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Convergent mutations and single nucleotide variants in mitochondrial genomes of modern humans and Neanderthals

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Abstract

Genetic contributions of Neanderthals to the modern human genome have been evidenced by comparison of present-day human genomes with paleogenomes suggesting that the Neanderthal introgression is higher in Asians and Europeans and lower in Africans. Neanderthal signatures in extant human genomes are attributed to intercrosses between Neanderthals and archaic Anatomically Modern Humans (AMH). Although Neanderthal signatures are well documented in the nuclear genome, it has been proposed that there is no contribution of Neanderthal mitochondrial DNA to contemporary human genomes. Here we show that modern human mitochondrial genomes contain potential 66 Neanderthal signatures, or Neanderthal single nucleotide variants (N-SNVs) being 36 in coding regions of which 7 are nonsynonymous. Also, 7 N-SNVs are associated with traits such as cycling vomiting syndrome, Alzheimer's disease, Parkinson's disease and 2 N-SNVs are associated with intelligence quotient. Based on recombination tests, Principal Component Analysis (PCA) and the complete absence of these N-SNVs in 41 archaic AMH mitogenomes we conclude that convergent evolution due to homoplasy and not recombination, explains the presence of N-SNVs in present-day human mitogenomes.

Keywords

Human genome, Mitochondrial Genome, Neanderthal admixture, SNPs, Ancient DNA.

1. Introduction

Comparative analyses of present day human genomes and paleogenomes led to the proposal that Neanderthals contributed to the modern makeup of the human genome [1–4]. The current interpretation of genomic signatures is that Neanderthal contributions are different in European, East Asian and African lines of descent, with a higher frequency of Neanderthal segments in Asians and Europeans and lower frequencies in Africans [1]. Intercrosses between Neanderthals and ancient *Homo sapiens* lineages, or archaic Anatomically Modern Humans (AMH) who migrated from Africa into the Middle East and Europe in the last 50,000 years might explain the presence of Neanderthal signatures in extant human genomes [3,4]. The spatio-temporal overlap of Neanderthals and archaic AMH is estimated to be approximately 22,000 years since the first AMH arrived in Europe around 50,000 years ago and the last Neanderthal remains (in Spain) date back to 28,000 years [5,6].

Although there is evidence for Neanderthal contributions to the modern nuclear genome, it has been proposed that there is no Neanderthal contribution to contemporary human mitochondrial genomes (mitogenomes) [7]. It is widely accepted that human mitochondrial DNA is exclusively inherited from the mother and lacks recombination [8]. Because of mitogenome matrilineal inheritance this implies that the intercrosses occurred exclusively between Neanderthal males and AMH females or that crosses between AMH males and Neanderthal females were extremely rare. Another possibility is that crosses between AMH males and Neanderthal females produced such unfavorable trait combinations, due to mitonuclear incompatibility [9], that none of their descendants left marks in present day human populations.

The lack of recombination in human mtDNA is still a matter hotly debated, with published evidence supporting it and rejecting it [10,11]. The main problem posed by a complete lack of human mtDNA recombination is the consequential Müller ratchet, the theoretical concept, in population genetics, that explains the accumulation of harmful mutations in a population over time, which helps to understand genetic deterioration and extinction [12,13]. Although the Müller ratchet is a theoretical concept it highlights the importance of recombination in the long-term maintenance of genetic diversity and the prevention of genetic deterioration in populations. Recombination allows for the removal of harmful mutations and the shuffling of genetic material and therefor alleviate the effect of the Müller ratchet [14].

Analysis of mitogenomes of present-day humans, archaic AMH and Neanderthals revealed 66 variants (N-SNVs), or signatures, in contemporary human mitogenomes that were also present in Neanderthals but not in archaic AMH mitogenomes. The archaic AMH samples

selected for analysis are approximately the same age as Neanderthal mitogenomes, and therefore represent, in theory, an archaic AMH ensemble that could have overlapped with Neanderthals in Europe, Middle East and Central Asia. This also allows for assessment of homoplasy because Neanderthal and archaic AMH sequences here considered are of approximately the same age. The distribution of these N-SNVs in different human haplogroups was investigated and Principal Component Analysis (PCA) and recombination tests were performed to evaluate whether recombination or homoplasy might explain the N-SNVs in present human mitogenomes.

2. Material and Methods

2.1. Mitochondrial genome sequences

Comparative analysis of the mitochondrial DNA from present day humans (52 sequences), ancient *Homo sapiens*, or archaic AMH (41 sequences) and Neanderthals (9 sequences) were aligned to identify single nucleotide variants. Paleogenomes mtDNA sequences were downloaded from GenBank for 41 archaic AMH (*H. sapiens*) and 9 Neanderthal mtDNA (Table 1). Neanderthal signatures, or N-SNVs, were determined as positions that were identical between present day sequences and Neanderthal sequences at the exclusion of archaic AMH. The 52 sequences of present day human mtDNA, representing all major mitochondrial haplogroups (Table 2), were selected from the PhyloTREE database [15] and downloaded from GenBank.

2.2. Sequence assembly and alignments

The Ust-Ishim sequence was assembled using reads downloaded from Study PRJEB6622 at the European Nucleotide Archive (EMBL-EBI) and assembled using the CLC Genomics Workbench 7 program (https://www.qiagenbioinformatics.com). To maintain the reference numbering, sequences were aligned to the revised Cambridge Reference Sequence (rRCS; GenBank accession number NC012920) [16], totalizing 103 sequences using the map to reference option implemented in Geneious 10 program [17]. Variants were called using Geneious 10 program. A total of 918 polymorphic positions were found. Neanderthals, ancient and modern humans were screened for disease associations at MitoMap (http://www.mitomap.org/MITOMAP) [18].

2.3. Phylogenetic inference

Position specific similarities between modern haplogroups and Neanderthals were depicted by cladograms for each of the single 66 variant positions present only in Neanderthals and modern humans and excluding archaic AMH, were generated using parsimony heuristic search implemented in PAUP v4.1a152 with the default parameters [19]. Proximity of mitochondrial haplogroups in Ancient *H. sapiens* and Neanderthals were inferred using Haplogrep 2.1.0 [20]. All 66 cladograms, corresponding to each N-SNV, are available upon request.

2.4. Recombination analysis

Potential recombination between Neanderthals and ancient *H. sapiens* sequences was inferred by a phylogenetic based method implemented by manual bootscan in the Recombination Detection Program (RDP) v.4.87. Parameters for bootscan analysis were: window size = 200; step size = 20; bootstrap replicates = 1,000; cutoff percentage = 70; use neighbor joining trees; calculate binomial p-value; model option = Kimura 1980 [21]. For each analysis, a single alignment was created which included the modern haplogroup, all 9 Neanderthal and all 6 Ancient *H. sapiens* sequences. When rCRS was used as query, two sets of possible parental sequences were selected: either Neanderthals Mezmaiskaya and Altai and ancient *H. sapiens* Fumane and Ust Ishim or only Neanderthals Feldhofer1, Mezmaiskaya and Vindija 33.16. For haplogroups L0d1a and L3d3b possible parental sequences were Neanderthals Feldhofer1, Mezmaiskaya and Vindija 33.16 and Ancient *H. sapiens* Kostenki 14, Fumane, Doni Vestonice 14 and Tianyuan. For haplogroups M29a and R0a possible parental sequences were Neanderthals Mezmaiskaya and Altai and Ancient *H. sapiens* Kostenki 14 and Doni Vestonice 14. For haplogroup N1b1a3 possible parental sequences were Neanderthals Feldhofer1 and Vindija 33.16 and Ancient *H. sapiens* Kostenki 14 and Doni Vestonice 14.

2.5. Statistical analysis

For variants (SNVs) calling three different datasets were used: (1) the whole mitogenome from the 102 sequences alignment; (2) the 128 to 315bp fragment and (3) the 6,950 to 7,660bp fragment in the same alignment. All fasta alignments were processed using the MSA2VCF software to generate the VCF files [22]. The options used on msa2vcf were: --haploid --output. To convert the VCF files to Plink format we used the vcftools package [23]. The whole mitogenome alignment with 103 sequences had 785 SNPs (positions containing gaps in at least one sequence were excluded from the analysis). Both 128 – 315 and 6,950-7,660 fragments had 24 SNPs.

Principal component analysis (PCA) was performed using the PLINK software v1.90b4

[24,25]. PCA figure plotting was made using Genesis PCA and admixture plot viewer (http://www.bioinf.wits.ac.za/software/genesis/). The first two principal components were chosen for the Neanderthal - *H. sapiens* comparison.

3. Results

3.1. Distribution of Neanderthal SNVs

An alignment of 102 sequences was produced which contained the mitogenomes of 41 archaic AMH (Table 1), 9 *Homo sapiens neanderthalensis* (Table 1) and 52 contemporary *Homo sapiens sapiens* with representatives of the major worldwide mitochondrial haplogroups (Table 2). This alignment contained the revised Cambridge Reference Sequence (rCRS) used as numbering reference for all polymorphisms identified [16]. We found 66 positions in which contemporary *Homo sapiens sapiens* were identical with at least one *Homo sapiens neanderthalensis* sequence position (Figure 1, Tables 3 and 4). In 13 positions just a subset of contemporary *Homo sapiens sapiens* were identical with at least one *Homo sapiens neanderthalensis* sequence position and at least one sequence of archaic AMH. In 175 positions *Homo sapiens neanderthalensis* differed from contemporary *Homo sapiens sapiens* and archaic AMH. In 11 positions the archaic AMH differed from other sequences and in 653 positions the contemporary *Homo sapiens sapiens* presented expected variants among haplogroups that were not relevant for this analysis.

To depict the distribution of Neanderthal SNVs, or N-SNVs, in different human mitochondrial haplogroups, we constructed a heat map of N-SNVs (Figure 2). Most N-SNVs are concentrated in the D-loop, followed by 12SrDNA and 16SrDNA. Among tRNAs N-SNVs were found only in isoleucine, asparagine and cysteine tRNAs. In *COX2* only one N-SNV was found in haplogroup R0a.

Of the 66 N-SNVs identified, 20 are common to modern African and Eurasian haplogroups, 25 are exclusive to African haplogroups and 21 are exclusive to Eurasian haplogroups. In Figure 3 the distribution of N-SNVs is depicted in the human mitogenome map. The distribution reveals that 11 Eurasian N-SNVs are in coding regions, 3 in rRNA genes and 1 in a tRNA gene.

The distribution of N-SNVs in modern haplogroups and archaic AMH can be summarized as possible six introgression routes with predicted consequences (Figure 4). In hypothesis (1) SNVs conserved between Neanderthals, archaic AMH and present-day humans should be observed in

all sequences; in (2) if the introgression crosses occurred between Neanderthals and archaic AMH prior to divergence that separated archaic AMH from lineages of African and Eurasian haplogroups it is expected that N-SNVs would be present in all sequences, as observed for 13 N-SNVs. In (3) the crosses would have occurred only with archaic AMH who did not contribute to present day haplogroups while hypotheses (4), (5) and (6) would represent crosses between Neanderthals and lineages who contributed to present day haplogroups. The 66 N-SNVs are consistent with these hypotheses, which exclude their presence in archaic AMH and thus are likely signals of crosses between these two subspecies of humans. More importantly no SNVs are identical between Neanderthals and archaic AMH at the exclusion of present day mitogenomes of either African and Eurasian haplogroups which suggests that the presence of N-SNVs must be a signal of horizontal transfer or a very high number of reverse/convergent substitutions.

