RESEARCH ARTICLE

Blood groups of Neandertals and Denisova decrypted

Silvana Condemi1,*, Stéphane Mazières1, Pierre Faux1, Caroline Costedoat1, Andres Ruiz-Linares1,2,3, Pascal Bailly1,4, Jacques Chiaroni1,4*

1 Aix Marseille Univ, CNRS, EFS, ADES, UMR 7268, Marseille, France, 2 Department of Genetics, Evolution and Environment, and UCL Genetics Institute, University College London, London, United Kingdom, 3 Ministry of Education Key Laboratory of Contemporary Anthropology and Collaborative Innovation Center of Genetics and Development, School of Life Sciences and Human Phenome Institute, Fudan University, Yangpu District, Shanghai, China, 4 Etablissement Français du Sang PACA-Corse, « Biologie des Groupes Sanguins », Marseille, France

* silvana.condemi@univ-amu.fr (SC); jacques.chiaroni@efs.sante.fr (JC)

Abstract

Blood group systems were the first phenotypic markers used in anthropology to decipher the origin of populations, their migratory movements, and their admixture. The recent emergence of new technologies based on the decoding of nucleic acids from an individual's entire genome has relegated them to their primary application, blood transfusion. Thus, despite the finer mapping of the modern human genome in relation to Neanderthal and Denisova populations, little is known about red cell blood groups in these archaic populations. Here we analyze the available high-quality sequences of three Neanderthals and one Denisovan individuals for 7 blood group systems that are used today in transfusion (ABO including H/Se, Rh (Rhesus), Kell, Duffy, Kidd, MNS, Diego). We show that Neanderthal and Denisova were polymorphic for ABO and shared blood group alleles recurrent in modern Sub-Saharan populations. Furthermore, we found ABO-related alleles currently preventing from viral gut infection and Neanderthal RHD and RHCE alleles nowadays associated with a high risk of hemolytic disease of the fetus and newborn. Such a common blood group pattern across time and space is coherent with a Neanderthal population of low genetic diversity exposed to low reproductive success and with their inevitable demise. Lastly, we connect a Neanderthal RHD allele to two present-day Aboriginal Australian and Papuan, suggesting that a segment of archaic genome was introgressed in this gene in non-Eurasian populations. While contributing to both the origin and late evolutionary history of Neanderthal and Denisova, our results further illustrate that blood group systems are a relevant piece of the puzzle helping to decipher it.

Introduction

Over the last decade, technological progress has allowed generation of data from the entire genome of some fifteen extinct Neanderthal and Denisova hominins who lived 40,000 to 100,000 years ago from Western Europe to Siberia [1]. It reveals population structure, several
demographic fluctuations and gene flows across hominin populations, worldwide dispersal of archaic genes by admixed *Homo sapiens*, and even the existence of a super-archaic ‘ghost’ population [2]. In addition, the availability of Neanderthal and Denisova DNA sequences provides a phylogenetic status and chronological depth that have significantly enhanced the understanding of gene variation in modern humans for phenotype, metabolic, and immune traits (reviewed in [3]).

Red cell blood groups are powerful anthropological markers. Phenotype and genotype geographical distribution mirrors past human migrations and natural selection [4–7] and comparison with primates makes it possible to evoke their evolutionary and migration trajectory with accuracy [8, 9]. Red cell blood groups are also crucial in medicine to ensure transfusion safety, transplants, and foeto-maternal compatibility [10]. To date, the International Society of Blood Transfusion (retrieved from the ISBT website, [http://www.isbtweb.org](http://www.isbtweb.org)) has recorded more than 380 blood group specificities grouped into 40 systems. In transfusion, it is routine practice to scrutinize six blood groups: ABO, Rh, Kell, Duffy, Kidd and MNS (reviewed in [10]). Curiously, despite their significance and the amount of available genotypic data on modern Humans that is continuously accumulating [11], almost no attention has been paid to these major red cell blood polymorphisms in palaeogenetic studies [12].

In the present study, we analyze Neanderthal and Denisovan blood groups in order to trace back the current human diversity and to discuss health aspects and vulnerabilities of archaic populations. For that purpose, we investigated the high-quality nuclear genomes previously published from three Neanderthals one Denisovan.

**Material and methods**

**Selection criteria of the probands**

To ensure genotype calling rate, consistency across individuals and phylogenetic positioning in relation to anatomically modern humans, we did not consider contaminated, admixed, low-depth and archaic genomes with abundant uncalled positions in the loci understudy. We hence retained only high-quality genomes from one Denisovan (Denisova 3) and three Neanderthal individuals i.e., Altai Neanderthal (Denisova 5), Vindija 33.19, and Chagyrskaya 8 [13–16] (S1 Table). These four probands are representative of the two archaic human-related species that spanned over 50,000 years of the Late Pleistocene and across approximately 5,000 km of Eurasia.

**Presentation of the blood groups under study**

We studied 7 blood group systems covering 11 genes: ABO including H system and Secretor status (ISBT 001 and 018, *ABO, FUT1 and FUT2* genes), Rh (ISBT 004, *RHD* and *RHCE* genes), Kell and the covalently linked Kx protein (ISBT 006, *KEL* and *XK* genes), Duffy (ISBT 008, *ACKR1* gene), Kidd (ISBT 009, *SLC14A1* gene), MNS (ISBT 002, *GYPB* gene), and Diego and its Band 3-Memphis variant (ISBT 010, *SLC4A1* gene) (S2 Table).

**Exploration procedure for blood group alleles**

For the probands and blood groups under study, we downloaded the already published [13–16] and curated `.vcf` and `.bam(.bai)` chromosome files available at the Genome Projects website of the Max Planck Department of Evolutionary Genetics ([https://www.eva.mpg.de/genetics/genome-projects.html](https://www.eva.mpg.de/genetics/genome-projects.html), S1 Table). For genotype calling filters, see the Supplementary Information of [13–16] and the readme files at [http://cdna.eva.mpg.de/neandertal/](http://cdna.eva.mpg.de/neandertal/). Briefly, the
filters included a coverage filter stratified by GC content, minimum coverage of 10, Heng Li’s Mappability 35, Mapping Quality (MQ) of 25, no tandem repeats and no indels.

Then, we briefly proceeded in a two-step screening of the blood group loci. First, we first gathered the genotypes at the key functional changes with depth, allele counts, quality and Phred scores probability using vcftools [17] (S2 Table, S1 File). Second, we browsed all exomes regions within the coding bounds (i.e. from the initiation ATG to the stop codons), in search for additional variation from hg19 (S3 Table, S1 File). While doing so, we paid specific attention to the following five points.

Consideration of the reference sequence. We aligned with the reference blood group gene sequences used by the ISBT against the GRCh37 (hg19) with nucleotide labelling according to the sense (5’-3’) strand (S2 Table). We noticed that for six loci we studied (ABO, KEL, GYPB, RHCE, SCL4A1 and FUT1) the hg19 reference sequence opens by default onto the antisense strand (3’ → 5’) in the NCBI graphic window and the ancient genome browser, although their cDNA is conventionally the sense strand (5’ → 3’). This has two consequences: the chromosomal coordinates are decreasing as we progress throughout the coding strand (5’ → 3’) of these genes (from exon ‘n’ to exon ‘n+1’) and overall, nucleotides should be reversed-complemented.

