying levels of sub-Saharan or basal Eurasian ancestry may explain the elevated variation in allele sharing with Neanderthals among Western Asian genomes. This scenario would be consistent with the results of a previous study that found different levels of Neanderthal ancestry among three Qatari populations (Rodriguez-Flores et al. 2016), possibly due to differences in their levels of sub-Saharan and basal Eurasian ancestries (Scott et al. 2016).

To investigate this notion, we studied ancestral components in the Human Origins data set genomes using ADMIXTURE (Alexander et al. 2009). This revealed conspicuous heterogeneity among Western Asian populations (fig. 1E), with varying levels of African and Asian contributions within and among Western Asian populations (e.g., higher East Asian ancestry in Turkish genomes, and higher African ancestry in Palestinian genomes, compared with the Druze). We then tested the hypothesis that Neanderthal ancestry estimated for Western Asian individuals would be negatively correlated with their estimated sub-Saharan ancestral components. Testing this within each population, we found no significant negative correlation except for Yemenis (P < 0.05, Spearman nonparametric correlation test, supplementary table S2, Supplementary Material online). However, when we pooled data for each continental group and compared the sub-Saharan component across all genomes within that group with the estimated Neanderthal ancestry in the same genomes, we found a highly significant negative correlation for Western Asia (rho = −0.234, P < 0.0002; supplementary table S2, Supplementary Material online). We were able to see the same significant trend when we compared the average sub-Saharan component in each Western Asian population with the average Neanderthal ancestry in the same population across populations (rho = −0.739, P < 0.003, supplementary fig. S4 and table S2, Supplementary Material online). As such, our results support the notion that varying levels of sub-Saharan ancestry among Western Asian genomes contributed to the observed differences in Neanderthal ancestry among these populations.
Neanderthal-Introgressed Haplotypes in the Turkish Population

Next, we aimed to delineate the genomic impact of Neanderthal-introgressed haplotypes in Western Asian populations. As a case example, we focused on the 16 present-day Turkish individuals for whom we have access to high-quality genomes (due to sample size restrictions we could not include the Druze individuals in this analysis). We followed the $S^*$-based pipeline recently fine-tuned for identifying introgressed haplotypes (Vernot and Akey 2014). Briefly, we determined putatively introgressed haplotypes in 16 Turkish genomes (i.e., 32 sets of phased chromosomes), as well as in 16 Western European and in 16 East Asian genomes for comparative purposes (fig. 2A and Supplementary Material online). Briefly, using a combination of empirical analyses and simulation-based modeling, this pipeline identifies stretches of DNA carrying unusually high numbers of proximate, derived single nucleotide variants present in Eurasian genomes, but not found in sub-Saharan African genomes (see supplementary methods, Supplementary Material online, for details).

Using our pipeline, we identified 6,599 derived haplotypes in 16 Turkish genomes with an average size of ~72 kb (supplementary table S3, Supplementary Material online). Because the $S^*$ statistic does not consider information from Neanderthal genomes, we further refined our data set by probabilistically categorizing the derived haplotypes into those that harbor higher than expected numbers of derived Neanderthal alleles (introgressed), and those do not (ancestral) (see Supplementary Material online for details). Not surprisingly, we found that 3,378 (~51%) of these divergent haplotypes are not enriched in Neanderthal derived alleles and are thus likely remnants of the ancestral genetic structure in Africa, rather than an introgression from Neanderthals (Yang et al. 2012).

The remaining 3,221 (~49%) derived haplotypes were likely introgressed from Neanderthals (FDR < 0.05), of which 1,790 (~56%) are found in more than one copy (supplementary table S3, Supplementary Material online). The 3,221 putatively Neanderthal-introgressed haplotypes observed in 16 Turkish genomes cover close to 280 Mb (~9%) of sequence, corresponding on an average to 18 Mb of Neanderthal-introgressed DNA in each genome. Among these, 1,431 (~42%) are singletons, that is, observed in 1 out of 32 copies, whereas the rest of the haplotypes are observed at least twice. As expected, Neanderthal-introgressed haplotypes are larger but less frequent than ancestral haplotypes (fig. 2B and supplementary fig. S5, Supplementary Material online).