3.2. Disease associated N-SNVs

Among the 66 N-SNVs, 7 are associated with diseases as depicted in Table 5. Of note, 4 of these disease-associated N-SNVs were observed in African haplogroups (L0, L1, L2, L3, L4, L5 and L6) and 3 were observed exclusively in Eurasian haplogroups. The most common diseases associated with N-SNVs are neurological disorders and tumors. One N-SNVs, in position 15,043 and associated with depression, was also found in one archaic AMH. Although not considered a *bona fide* N-SNV based on our exclusion criteria, it is relevant because chronic depression has been associated with Neanderthal introgression [26]. Among protein coding genes an important N-SNV was found in *ND2* (position 5,460) causing an amino acid change from alanine to threonine that has been associated with Alzheimer's disease and Parkinson's disease due to its high prevalence in the brains of Alzheimer's and Parkinson's patients [27–29]. This amino acid substitution changes from a nonpolar amino acid to a polar amino acid, which promotes destabilizing effects in the encoded NADH dehydrogenase. Nonpolar to polar amino acid changes are associated with amyloid diseases [30–32]. Other disease associated N- SNVs are in the D-loop, 16S rRNA and tRNA-Cys (Table 2). The prevalence of diseases associated with N-SNVs in Table 2 are (data from https://vizhub.healthdata.org/gbd-compare/, accessed on August 31 2019): Bipolar disorder = 596 cases in 100,000 persons (596/100,000), Parkinsons' disease = 111/100,000, Alzheimer's disease = 588/100,000, Melanoma = 30.42/100,000, Ovarian cancer = 17.71/100,000, Lung cancer = 43/100,000, Prostate cancer = 129/100,000, Stomach cancer = 36.9/100,000. Also, Cycling vomiting syndrome 3.2/100,000 [33], Deafness = 110/1,000 [34], Glioblastoma = 10 /100,000 [35]

3.3. Haplogroups of paleogenomes

Mitogenomes of Neanderthals and archaic AMH were classified in haplogroups according to sequence similarity with extant human mitogenomes using Haplogrep 2 [20] (Table 1). 83% of archaic AMH mitogenomes belong to haplogroup U (45% haplogroup U5 and 16% to haplogroup U2) which is consistent as U being the oldest European haplogroup. Among the 9 Neanderthal mitogenomes, the 7 more recent genomes can be classified as haplogroup H1 (European) while the two oldest can be classified as haplogroup L (African) (Table 1). N-SNVs at positions 16,278 and 16,298 are associated with intelligence quotient [36]. N-SNV 16,278 is found in African haplogroups (L0, L1, L2, L5 and L6) and two Eurasian haplogroups (X3, U2c and P2) and in all Neanderthal sequences while N-SNV 16.298 is found only in Eurasian-Native American haplogroups (V1, V2, M8, C1, C4, C7 and Z1) and only in the Altai Neanderthal.

3.4. PCAs of Neanderthal and Human mitogenomes

Principal component analysis (PCA) of the whole mitochondrial genome shows four clusters: (1) the modern haplogroups including the ancient *H. sapiens* (Table 1), (2) the L haplogroup cluster, (3) the Neanderthal Altai-Mezmaskaya L-like cluster (Table 1) and (4) the Neanderthal H-like group (Figure 5). However, the PCA of the segment corresponding to the ribosomal RNA gene proximal half produces a pattern that approximates the L haplogroup cluster to the Neanderthal Altai-Mezmaskaya L-like cluster suggesting the introgression point. The PCA of ribosomal RNA gene distal half suggests an opposite pattern with L cluster closer to Neanderthal although not as close as shown in Figure 5B.

3.5. Bootscan analysis of mitogenomes

We tested potential recombination in our dataset with bootscan. We used a different set of parental sequences depending on the query mitogenome (Figure 6). The bootscan analysis indicates that there are potential recombination points. Upon deeper analysis we observed that bootscan considers the Neanderthal specific signatures, such as in L haplogroups, as recombination points. Although the bootscan putative recombination segments are above the bootstrap threshold we do not consider this as definitive evidence of recombination since the segments between the Neanderthal signatures are almost identical. Bootscan analysis excluded Human-Neanderthal recombination in rCRS sequence (Figure 7). The sensivity of bootscan to substitution models and alignment methods was assessed by comparing the same set query-parentals with different parameters (Figure 8), revealing minor profile alterations. The alignment

parameters are not so critical in this case because the sequences are extremely conserved (918 polymorphic positions in 16,565bp). Although indels are present in the alignments, 99% are located near the H promoter in the D-loop region. These are automatically excluded in phylogeny inference algorithms and therefore have no weight in bootscan results. The "positional homology" is therefore solid, particularly in coding domains and regions without repeats in non-coding domains. The Neanderthal signatures are in unambiguously aligned segments.

4. Discussion

In the present study we observe Neanderthal signatures in modern human mitochondrial genomes. Recombination test data here presented (bootscan analysis) shows that recombination does not explain the presence of all these 66 SNVs, or N-SNVs, in all modern mitochondrial haplogroups. The topic of recombination in mtDNA is hotly debated and evidence supporting it as well as evidence denying it are published [10,11]. It can be argued that recombination in human mtDNA is extremely rare, just sufficient for mtDNA to escape the Müller's ratchet, but not enough to distinguish clear recombination blocks. We analyzed an alignment of 103 mitogenomes, which contained 918 polymorphic positions, by bootscan tests and PCA. The idea was to evaluate whether the presence of N-SNVs could be explained solely by homoplasy or recombination. We show that there are N-SNVs in present day human mtDNA that are completely absent in archaic modern Human mtDNA (41 mitogenomes). From our data we tested the possibility that rare recombination events might, at least in part, explain these results. Although an intense homoplasy rate could explain the N-SNVs in the D-loop, it is interesting to test if recombination can be associated with N-SNVs in coding regions, especially the nonsynonymous changes in positions 5460 (*ND2*, rs3021088), 7146 (*COX1*, rs372136420), 7650 (*COX2*, without rs, ACC>ATC), 9053 (*ATP6*, rs199646902), 13105 (*ND5*, rs2853501), 13276 (*ND5*, rs2853502) and 14178 (*ND6*, rs28357671). Our bootscan analyses suggest that all these N-SNVs could be explained by homoplasy in the last 40,000 years and that these changes occurred only in the present-day lineages and not in any of the archaic AMH mitogenomes analyzed.

The mitogenomes analyzed were from modern *Homo sapiens*, archaic AMH and Neanderthals as detailed above. Based on populational data Sykes (2001) estimated that a single observed change in comparative mitogenomics corresponds to 10,000 years of divergence. More modern estimates of the mitogenome clock, based on ancient DNA data, ranges from 2.14×10^{-8} to 2.74×10^{-8} substitutions per site per year [37–40], which gives approximately 4.14 substitutions

in mitogenomes in 10,000 years. Therefore, according to populational estimates the 918 polymorphisms would have occurred in a period of 9.18 million years and the 66 N-SNVs for 660,000 years. If ancient DNA estimates are considered, the 918 polymorphisms would correspond to 2.21 million years of evolution and the 66 N-SNVs would have occurred in the last 159,420 years. Therefore all 66 N-SNVs are a product of random changes or simple homoplasy not observed in any of the 41 samples of archaic AMH. This suggests that homoplasy, parallel with Neanderthals, occurred only in the modern lineage, and none of those observed in archaic AMH. Tests for recombination using bootscan (Figure 6) indicate that in 11 positions the bootstrap support is significant for recombination which is not sufficient to explain all N-SNVs. It is important to notice that Posada and Crandall [41] explicitly tested how homoplasy could confound recombination tests and concluded that in extreme levels of rate variation (α=0.05) recombination tests would produce false positives which fits with mtDNA mutational load and among-site rate variation.

The analysis of Neanderthal mitochondrial genomes here presented revealed four derived amino acid changes that modern humans carry in the *COX2* gene as compared to Neanderthals and other Ape outgroups [42]. However, the same four amino acid changes can also be found in macaques which suggests that there is no need to invoke mitochondrial recombination, which is exceedingly rare, if present at all, or interbreeding events, to explain the presence of N-SNVs in modern human mitochondrial genomes and therefore recurrent mutations would be sufficient to explain N-SNVs. The divergence between the *H. sapiens* lineage and macaques lineage is 30.5 (26.9-36.4) million years [43] while the divergence we are dealing here is between 300,000 to 28,000 years. As is known, homoplasy significantly increase with long divergence times which produces the effect of long branch attraction in phylogenies [44]. For example, one of the mutations would be the macaques' parallel mutation in *COX2* gene m.7650C>T. This mutation is found in Neanderthals (except Mezmaskaya), Denisovans, modern R0 haplogroup, Gorilla, Chimpanzee and Bonobo. It was not found in any archaic AMH samples. This pattern more likely suggests that it is highly conserved, inherited by early hominins from apes and secondarily lost in one Neanderthal lineage and in almost all *Homo sapiens* lineages, expect for the R0 haplogroup. The reappearance of this mutation in the very old R0 haplogroup has two possible explanations: (1) a back mutation reverting to exact the same ancestral condition instead of changing to any of the other three possible bases or (2) acquired by recombination *via* a Neanderthal female with introgression in one of the *Homo sapiens* lineages. In our study we present a bootstrap recombination analysis (bootscan) that shows a recombination point with bootstrap support in the region encompassing m.7650C>T (Figures 6A, 6E and 6F). Based on this test alone it seems that

at least regarding this mutation a very rare recombination event could have happened although a back mutation cannot be completely excluded.

Another possible argument is that the Neanderthal signatures are in fact character states conserved since the last common ancestor of Neanderthals and present-day *Homo sapiens* (e.g. *Homo erectus* but this would not be consistent with the absence of these signatures in ancient *H. sapiens* mitochondrial genomes (Figure 1).

In regard to presence of N-SNVs in African haplogroups it is interesting to note that a back to Africa hypothesis has been proposed in which humans from Eurasia returned to Africa and impacted a wide range of sub-Saharan populations [45]. Our data suggest that Neanderthal signatures might be present in all major African haplogroups which is consistent with the "Back to Africa" contribution to the modern mitochondrial African pool. The preponderance of N-SNVs in the D-loop is observed mostly in African haplogroups. In Eurasian haplogroups we observe important changes in coding regions as demonstrated in Figures 2 and 3. Although our data suggest that convergent mutations explains the N-SNVs here observed, mitochondrial recombination is not so rare as to completely exclude it as a potential mechanism in human mtDNA mutational patterns [46,47].

Our observations suggest that crosses between AMH males and Neanderthal females left significantly less descendants than the reverse crosses (Neanderthal males and AMH females), which seems to be the dominant pattern. Although it has been generally accepted that recombination does not occur in the human mitochondrial genome, evidence of mitochondrial recombination has been reported [10,48]. A scenario with complete absence of recombination presents a problem to explain how the human mitochondrial genome would escape the Müller's ratchet and therefore avoiding its predicted "genetic meltdown" [14]. It has been shown that even minimal recombination is sufficient to allow the escape from the Müller's ratchet [49] and this could be the case regarding the human mitochondrial genome. For example, recombination has been simulated along a chromosome of 1000 *loci* to estimate the amounts of recombination required to halt Müller's ratchet and the drift-catalyzed fixation of deleterious mutations. It has been found that for a population size of $N<100$, a recombination rate equivalent to one crossover per chromosome per 100 generations (10^{-5}/locus/generation) countered Müller's ratchet effectively. This is much lower than the minimum of one crossover per chromosome arm per generation that is assumed to occur in sexual taxa. A higher recombination rate of 10^{-4} can impede the selective interference that would otherwise enhance the fixation of deleterious mutations due to genetic drift [50].

