1st round

Reviewer 1

He et al. presented exciting and vital work focused on the advances of ancient DNA research in East Asia, which systematically summarized fragmented previous research on ancient Chinese DNA, providing detailed insights into the dynamic evolutionary processes among ancient human populations in China spinning from the past 160,000 years to the historical period. This comprehensive review encompassed both temporal and spatial dimensions, including regions such as the Tianshan Mountains, Yellow River Basin, Yangtze River Basin, Amur River Basin, Tibetan Plateau, and Tibetan-Yi Corridor. This review elucidated the evolutionary trajectories of genetic interactions and admixture histories, as well as multiple genetic introgressions and their biological implications for modern human health and traits. Generally, this work is well-designed, and the picture is carefully prepared. After reading the manuscript, I found this manuscript to be insightful and to be a valuable contribution to the field, but I have the following concerns before its final publication:

Major concerns.

My main concern is that this review is too long. It will take a lot of patience for reviewers and readers to read this paper. So, I suggest simplifying the article and some sentences.

Another concern is the abbreviations; there are too many abbreviations, so consider only using abbreviations for the most important descriptions.

All figures should be concise and informative.

Some language errors need to be corrected.

Title

I think not only focus on Chinese history, should be East Asia.

Introduction

There should be a description of the origin and migrant for humans, then introduce East Asians.

Minor concerns

1. As mentioned in this manuscript, the farming-language-people codispersal model among ASEA farmers, changes in subsistence strategies among ANA, and the adoption of barley-based agriculture in ancient TP people are all factors that may have influenced the evolution of past populations. Previous studies have demonstrated that the domestication of animals and plants has enabled humans to effectively exploit grassland resources, leading to migration to new environments. In other words, the adaptational evolution of animals and plants to the environment, to some extent, also reflects past Chinese population dynamics. My first concern is that it is essential to appropriately expand discussions on the evolution of animals and plants to provide more comprehensive insights into China's past population dynamics and biological adaptations. This interdisciplinary approach will enrich the manuscript and contribute to a more comprehensive understanding of the complex environmental adaptation of human populations.

2. Another concern is that you have expounded on the genetic interaction between populations with trans-Eurasian herder-related ancestry and Chinese farmer-related ancestry populations, and previous studies have suggested that the rise of genetic risk for disease in modern European populations was associated with Yamnaya-related ancestry populations' migration approximately 5,000 years ago, such as multiple sclerosis (Barrie et al., 2024; Nature). Please clarify whether similar genetic admixture/introgression events occurred in ancient

Chinese populations and investigate their potential implications for disease susceptibility and population dynamics, which would significantly advance our understanding of human evolutionary history.

3. Line 61: “evolution” should be “evolutionary”.

4. Lines 103-106: “The aDNA data revolutionized our previous understanding of European genetic diversity that three ancestral sources related to the Near East farmers, Yamnaya-related pastoralists, and local hunter-gatherers generally contributed to the formation of the gene pool of modern Europeans [13]”. Recent studies using a large ancient genome dataset highlighted the Neolithic period’s and Bronze Age’s critical importance as determinants of immune responses for modern Europeans. Specifically, Yamnaya-related migration has introduced a high genetic risk of multiple sclerosis into Europe. My concern is that since you have comprehensively discussed population evolution and biological adaptation, it is imperative to elucidate the impact of past population admixture/migration events on the genetic basis of biological adaptation.

5. Lines 180-183: Change “The following investigations on archaic DNA introgression have supported the genetic interaction between archaic and modern humans and the acquisition of beneficial variants, which improved survival in new environments from archaic humans” to “The following investigations on archaic DNA introgression have supported the genetic interaction between archaic and modern humans, and the acquisition of beneficial variants from archaic humans improved the survival of modern humans in new environments” for clarity.

6. Line 185: Clarify the “separation” of specific groups.

7. Lines 268-271: The language of the paper has been polished well, but long sentences will affect the understanding of the content, such as “Sagart et al. also reconstructed the phylogeny topology with estimated divergence times, which confirmed the direct connection between the Sino-Tibetan language and late Cishan and the early Yangshao millet farmers in northern China from 7,200 before present (BP) [36]”.

8. Lines 286-287: The sentence “mixed” is repeated with “with each other”.

9. Lines 336-339: Clarify who is the “Austronesian-related ancients”.

10. Lines 378-383: Change “The Paleolithic separation between western Eurasian Steppe and eastern MP hunter-gatherer and Bronze Age large-scale westward migrations of Yamanaya, Afanasievo, Chemurchek, Sintashta and even involving Bactria-Margiana Archaeological Complex (BMAC)-related barley farmers, and of post-Bronze Age westward migrations of the nomadic regime of Xiongnu, Xianbei, Rouran, Türk, Mongolian and others were the major themes across Eurasian Steppe” to “The Eurasian Steppe is characterized by population separation between western Eurasian Steppe and eastern MP hunter-gatherer during the Paleolithic period. Bidirectional migration within the Eurasian Steppe has mainly occurred after the start of the Bronze Age, including Bronze Age large-scale eastward migrations of Yamanaya, Afanasievo, Chemurchek, Sintashta and even involving Bactria-Margiana Archaeological Complex (BMAC)-related barley farmers, and post-Bronze Age westward migrations of the nomadic regime of Xiongnu, Xianbei, Rouran, Türk, Mongolian and others".