ABO genotype calls. We inferred the ABO alleles according to the functional approach developed by [18, 19] for pure and chimeric A-B transferase cDNAs. In conformity with this approach, we identified the ABO alleles by 4 letters corresponding to the 4 main amino acid changes in the catalytic site of the glycosyltransferase of pure A or B allele positions, preceded by the presence or not of the G in position c.261 (rs8176719) (i.e. G-AAAA meaning 4 SNPs alleles [20] (S2 Table; Fig A in S1 File). Special attention has been taken to FUT2 whose amino acid numbering in NBCI and hg19 should be rectified to get the correct amino-acid changes as mentioned by the ISBT. This is due to the fact that the initiation codon is the third ATG at the beginning of exon 2 (19: 49,206,247) [21].

RHCE genotype calls. For RHCE, while browsing the exons in search for variation with hg19, we gathered the genotypes of the key changes of the initiation codon is the third ATG at the beginning of exon 2 (19: 49,206,247) [21].

RHD and RHCE genotype calls. For RHD and RCE, while browsing the exons in search for variation with hg19, we gathered the genotypes of the key changes of the RH+ Ce, ‘CE, ‘Ce, and ‘cE alleles. Any variation with hg19 was consolidated with The Human Rhesusbase.com [22], Erythrogene.com [11] and screenshots of the bam sequences (Figs B-D in S1 File).

For any identification of a variant, we searched for it in all four archaic genomes. In addition, for any call at two key variants of our findings, namely c.733G>C (RHD) and c.712A>G (RHCE), we searched for the other polymorphisms that usually constitute the haplotypes made with them, respectively RHD’ DBU, ‘DLX, ‘DV, ‘DVI ‘DBS, ‘DBT, ‘DUC2 and ‘ceAR, ‘ceEK, ‘cebI, ‘ce’ SM, and ‘ceHAR. For this, we browsed both vcf and bam alignment by varying the MQ threshold (S2 Table; Fig D in S1 File).

Identification of indels. Because indels could have been filtered out in the making the vcf files, all ABO, RHD and RHCE, notably the ABO c.261delG, c.1061delC, and RHCE 209bp insert have been double-checked from the specific indels vcf files (http://ftp.eva.mpg.de/neandertal/Vindija/VCF/indels/) and bam alignments using Integrative Genomics Viewer (IGV [23]) (S2 Table and Fig A in S1 File).

Low-mapped variants. The screenshots of the bam alignments in simultaneously the four archaic individuals have highlighted a difference in depth and MQ between reference and alternate alleles. This is especially true for variants with very low frequency in modern humans reference panel such as rs17418085 (RHD), rs150073306 (RHD), rs1132763 (RHCE), and rs1132764 (RHCE) in the 3 Neandertals (alternate allele) in comparison with Denisova 3,
homozygous for the reference alleles (Figs C and D in S1 File). Hence, these loci may suffer from reference bias, which is known to strongly reduce the depth and mapping of the reads with the alternate alleles [24, 25], and consequently, the genotype accuracy indexes at these loci. Hence, in cases where variants have been called in the released VCFs of some probands but filtered out in the others, we screenshoted the indexed alignments with hg19 using IGV [23] to manually call genotypes with allocated reads count and MQ cut-off (i.e. value above which the reads are not visualized) (S1 File).

Results

Detailed information for the blood group systems, genotypes and phenotypes as well as for other polymorphisms identified in these archaic hominins is presented in Tables 1 and 2 and the principal information is shown in Figs 1 and 2.

The results of the analysis for each blood group system can be resumed as follows:

ABO system. We found the most common phenotypes present in modern human populations: A1, B and O resulting from the combination of 3 different alleles (Fig 1). Two samples are heterozygous: Chagyrskaya 8 with group A1 (delG-AAAA/G- BAAA without C deletion at position 1061) and Vindija 33.19 with group B (delG-AAAA/G-BABB) and two are homozygous: Altaï with group A1 (G-BAAA/G-BAAA without C deletion at position 1061) and Denisova-3 with group O (delG-AAAA/delG-AAAA).

H/Se system. All the samples present a common FUT1 allele. Concerning FUT2 locus, 3 alleles are found: the conventional functional FUT2\(^{01}\) allele and two non-functional alleles: FUT2\(^{01N.04}\) and FUT2\(^{01N.}\).c.714A, p.238stop. Although FUT2\(^{01N.04}\) (Neanderthal Vindija and Chagyrskaya) is presently found in South East Asian and Oceanian populations [26–28], the latter allele, present in a single dose in Denisova-3, has never been described in modern humans [10, 11].

RH system. All of the backgrounds are based on the Dce (R\(^{0}\)) haplotype as described in Africa [8] and we found only one complete Dce (R\(^{0}\)) haplotype corresponding to the wild type described in modern human populations [8]. This complete R\(^{0}\) haplotype is present, at a single dose, in the Denisova-3 sample. The other haplotypes are variants encoding partial antigens D, c and e, which are missing some epitopes. All of these haplotypes, whether wild or variant, are related, phylogenetically speaking, to the African RH\(^{DIII}DIII\)a cluster [8]. From the three Neanderthal genomes, we have identified one potential novel RH\(^{DIII}DIII\) variant sharing typical SNP combinations assigned to this cluster (Fig 2). This variant exhibited the three SNPs that characterize the partial RH\(^{DIII}DIII\) type4 (or RH\(^{DIII}DIII\) 03.04 defined by c.186T, c.410T and c.455C), combined with two additional SNPs, c.602C>G and c.733G>C. This latter SNP defining the partial RH\(^{DIII}DUC2\) allele. This new combination has been reported for the first time in only one modern Australian Aborigine [29] and occurred at a homozygous level in Altaï, and at least at a heterozygous level in Vindija and Chagyrskaya, with a normal RH\(^{DIII}DIII\) sequence (c.1170T, c.1193A) for all of them. This new RH\(^{DIII}DIII\) variant segregated with RHCE\(^{ce}\) allele variants although the RH\(^{DIII}DIII\) 03.04 has been reported to segregate with RHCE\(^{Ce}\) in modern humans [30].