Of the common Neanderthal-introgressed haplotypes, 584 (33%) were not found in previous studies (Vernot and Akey 2014) and could be region- or population-specific. This observation is consistent with the observation that the frequencies of Neanderthal-introgressed haplotypes can be highly variable both within and among Eurasian populations (Sankararaman et al. 2014; Vernot et al. 2016). In our further analyses, we focused on understanding the genomic and evolutionary impact of the Neanderthal introgression in the Turkish population using the ancestral derived haplotypes as an internal control.

Neanderthal Introggression Shapes Variation Related to Innate Immunity and Immune-Mediated Disorders in Turkish Populations

To determine the functional relevance of Neanderthal-introgressed haplotypes in the Turkish samples, we first analyzed the annotated functional sequences that overlap with Neanderthal-introgressed haplotypes. Neanderthal haplotypes found in the Turkish population are depleted for exonic sequences, where we observe that only 1.1% of the Neanderthal-introgressed haplotypes are covered by exons, as compared with the expected 1.5% for the similar-sized regions ($P < 10^{-15}$, chi-squared test). This result is consistent

Fig. 2.—$S^*$ statistics workflow and haplotypes. (A) $S^*$ analysis workflow. The boxes show each major computational step to determine Neanderthal-introgressed haplotypes. The number of haplotypes that we found in our pipeline at each step was indicated within the respective boxes. (B) Distribution of Neanderthal-introgressed haplotypes detected by $S^*$ over the Turkish genomes ($n = 16$) sequenced for the Turkish Genome Project (TGP) (Alkan et al. 2014). The x-axis shows the chromosomal locations of the haplotypes. Y-axis indicates the allele frequency observed in the Turkish sample ($n = 16$ individuals). Haplotypes labeled by red show haplotypes carrying GWAS variants where the Neanderthal carries the derived allele. Arrows show the haplotypes carrying likely introgressed from Neanderthals (FDR < 0.05), of which 1,790 (~56%) are found in more than one copy (supplementary table S3, Supplementary Material online). The 3,221 putatively Neanderthal-introgressed haplotypes observed in 16 Turkish genomes cover close to 280 Mb (~9%) of sequence, corresponding on an average to 18 Mb of Neanderthal-introgressed DNA in each genome. Among these, 1,431 (~42%) are singletons, that is, observed in 1 out of 32 copies, whereas the rest of the haplotypes are observed at least twice. As expected, Neanderthal-introgressed haplotypes are larger but less frequent than ancestral haplotypes (fig. 2B and supplementary fig. S5, Supplementary Material online).
with the previously reported notion that there is widespread negative selection against functional Neanderthal alleles (Harris and Nielsen 2016; Juric et al. 2016).

Earlier work has also shown that a small number of introgressed Neanderthal haplotypes with phenotypic and biomedical effects have reached high frequencies in non-African populations. For example, a previously reported (Dannemann et al. 2016) Neanderthal-introgressed haplotype that is common in Eurasia and overlaps multiple Toll-like receptor genes (supplementary fig. S6, Supplementary Material online) is found in 31% of the sampled Turkish chromosomes. Looking at the tag variants for this haplotype we found that it has 15% frequency in the Western European populations, but reaches 50% in the East Asian populations. It is possible that this Neanderthal-introgressed haplotype increased in frequency in the Turkish population due to recent Asian migrations into Anatolia (Di Benedetto et al. 2001; Berkman et al. 2008).

To further investigate putatively functional introgressed Neanderthal haplotypes in the Turkish population, we searched for introgressed haplotypes that harbor single nucleotide variants associated with human traits in genome-wide association studies (GWAS) (MacArthur et al. 2017). Specifically, we searched in the GWAS database for derived single nucleotide variants within the introgressed haplotype regions that are also derived in the Altai Neanderthal genome. We found 55 such GWAS variants (supplementary table S4, Supplementary Material online), 42 of which were observed among at least 2 Turkish chromosomes (>6.25% allele frequency).

