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Neanderthal DNA and modern human origins

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ABSTRACT

Neanderthals are an extinct hominid lineage that coexisted with modern humans in Eurasia. The Neanderthal's role in the evolutionary history of modern humans is a well concerned topic. Mitochondrial DNA sequences from Neanderthal fossils support the "Out of Africa" theory of human evolution, suggesting that modern human ancestors replaced Neanderthal populations in Eurasia. Recent analyses of Neanderthal genome draft sequences indicate minor gene flow between Neanderthals and modern humans in Eurasia but not in Africa. However, these conclusions are quite controversial due to data quality and insufficient sampling, especially about when and where the genetic admixture took place, and the direction of the assumed gene flow.

1. Introduction

The debate on the origin of anatomically modern human (*Homo sapiens sapiens*) has lasted for decades. The well known "out-of-Africa" replacement model postulates that modern human ancestors evolved in Africa, spread throughout the globe and subsequently replaced all other hominids (Stringer and Andrews, 1988). However, the multiregional origin model suggests that the gradual transition from *Homo erectus* to modern human took place on different continents in the Old World (Wolpoff et al., 1984). Other, intermediate models acknowledge an African origin of modern humans and theorize that archaic hominids outside of Africa also made genetic contributions (Smith et al., 2005).

Now, the debate focuses on the evolutionary relationship between archaic hominids and anatomically modern humans. It also seeks to address whether gene flow could have occurred between archaic hominids and modern humans, thereby leaving a footprint on the modern humans' current gene pool.

Neanderthals (*Homo neanderthalensis*) are archaic hominids, supposed to be most similar to modern humans. These hominids, extinct members of the *Homo* genus, populated Europe and parts of western and central Asia before their disappearance 25,000 years ago (Tattersall, 1995). Fossil evidence suggests that Neanderthals probably coexisted with anatomically modern humans (i.e. Cro-Magnon) for 20,000 years (Finlayson et al., 2006). Although there is no evidence of contemporaneous cohabitation at any single archeological site, their long period of coexistence (including cohabitation in the Middle East and Europe) raises the possibility of genetic admixture between Neanderthals and modern humans. The shared morphological features between Neanderthals and early modern Europeans also have been interpreted as evidence for the gene flow between Neanderthals and modern Europeans (Wolpoff et al., 2001; Voisin, 2006). Since new developments in fossil-preserved ancient DNA analysis arose in the late 1980s, genetic evidence has shed more light on the relationship between Neanderthals and modern humans.

2. Neanderthal mitochondrial DNA

In 1997, a segment of hypervariable control region I (HVRI) of maternally inherited mitochondrial DNA (mtDNA) was determined from a Neanderthal-type specimen found in 1856 in Neander Valley, near Düsseldorf, Germany (Krings et al., 1997). Since then, mtDNA sequences have been determined from multiple Neanderthal samples (Krings et al., 1999, 2000; Ovchinnikov et al., 2000; Gutierrez et al., 2002; Schmitz et al., 2002; Serre et al., 2004; Beauval et al., 2005; Caramelli et al., 2006; Lalueza-fox et al., 2006; Orlando et al., 2006; Krause et al., 2007; Green et al., 2008; Briggs et al., 2009).

The successful retrieval of mtDNA sequences from Neanderthal fossils made it possible to compare DNA sequences from extinct hominids with those from modern humans. Analyses showed that Neanderthal mtDNA sequences fall outside the variation for modern human mtDNA. Also, these sequences were not more similar to contemporary European sequences than the sequences of the earliest modern European specimens. These results clearly reveal that Neanderthals made no contribution to the modern

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human mtDNA gene pool; the results thus support the "Out of Africa" model of modern human evolution. However, since the mtDNA genome is inherited maternally, mtDNA analyses could only disprove the Neanderthals' maternal contribution to modern humans. While these findings might reflect the extinction of Neanderthals, they could also show the effects of stochastic genetic drift or the sex bias in reproduction; Neanderthals might have exchanged other parts of their genomes with modern humans (Serre et al., 2004; Wall and Hammer, 2006).