Furthermore, we found the presence of at least three RHCE\(^{ceEK}\) variant alleles (c.48G>C, c.712A>G, c.787A>G and possibly c.800T>A) in Altaï Neandertal, Vindija and Chagyrskaya, and two wild-type RHCE\(^{ce}\) in Denisova-3. Each of the Neanderthal archaic genomes has one other African RHCE\(^{ce}\) variant in cis. The RHCE\(^{ceEK}\) allele is mostly encountered in people of African descent [31]. When present in double dose, it encodes a rare phenotype defined by the absence of public antigen RH:-18. The anti-RH18 antibody has been reported to be
Table 1. Blood group systems, genotypes and phenotypes of four Denisova and Neanderthal archaic genomes. Each occurrence of a SNP is scored by the “+” character.

| System, ISBT | Genotype: nt, aa | Phenotype | hg19 | NCBI | “Pinky” Denisova3 | Altai | Vindija 33.19 | Chagyrskaya 8 |
|--------------|------------------|-----------|------|------|------------------|-------|---------------|---------------|
| ABO, 001     | c.261G           | A1, A2, B, O2 | uc004cda.1 (no-patched) | NM_020469.3 | + | + | + | + |
|              | c.261G, p.H86fs118stop | O1 | + | + | + | + | + | + |
|              | c.526C, p.176Arg | A1, A2, O1 | + | + | + | + | + | + |
|              | c.526G, p.176Gly | B, O2 | + | + | + | + | + | + |
|              | c.703G, p.235Gly | A1, A2, O1, O2 | + | + | + | + | + | + |
|              | c.703A, p.235Ser | B | + | + | + | + | + | + |
|              | c.796C, p.296Leu | A1, A2, O1, O2 | + | + | + | + | + | + |
|              | c.796A, p.296Met | B | + | + | + | + | + | + |
|              | c.802/3G, p.268Gly | B | + | + | + | + | + | + |
|              | c.802/3G, p.268Ala | O2 | + | + | + | + | + | + |
|              | c.1061delC, p. Pro53Argfs21 | A2 | + | + | + | + | + | + |

Phenotype

- aa
- O

| Genotype       | delG. AAAA | G. AAAA | delG. AAAA / delG. AAAA | G-BAAA / G-BAAA | delG. AAAA / G-BABB | delG. AAAA / G-BAAA |
|----------------|------------|---------|--------------------------|----------------|---------------------|---------------------|
| Phenotype      | O³         | A³      | O¹                      | A³            | B                   | A³                  |
| H, 018         | FUT1* 01* : c.35C, p.12Ala | H+ | uc002pkk.3 | + | NM_000148.3 | + | + | + | + |
|                | FUT2* 01* : c.4G, p.2Ala | Se | uc002pke.4 (short) | + | NM_000511.5 (short) | + | + | + | + |
|                | FUT2* 01* : c.571C, p.191Arg | Se | + | + | + | + | + | + |
|                | FUT2* 01* : c.714T, p.238Cys | Se | + | + | + | + | + | + |

Phenotype

- HH, SeSe
- HH, SeSe
- HH, SeSe &
- HH, SeSe
- HH, SeSe

| MNS, 002       | GYPB* 04* : c.143C, p.48Thr | s | uc003ijm.1 | + | NM_002100.4 | + | + | + | + |
|                | c.251C, p.84Thr | + |
|                | c.251G, p.84Ser | + | + | + | + | + | + |

Phenotype

- RHD* 01
- RHD* 01
- RHD* 01

| RHD, 004       | c.186G, p.62Leu | RhD | uc001bhz.3 | + | MN_016124.3 | + | + | + | + |
|                | c.141T, p.62Phs | + | + | + | + | + | + | + | + |
|                | c.440C, p.137Ala | RhD | + | + | + | + | + | + | + | + |
|                | c.441T, p.137Val | RhD | + | + | + | + | + | + | + | + |
|                | c.455A, p.152An | RhD | + | + | + | + | + | + | + | + |
|                | c.455C, p.152Thr | RhD | + | + | + | + | + | + | + | + |
|                | c.602C, p.201Thr | RhD | + | + | + | + | + | + | + | + |
|                | c.602G, p.201Arg | RhD | + | + | + | + | + | + | + | + |
|                | c.733G, p.245Val | RhD | + | + | + | + | + | + | + | + |
|                | c.733C, p.245Leu | RhD | + | + | + | + | + | + | + | + |
|                | c.929A, p.310Gys | RhD | + | + | + | + | + | + | + | + |
|                | c.929G, p.310Arg | + | + | + | + | + | + | + | + | + |

Phenotype

- RHD
- RhD
- RhD
- probably partial RhD
- probably partial RhD
- probably partial RhD

(Continued)
responsible for haemolytic transfusion reactions and haemolytic disease of the foetus and new-born (HDFN) [32].

MNS, KEL, Duffy, Kidd and Diego systems. All four archaic samples present the deduced phenotype S-s+, K negative, Fy(a-b+), Jk (a+b-) and Di (a-b+) due to double-dose of MNS*04, KEL*02, FY*02, JK*01, DI*02 respectively. The three Neanderthals present the deduced

| Table 1. (Continued) |
|----------------------|
| System, ISBT        | Genotype: nt, aa | Phenotype                                                                 |
| RHCE, 004           | c.48G, p.16Thr  | RhCE, RhCE                                                                 |
|                     | c.48C, p.16Cys  | RhCE, RhCE                                                                 |
|                     | c.178A, p.60leu | RhCE, RhCE                                                                 |
|                     | c.178C, p.60Leu | RhCE, RhCe                                                                  |
|                     | c.203G, p.68Ser | RhCE, RhCE                                                                 |
|                     | c.203A, p.68Asn | RhCE, RhCe                                                                  |
|                     | c.307T, p.103Ser| RhCE, RhCe                                                                 |
|                     | c.307C, p.103Pro| RhCE, RhCe                                                                  |
|                     | IVS2.ins+109bp  | RhC                                                                        |
|                     | c.676C, p.226Pro| RhCE, RhCe                                                                  |
|                     | c.676G, p.226Ala| RhCe, Rhce                                                                 |
|                     | c.410T, p.137Val| new variant                                                               |
|                     | c.410C, p.137Ala| RhCE, Cc, Ce, cE                                                          |
|                     | c.712G, p.238Val| partial and weak antigens                                                 |
|                     | c.712A, p.238Met| RhCE, Ce, Cc, cE                                                          |
|                     | c.744C, p.248Ser| partial and weak antigens                                                 |
|                     | c.744T, p.248Ser| RhCE, Ce, Cc, cE                                                          |
|                     | c.787G, p.263Gly| partial and weak antigens                                                 |
|                     | c.787A, p.263Arg| RhCE, Ce, Cc, cE                                                          |
|                     | c.800A, p.267Lys| partial and weak antigens                                                 |
|                     | c.800T, p.267Met| RhCE, Ce, Cc, cE                                                          |
| KEL, 006            | KEL*02/*02: c.578C, p.193Thr| k uc003wcb.3 | NM_000420.2                     | +    | +    | +    | +    |
|                     | KEL*02/*07: c.1790T, p.5571Leu| Js uc012xcn.2 | +    | +    |
|                     | KEL*06/*06: c.1790C, p.5579Pro| Js uc012xcn.2 | +    | +    |
| FY, 008             | FY*01/*01: c.125G, p.42Gly, c.67T| Fy uc001fso.3 | NM_002036.3                     | +    |
|                     | FY*02/*02: c.125A, p.42Asp, c.67T| Fy uc001fso.3 | +    | +    | +    | +    |
| JK, 009             | JK*01/*01: c.838G, p.280Asp| Jk uc010scm.2 | NM_015865.6                     | +    | +    | +    | +    |
| DI, 010             | DI*02/*02: c.2561C, p.854Pro| Di uc002igf.4 | NM_000342.3                     | +    | +    | +    | +    |
|                     | c.166G, p.56Gla | +    | +    | +    | +    |
|                     | c.166A, p.56lys | +    | +    | +    | +    |

https://doi.org/10.1371/journal.pone.0254175.1001
Table 2. Other polymorphisms identified in blood group genes from Denisova and Neanderthals.