Our data is compatible with a scenario in which the AMH-Neanderthal crosses occur in

Europeans, East Asians and African lines of descent which is consistent with recent findings in nuclear genomes [51]. However, in the African haplogroups the crosses between AMH males and Neanderthal females would have a higher frequency than in European lines of descent, where the reverse crosses would be predominant. Based on the comparison of Neanderthal signatures in nuclear and mitochondrial genome haplogroups we hypothesize that the African lines of descent would have a higher female Neanderthal contribution whereas European lines of descent would have higher male Neanderthal contribution. The fact that AMH and Neanderthals crossed and produced fertile descendants is evidence that they belong to the same species [2]. Some authors propose that this suggests that *Homo sapiens* emerged independently in Africa, Europe and Asia [52]. The intercrosses of these three *Homo sapiens* subgroups, and other even deeper ancestors such as Denisovans, in their different proportions and specific signatures, would have produced the extant human genomes.

5. Conclusions

The analyses here presented suggest that Neanderthal genomic signatures might have been a product of convergent evolution due to homoplasy and that rare mtDNA recombination events cannot explain the presence of these signatures in modern mtDNA haplogroups. Although there is some evidence for mtDNA recombination, its weight in phylogenies and mutational patterns in comparative analysis remain controversial. Some authors contend that due to the high mutation rate in mtDNA, reverse compensatory mutations can be confounded with recombination. Our data is consistent with a scenario in which mtDNA recombination is not sufficient to support the contribution of Neanderthal mtDNA to modern human genomes, although rare recombination events might occur in human mtDNA to alleviate the effects of Müller rachet.

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Author contributions

R.C.F. and C.R.R. planned and performed analyses. M.R.S.B. performed preliminary analysis

and wrote the manuscript. R.C.F., C.R.R., J.R.B and M.R.S.B. discussed data and analyses and edited the manuscript.

Competing interests

The authors declare no competing interests.

Data and materials availability

All data and files of analyses here presented are available upon request to the corresponding author.

References

1. Green RE, Krause J, Briggs AW, Maricic T, Stenzel U, Kircher M, et al. A Draft Sequence of the Neandertal Genome. Science. 2010;328: 710–722. doi:10.1126/science.1188021

2. Ko KH. Hominin interbreeding and the evolution of human variation. J Biol Res-Thessalon. 2016;23: 17. doi:10.1186/s40709-016-0054-7

3. Prüfer K, Racimo F, Patterson N, Jay F, Sankararaman S, Sawyer S, et al. The complete genome sequence of a Neanderthal from the Altai Mountains. Nature. 2014;505: 43–49. doi:10.1038/nature12886

4. Simonti CN, Vernot B, Bastarache L, Bottinger E, Carrell DS, Chisholm RL, et al. The phenotypic legacy of admixture between modern humans and Neandertals. Science. 2016;351: 737–741. doi:10.1126/science.aad2149

5. Gibbons A. Close Encounters of the Prehistoric Kind. Science. 2010;328: 680–684. doi:10.1126/science.328.5979.680

6. Higham T, Douka K, Wood R, Ramsey CB, Brock F, Basell L, et al. The timing and spatiotemporal patterning of Neanderthal disappearance. Nature. 2014;512: 306–309. doi:10.1038/nature13621

7. Serre D, Langaney A, Chech M, Teschler-Nicola M, Paunovic M, Mennecier P, et al. No Evidence of Neandertal mtDNA Contribution to Early Modern Humans. PLOS Biol. 2004;2: e57. doi:10.1371/journal.pbio.0020057

8. Kivisild T. Maternal ancestry and population history from whole mitochondrial genomes. Investig Genet. 2015;6: 3. doi:10.1186/s13323-015-0022-2

9. Hill GE, Johnson JD. The mitonuclear compatibility hypothesis of sexual selection. Proc R Soc B. 2013;280: 20131314. doi:10.1098/rspb.2013.1314

10. Kraytsberg Y, Schwartz M, Brown TA, Ebralidse K, Kunz WS, Clayton DA, et al. Recombination of Human Mitochondrial DNA. Science. 2004;304: 981–981. doi:10.1126/science.1096342

11. Piganeau G, Eyre-Walker A. A reanalysis of the indirect evidence for recombination in human mitochondrial DNA. Heredity. 2004;92: 282–288. doi:10.1038/sj.hdy.6800413

12. Muller HJ. Some Genetic Aspects of Sex. Am Nat. 1932;66: 118–138. doi:10.1086/280418

13. Loewe L. Quantifying the genomic decay paradox due to Muller's ratchet in human mitochondrial DNA. Genet Res. 2006;87: 133–159. doi:10.1017/S0016672306008123

14. Felsenstein J. The evolutionary advantage of recombination. Genetics. 1974;78: 737–756.

15. Oven M van, Kayser M. Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum Mutat. 2009;30: E386–E394. doi:10.1002/humu.20921

16. Andrews RM, Kubacka I, Chinnery PF, Lightowlers RN, Turnbull DM, Howell N. Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat Genet. 1999;23: 147. doi:10.1038/13779

17. Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, et al. Geneious Basic: An integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012;28: 1647–1649. doi:10.1093/bioinformatics/bts199

18. Lott MT, Leipzig JN, Derbeneva O, Xie HM, Chalkia D, Sarmady M, et al. mtDNA Variation and Analysis Using Mitomap and Mitomaster. Curr Protoc Bioinforma. 2013;44: 1.23.1-1.23.26. doi:10.1002/0471250953.bi0123s44

19. Swofford DL. PAUP*: Phylogenetic Analysis Using Parsimony (and other methods) 4.0.b5. 2001.

20. Weissensteiner H, Pacher D, Kloss-Brandstätter A, Forer L, Specht G, Bandelt H-J, et al. HaploGrep 2: mitochondrial haplogroup classification in the era of high-throughput sequencing. Nucleic Acids Res. 2016;44: W58–W63. doi:10.1093/nar/gkw233

21. Kimura M. A simple method for estimating evolutionary rates of base substitutions through comparative studies of nucleotide sequences. J Mol Evol. 1980;16: 111–120.

22. Lindenbaum P. JVarkit: java-based utilities for Bioinformatics. 2015. doi:10.6084/m9.figshare.1425030.v1

23. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011;27: 2156–2158. doi:10.1093/bioinformatics/btr330

24. Chang CC, Chow CC, Tellier LC, Vattikuti S, Purcell SM, Lee JJ. Second-generation PLINK: rising to the challenge of larger and richer datasets. GigaScience. 2015;4: 7. doi:10.1186/s13742-015-0047-8

25. Purcell S, Neale B, Todd-Brown K, Thomas L, Ferreira MAR, Bender D, et al. PLINK: A Tool Set for Whole-Genome Association and Population-Based Linkage Analyses. Am J Hum Genet. 2007;81: 559–575.

26. Racimo F, Marnetto D, Huerta-Sánchez E. Signatures of Archaic Adaptive Introgression in Present-

Day Human Populations. Mol Biol Evol. 2017;34: 296–317. doi:10.1093/molbev/msw216

27. Lin F-H, Lin R, Wisniewski HM, Hwang Y-W, Grundke-Iqbal I, Healy-Louie G, et al. Detection of point mutations in codon 331 of mitochondrial NADH dehydrogenase subunit 2 in alzheimer's brains. Biochem Biophys Res Commun. 1992;182: 238–246. doi:10.1016/S0006-291X(05)80136-6

28. Petruzzella V, Chen X, Schon EA. Is a point mutation in the mitochondrial ND2 gene associated with alzheimer's disease? Biochem Biophys Res Commun. 1992;186: 491–497. doi:10.1016/S0006-291X(05)80834-4

29. Schnopp NM, Kösel S, Egensperger R, Graeber MB. Regional heterogeneity of mtDNA heteroplasmy in parkinsonian brain. Clin Neuropathol. 1996;15: 348–352.

30. Alvares RDA, Tulumello DV, Macdonald PM, Deber CM, Prosser RS. Effects of a polar amino acid substitution on helix formation and aggregate size along the detergent-induced peptide folding pathway. Biochim Biophys Acta BBA - Biomembr. 2013;1828: 373–381. doi:10.1016/j.bbamem.2012.09.024

31. Podoly E, Hanin G, Soreq H. Alanine-to-threonine substitutions and amyloid diseases: Butyrylcholinesterase as a case study. Chem Biol Interact. 2010;187: 64–71. doi:10.1016/j.cbi.2010.01.003

32. Volkenstein MV. Coding of Polar and Non-polar Amino-acids. Nature. 1965;207: 294–295. doi:10.1038/207294a0

33. Fitzpatrick E, Bourke B, Drumm B, Rowland M. The incidence of cyclic vomiting syndrome in children: population-based study. Am J Gastroenterol. 2008;103: 991–995; quiz 996. doi:10.1111/j.1572-0241.2007.01668.x

34. Lancet T. Hearing loss: an important global health concern. The Lancet. 2016;387: 2351. doi:10.1016/S0140-6736(16)30777-2

35. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma Multiforme: A Review of its Epidemiology and Pathogenesis through Clinical Presentation and Treatment. Asian Pac J Cancer Prev APJCP. 2017;18: 3–9. doi:10.22034/APJCP.2017.18.1.3

36. Thomas MG, Miller KWP, Mascie-Taylor CGN. Mitochondrial DNA and IQ in Europe. Intelligence. 1998;26: 167–173. doi:10.1016/S0160-2896(99)80060-9

37. Fu Q, Mittnik A, Johnson PLF, Bos K, Lari M, Bollongino R, et al. A Revised Timescale for Human Evolution Based on Ancient Mitochondrial Genomes. Curr Biol. 2013;23: 553–559. doi:10.1016/j.cub.2013.02.044

38. Fu Q, Li H, Moorjani P, Jay F, Slepchenko SM, Bondarev AA, et al. Genome sequence of a 45,000-year-old modern human from western Siberia. Nature. 2014;514: 445–449. doi:10.1038/nature13810

39. Posth C, Renaud G, Mittnik A, Drucker DG, Rougier H, Cupillard C, et al. Pleistocene Mitochondrial Genomes Suggest a Single Major Dispersal of Non-Africans and a Late Glacial Population Turnover in Europe. Curr Biol. 2016;26: 827–833. doi:10.1016/j.cub.2016.01.037

40. Rieux A, Eriksson A, Li M, Sobkowiak B, Weinert LA, Warmuth V, et al. Improved Calibration of the Human Mitochondrial Clock Using Ancient Genomes. Mol Biol Evol. 2014;31: 2780–2792. doi:10.1093/molbev/msu222

41. Posada D, Crandall KA. Evaluation of methods for detecting recombination from DNA sequences: Computer simulations. Proc Natl Acad Sci. 2001;98: 13757–13762. doi:10.1073/pnas.241370698

42. Green RE, Malaspinas A-S, Krause J, Briggs AW, Johnson PLF, Uhler C, et al. A Complete Neandertal Mitochondrial Genome Sequence Determined by High-Throughput Sequencing. Cell. 2008;134: 416–426. doi:10.1016/j.cell.2008.06.021

43. Steiper ME, Young NM. Primate molecular divergence dates. Mol Phylogenet Evol. 2006;41: 384–394. doi:10.1016/j.ympev.2006.05.021

44. Bergsten J. A review of long-branch attraction. Cladistics. 2005;21: 163–193. doi:10.1111/j.1096-0031.2005.00059.x

45. Henn BM, Botigué LR, Gravel S, Wang W, Brisbin A, Byrnes JK, et al. Genomic Ancestry of North Africans Supports Back-to-Africa Migrations. PLOS Genet. 2012;8: e1002397. doi:10.1371/journal.pgen.1002397

46. D'Aurelio M, Gajewski CD, Lin MT, Mauck WM, Shao LZ, Lenaz G, et al. Heterologous mitochondrial DNA recombination in human cells. Hum Mol Genet. 2004;13: 3171–3179. doi:10.1093/hmg/ddh326

47. Mita S, Rizzuto R, Moraes CT, Shanske S, Arnaudo E, Fabrizi GM, et al. Recombination via flanking direct repeats is a major cause of large-scale deletions of human mitochondrial DNA. Nucleic Acids Res. 1990;18: 561–567.