3. Neanderthal nuclear DNA

The development of high-throughput DNA sequencing technology and improved computational and statistical methodology have allowed for the large-scale sequencing of nuclear DNA from Neanderthal fossils. Noonan et al. (2006) used a cell-based approach to create meta-genomic libraries of fossil DNA extracts by performing traditional Sanger sequencing and 454 pyrosequencing. They obtained 65,250 bp of Neanderthal genomic sequences. Green et al. (2006) used an emulsionbead-based approach and obtained more than 1 Mb of genomic sequences by direct 454 pyrosequencing from the same Neanderthal fossil. Surprisingly, the two teams came to very different conclusions. Noonan et al. (2006) estimated that Neanderthals and modern humans diverged 706,000 years ago, thus supporting the earlier conclusion of Neanderthal's negligible DNA contribution to the modern European gene pool. In contrast, Green et al. (2006) calculated the average sequence divergence time between Neanderthals and modern human as 516,000 years ago and found a considerable level of admixture between Neanderthals and modern humans.

However, further analysis of the Green et al. (2006) data detected contamination from modern human DNA to an extent of nearly 80% (Wall and Kim, 2007). Pääbo and his colleagues, however, contended that it was only 11–40% (Green et al., 2010). This incident indicated that even extremely stringent protocols cannot eliminate possible contamination and mapping errors.

In 2010, Pääbo's team used tagged sequence adaptors and other technical improvements in Neanderthal whole genome sequencing to minimize the risk of contamination. They have completed a draft sequence for approximately 60% of the Neanderthal genome, composed of more than four billion nucleotides from three individuals. After comparing their draft sequence with the genomes of five geographically distant present-day humans, they demonstrated that the Neanderthal genome is remarkably similar to the modern human genome and estimated that Neanderthals and modern human ancestors most likely diverged 270,000 to 440,000 years ago. Noticeable differences in modern human genomic regions include changes in metabolism, cognition and skeletal development, showing signs of positive selection. They also found that Neanderthals share more derived polymorphisms with modern humans in Eurasia than with modern humans in Sub-Saharan Africa; therefore, they concluded that about 1–4% of non-African genomes in Europeans are derived from Neanderthals. In addition, they claimed that they have detected gene flow from Neanderthals to modern humans but no gene flow from modern human to Neanderthals (Green et al., 2010). Still, these conclusions are quite controversial, especially issues pertaining to regional affinities and the assumed direction of gene flow.

One obvious drawback of this study is data quality. Draft sequences consist mainly of 30–100 bp-long reads that only provide 1.3 fold genomic coverage. De novo sequence assembly is not possible with such a low-coverage data (Noonan, 2010). All Neanderthal reads were aligned to the human and chimpanzee reference genomes using megablast (Green et al., 2010). These analyses have largely been limited to detecting lineage-specific substitutions and small insertions or deletions (indels). Sequencing errors are also inevitable due to low coverage. For instance, Neanderthal-specific substitutions are about 30 times higher than on human lineages, largely due to transitions caused by cytosine deamination in the Neanderthal DNA (Green et al., 2010). Green et al. disregarded transitions to reduce contamination and sequencing errors and to provide evidence of low contamination rates. Although removing transition would not cause a negative result to positive, this disregard most likely resulted in the loss of useful information.