| System, ISBT | Chr rs number | nt, aa                  | cDNA                  | hg19 | NCBI    | "Pinky" Denisova3 | Altai 33.19 | Vindija 33.19 | Chagyrskaya 8 |
|--------------|---------------|-------------------------|-----------------------|------|---------|-------------------|------------|--------------|---------------|
| **ABO, 001** | 9             | rs782228072 c.43T, p.15Tyr | uc004cda.1 (no-patched) | +    | +       | +                 | +          | +            | +             |
|              |               | c.45C, p.15His          |                       |      |         |                   |            |              |               |
|              |               | c.488C, p.163Thr        |                       | +    |         |                   | +          | +            | +             |
|              |               | rs55756402 c.488T, p.163M |                       | +    |         |                   |            |              |               |
|              |               | rs782713806 c.777T, p.259A |                       | +    |         |                   | +          |              |               |
|              |               | c.777C, p.259A           |                       | +    |         |                   | +          |              |               |
|              |               | rs578090581 c.799C, p.267A |                       | +    |         |                   | +          |              |               |
| **KEL, 006** | 7             | rs657G, p.219H           | uc003wcb.3             | +    | NM_000420.2 | +                 | +          | +            | +             |
|              |               | c.657A, p.219H           |                       |      |         |                   |            |              |               |
|              |               | rs139319150 c.936C, p.312P |                       | +    |         |                   | +          | +            | +             |
|              |               | c.936T, p.312P           |                       | +    |         |                   | +          |              |               |
|              |               | rs370938244 c.1963C, p.665A |                       | +    |         |                   | +          | +            | +             |
|              |               | c.1963T, p.665A          |                       | +    |         |                   | +          |              |               |
|              | no rs          | rs1967G, p.656A          |                       | +    |         |                   | +          |              |               |
|              |               | c.1967A, p.656A          |                       | +    |         |                   | +          |              |               |
| **JK, 009**  | 18            | rs11357896 c.196A, p.Met66 | uc010xca.2             | +    | NM_015865.6 | +                 | +          | +            | +             |
|              |               | rs113029149 c.196G, p.66Val |                       | +    |         |                   | +          | +            | +             |
|              |               | rs28994287 c.1471A, p.157Val |                      | +    |         |                   | +          |              |               |
|              |               | rs2298718 c.756G, p.252Pro |                      | +    |         |                   | +          |              |               |
|              |               | c.756A, p.252Pro         |                      | +    |         |                   | +          |              |               |
| **DI, 010**  | 17            | rs5015 c.1249T, p.417Leu | uc002igf.4             | +    | NM_000342.3 | +                 | +          | +            | +             |
|              |               | c.1249C, p.417Leu        |                      | +    |         |                   | +          | +            | +             |
|              |               | rs148115666 c.1272T, p.424Gly |                     | +    |         |                   | +          |              |               |
|              |               | c.1272C, p.424Gly        |                      | +    |         |                   | +          |              |               |

(Continued)
phenotype Js(a+b-) of the Kell system due to double-dose of the ancestral \textit{KEL06} allele, which is exclusive to African populations with 1% of Js(a+b-) phenotype [11]. On the contrary, Denisova 3 presents the deduced Js(a-b+) phenotype resulting from a double-dose of the antithetical \textit{KEL07} allele. Lastly, the three Neanderthals are carriers of the Band 3-Memphis variant (according to rs5036).

| System, ISBT | Chr | rs number | nt, aa | hg19 | NCBI | "Pinky" Denisova3 | Altai | Vindija 33.19 | Chagyrskaya 8 |
|--------------|-----|-----------|--------|-------|------|----------------|-------|--------------|--------------|
| H, 018       | 19  | no rs     | c.717G, p.Ser239 | uc002pkk.3 | +   | +   | +   | +   | +   |
|              |     | c.717A, p.Ser239 | + | + |
|              |     | rs180026  | c.375G, p. Glu125 | uc002pke.4 (short) | MN_000511.5 (short) | + | + | + | + |
|              |     | c.375A, p. Glu125 | + | + |
|              |     | rs370886251 | c.400G, p.Val134 | + | + | + | + | + | + |
|              |     | c.400A, p.Ile134 | + | + |
|              |     | rs752579948 | c.487A, p.163Met | + | + |
|              |     | c.487G, p.163Val | + | + | + | + | + | + |
|              |     | rs147479067 | c.807G, p.269Glu | + | + |
|              |     | rs.807C, p.269Gln | + | + | + | + |
|              |     | rs485186  | c.960G, p.320Thr | + | + | + | + |
|              |     | c.960A, p.320Thr | + | + | + | + | + | + |
| RH, 004      | 1   | c.19C, p.Arg7 | + | + | + | + | + | + |
|              |     | rs142037235 | c.19G, p.Gly7 | + | + | + | + | + | + |
| KX, 019      | X   | c.72G, p.Leu24 | + | + | + | + | + | + |
|              |     | rs1556440110 | c.72A, p.Leu24 | + | + | + | + | + |

Table 2. (Continued)

Fig 1. \textit{ABO} system. A, Nomenclature of the archaic \textit{ABO} alleles [18]. B, Different observed genotypes and inferred phenotypes in Neandertal and Denisova. Red: polymorphisms characteristic of the \textit{ABO*A} allele, blue: polymorphisms characteristic of the \textit{ABO*B} allele, brown: deletion characteristic of the \textit{ABO*O01} allele.

https://doi.org/10.1371/journal.pone.0254175.t002
https://doi.org/10.1371/journal.pone.0254175.g001
Discussion

Neanderthals are a human hunter-gatherer fossil population that lived in Eurasia between 250 kya and 38 kya before being totally replaced throughout their territory by *Homo sapiens*. Morphological features progressively evolved from their African ancestors (*Homo heidelbergensis*) and adapted to the cooler climate of Europe [33]. Their arrival in Europe marks a major cultural change with the importation of a new tool, well known in Africa since at least 1.5 mya and in the Levant, the handaxe [34]. The Denisovans are also an extinct human population but bone record is too fragmentary. Their “discovery” and phylogeny relies only on genetic studies [35, 36].