48. Schwartz M, Vissing J. Paternal Inheritance of Mitochondrial DNA. N Engl J Med. 2002;347: 576–580. doi:10.1056/NEJMoa020350

49. Dokianakis E, Ladoukakis ED. Different degree of paternal mtDNA leakage between male and female progeny in interspecific Drosophila crosses. Ecol Evol. 2014;4: 2633–2641. doi:10.1002/ece3.1069

50. Neiman M, Taylor DR. The causes of mutation accumulation in mitochondrial genomes. Proc R Soc B Biol Sci. 2009;276: 1201–1209. doi:10.1098/rspb.2008.1758

51. Chen L, Wolf AB, Fu W, Li L, Akey JM. Identifying and Interpreting Apparent Neanderthal Ancestry in African Individuals. Cell. 2020; S0092867420300593. doi:10.1016/j.cell.2020.01.012

52. Horai S, Hayasaka K, Kondo R, Tsugane K, Takahata N. Recent African origin of modern humans revealed by complete sequences of hominoid mitochondrial DNAs. Proc Natl Acad Sci U S A. 1995;92: 532–536.

53. Hervella M, Svensson EM, Alberdi A, Günther T, Izagirre N, Munters AR, et al. The mitogenome of a 35,000-year-old *Homo sapiens* from Europe supports a Palaeolithic back-migration to Africa. Sci Rep. 2016;6: 25501. doi:10.1038/srep25501

54. Formicola V, Pontrandolfi A, Svoboda J. The Upper Paleolithic triple burial of Dolní Věstonice:

Pathology and funerary behavior. Am J Phys Anthropol. 2001;115: 372–379. doi:10.1002/ajpa.1093

55. Gansauge M-T, Meyer M. Selective enrichment of damaged DNA molecules for ancient genome sequencing. Genome Res. 2014; gr.174201.114. doi:10.1101/gr.174201.114

56. Briggs AW, Good JM, Green RE, Krause J, Maricic T, Stenzel U, et al. Targeted Retrieval and Analysis of Five Neandertal mtDNA Genomes. Science. 2009;325: 318–321. doi:10.1126/science.1174462

Table 1. Archaic AMH and Neanderthal mitogenomes used in this study. Haplogroup inference quality** as calculated by Haplogrep 2 **[20]**.

Archaic AMH	**Haplogroup**	**Quality** (%)**	**Age (years)**	**Location**	**Reference**	**GenBank/ENA***
Berry Au Bac 1	U5b1a	98.78	7,160-7,319	France	[39]	KU534977
Bockstein	U5b1d1	98.72	8,016-8,329	Germany	[39]	KU534973
Cuiry Les Chaudardes 1	U5b1b	97.01	8,050-8,360	France	[39]	KU534975
Ofnet	U5b1d1	98.72	8,159-8,424	Germany	[39]	KU534974
Felsdach	U5a2c	97.43	8,380-8,980	Germany	[39]	KU534954
Hohlenstein Stadel	U5b2c1	94.77	8,446-8,809	Germany	[39]	KU534979
Falkenstein	U5b2a	92.19	8,993-9,409	Germany	[39]	KU534980
Mareuil Les Meaux 1	U5a2+16362	100	9,080-9,500	France	[39]	KU534959
Les Closeaux 3	U5a2	98.27	9,580-10,230	France	[39]	KU534958
Ranchot 88	U5b1	95.95	9,933-10,235	France	[39]	KU534978
Iboussieres 31-2	U5b1+16189	99.01	10,140	France	[39]	KU534976
Iboussieres 25-1	U5b2a	93.92	10,140	France	[39]	KU534981
Iboussieres 39	U5b2b	92.40	11,600-12,040	France	[39]	KU534972
Hohle Fels 10	U8a	96.92	12,700	Germany	[39]	KU534961
Rochedane	U5b2b	97.01	12,830-13,090	France	[39]	KU534971
Burkhardtshohle	U8a	96.92	14,150-15,080	Germany	[39]	KU534960
Hohle Fels 79	U8a	96.92	14,270-15,070	Germany	[39]	KU534962
Brillenhohle	U8a	97.66	14,400-15,120	Germany	[39]	KU534947
Oberkassel 998	U5b1+	99.45	14,000	Germany	[53]	KC521457
Goyet Q-2	U8a	96.92	14,780-15,230	Belgium	[39]	KU534963
Rigney 1	U2'3'4'7'8'9	87.68	15,240-15,690	France	[39]	KU534957
Hohle Fels 49	U8a	96.92	15,568-16,250	Germany	[39]	KU534964
Paglicci 71	U5b2b	97.97	18,197-18,973	Italy	[39]	KU534950
Dolni Vestonice 43	U5	97.44	25,000	Czech Rep.	[39]	KU534970
Goyet Q56-16	U2	91.40	26,040-26,600	Belgium	[39]	KU534965
Goyet 2878-21	U5	92.05	26,269-27,055	Belgium	[39]	KU534955
Goyet Q376-19	U2	89.43	27,310-27,720	Belgium	[39]	KU534967
Goyet Q55-2	U2	85.86	27,310-27,730	Belgium	[39]	KU534948
La Rochette	M	92.38	27,400-27,784	France	[39]	KU534951
Goyet Q53-1	U2	93.23	27,720-28,230	Belgium	[39]	KU534966
Paglicci 108	U2'3'4'7'8'9	92.40	27,831-28,961	Italy	[39]	KU534968
Paglicci 133	U8c	99.41	28,000-29,000	Italy	[39]	KU534956
Dolni Vestonice 16	U5	97.94	29,386-30,567	Czech Rep.	[39]	KU534949
Doni Vestonice 14	U5	100	31,000	Czech Rep.	[54]	KC521458
Cioclovina 1	U	96.72	32,519-33,905	Romania	[39]	KU534969
Goyet Q376-3	M	88.67	33,140-33940	Belgium	[39]	KU534953
Goyet Q116-1	M	93.64	34,430-35,160	Belgium	[39]	KU534952

Kostenki 14	U2	92.69	36-39,000	Russia	[53]	FN600416*
Fumane 2	R	94.43	39-41,000	Italy	[53]	KP718913
Tianyuan	B4’5	91.17	40,000	China	[37]	KC417443
Ust-Ishim	R	87.89	45,000	West Siberia	[53]	PRJEB6622*
Neanderthals						
Vindija 33.16	H1e	52.74	38,000	Croatia	[42]	AM948965
Vindija 33.17	H1e	52.75	Not dated	Croatia	[55]	KJ533544
Vindija 33.19	H1e	52.74	Not dated	Croatia	[55]	KJ533545
Vindija 33.25	H1as	52.84	Not dated	Croatia	[56]	FM865410
El Sidron 1253	H1e	52.81	39,000	Spain	[53,56]	FM865409
Feldhofer 1	H1as	52.84	40,000	Germany	[56]	FM865407
Feldhofer 2	H1e	52.81	40,000	Germany	[56]	FM865408
Altai Neanderthal	L1’2’3’4’5’6	55.96	50,000	Siberia	[3]	KC879692
Mezmaskaya 1	L1’2’3’4’5’6	52.12	65,000	Russia	[56]	FM865411

Table 2. Haplogroups of present-day *Homo sapiens sapiens* mitogenomes used in this study.

Mitochondrial Haplogroup	GenBank	Mitochondrial Haplogroup	GenBank
L0a1b1	AF381988	S1	AF346963
L1c3a	AF381992	Q1	AY289090
L2a1f	AY195776	Z1	AY519493
L3d3b	AF381998	O	AY289059
L4a1	FJ460531	Y1	KF540727
L5a1a	DQ341060	R0a	JX153281
L6a	EU092773	R0a1a3	GU592021
N1b1a3	AY195756	R1a	KC985147
N9a1	HM589048	I1	JQ245776
N2a	JF904935	W1	EU558696
A	AP013225	X1a	EU600318
B2	EF648602	X3	EF177437
F1a1	NA17963	U1a1d	EF692533
M29a	DQ137407	U2c	AY714010
M2b	EU443512	U6a7a2	AF382008
M3b	FJ383523	K	AF382005
M8a1	KF148510	K1	EU073969
M9a	HM346891	K2a2a	EU327986
M20	JX289112	H1a1	EU007858
G1a1	HM460792	H3	EU150187
E1	KF540505	H15	KC911292
D4	JQ704974	J1c	EU547187
C1a	EU007858	J2a2a	EF660967
C4	FJ951604	T1	JQ797976
C7	FJ951594	V1a	JQ702026
P2	AY289088	V2	JQ703647

Table 3. Complete list of mitogenome positions where present day sequences are identical with Neanderthals.

Position	Modern haplogroups	Neanderthals
146	L2a1f, L6a, K, K2a2a, R0a1a3, X1a, X3, Y1, M3b, N1b1a3, Q1	Altai, El Sidron 1253, Feldhofer 1, Feldhofer 2, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
152	L1c3a, L2a1f, L3d3b, L6a, I1, K1, K2a2a, T1, U2c, U6a7a2, N1b1a3, M29a, M2b, M20, C7, S1, Z1	El Sidron 1253, Feldhofer 1, Feldhofer 2, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
185	L0a1b1	Altai, Mezmaiskaya 1
189	L0a1b1, L5a1a, W1, C4	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
195	L2a1f, L4a1, L5a1a, L6a, W1, K, J2a2a, M2b	Altai Neanderthal
247	L0a1b1, L1c3a, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
709	L5a1a, L6a, W1, K2a2a, T1, Y1, N2a, G1a1, S1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
769	L0a1b1, L1c3a, L2a1f, L4a1, L5a1a, L6a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
825	L0a1b1, L1c3a, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
827	R0a1a3, B2	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
1018	L0a1b1, L1c3a, L2a1f, L4a1, L5a1a, L6a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
2706	U6a7a2, rCRS, H1a1, H3, H15	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
2758	L0a1b1, L1c3a	Altai Neanderthal, Mezmaiskaya 1
2885	L0a1b1, L1c3a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
3010	H1a1, J1c, D4	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
3594	L0a1b1, L1c3a, L2a1f, L5a1a, L6a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
4104	L0a1b1, L1c3a, L2a1f, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
4312	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
4688	L3d3b	Feldhofer 1, Vindija 33.25