The poor quality of the data makes it difficult to detect signals of gene flow and might cause bias in subsequent analyses. To estimate Neanderthal ancestry in European genomes, this study analyzes the biallelic SNPs, focusing on those SNPs with different alleles in modern Europeans and Africans and with derived allele in the Neanderthals. Although the sequenced Neanderthal genome has derived alleles, SNPs may be polymorphic in other Neanderthals. The detected Neanderthal-specific polymorphisms might be induced by damage after death. For sites with ancestral alleles but read as derived alleles in the sequenced Neanderthal genomes, the same derived allele in human populations would be considered as in the same lineage rather than novel mutations. Green et al. developed D statistics (Green et al., 2010; Durand et al., 2011) in comparing the relationship of both non-Africans and Africans to Neanderthals. Using D statistics when Neanderthal sites with derived alleles are controlled, the authors observed the same derived allele in modern humans as in Neanderthals, meaning that they share the same lineage. With low coverage in Neanderthals, derived alleles in heterozygotes tend to be lost. Since results between Africans and non-Africans within these sites are defined by demographic events, the lost data is not correlated with distribution of derived alleles in Africans and non-Africans. Taken this into consideration, low coverage will cause bias in conclusion of gene flow direction. In addition to this finding, Durand et al. argues that the D-statistic used by Green et al. to test for admixture was insensitive to confounding factors, such as sequencing errors, ancient DNA damage, and human/Neanderthal population sizes (Durand et al., 2011).

Furthermore, to explain regional affinities between Neanderthal DNA and modern non-Africans's DNA, Green et al. proposes that gene exchange between Neanderthals and ancestors of non-Africans occurred shortly after modern humans left Africa around 50,000 years ago. Therefore, there was no gene flow from Neanderthals to the ancestors of modern Africans. Based on this assumption, Green et al. estimated the proportion of Neanderthal ancestry in non-Africans between 1% and 4% (Green et al., 2010). However, Hodgson et al. shows that interbreeding between Neanderthals and modern humans may not have been possible during this time because they would not have made contact (i.e. The accepted southern route of out-of-Africa migration took Neanderthals through the southern Arabian Peninsula, thus preventing contact). Hodgson et al. also proposed that modern humans might have returned to Africa after initial admixture with Neanderthals due to climatic shifts (Hodgson et al., 2010).

According to Hodgson et al, signs of Neanderthal admixture in non-Africans were probably due to founder effects during modern human dispersal. Thus, some traces of Neanderthal DNA could also be detectable in East Africans, who have not been sampled by Green et al. (Hodgson et al., 2010). However, Hodgson's scenario was not supported by Yotova et al.'s analysis based on haplotypes of the dys44 segment in a sample of 6092 X-chromosomes. Specifically, haplotype B006, which is the closest to the ancestral one, is common outside Africa but virtually absent in sub-Saharan Africa. Yotova et al. concluded that Neanderthal admixture occurred very early or prior to the worldwide expansion of modern humans' African common ancestors (Yotova et al., 2011). However, neither of the two papers by Green et al. and Hodgson et al. could rule out that genetic

substructure of Neanderthals' and modern humans' African common ancestors was already formed by the time Neanderthal ancestors left Africa to settle western Eurasia. If this is so, some ancient African modern human populations might have more affinities with Neanderthals than others. If these populations were also the source of modern humans' out-of-Africa expansion, then present-day non-Africans would be more closely related to Neanderthals than Africans in some genomic regions (Green et al., 2010; Hodgson et al., 2010). If the ancient genetic substructure really existed in the African gene pool, then admixture from Neanderthals may not be necessary at all to understand the data. However, because the origin and dispersal of modern humans experienced severe bottlenecks (Lahr and Foley, 1994; Ambrose, 1998), the genetic divergence of Neanderthals and modern humans is greater than that of any two present-day humans, which is also observed in Green et al.'s data. Thus, theorizing that an ancient substructure exists within African populations might not be an appropriate interpretation. Better understanding of African genetic diversity is key point to solving the dispute of when and where admixture occurred.