Neanderthal populations were never large, structured in small interconnected groups (about 20 individuals) that never outnumbered 70,000 individuals at the time of their "golden age" (the "emian" interglacial at OIS 5, around 120 kya) [37]. The small size of the population has to be correlated with the partial geographic isolation of Neanderthals caused by European climatic fluctuations during Pleistocene. It was at this time that the Neanderthals, well identified by singular morphological features, spread eastwards carrying the same lithic assemblages and technology as far east as the Altai mountains [38] where they encountered the Denisovans. Genetic data has also pointed out the low genetic diversity of Neanderthals, with a demographic depression peak in the Altai where a very consanguineous individual was found [14], and genetic continuity across Europe from 120 kya until the disappearance of the population around 40 kya [39]. This low variability is also visible in the morphology of the Neanderthals, which remained the same during the last 100 kya of their existence throughout their territory from the Atlantic to the Altai [40]. In this view, our results provide four main points relevant for the origin, vulnerabilities and dispersion of Neanderthal and Denisova (Figs 3 and 4).

Consolidation of an African origin for Neanderthal and Denisova

First, the analysis of archaic blood groups anchors the lineage of Eurasian Neanderthals and Denisovans to Africa via: the absence of the antigen combination RhC, RhE, K, Fy\(a\), Jk\(b\) and S (14% of sub-Saharan African populations, 0.06% in non-African populations [10]), the
presence of a double-dose of ancestral forms of the RH, Kell, Duffy, Kidd, MNS and Diego blood groups (respectively the haplotype Dce/Dce, KEL’02’/’02, KEL’06’/’06, FY’02’/’02, JK’01’/’01, MNS’04’/’04 and Memphis form of Band 3 system [8, 41, 42], and RHD, RHCE variants phylogenetically linked or presently exclusive to African clusters and populations [31]. These features are in accordance with a Neanderthal and Denisovan gene pool pre-dating the exit of Homo sapiens from Africa [43].

Fig 3. Erythroid blood group distribution from Denisova and Neanderthal archaic genomes. Branching matches nuclear DNA tree topology [13]. Blue, Neanderthal lineage; red, Denisovan lineage. Made with Natural Earth. Free vector and raster map data @ naturalearthdata.com.

https://doi.org/10.1371/journal.pone.0254175.g003

Fig 4. Introgression scan of modern humans in the RHD region. Introgression tracts were called at confidence > = 0.2 and < 0.5 (15.7 Kb, light purple) and confidence > = 0.5 and < 0.9 (4.6 Kb, dark purple) in 5 haplotypes from 2 HGDP-CEPH populations (1: Papuans Sepik; 2: Brahuis). Alleles carried by these haplotypes at 5 key SNPs for the RHD*DIII type 4 with c.602G and DUC2 are shown in green or orange, whether they are the ancestral or derived allele in the human lineage. The bottom panel shows the position of these SNPs on the RHD map.

https://doi.org/10.1371/journal.pone.0254175.g004
A (mysterious) genetic link between Neanderthals and Australia

The new partial \textit{RHD} allele, \textit{RHD}$^*$\textit{DIII type4} with c.602G and c.733C, reported in the three Neanderthal individuals, and not in Denisova 3, was unknown in modern humans until 2019 and its description as a new variant in an individual from the First Nation of Australia with a \textit{RHD} Exon-9 deletion or rearrangement with \textit{RHCE Exon-9} \cite{29}. Thus, this polymorphism is not a new variant in the historical sense of the term, as it was already present around 100 kya in Neanderthals. This result fuels the discussion about admixture events between the different lineages and also about the early dispersal of \textit{Homo sapiens} via the Arabian Peninsula \cite{44} towards Australia \cite{45} and Oceania \cite{46}. In order to assess whether the Neanderthal \textit{RHD}$^*$\textit{DIII type4} with c.602G and c.733C arose in modern humans from shared ancestry or introgression, we have modelled high-coverage modern chromosomes from Asia and Oceania (HGDP-CEPH collection, publicly available, \cite{47}) as a mosaic of modern and archaic states using a hidden-Markov model (see S2 File for detailed methods). We found a probable introgressed tract of 15.7 Kb spanning over 4 exons of \textit{RHD} in 5 individuals: 2 HGDP-Brahuis and 3 HGDP-Sepik Papuans (Fig 4). The 3 Sepik Papuans are heterozygous carriers of the derived allele at rs150073306 which defines the DUC2 allele (raising thus the MAF of the derived allele nearly to 19\% in that population—see S2 File), for which Altai is homozygous. When phased, there is an almost identical haplotype to Altai and the Australian Aborigine in Papuan HGDP00546. A parsimonious hypothesis to explain the relic of a \textit{RHD} haplotype common amongst all Neanderthals in 2 modern Oceanians only, would suggest that the \textit{RHD}$^*$\textit{DIII type4} with c.602G and c.733C profile have been carried by Levantine Neanderthals and passed to modern humans before 65 kya and the route towards SouthEast Asia \cite{48, 49}. This assumption remains however speculative as the archaic tract was called at a low confidence level (>20\%). A shorter tract (4.6Kb) was still called at a confidence level of >50\% but not at higher confidence thresholds (>90\%). Further analyses would be required to validate our assumption.

The signature of environmental pressure

Our analysis of the \textit{ABO} and \textit{FUT2} genes supports the fact that pathogens have played a major role in the survival and selection of genetic variants in modern and archaic hominins \cite{50}. The molecular bases of the archaic ABO phenotypes, particularly for A and B groups, are identical to those found in non-human primates, which confirms an ancient balanced selection shared by Primate species \cite{51}. Noteworthy is the presence of two non-secretor \textit{FUT2} polymorphisms in Neanderthal and Denisovan individuals. To date, the non-secretor phenotype has not been found in non-human primates \cite{51} and shown to confer more resistance to some viruses with a notable resistance to Norovirus (Norwalk-like, \textit{Caliciviridae} family) infection \cite{52–54}, which is a major cause of acute gastroenteritis worldwide. Given the short existence of the Norwalk-like virus in humans (post-Neolithic \cite{55}), two scenarios would explain the occurrence of the non-secretor trait in archaic humans. \textit{FUT2} could have evolved neutrally in hominins before the late occurrence of selective pressure, in the same way as for glucose transporters (GLUT 2 and 12) and G6PD2 with malaria \cite{56}. Or, given that hominids likely hosted some viruses \cite{57}, a Norwalk-like virus (or a sister group) could have already existed 90,000 years ago across Eurasia, causing enough mortality in young children to select two convergent \textit{FUT2} alleles. Note that the occurrence of the Neanderthal non-secretor allele in Oceanians \cite{26–28} remains open.