5460	L0a1b1, L4a1, W1, Q1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
5471	U6a7a2, N1b1a3	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
5821	C7	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
6962	F1a1	Mezmaiskaya 1
7146	L0a1b1, Lc3a	Altai Neanderthal, Mezmaiskaya 1
7256	L0a1b1, L1c3a, L2a1f, L5a1a, L6a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
7424	L3d3b, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
7521	L0a1b1, L1c3a, L2a1f, L5a1a, R0a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
7650	R0a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
8386	J2a2a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
8468	L0a1b1, Lc3a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
8655	L0a1b1, L1c3a, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
8701	L0a1b1, L1c3a, L2a1f, L3d3b, L4a1, L5a1a, L6a, M29a, M2b, M3b, M8a1, M9a, M20, G1a1, E1, Q1, Z1, C1a, C4, C7	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
9053	F1a1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
9090	Z1	Altai Neanderthal
9540	L0a1b1, L2a1f, L3d3b, L4a1, L5a1a, L6a, C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, Q1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
9755	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10310	F1a1, A	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10373	L4a1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10398	L0a1b1, L1c3a, L2a1f, L3d3b, L4a1, L5a1a, L6a, J1c, I1, J2a2a, K, K1, C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, Q1, Y1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10586	L1c3a	Feldhofer 2
10664	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25

10688	L0a1b1, L1c3a, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10810	L0a1b1, L1c3a, L5a1a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10873	L0a1b1, L1c3a, L2a1f, L3d3b, L4a1, L5a1a, L6a, Q1, Z1, C1a, C4, C7, M29a, M2b, M3b, M8a1, M9a, M20, G1a1, E1, D4	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
10915	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
11914	L0a1b1, L2a1f, K2a2a, C1a, C4, C7, M20	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
12366	M29a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
12406	F1a1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
12705	L0a1b1, L1c3a, L2a1f, L3d3b, L4a1, L5a1a, L6a, W1, X1a, I1, X3, A, C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, N1b1a3, N2a, N9a1, O, Q1, S1, Y1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
12810	L1c3a, K1	Mezmaiskaya 1
13105	L0a1b1, L1c3a, L3d3b, L5a1a, V2	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
13276	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
14178	L1c3a, Y1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
14560	L1c3a, X3	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
15043	U6a7a2, U1a1d, I1, C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, Q1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
15148	U1a1d, M8a1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
15244	L6a, V2	Altai Neanderthal
15301	L2a1f, L3d3b, L4a1, L6a, K C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, Q1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
15355	M8a1, B2	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16086	M8a1, M20	Mezmaiskaya 1
16093	L4a1, R0a, K1, H3, T1, A, C1a	Feldhofer 1, Vindija 33.25
16148	L0a1b1, L5a1a, Q1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16170	U2c	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25

16182	X1a, X3, U1a1d, M2b, M29a, O	Feldhofer 2, Mezmaiskaya 1, Vindija 33.19
16183	X1a, X3, U1a1d, B2, M2b, M29a, O	El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16209	H1a1, S1, M20	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16223	L0a1b1, L1c3a, L2a1f, L3d3b, L4a1, L5a1a, L6a, X3, I1, W1, X1a, A, C1a, C4, C7, D4, E1, G1a1, M20, M29a, M2b, M3b, M8a1, M9a, N1b1a3, N2a, N9a1, O, Q1, S1, Z1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16230	L0a1b1	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16234	U2c, M9a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16278	L0a1b1, L1c3a, L2a1f, L5a1a, L6a, X3, U2c, P2	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16298	V1a, V2, Z1, M8a1, C1a, C4, C7	Altai Neanderthal
16311	L0a1b1, L1c3a, L4a1, I1, L5a1a, L6a, V1a, K, K1, K2a2a, R1a, O, Q1, M29a	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16320	L0a1b1, M2b	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16362	L4a1, L5a1a, L6a, V2, K1, U1a1d, I1, R0a, R0a1a3, R1a, G1a1, E1, D4, M9a, A	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25
16519	L1c3a, L2a1f, L6a, H15, H1a1, H3, I1, K, K1, K2a2a, R0a1a3, R1a, U2c, V1a, W1, X1a, X3, B2, C4, C7, E1, F1a1, G1a1, M20, M29a, M2b, M3b, N1b1a3, N2a, O, P2	Altai Neanderthal, El Sidron 1253, Feldhofer 1, Feldhofer 2, Mezmaiskaya 1, Vindija 33.16, Vindija 33.17, Vindija 33.19, Vindija 33.25

Table 4. Full list of all mitogenome N-SNVs analysed in this study. Ts=transition, Tv=transversion, AA=amino acid, Nt=nucleotide, Freq=Frequency, Alig.=alignment, 1K Gen.=1000 genomes project database, Prot. Effect=Protein effect.

Position	DbSNP	Change	Change	Change	Type	Prot. Effect	Alignment.	1K Gen.	Gene	Codon
	rs	AA	Nt	Codon			Freq (%)	Freq (%)		Position
146	rs370482130	-	T>C	-	Ts	-	28.40%	21.40%	-	-
150	rs62581312	-	C > T	-	Ts	-	20.90%	19.10%	-	-
152	rs117135796	-	T>C	-	Ts	-	38.80%	33%	-	-
185	rs879015046	-	G>A	-	Ts	-	4.50%	3.70%	-	-
189	rs371543232	-	A>G	-	Ts	-	19.40%	7.70%	-	-
195	rs2857291	-	T>C	-	Ts	-	13.40%	24.70%	-	-
247	rs41334645	-	G>A	-	Ts	-	17.90%	7.00%	-	-
709	rs2853517	-	G>A	-	Ts	-	26.90%	16.70%	-	-
769	rs2853519	-	G>A	-	Ts	-	22.40%	16.40%	-	-
825	rs2853520	-	T>A	-	Tv	-	17.90%	7.10%	-	-
827	rs28358569	-	A>G	-	Ts	-	16.40%	4.30%	-	-
1018	rs2856982	-	G>A	-	Ts	-	22.40%	16.50%	-	-
2706	rs2854128	-	A>G	-	Ts	-	80.60%	89.00%	-	-
2758	rs2856980	-	G>A	-	Ts	-	6.00%	6.60%	-	-
2885	rs2854130	-	T>C	-	Ts	-	16.40%	6.80%	-	-
3010	rs3928306	-	G>A	-	Ts	-	17.90%	11.20%	-	-
3594	rs193303025	-	C>T	GTC>GTT	Ts	No change	20.90%	15.90%	*ND1*	3
4104	rs1117205	-	A>G	CTA>CTG	Ts	No change	19.40%	15.80%	*ND1*	3
4312	rs193303033	-	C>T	-	Ts	-	14.90%	1.80%	*tRNA*	-
4688	rs878853056	-	T>C	GCT>GCC	Ts	No change	4.50%	0.40%	*ND2*	3
5460	rs3021088	A>T	G>A	GCC>ACC	Ts	AA change	19.40%	7.50%	*ND2*	1
5471	rs879108598	-	G>A	ACG>ACA	Ts	No change	16.40%	0.50%	*ND2*	3
5821	rs56133209	-	G>A	-	Ts	-	14.90%	0.50%	*tRNA*	-
6962	rs1970771	-	G>A	CTG>CTA	Ts	No change	3.00%	3.00%	*COX1*	3
7146	rs372136420	T>A	A>G	ACT>GCT	Ts	AA change	6.00%	6.10%	*COX1*	1
7256	-	-	C>T	AAC>AAT	Ts	No change	20.90%	15.80%	*COX1*	3
7424	-	-	A>G	GAA>GAG	Ts	No change	16.40%	2.30%	*COX1*	3
7521	rs199474817	-	G>A	-	Ts	-	20.90%	16.00%	*tRNA*	-
7650	-	T>I	C>T	ACC>ATC	Ts	AA change	13.40%	0.00%	*COX2*	2
8386	-	-	C>T	ACC>ACT	Ts	No change	14.90%	0.00%	*ATP8*	3
8468	rs1116907	-	C>T	CTA>TTA	Ts	No change	16.40%	6.70%	*ATP8*	1
8655	-	-	C>T	ATC>ATT	Ts	No change	17.90%	7.10%	*ATP6*	3
9053	rs199646902	S>N	G>A	AGC>AAC	Ts	AA change	14.90%	2.60%	*ATP6*	2
9090	rs386829064	-	T>C	TCT>TCC	Ts	No change	3.00%	0.30%	*ATP6*	3
9755	rs2856985	-	G>A	GAG>GAA	Ts	No change	14.90%	2.00%	*COX3*	3
10310	rs41467651	-	G>A	CTG>CTA	Ts	No change	16.40%	4.00%	*ND3*	3

10373	rs28358277	-	G>A	GAG>GAA	Ts	No change	14.90%	3.00%	*ND3*	3
10586	rs28358281	-	G>A	TCG>TCA	Ts	No change	3.00%	2.60%	*ND4L*	3
10664	-	-	C>T	GTC>GTT	Ts	No change	14.90%	1.80%	*ND4L*	3
10688	rs2853488	-	G>A	GTG>GTA	Ts	No change	17.90%	7.00%	*ND4L*	3
10810	rs28358282	-	T>C	CTT>CTC	Ts	No change	17.9	7.10%	*ND4*	3
10915	rs2857285	-	T>C	TGT>TGC	Ts	No change	14.90%	2.30%	*ND4*	3
11914	rs2853496	-	G>A	ACG>ACA	Ts	No change	23.90%	12.70%	*ND4*	3
12366	-	-	A>G	CTA>CTG	Ts	No change	14.90%	0.20%	*ND5*	3
12810	rs28359174	-	A>G	TGA>TGG	Ts	No change	4.50%	2.40%	*ND5*	3
13105	rs2853501	I>V	A>G	ATC>GTC	Ts	AA change	20.90%	13.70%	*ND5*	1
13276	rs2853502	M>V	A>G	ATA>GTA	Ts	AA change	14.90%	1.80%	*ND5*	1
14178	rs28357671	I>V	T>C	ATT>GTT	Ts	AA change	16.40%	5.20%	*ND6*	1
14560	rs28357676	-	G>A	GTC>GTT	Ts	No change	16.40%	5.40%	*ND6*	3
15148	rs527236206	-	G>A	CCG>CCA	Ts	No change	16.40%	1.10%	*CYTB*	3
15244	rs28357369	-	A>G	GGA>GGG	Ts	No change	4.50%	2.10%	*CYTB*	3
15355	rs527236181	-	G>A	ACG>ACA	Ts	No change	16.40%	0.60%	*CYTB*	3
16086	rs386420030	-	T>C	-	Ts	-	4.50%	2.10%	-	-
16093	rs2853511	-	T>C	-	Ts	-	13.40%	5.60%	-	-
16148	rs201893071	-	C>T	-	Ts	-	17.90%	3.10%	-	-
16169	rs879121566	-	C>T	-	Ts	-	1.50%	0.80%	-	-
16182	rs879044186	-	A>C	-	Tv	-	15.20%	2.70%	-	-
16183	rs28671493	-	A>G	-	Ts	-	1.50%	0.40%	-	-
16209	rs386829278	-	T>C	-	Ts	-	17.90%	3.00%	-	-
16230	rs2853514	-	A>G	-	Ts	-	14.90%	2.10%	-	-
16234	rs368259300	-	T>C	-	Ts	-	16.40%	1.90%	-	-
16278	rs41458645	-	C>T	-	Ts	-	25.40%	20.80%	-	-
16298	rs148377232	-	T>C	-	Ts	-	11.90%	5.00%	-	-
16311	rs34799580	-	T>C	-	Ts	-	34.30%	18.70%	-	-
16320	rs62581338	-	C>T	-	Ts	-	16.40%	5.20%	-	-
16519	rs3937033	-	T>C	-	Ts	-	61.20%	62.30%	-	-

Table 5. Disease associated Neanderthal SNVs. Associations as compiled and summarized in MITOMAP [18].