Concordant with previous observations (Khrameeva et al. 2014; Deschamps et al. 2016), 18 of the 41 common GWAS variants on Neanderthal-derived haplotypes in the Turkish population were immune- and metabolism-related. One of these haplotypes is also the largest (>500 kb) among all Neanderthal haplotypes we found in our analysis (fig. 3A). It is found in \(n = 3\) (9%) of the Turkish chromosomes and has remained fully intact in one individual, which appears unlikely assuming at least 55,000 years, or 2,200 generations since admixture and given the recombination rate of this region (\(P < 10^{-15}\); see Supplementary Material online). The haplotype harbors rs13098911, a variant associated with celiac disease (Dubois et al. 2010). This haplotype overlaps with the C-C motif chemokine receptor (CCR) gene family with a known role in HIV infection (Choe et al. 1996). Intriguingly, other variants within the same region, which are not linked to the Neanderthal haplotype, were associated with Behcet’s disease (Kirino et al. 2013), an autoimmune disorder highly common in Western Asia. Collectively, its functional relevance and unusual size raise the possibility that this haplotype may have been maintained in Western Asian populations through adaptive forces, making it an ideal candidate for future studies.

We found another similarly large (>250 kb) Neanderthal-derived haplotype, which harbors rs12788102, a variant strongly associated with severity of malaria (Band et al. 2013) (fig. 3B). This haplotype overlaps with multiple olfactory receptor 5 (OR5) subfamily genes and is found at 20% frequency among the 32 sets of Turkish chromosomes investigated. To our knowledge, this is the first time this haplotype

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**Fig. 3.**—Neanderthal-introgressed haplotypes with putative functional effects. The graphs show genome-browser snapshots of genomic regions where we detected Neanderthal-introgressed haplotypes harboring GWAS variants. The upper ruler indicates a scale for the region. The “Neanderthal Alleles” track denotes the variants used in our S* pipeline and the colors indicate whether the variants are derived (red) in the Neanderthal genome or not (black). The “UCSC Genes” track shows the location of exons (thick blue sticks) and introns (thin blue sticks). (A) Neanderthal-introgressed haplotype carrying GWAS variants associated with celiac disease. The haplotype carries multiple C-C motif chemokine receptor (CCR) genes. The track on the bottom shows the GWAS variants in the region. The arrows show the GWAS variants, which are linked with celiac disease and linked with the Neanderthal haplotype. (B) Neanderthal-introgressed haplotype carrying multiple olfactory receptor 5 (OR5) genes. The haplotype also carries a GWAS variant (rs12788102) associated with the severity of malaria infections (Band et al. 2013). The arrow shows the GWAS variant associated with the severity of malaria infections.
has been highlighted to be introgressed from Neanderthals into humans. Overall, Neanderthal-introgressed haplotypes may have observable effects on immunity-related variation within the Turkish population.

Neanderthal-Introgressed Haplotypes Affect Multiple Metabolism Genes in the Turkish Population

Previous studies have shown that the genes related to metabolism may have been particularly affected by Neanderthal-introgressed haplotypes (Khrameeva et al. 2014). Here, we identified two independent Neanderthal-variants (rs13201877, rs10540) common in this Turkish sample (at ~9% and ~6% frequency, respectively) and associated with Body Mass Index (BMI). Among these, the haplotype block that carries rs10540 is >80 kb and harbors three genes: PTSS2, RNH1, and HRAS (fig. 4). The haplotype is defined by hundreds of alleles and, consequently, the causal variant(s) that lead to the change in BMI remain unknown. The haplotype is intact (i.e., not recombined) in >5,000 human haplotypes phased in the 1000 Genomes data set (1000 Genomes Project Consortium 2012). In this data set, the haplotype’s allele frequency is between 7% and 11% in European populations. It is absent in sub-Saharan African populations and found only rarely in some East Asian populations.