Demographic ‘fragility’

Lastly, our study highlights unfavorable characteristics that can lead to “demographic fragility”. This fragility can be evoked on the basis of two elements: a low genetic diversity and the
possible presence of HDFN. Indeed, the large number of shared alleles by the four archaic genomes despite their geographical and temporal distribution may be related to the deduced inbreeding situation in Neanderthals [14–16, 58], known to be a source of low adaptability. Meanwhile, the Neanderthal RH allele variants encode for partial RhD, Rhc and Rhe antigens, only Denisova 3 presents a complete form in terms of epitopes, such as they are described in their “wild” forms in modern humans. Partial RhD, Rhc and Rhe antigens lacking epitopes may induce an immune response when exposed to complete antigens [59, 60]. Moreover, when the RHCE’ceEK allele is present in a double dose (a situation which may turn out to be frequent in view of its presence in the 3 Neanderthals), in addition to the presence of partial Rhc and Rhe antigens, it encodes a phenotype defined by the absence of an Rh antigen named RH18. Today, this antigen is considered to be a high frequency antigen in the modern human population. Thus, a Neanderthal mother with partial RhD, Rhc, and Rhe phenotypes and sometimes RH:-18, carrying a Denisovan foetus expressing complete forms of RhD, Rhc and Rhe antigens and expressing the RH18 antigen, would have been prone to be immune to missing epitopes and synthesize anti-RhD, anti-Rhc, anti-Rhe and even anti-RH18 antibodies. These antibodies are known to have an important clinical significance in terms of HDFN [32]. These elements could have contributed to weakening the descendants to the point of leading to their demise especially combined with the competition with Homo sapiens for the same ecological niche [61].

Conclusions
Analyses of blood group systems of Neanderthals and Denisovans contributed to a better understanding of their origin, expansion and encounters with Homo sapiens. Blood group profiles revealed polymorphism at the ABO locus, ancestral and African-origin alleles, and a RH haplotype presently secluded in Oceania, plausible relic of introgression events into modern humans prior their expansion towards Southeast Asia. An additional contribution is the reduced variability of many alleles and the possible presence of haemolytic disease of the foetus and new-born, which reinforces the notion of high inbreeding, weak demography and endangered reproductive success of the late Neanderthals, giving to our species the great opportunity to spread throughout the world.

Supporting information
S1 Fig. (TIF)
S1 File. This file contains 5 figures showing screenshots of bam sequence alignments of loci for which vcf data are not available for at least one individual. (PPTX)
S2 File. Introgression scan of modern humans in RHD region. (DOCX)
S1 Table. Information about the individual analyzed in this study. (DOCX)
S2 Table. ISBT nomenclatures of the blood group systems presented in Table 1, with archaic genotypes and Phred-score posterior probability. REF: reference base, ALT: alternate base, GT: (unphased) genotype. DP: read depth, GQ: Genotype Quality, PL: phred-scaled genotype likelihoods, A:C;G:T: number of A,C,G,T bases. IR: Number of reads with InDel starting at this position, PP = Phred-scaled posterior probability for genotypes AA, CC, GG,
TT, AC, AG, AT, CG, CT, GT, in this order; 0/0: homozygous reference allele, 1/1: homozygous alternate allele, 0/1: heterozygous. Blue font: ABO key functional changes [18].

S3 Table. Genomic information and genotype posterior probability of the other red cell blood group polymorphisms observed in the Denisovan and Neanderthal individuals. REF: reference base, ALT: alternate base, GT: (unphased) genotype. DP: read depth, GQ: Genotype Quality, PL: phred-scaled genotype likelihoods, A:C:G:T: number of A,C,G,T bases, IR: Number of reads with InDel starting at this position, PP = Phred-scaled posterior probability for genotypes AA, CC, GG, TT, AC, AG, AT, CG, CT, GT, in this order; 0/0: homozygous reference allele, 1/1: homozygous alternate allele, 0/1: heterozygous.

Acknowledgments

We thank the Centre de Calcul Intensif d’Aix-Marseille for granting access to its high performance computing resources. We are also grateful to Céline Bon, Laure Ségurel and the two anonymous reviewers for their fruitful comments.

Author Contributions

Conceptualization: Silvana Condemi, Pascal Bailly, Jacques Chiaroni.

Formal analysis: Stéphane Mazières, Pierre Faux, Caroline Costedoat.

Funding acquisition: Stéphane Mazières, Caroline Costedoat.

Investigation: Pierre Faux, Caroline Costedoat, Pascal Bailly, Jacques Chiaroni.

Methodology: Pierre Faux, Andres Ruiz-Linares, Pascal Bailly.

Project administration: Silvana Condemi, Stéphane Mazières.

Resources: Andres Ruiz-Linares.

Software: Pierre Faux, Andres Ruiz-Linares.

Supervision: Silvana Condemi, Pascal Bailly, Jacques Chiaroni.

Validation: Silvana Condemi, Pierre Faux, Andres Ruiz-Linares, Pascal Bailly.

Writing – review & editing: Silvana Condemi, Stéphane Mazières, Caroline Costedoat, Pascal Bailly, Jacques Chiaroni.

References

1. Slatkin M, Racimo F. Ancient DNA and human history. Proc Natl Acad Sci U S A. 2016; 113(23):6380–7. https://doi.org/10.1073/pnas.1524306113 PMID: 27274045

2. Rogers AR, Harris NS, Achenbach AA. Neanderthal-Denisovan ancestors interbred with a distantly related hominin. Sci Adv. 2020; 6(8):eaay5483. https://doi.org/10.1126/sciadv.aay5483 PMID: 32128408

3. Rees JS, Castellano S, Andrés AM. The Genomics of Human Local Adaptation. Trends Genet. 2020; 36(6):415–28. https://doi.org/10.1016/j.tig.2020.03.006 PMID: 32396835

4. Bégat C, Bailly P, Chiaroni J, Mazieres S. Revisiting the Diego Blood Group System in Amerindians: Evidence for Gene-Culture Conmigration. PLoS One. 2015; 10(7):e0132211. https://doi.org/10.1371/journal.pone.0132211 PMID: 26148209

5. Anstee DJ. The relationship between blood groups and disease. Blood. 2010; 115(23):4635–43. https://doi.org/10.1182/blood-2010-01-261859 PMID: 20308598
6. Fumagalli M, Cagliani R, Pozzoli U, Riva S, Comi GP, Menozzi G, et al. Widespread balancing selection and pathogen-driven selection at blood group antigen genes. Genome Res. 2009; 19(2):199–212. https://doi.org/10.1101/gr.082768.108 PMID: 18997004

7. Cavalli-Sforza LL, Menozzi P, Piazza A. The history and geography of human genes. Press PU, editor. Princeton: Princeton University Press; 1994. 432 p.

8. Carrith B, Kemp TJ, Pouliot M. Evolution of the human RH (rhesus) blood group genes: a 50 year old prediction (partially) fulfilled. Hum Mol Genet. 1997; 6(6):843–50. https://doi.org/10.1093/hmg/6.6.843 PMID: 9175729

9. Kitano T, Blancher A, Saitou N. The functional A allele was resurrected via recombination in the human ABO blood group gene. Mol Biol Evol. 2012; 29(7):1791–6. https://doi.org/10.1093/molbev/mss021 PMID: 22319172

10. Reid ME, Lomas-Francis C, Olsson ML. The Blood Group Antigen Factsbook. 3rd ed. New York: Elsevier Academic Press; 2012. 758 p.