Position	195	3,010	5,460	5,821	16,093	16,183	16,519
Region / gene	D-loop	16S rRNA	*ND2*	tRNA-Cys	D-loop	D-loop	D-loop
dbSNP rs	rs2857291	rs3928306	rs3021088	rs56133209	rs2853511	rs28671493	rs3937033
DNA change	T>C	G>A	G>A	G>A	T>C	A>C	T>C
Type	Transition	Transition	Transition	Transition	Transition	Transversion	Transition
Codon position	-	-	1	-	-	-	-
Codon effect	-	-	Non Syn.	-	-	-	-
Codon change	-	-	GCC>ACC	-	-	-	-
Protein change	-	-	Ala>Thr	-	-	-	-
Disease associations	Bipolar disorder / melanoma patients	Cyclic Vomiting Syndrome with Migraine	Alzheimer`s disease / Parkinson`s disease	Deafness helper mutation	Cyclic Vomiting Syndrome	Melanoma patients	Cyclic Vomiting Syndrome with Migraine /metastasis / glioblastoma, gastric, lung, ovarian, prostate tumors
Modern haplogroups	L2a1f, L4a1, L5a1a, L6a, W1, K, J2a2a, M2b	H1a1, J1c, D4	L0a1b1, L4a1, W1, Q1	C7	L4a1, R0a, K1, H3, T1, A, C1a	X1a, X3, U1a1d, B2, M2b, M29a, O	L1c3a, L2a1f, L6a, H15, H1a1, H3, I1, K, K1, K2a2a, R0a1a3, R1a, U2c, V1a, W1, X1a, X3, B2, C4, C7, E1, F1a1, G1a1, M20, M29a, M2b, M3b, N1b1a3, N2a, O, P2
Neanderthal	Altai	Altai El Sidron 1253 Feldhofer 1 Feldhofer 2 Mezmaiskaya1 Vindija 33.16 Vindija 33.17 Vindija 33.19 Vindija 33.25	Altai El Sidron 1253 Feldhofer 1 Feldhofer 2 Mezmaiskaya1 Vindija 33.16 Vindija 33.17 Vindija 33.19 Vindija 33.25	Altai El Sidron 1253 Feldhofer 1 Feldhofer 2 Mezmaiskaya1 Vindija 33.16 Vindija 33.17 Vindija 33.19 Vindija 33.25	Feldhofer1 Vin. 33.25	El Sidron 1253 Feldhofer 1 Feldhofer 2 Mezmaiskaya1 Vindija 33.16 Vindija 33.17 Vindija 33.19 Vindija 33.25	Altai El Sidron 1253 Feldhofer 1 Feldhofer 2 Mezmaiskaya1 Vindija 33.16 Vindija 33.17 Vindija 33.19 Vindija 33.25

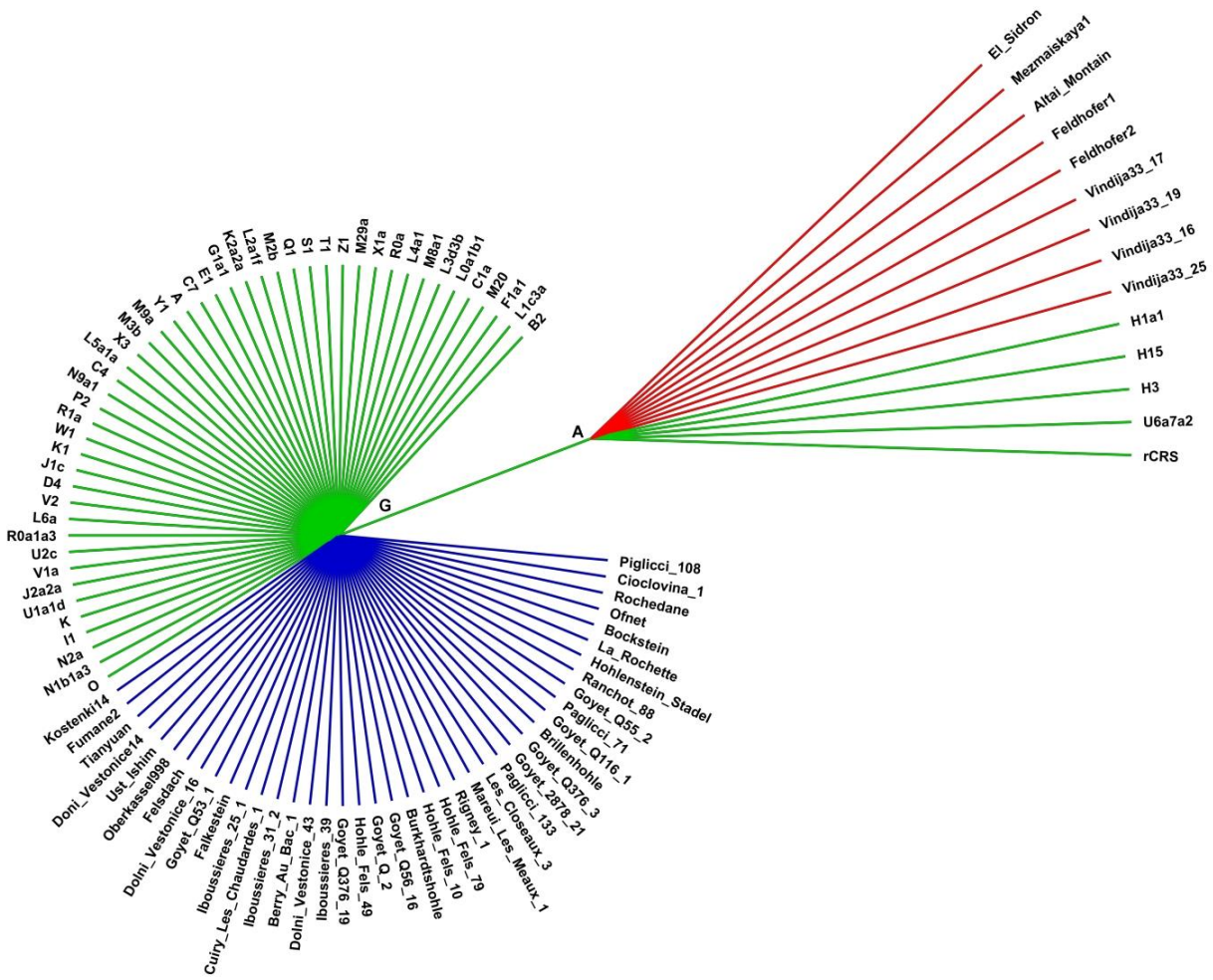

Fig. 1. Cladogram of mitochondrial genome position 2,706. This N-SNV is a G-A transition in the 16S rRNA gene. This genetic signature (Neanderthal signature 2706G) is present in all Neanderthal sequences (Red branches) and in European haplogroups U (U5a7a2) and H (H1a1, H3, H15), including the Revised Cambridge Reference Sequence (rCRS, haplogroup H2a2a1). The Neanderthal signature 2706G is absent in all archaic AMH (Anatomically Modern Humans from Europe in temporal overlap with Neanderthals - Blue branches) and other modern mitochondrial haplogroups. Position numbering corresponds to rCRS positions. This cladogram is representative of the 66 cladograms generated for each N-SNV giving similar topologies and which are available upon request.

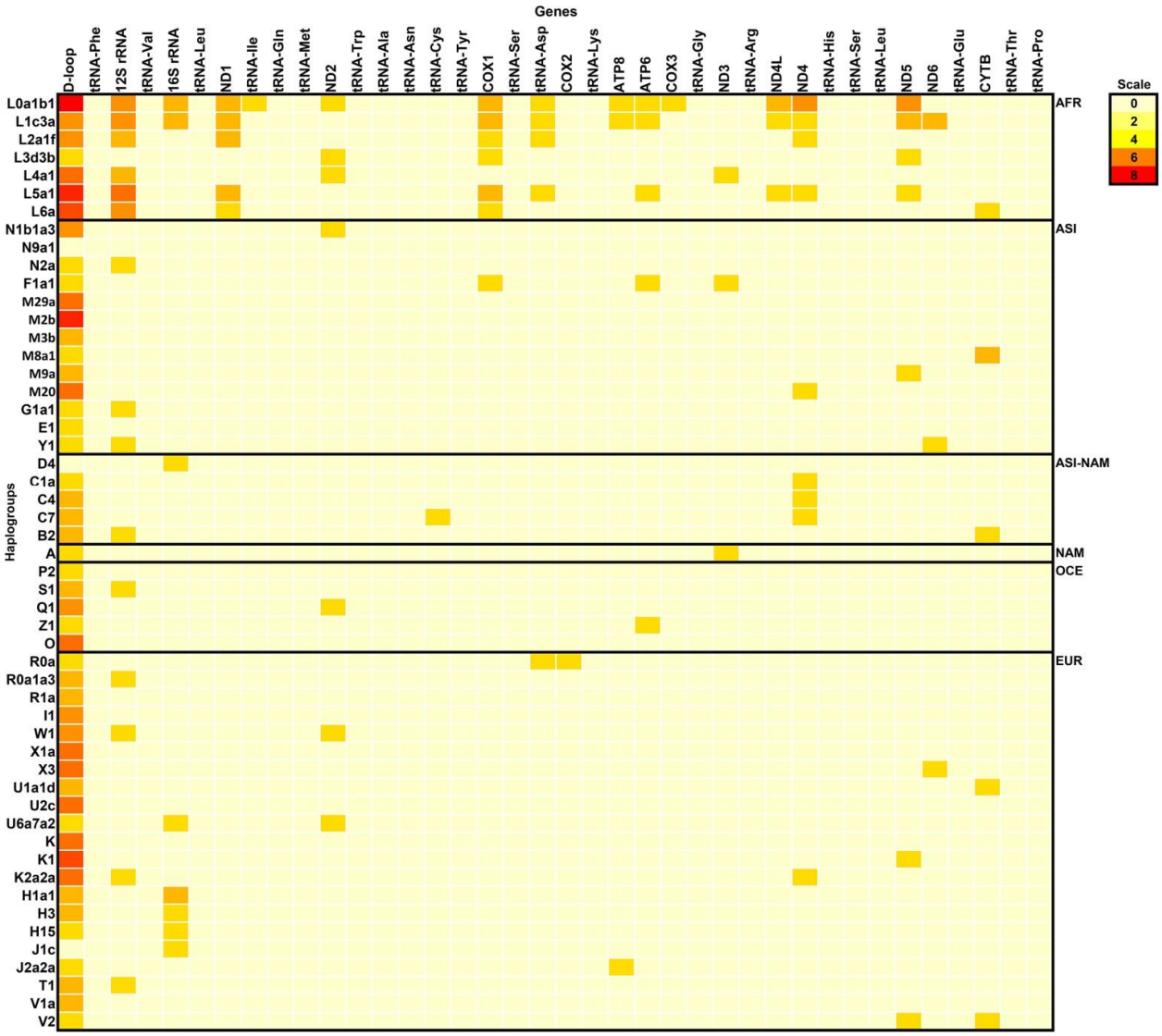

Fig. 2. Heatmap of Neanderthal signatures along the mitochondrial genome in different haplogroups. The color scale indicates the number of Neanderthal signatures (N-SNVs) present in modern human mitochondrial haplogroups. These signatures are absent in Ancient *H. sapiens* whose time range overlapped with Neanderthals in Europe (from approximately 50,000 to 28,000 years ago, Table 1). AFR=African, ASI=Asian, MDE=Middle East, NAM=Native American, OCE=Oceania and EUR=European.