We then investigated the cis-regulatory influence of this haplotype using the GTEx data set (The GTEx Consortium 2015). This investigation revealed a general inhibitory effect (supplementary table S4, Supplementary Material online): PTSS2, RNH1, and HRAS, three highly expressed genes in multiple adult tissues that overlap with this haplotype, are all significantly downregulated in the tissues we could assess (fig. 4). RNH1 is an RNase inhibitor and has been shown to regulate angiogenin (Lee et al. 1988). HRAS is a well-studied proto-oncogene (Krontiris et al. 1985; Bos 1989). It is developmentally important with variants associated with the Costello syndrome (Aoki et al. 2005), a drastic developmental disorder and myopathy with excess of muscle spindles (van der Burgt et al. 2007). Finally, PTSS2 is the main enzyme in the biosynthesis of phosphatidylserine, which accounts for up to 10% of cell membrane phospholipids (Tomohiro et al. 2009). In summary, this Neanderthal-introgressed haplotype has broad effects on the expression of multiple genes and a well-established association to BMI. The adaptive significance of these biological effects (or lack thereof) remains to be investigated.

Conclusion

The nature and biological impact of gene flow from Neanderthals to modern human ancestors have lately
attracted major interest in anthropological genomics. In this study, we measured the levels of Neanderthal introgression in Western Asian populations, and mapped, for the first time, the haplotype-level impact of Neanderthal introgression in Turkish genomes. Our study provides a first look at the functional impact of Neanderthal introgression in a Western Asian population, paving the way for future population comparisons.

We replicated previous studies (Lazaridis et al. 2016; Rodriguez-Flores et al. 2016) showing that contemporary Western Asian populations have similar or lower levels of Neanderthal introgression than other Eurasian populations. The presence of sub-Saharan African ancestry and possible ancestry from a basal Eurasian lineage with lower (or no) signatures of Neanderthal introgression are the most parsimonious explanations for this observation. We also find considerable variation in the levels of Neanderthal introgression among Western Asian populations (even between one sample-set to another from the Druze population), which we also attribute to variable sub-Saharan African ancestry. As such, it is important here to note that Western Asia has a complex population history and the currently available genome data may not be fully representative of the region’s population diversity.

In this study, we also describe the first haplotype-level Neanderthal introgression map for a Western Asian population. Despite a general depletion of functional sequences among Neanderthal-introgressed haplotypes, we still identified dozens of haplotypes common within our Turkish sample and previously associated with phenotypic variations, including multiple immunity- and metabolism-related variants. These included a haplotype >500 kb that harbors multiple CCR genes, which appears to have remained intact solely through neutral processes given the recombination rate in this region. This haplotype has been associated with celiac disease (Dubois et al. 2010). Moreover, variants within this haplotype have been discussed within the context of HIV and plague resistance (Galvani and Novembre 2005), as well as Behcet’s disease, an immune-mediated disorder especially common in the Turkish population (Kirino et al. 2013). Some of these variants could have been maintained adaptively, although this notion needs to be further scrutinized using larger data sets than those used here. Irrespective of their adaptive nature, our results indicate that Neanderthal-derived alleles have contributed to functional variation in Western Asia.

In addition to functionally annotated and common variants, we found hundreds of putatively functional (e.g., overlapping coding sequence) Neanderthal-introgressed haplotypes in the Turkish population that could be region- or population-specific. Since few genome-wide association studies have been conducted in Turkey and the Middle East, we are yet to discern the exact phenotypic impact of these population-specific haplotypes. This premise is exciting for future studies, as population-specific genomewide association studies in various populations have been identifying Neanderthal variants of functional significance, including variants linked to metabolic disorders (SIGMA Type 2 Diabetes Consortium et al. 2014). We, therefore, argue that the next step toward understanding the phenotypic impact of Neanderthal introgression is to focus on the impact of population-specific variants inherited from Neanderthals.

### Supplementary Material

Supplementary data are available at Genome Biology and Evolution online.

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