11. Möller M, Jöud M, Storry JR, Olsson ML. Erythrogene: a database for in-depth analysis of the extensive variation in 36 blood group systems in the 1000 Genomes Project. Blood Advances. 2016; 1(3):240–9. https://doi.org/10.1182/bloodadvances.2016001867 PMID: 29296939

12. Lalueza-Fox C, Gigli E, de la Rasilla M, Fortea J, Rosas A, Bertranpetit J, et al. Genetic characterization of the ABO blood group in Neandertals. BMC Evol Biol. 2008; 8:342. https://doi.org/10.1186/1471-2148-8-342 PMID: 19108732

13. Meyer M, Kircher M, Gansauge MT, Li H, Racimo F, Mallick S, et al. A high-coverage genome sequence from an archaic Denisovan individual. Science. 2012; 29(6104):222–6. https://doi.org/10.1126/science.1224344 PMID: 22936568

14. 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(7481):43–9. https://doi.org/10.1038/nature12886 PMID: 24352235

15. Prüfer K, de Filippo C, Grote S, Mafessoni F, Korlević P, Hajdinjak M, et al. A high-coverage Neanderthal genome from Vindija Cave in Croatia. Science. 2017; 358(6363):655–8. https://doi.org/10.1126/science.aao1887 PMID: 28982794

16. Mafessoni F, Grote S, de Filippo C, Slon V, Kolobova KA, Viola B, et al. A high-coverage Neanderthal genome from Chagyrskaya Cave. Proc Natl Acad Sci U S A. 2020. https://doi.org/10.1073/pnas.2004944117 PMID: 32546518

17. Danecek P, Auton A, Abecasis G, Albers CA, Banks E, DePristo MA, et al. The variant call format and VCFtools. Bioinformatics. 2011; 27(15):2156–92. https://doi.org/10.1093/bioinformatics/btq265 PMID: 21653522

18. Yamamoto F, Clausen H, White T, Marken J, Hakomori S. Molecular genetic basis of the histo-blood group ABO system. Nature. 1990; 345(6272):229–33. https://doi.org/10.1038/345229a0 PMID: 2333095

19. Yamamoto F, Hakomori S. Sugar-nucleotide donor specificity of histo-blood group A and B transferases is based on amino acid substitutions. J Biol Chem. 1990; 265(31):19257–62. PMID: 2121736

20. Yip SP. Sequence variation at the human ABO locus. Ann Hum Genet. 2002; 66(Pt 1):1–27. https://doi.org/10.1017/S0003480001008995 PMID: 12014997

21. Kelly RJ, Rouquier S, Giorgi D, Lennon GG, Lowe JB. Sequence and expression of a candidate for the human Secretor blood group alpha(1,2)fucosyltransferase gene (FUT2). Homozygosity for an enzyme-inactivating nonsense mutation commonly correlates with the non-secretor phenotype. J Biol Chem. 1995; 270(9):4640–9. https://doi.org/10.1074/jbc.270.9.4640 PMID: 7876235

22. Wagner FF, Flegel WA. The Rhesus Site. Transfus Med Hemother. 2014; 41(5):357–63. https://doi.org/10.1159/000366176 PMID: 25538538

23. Thorvaldsdóttir H, Robinson JT, Mesirov JP. Integrative Genomics Viewer (IGV): high-performance genomics data visualization and exploration. Brief Bioinform. 2013; 14(2):178–92. https://doi.org/10.1093/bib/bts017 PMID: 23657479

24. Sousa V,Hey J. Understanding the origin of species with genome-scale data: modelling gene flow. Nat Rev Genet. 2013; 14(6):404–14. https://doi.org/10.1038/nrg3446 PMID: 23657479

25. Günther T, Nettelblad C. The presence and impact of reference bias on population genomic studies of prehistoric human populations. PloS Genet. 2019; 15(7):e1008302. https://doi.org/10.1371/journal.pgen.1008302 PMID: 31348818

26. Koda Y, Ishida T, Tachida H, Wang B, Pang H, Soejima M, et al. DNA sequence variation of the human ABO-secretor locus (FUT2) in New Guinean populations: possible early human migration from Africa. Hum Genet. 2003; 113(6):534–41. https://doi.org/10.1007/s00439-003-1013-6 PMID: 14569463
27. Ferrer-Admetlla A, Sikora M, Laayouni H, Esteve A, Roubinet F, Blancher A, et al. A natural history of FUT2 polymorphism in humans. Mol Biol Evol. 2009; 26(9):1993–2003. https://doi.org/10.1093/molbev/msp108 PMID: 19487333

28. Consortium GK. The GenomeAsia 100K Project enables genetic discoveries across Asia. Nature. 2019; 576(7785):106–11. https://doi.org/10.1038/s41586-019-1793-z PMID: 31802016

29. Schoeman EM, Roulis EV, Perry MA, Flower RL, Hyland CA. Comprehensive blood group antigen profile predictions for Western Desert Indigenous Australians from whole exome sequence data. Transfusion. 2019; 59(2):768–78. https://doi.org/10.1111/trf.15047 PMID: 30520525

30. Wagner FF, Fieleg WA. RHD gene deletion occurred in the Rhesus box. Blood. 2000; 95(12):3662–8. PMID: 10845894

31. Deleers M, Thonier V, Claes V, Daelemans C, Peyrard T, El Kenz H. A Tutsi family harbouring two new RHCE variant alleles and a new haplotype in the Rh blood group system. Vox Sang. 2020. https://doi.org/10.1111/vox.12905 PMID: 32196693

32. Moores P. Rh18 and hrS blood groups and antibodies. Vox Sang. 1994; 66(3):225–30. https://doi.org/10.1111/j.1423-0410.1994.tb00314.x PMID: 8036793

33. Mounier A, Marchal F, Condemi S. Is Homo heidelbergensis a distinct species? New insight on the Mauer mandible. J Hum Evol. 2009; 56(3):219–46. https://doi.org/10.1016/j.jhevol.2008.12.006 PMID: 19249816

34. Bar-Yosef O, Goren-Inbar N. The lithic assemblages of ‘Ubeidiya: A lower palaeolithic site in the Jordan Valley (Qedem). Jerusalem. THUo, editor. Jerusalem: Institute of Archaeology, Hebrew University of Jerusalem; 1993. 266 p.