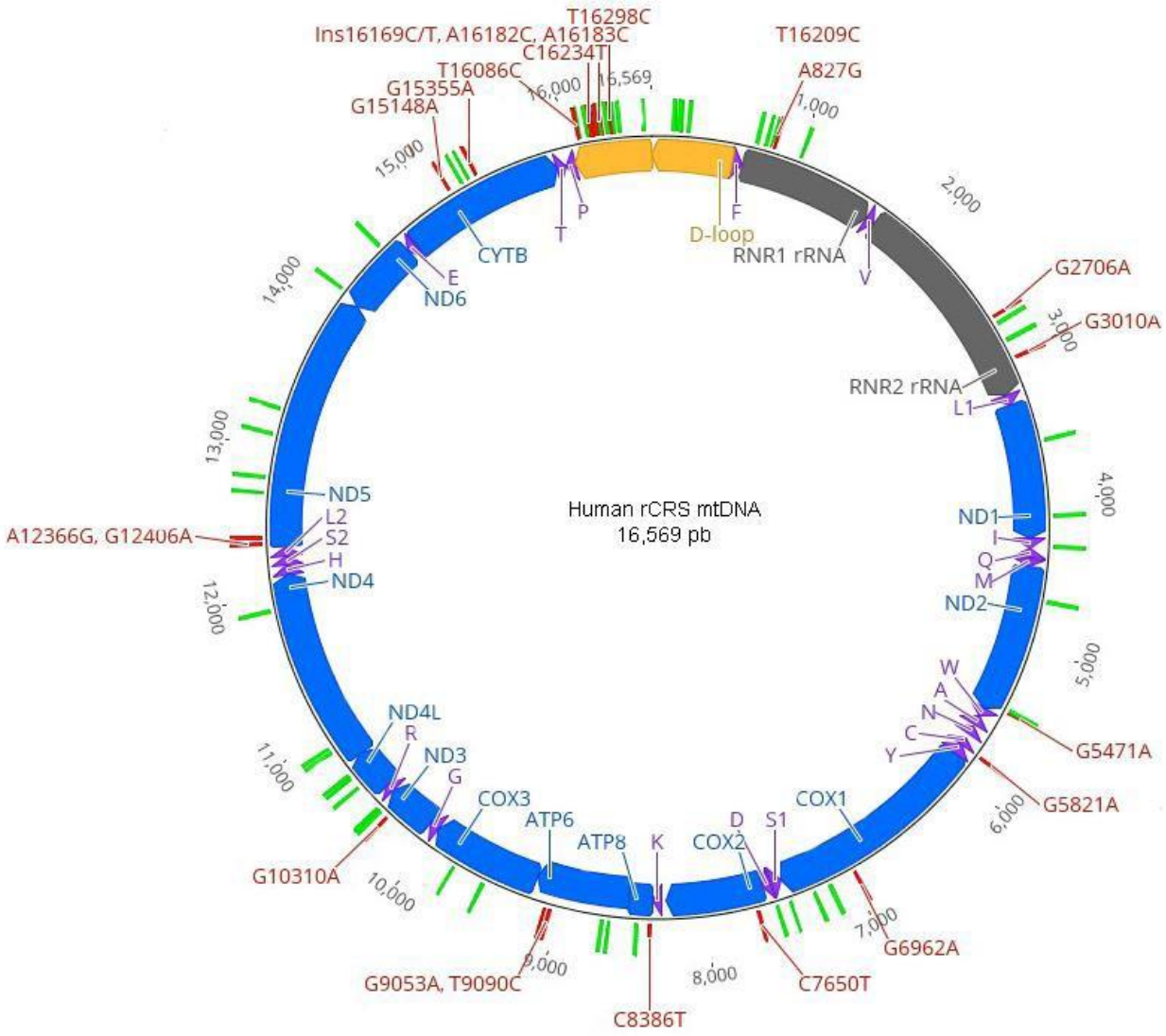

Fig. 3. Position of Neanderthal SNVs (N-SNVs) in the mitochondrial genome. Blue bars indicate protein coding genes, gray bars indicate ribosomal RNA genes, purple arrows indicate tRNAs, yellow bars indicate the D-loop, green bars indicate Neanderthal SNVs present in African haplogroups and red bars indicate N-SNVs exclusive of Eurasian haplogroups.

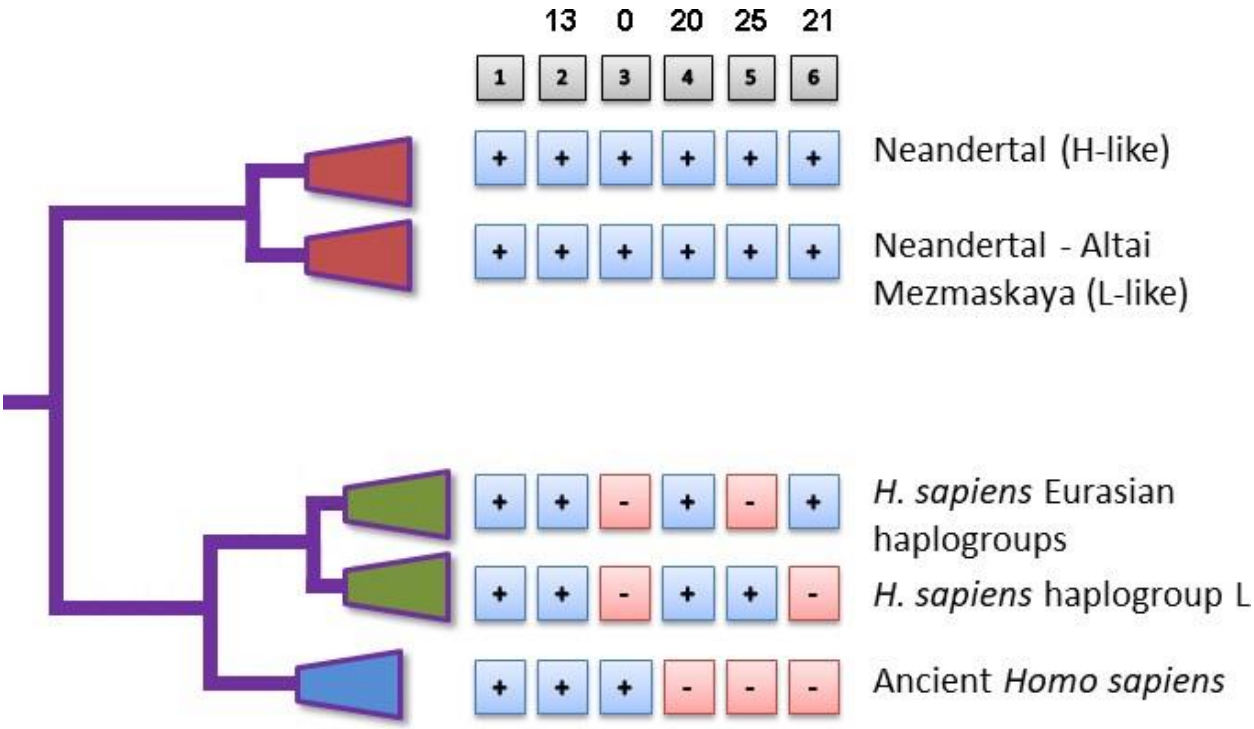

Fig. 4. Phylogenetic distribution of Neanderthal variants in modern humans and archaic AMH. The diagram depicts six possible introgression routes, or intercross events, between Neanderthals, archaic AMH and lineages leading to present day *Homo sapiens* haplogroups. Numbers above gray boxes indicate the number of specific N-SNVs in that particular clade. Plus (+) and minus (-) signs within blue and red boxes indicate N-SNVs presence or absence in that particular clade.

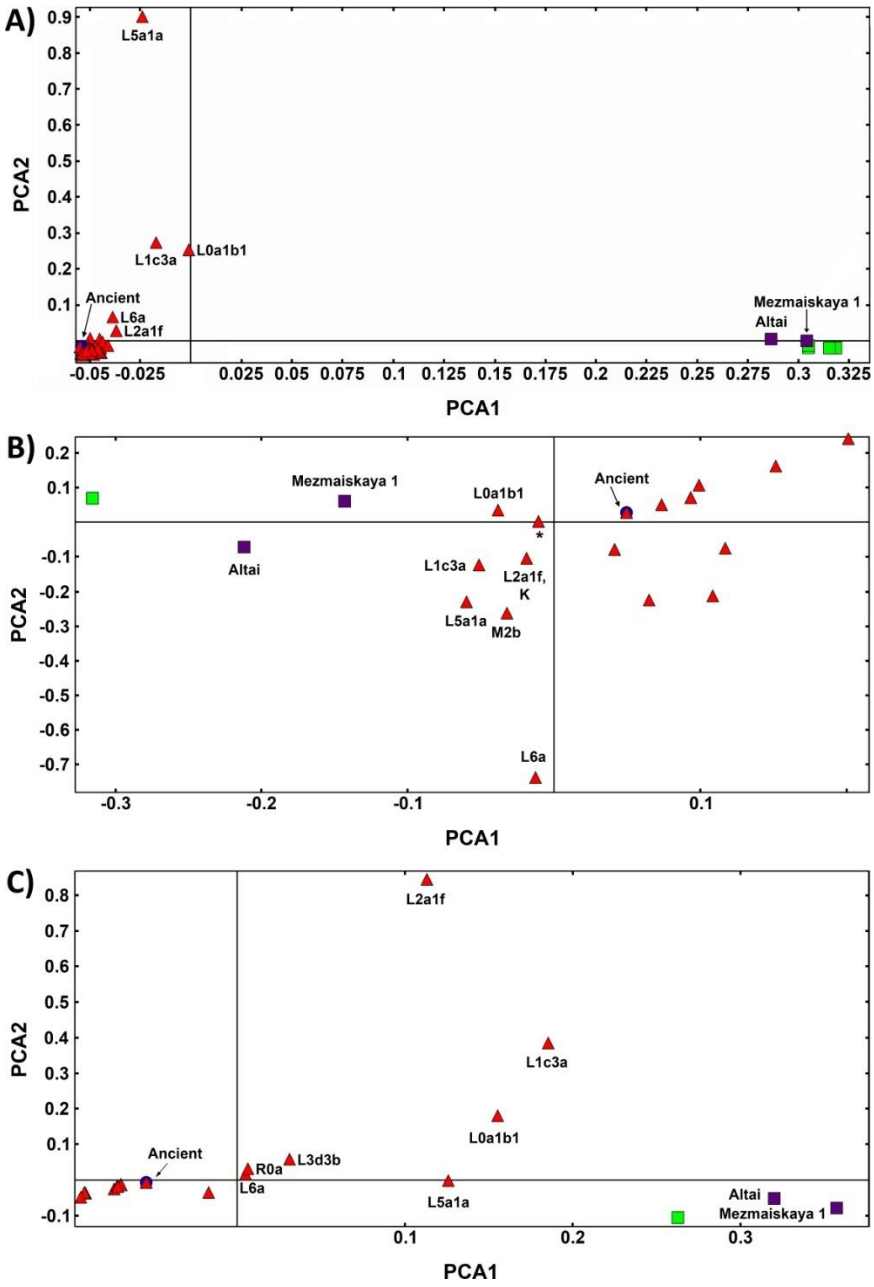

Fig. 5. Principal Component Analysis (PCA) of Human and Neanderthal mitochondrial genomes. In (A) PCA results for all 16,569 positions of 103 mitogenomes being 9 Neanderthals (green squares for H-like sequences and purple squares for L-like sequences), 42 archaic AMH (blue circles) and 53 modern *H. sapiens* haplogroups (red triangles). X-Axis denotes the value for PC1, while y-Axis denotes values for PC2. Each dot in the figure represents one or more individuals. In (B) PCA results for the segment comprising positions 128 to 315 extracted from the 103 mitogenomes alignment and in (C) PCA results for the segment comprising positions 6,950 to 7,660 extracted from the 103 mitogenomes alignment.