For the unidirectional gene flow from Neanderthals to modern humans, Green et al. suggested a "resident vs. colonizing" model. They think that detectable gene flow resulted from the resident population (Neanderthals) to the colonizing population (anatomically modern human) during the colonization process (Green et al., 2010). Mason and Short hypothesized that gene flow from Neanderthals to modern human and the lack of Neanderthal mitochondrial DNA in modern human populations were due to male Neanderthals mating with female modern humans to produce fertile female hybrids when taken the Haldane's Law into consideration (Mason and Short, 2011). However, Hofreiter argues that because all Neanderthal samples analyzed by Green et al. dated back to at least 38,000 years BP, which predated the first direct evidence for anatomically modern human in Europe, there was no trace of any gene flow from modern humans to Neanderthals that could be found in these samples (Hofreiter, 2011). We believe that the reciprocal gene flow from modern humans to Neanderthals might have been erased as any traces of modern human DNA in the data of Green et al. are interpreted as signs of contamination. Green et al. used the sex-determination method to estimate modern human DNA contamination. They checked for the presence of Y chromosomal DNA fragments in the extracts from the suggested three female Neanderthals. However, the sex-determination method used by Green et al. could lead to false-positive results for females especially when the genomic coverage of Neanderthals is low, because the absence of amplification can demonstrate either the absence of a Y chromosome or low coverage in a particular sample.

The draft sequence of the Neanderthal genome might have provided evidence against strict versions of both multiregional continuity and Out-of-Africa replacement models. It suggested the model emphasizing the role of Out-of-Africa expansion could be coupled with the low levels of assimilation of regional late archaic hominids. Such a model might be realistic if the hypothesis of regional affinities and the relevant data was reliable (Green et al., 2010). However, for a better understanding of Neanderthal to modern human gene flow, deeper sequencing as well as more sampling representing population structure on Neanderthals is necessary.

References

Ambrose, S., 1998. Late Pleistocene human population bottlenecks, volcanic winter, and differentiation of modern humans. Journal of Human Evolution 34, 623–651.

Beauval, C., Maureille, B., Lacrampe-cuyaubere, F., Serre, D., Peressinotto, D., Bordes, J.G., Cochard, D., Couchoud, I., Dubrasquet, D., Laroulandie, V., 2005. A late Neanderthal femur from Les Rochers-de-Velleneuve. Diogenes 54, 147–166.

Briggs, A.W., Good, J.M., Green, R.E., Krause, J., Maricic, T., Stenzel, U., Lalueza-Fox, C., Rudan, P., Brajkovic, D., Kucan, Z., Gusic, I., Schmitz, R., Doronichev, V.B., Golovanova, L.V., de la Rasilla, M., Fortea, J., Rosas, A., Pääbo, S., 2009. Targeted retrieval and analysis of five Neanderthal mtDNA genomes. Science 325, 318–321.

Caramelli, D., Lalueza-Fox, C., Condemi, S., Longo, L., Milani, L., Manfredini, A., de Saint Pierre, M., Adoni, F., Lari, M., Giunti, P., Ricci, S., Casoli, A., Calafell, F., Mallegni, F., Bertranpetit, J., Stanyon, R., Bertorelle, G., Barbujani, G., 2006. A highly divergent mtDNA sequence in a Neanderthal individual from Italy. Current Biology 16, R630–R632.

Durand, E., Patterson, N., Reich, D., Slatkin, M., 2011. Testing for ancient admixture between closely related species. Molecular Biology and Evolution. doi:10.1093/molbev/msr048.

Finlayson, C., Pacheco, F.G., Rodriguez-Vidal, J., Fa, D.A., Lopez, J.M.G., Perez, A.S., Finlayson, G., Allue, E., Preysler, J.B., Caceres, I., Carrion, J.S., Jalvo, Y.F., Gleed-Owen, C.P., Espejo, F.J.J., Lopez, P., Saez, J.A.L., Cantal, J.A.R., Marco, A.S., Guzman, F.G., Brown, K., Fuentes, N., Valarino, C.A., Villalpando, A., Stringer, C.B., Ruiz, F.M., Sakamoto, T., 2006. Late survival of Neanderthals at the southernmost extreme of Europe. Nature 443, 850–853.

Green, R.E., Krause, J., Ptak, S.E., Briggs, A.W., Ronan, M.T., Simons, J.F., Du, L., Egholm, M., Rothberg, J.M., Paunovic, M., Pääbo, S., 2006. Analysis of one million base pairs of Neanderthal DNA. Nature 444, 330–336.