35. Reich D, Green RE, Kircher M, Krause J, Patterson N, Durand EY, et al. Genetic history of an archaic hominin group from Denisova Cave in Siberia. Nature. 2010; 468(7327):1053–60. https://doi.org/10.1038/nature09710 PMID: 21179161

36. Sawyer S, Renaud G, Viola B, Hublin JJ, Gansauge MT, Shukov MV, et al. Nuclear and mitochondrial DNA sequences from two Denisovan individuals. Proc Natl Acad Sci U S A. 2015; 112(51):15696–700. https://doi.org/10.1073/pnas.1519905112 PMID: 26630009

37. Bocquet-Appel J-P, Degioanni A. Neanderthal Demographic Estimates. Current Anthropology. 2013; NS 8:S202–S13.

38. Kolobova KA, Roberts RG, Chabai VP, Jacobs Z, Krajcarz MT, Shalagina AV, et al. Archaeological evidence for two separate dispersals of Neanderthals into southern Siberia. Proc Natl Acad Sci U S A. 2020; 117(6):2879–85. https://doi.org/10.1073/pnas.1918047117 PMID: 31988114

39. Bokelman L, Hajdinka M, Peyrégné S, Brace S, Essel E, de Filippo C, et al. A genetic analysis of the Gibraltar Neanderthals. Proc Natl Acad Sci U S A. 2019; 116(31):15610–5. https://doi.org/10.1073/pnas.1903984116 PMID: 31308224

40. Trinkaus E. An abundance of developmental anomalies and abnormalities in Pleistocene people. Proc Natl Acad Sci U S A. 2018; 115(47):11941. https://doi.org/10.1073/pnas.1814989115 PMID: 30397116

41. Tournamille C, Blancher A, Le Van Kim C, Gane P, Apoil PA, Nakamoto W, et al. Sequence, evolution and ligand binding properties of mammalian Duffy antigen/receptor for chemokines. Immunogenetics. 2004; 55(10):682–94. https://doi.org/10.1007/s00251-003-0633-2 PMID: 14712331

42. Jarolim P, Rubin HFau—Zhai S, Zhai S Fau—Sahr KE, Sahr Ke Fau—Liu SC, Liu Sc Fau—Mueller TJ, Mueller Tj Fau—Palek J, et al. Band 3 Memphis: a widespread polymorphism with abnormal electrophoretic mobility of erythrocyte band 3 protein caused by substitution AAG——GAG (Lys——Glu) in codon 56. Blood. 1992; 80(0006–4971 (Print)):1592–8. PMID: 1520883

43. Posth C, Wißing C, Kitagawa K, Pagani L, van Holstein L, Racimo F, et al. Deeply divergent archaic mitochondrial genome provides lower time boundary for African gene flow into Neanderthals. Nature Communications. 2017; 8:16046. https://doi.org/10.1038/ncomms16046 PMID: 28675384

44. Groucutt HS, Grün R, Zalmout IAS, Drake NA, Armitage SJ, Candy I, et al. Homo sapiens in Arabia by 85,000 years ago. Nat Ecol Evol. 2018; 2(5):800–9. https://doi.org/10.1038/s41559-018-0518-2 PMID: 29632352

45. Clarkson C, Jacobs Z, Marwick B, Fullagar R, Wallis L, Smith M, et al. Human occupation of northern Australia by 65,000 years ago. Nature. 2017; 547(7663):306–10. https://doi.org/10.1038/nature22968 PMID: 28726833

46. Choin J, Mendoza-Revilla J, Arauña LR, Cuadros-Espinoza S, Cassar O, Larena M, et al. Genomic insights into population history and biological adaptation in Oceania. Nature. 2021; 592(7855):583–9. https://doi.org/10.1038/s41586-021-03236-5 PMID: 33854233

47. Bergström A, McCarthy SA, Hui R, Almarri MA, Ayub Q, Danecek P, et al. Insights into human genetic variation and population history from 929 diverse genomes. Science. 2020; 367(6484). https://doi.org/10.1126/science.aay5012 PMID: 32193295
48. Sankararaman S, Mallick S, Dannemann M, Prüfer K, Kelso J, Pääbo S, et al. The genomic landscape of Neanderthal ancestry in present-day humans. Nature. 2014; 507(7492):354–7. https://doi.org/10.1038/nature12961 PMID: 24476815

49. Vernoil B, Akey JM. Resurrecting surviving Neandertal lineages from modern human genomes. Science. 2014; 343(6174):1017–21. https://doi.org/10.1126/science.1245938 PMID: 24476670

50. Barreiro LB, Quintana-Murci L. Evolutionary and population (epi)genetics of immunity to infection. Hum Genet. 2020. https://doi.org/10.1007/s00439-020-02167-x PMID: 32885198

51. Ségurel L, Gao Z, Przeworski M. Ancestry runs deeper than blood: the evolutionary history of ABO points to cryptic variation of functional importance. Bioessays. 2013; 35(10):862–7. https://doi.org/10.1002/bies.201300030 PMID: 23836453

52. Nordgren J, Svensson L. Genetic Susceptibility to Human Norovirus Infection: An Update. Viruses. 2019; 11(3). https://doi.org/10.3390/v11030226 PMID: 30845670

53. Cooling L. Blood Groups in Infection and Host Susceptibility. Clin Microbiol Rev. 2015; 28(3):801–70. https://doi.org/10.1128/CMR.00109-14 PMID: 26085552

54. Lindesmith L, Moe C, Marionneau S, Ruvoen N, Jiang X, Lindblad L, et al. Human susceptibility and resistance to Norwalk virus infection. Nat Med. 2003; 9(5):548–53. https://doi.org/10.1038/nm860 PMID: 12692541

55. van Der Poel WH, Vinjé J, van Der Heide R, Herrera MI, Vivo A, Koopmans MP. Norwalk-like calicivirus genes in farm animals. Emerging Infectious Diseases. 2000; 6(1):36–41. https://doi.org/10.3201/eid0601.000106 PMID: 10653567

56. Gouy A, Excoffier L. Polygenic Patterns of Adaptive Introgression in Modern Humans Are Mainly Shaped by Response to Pathogens. Mol Biol Evol. 2020; 37(5):1420–33. https://doi.org/10.1093/molbev/msz306 PMID: 31935281

57. Van Blerkom LM. Role of viruses in human evolution. Am J Phys Anthropol. 2003; Suppl 37(Suppl):14–46. https://doi.org/10.1002/ajpa.10384 PMID: 14665532

58. Ríos L, Kivel T, Lalavea-Fox C, Estalrich A, García-Taberner A, Huguet R, et al. Skeletal Anomalies in The Neandertal Family of El Sidrón (Spain) Support A Role of Inbreeding in Neandertal Extinction. Sci Rep. 2019; 9(1):1697. https://doi.org/10.1038/s41598-019-38571-1 PMID: 30737446

59. Denommée GA, Wagner FF, Fernandes BJ, Li W, Flegel WA. Partial D, weak D types, and novel RHD alleles among 33,864 multiethnic patients: implications for anti-D alloimmunization and prevention. Transfusion. 2005; 45(10):1554–60. https://doi.org/10.1111/j.1537-2995.2005.00586.x PMID: 16181204

60. Jakobsen MA, Nielsen C, Sprogøe U. A case of high-titer anti-D hemolytic disease of the newborn in which late onset and mild course is associated with the D variant, RHD-CE(9)-D. Transfusion. 2014; 54(10):2463–7. https://doi.org/10.1111/trf.12673 PMID: 24749928

61. Degioanni A, Bonenfant C, Cabot S, Condemi S. Living on the edge: Was demographic weakness the cause of Neanderthal demise? PLoS One. 2019; 14(5):e0216742. https://doi.org/10.1371/journal.pone.0216742 PMID: 31141515