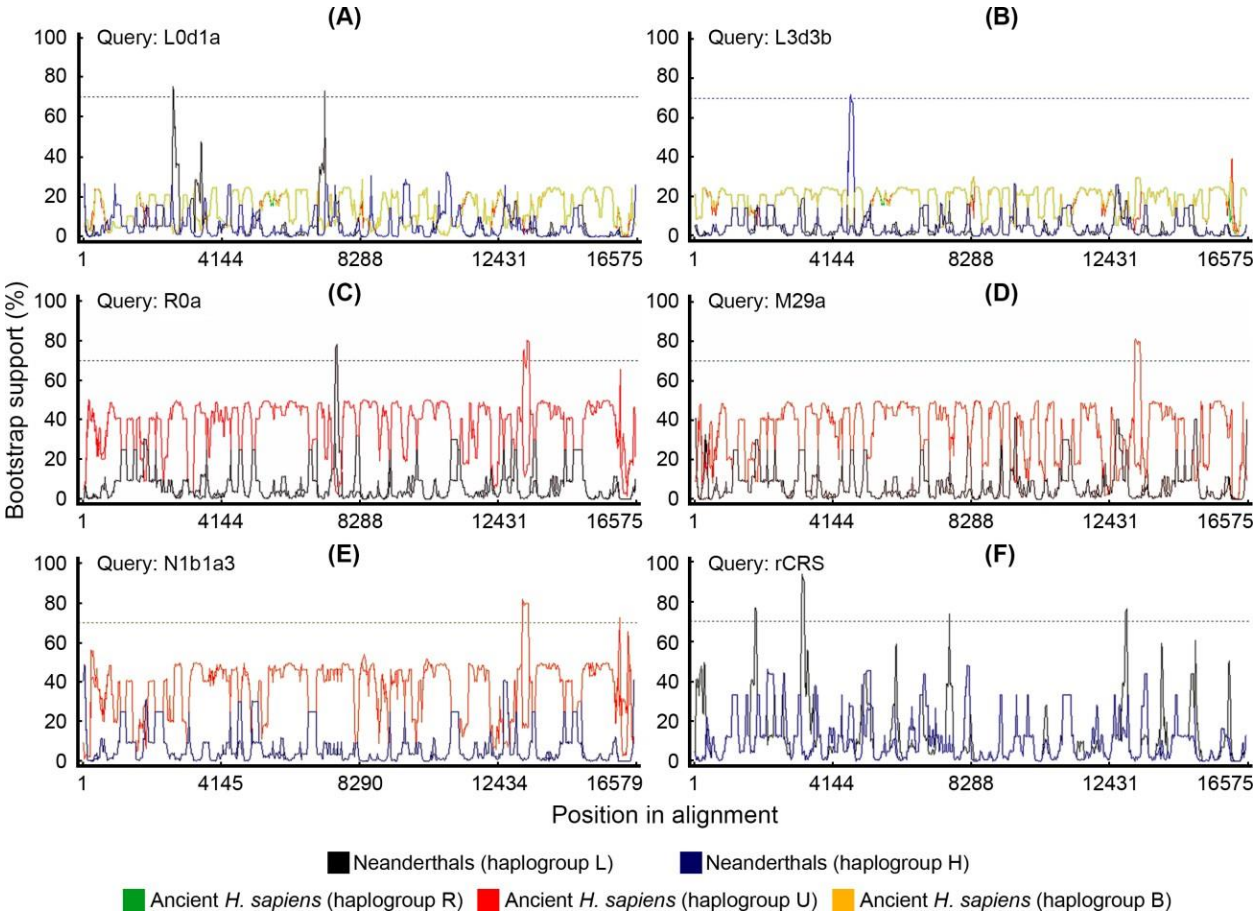

Fig. 6. Bootscan recombination analysis. Possible recombination points in were detected using different modern haplogroups as queries and Ancient *H. sapiens* or Neanderthals as putative parental sequences.

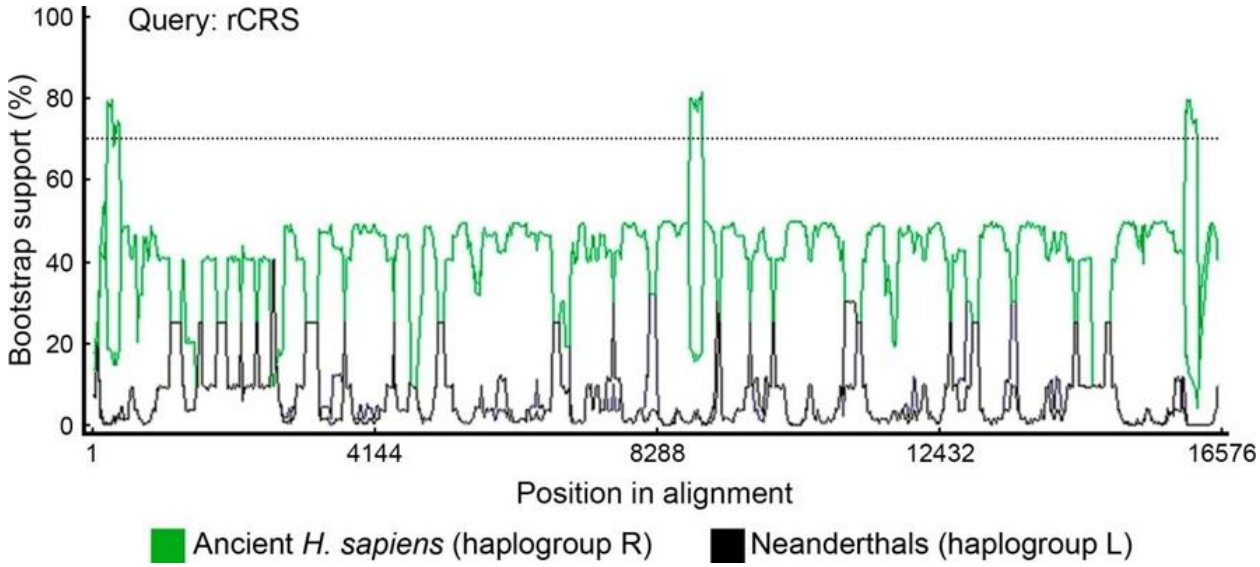

Fig. 7. Bootscan analysis of the rCRS sequence (haplogroups H2a2a1) using Ancient *H. sapiens* and Neanderthals as putative parentals.

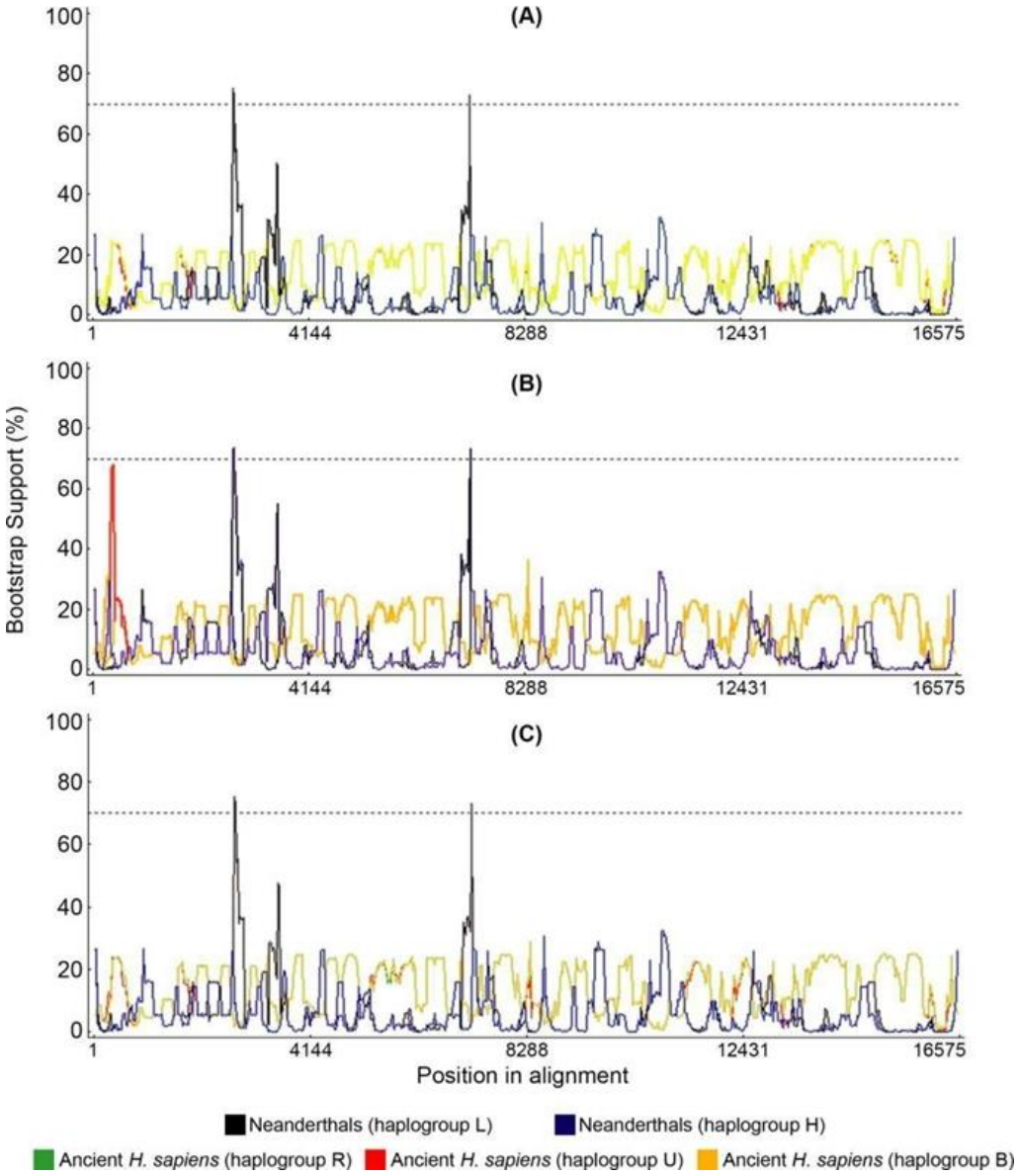

Fig. 8. Bootscan/RDP analysis of haplogroup L0d1a showing the profile consistency regardless the alignment algorithm or bootscan model option. (A) Map to reference aligment and Felsenstein model on bootscan; (B) MAFFT alignment with Kimura two parameters model on bootscan; (C) Map to reference aligment and Kimura two parameters model on bootscan. Neanderthal sequences from haplogroup L (black line) and haplogroup H (blue line) and Ancient *H. sapiens* from haplogroup R (green line), haplogroup U (red line) and haplogroup B (orange line) were used as parentals. Neanderthal sequences were Feldhofer 1, Mezmaiskaya and Vindija33_16 and ancient *H. sapiens* sequences were Kostenki, Fumane, Doni Vestonice 14 and Tianyuan.