Green, R.E., Malaspinas, A.S., Krause, J., Briggs, A.W., Johnson, P.L., Uhler, C., Meyer, M., Good, J.M., Maricic, T., Stenzel, U., Prüfer, K., Siebauer, M., Burbano, H.A., Ronan, M., Rothberg, J.M., Egholm, M., Rudan, P., Brajković, D., Kućan, Z., Gusić, I., Wikström, M., Laakkonen, L., Kelso, J., Slatkin, M., Pääbo, S., 2008. A complete Neanderthal mitochondrial genome sequence determined by high-throughput sequencing. Cell 134, 416–426.

Green, R.E., Krause, J., Briggs, A.W., Maricic, T., Stenzel, U., Kircher, M., Patterson, N., Li, H., Zhai, W.W., Fritz, M.H.Y., Hansen, N.F., Durand, E.Y., Malaspinas, A.S., Jensen, J.D., Marques-Bonet, T., Alkan, C., Prüfer, K., Meyer, M., Burbano, H.A., Good, J.M., Schultz, R., Aximu-Petri, A., Butthof, A., Hber, B., Hffner, B., Siegemund, M., Weihmann, A., Nusbaum, C., Lander, E.S., Russ, C., Novod, N., Affourtit, J., Egholm, M., Verna, C., Rudan, P., Brajkovic, D., Kucan, Ž., Gušic, I., Doronichev, V.B., Golovanova, L.V., Lalueza-Fox, C., Rasilla, M., Fortea, J., Rosas, A., Schmitz, R.W., Johnson, P.L.F., Eichler, E.E., Falush, D., Birney, E., Mullikin, J.C., Slatkin, M., Nielsen, R., Kelso, J., Lachmann, M., Reich, D., Pääbo, S., 2010. A draft sequence of the Neanderthal genome. Science 328, 710–722.

Gutierrez, G., Sanchez, D., Marin, A., 2002. A reanalysis of the ancient mitochondrial DNA sequences recovered from Neanderthal bones. Molecular Biology and Evolution 19, 1359–1366.

Hodgson, J.A., Bergey, C.M., Disotell, T.R., 2010. Neanderthal genome: the ins and outs of African genetic diversity. Current Biology 20 (12), R517–R519.

Hofreiter, M., 2011. Drafting human ancestry: what does the Neanderthal genome tell us about hominid evolution? Commentary on Green et al. Human Biology 83 (1), 1–11.

Krause, J., Orlando, L., Serre, D., Viola, B., Prüfer, K., Richards, M.P., Hublin, J.J., Hänni, C., Derevianko, A.P., Pääbo, S., 2007. Neanderthals in central Asia and Siberia. Nature 449, 902–904.

Krings, M., Stone, A., Schmitz, R.W., Krainitzki, H., Stoneking, M., Pääbo, S., 1997. Neanderthal DNA sequences and the origin of modern humans. Cell 90 (1), 19–30.

Krings, M., Geisert, H., Schmitz, R.W., Krainitzki, H., Pääbo, S., 1999. DNA sequence of the mitochondrial hypervariable region II from the Neanderthal type specimen. Proceedings, National Academy of Science USA 96, 5581–5585.

Krings, M., Capelli, C., Tschentscher, F., Geisert, H., Meyer, S., Haeseler, A., Grossschmidt, K., Possnert, G., Paunovic, M., Pääbo, S., 2000. A view of Neanderthal genetic diversity. Nature Genetics 26, 144–146.

Lahr, M., Foley, R., 1994. Multiple dispersals and modern human origins. Evolutionary Anthropology 3, 48–60.

Lalueza-fox, C., Krause, J., Caramelli, D., Catalano, G., Milani, L., Sampietro, L., Calafell, F., Martinez-maza, C., Bastir, M., Garcia-tabernero, A., 2006. The mitochondrial hypervariable region I of an Iberian Neanderthal suggests a population affinity with other European Neanderthals. Current Biology 16, 29–30.

Mason, P.H., Short, R.V., 2011. Neanderthal-human hybrids. Hypothesis 9 (1), e1.

Noonan, J.P., 2010. Neanderthal genomics and the evolution of modern humans. Genome Research 20 (5), 547–553.

Noonan, J.P., Coop, G., Kudaravalli, S., Smith, D., Krause, J., Alessi, J., Chen, F., Platt, D., Pääbo, S., Pritchard, J.K., Rubin, E.M., 2006. Sequencing and analysis of Neanderthal genomic DNA. Science 314, 1113–1118.

Orlando, L., Darlu, P., Toussaint, M., Bonjean, D., Otte, M., Hänni, C., 2006. Revisiting Neanderthal diversity with a 100,000 year old mtDNA sequence. Current Biology 16, R400–R402.

Ovchinnikov, I.V., Götherström, A., Romanova, G.P., Kharitonov, V.M., Lidén, K., Goodwin, W., 2000. Molecular analysis of Neanderthal DNA from the northern Caucasus. Nature 404 (6777), 490–493.

Schmitz, R.W., Serre, D., Bonani, G., Feine, S., Hillgruber, F., Krainitzki, H., Pääbo, S., Smith, F.H., 2002. The Neanderthal type site revisited: interdisciplinary investigations of skeletal remains from the Neander Valley, Germany. Proceedings, National Academy of Science USA 99, 13342–13347.

Serre, D., Langaney, A., Chech, M., Teschler-Nicola, M., Paunovic, M., Mennecier, P., Hofreiter, M., Possnert, G., Pääbo, S., 2004. No evidence of Neanderthal mtDNA contribution to early modern humans. PLoS Biol 2, E57.

Smith, F.H., Jankoviæ, I., Karavaniæ, I., 2005. The assimilation model, modern human origins in Europe, and the extinction of the Neanderthals. Quaternary International 137, 7–19.

Stringer, C.B., Andrews, P., 1988. Genetic and fossil evidence for the origin of modern humans. Science 239, 1263–1268.
Tattersall, I., 1995. The Last Neanderthal: The Rise, Success, and Mysterious Extinction of Our Closest Human Relatives. Macmillan, New York.
Voisin, J.L., 2006. Speciation by distance and temporal overlap: a new approach to understanding Neanderthal evolution. In: Harvati, K., Harrison, T. (Eds.), Neanderthals Revisited: New Approaches and Perspectives. Springer, Dordrecht, pp. 299–314.
Wall, J.D., Hammer, M.F., 2006. Archaic admixture in the human genome. Current Opinions in Genetic Developments 16, 606–610.
Wall, J.D., Kim, S.K., 2007. Inconsistencies in Neanderthal genomic DNA sequences. PLoS Genetics 3 (10), e175.
Wolpoff, M.H., Wu, X.Z., Thorne, A., 1984. Modern *Homo sapiens* origins: a general theory of hominid evolution involving the fossil evidence from East Asia. In: Smith, F.H., Spencer, F. (Eds.), The Origins of Modern Humans. Alan R Liss Inc, New York, pp. 411–483.
Wolpoff, M.H., Hawks, J., Frayer, D.W., Hunley, K., 2001. Modern human ancestry at the peripheries: a test of the replacement theory. Science 291, 293–297.
Yotova, V., Lefebvre, J.F., Moreau, C., Gbeha, E., Hovhannesyan, K., Bourgeois, S., Bédarida, S., Azevedo, L., Amorim, A., Sarkisian, T., Avogbe, P.H., Chabi, N., Dicko, M.H., Kou' Santa Amouzou, E.S., Sanni, A., Roberts-Thomson, J., Boettcher, B., Scott, R.J., Labuda, D., 2011. An X-linked haplotype of Neandertal origin is present among all non-African populations. Molecular Biology and Evolution 28 (7), 1957–1962.