11. Lines 551 and 552: The title needs to be revised according to the context.

12. Lines 571-576: Although ancient people in the plateau region have relatively high genetic continuity, some ancient individuals on the plateau are closely related to ancient people outside the plateau. For example, individuals in the 5th century AD and the 12th century AD in Shigatse on the southern plateau showed signals of interaction with ancient groups in Central Asia. Therefore, the complex communication history of people inside and outside the plateau should be described in detail.

13. Line 719: “the gene and variant spectrum of immunity genes” needs to be improved for readability.

14. Line 767: “benefit” should be deleted.

15. The abbreviations in the text are quite numerous. It would be helpful to present them appropriately to avoid confusion for the readers.

Figures
16. Fig. 1: I appreciate the thorough insights that the manuscript delineated the genetic introgression from archaic humans during the Paleolithic and the admixture-introduced population dynamics from the Neolithic to the historical period. I think depicting these two distinct patterns in Fig. 1a using different styles for ancient/archaic human genomic resources would significantly enhance the clarity and visual representation.

17. Fig. 2: Based solely on the legends and the picture, I find it difficult to distinguish between parts a, b, and c.

18. I noticed that you have performed the genetic analyses, which include PCA and ADMIXTURE analyses, and this manuscript lacked detailed information regarding the sources of the data used in the abovementioned analyses. Thus, providing comprehensive information about the data sources in the figure legends is essential.

Reviewer 2
He and colleagues performed a review of human demographic history in East Asia based on ancient DNA evidence. They base their interpretations on existing literature as well as a basic analysis of publicly available ancient DNA data (i.e. PCA, admixture plots, allele frequencies). Notwithstanding my previous comment, I commend the authors for trying to summarise ancient DNA research performed in East Asia to date. However, I consider that some major changes are needed before accepting the manuscript for publication.

I strongly suggest avoiding a focus on China in the title, and rather considering East Asia. Indeed, the population history starts long before China, and includes archaeogenomic evidence and several episodes of gene flow from many Eurasian sources.

The manuscript looks and reads like a review, but the authors also performed some basic population genetics analyses (PCA, admixture, allele counts) to summarise the data. However, they provide no details about data or methods used to build Figure 2, 3, and 5, and I strongly suggest including a material and methods section in the main text or as supplementary material.

The use of many acronyms makes reading the manuscript quite challenging, even if these acronyms are explicitly explained in the introduction. The structure of many sentences and the

sometimes poor choice of words also make reading the manuscript very challenging. In many places, I think I could understand what the authors meant, but I am concerned that I had to interpret the text in light of my own knowledge of the literature and many readers may not be able to do so. I acknowledge that none of the authors may be native English speakers, and I know it is unfair for non-English speakers to have to publish in English, but I am afraid the services of a professional translator will be needed to improve readability.

Some of the conclusions seem superfluous or just a repeat of what has been shown or suggested, without showing relevance to the demographic history of East Asia. For example, how would haplotype-based methods improve research? What would be the benefits of studying large pedigrees in historical burial sites in the context of Chinese dynasties? It also seems that the authors suggest several times that current databases and methods are not adequate to answer questions related to the ancestry of people currently living in China. It is a valuable point, and something that would need to be consolidated in the manuscript and discussed more comprehensively.

I am not sure the authors make a convincing argument when they summarise EPAS1, EDAR, SLC42A5 and OCA2 allele frequencies to demonstrate that aDNA evidence is important to identify or characterise determinants of health and disease in the present-day Chinese population (of note, Figure 5 is rather confusing given that the time periods are different in panels b-e). I understand that the authors argue for a more comprehensive spatiotemporal coverage of genetic diversity in East Asia, but they may need to demonstrate better why and how aDNA could actually lead to evolutionary medicine insights given the many ancestral gene flows described in previous sections.

Authors' response

Reviewer 1

He et al. presented exciting and vital work focused on the advances of ancient DNA research in East Asia, which systematically summarized fragmented previous research on ancient Chinese DNA, providing detailed insights into the dynamic evolutionary processes among ancient human populations in China spinning from the past 160,000 years to the historical period. This comprehensive review encompassed both temporal and spatial dimensions, including regions such as the Tianshan Mountains, Yellow River Basin, Yangtze River Basin, Amur River Basin, Tibetan Plateau, and Tibetan-Yi Corridor. This review elucidated the evolutionary trajectories of genetic interactions and admixture histories, as well as multiple genetic introgressions and their biological implications for modern human health and traits. Generally, this work is well-designed, and the picture is carefully prepared. After reading the manuscript, I found this manuscript to be insightful and to be a valuable contribution to the field, but I have the following concerns before its final publication:

***Our response**: Thanks. We sincerely appreciate your positive feedback on our work. Thank you for recognizing that our review provides a much-needed synthesis of previously fragmented research on ancient Chinese DNA and illuminates the dynamic evolutionary processes that shaped ancient human populations in China over the last 160,000 years. By examining both temporal and spatial dimensions, including the Tianshan Mountains, Yellow River Basin, Yangtze River Basin, Amur River Basin, Tibetan Plateau, and Tibetan-Yi Corridor, we aimed to present a comprehensive perspective on how genetic interactions, admixture histories, and multiple introgression events influenced modern human health and traits. We carefully revised our manuscript based on your suggestions. In response to your comments, we have refined the main sections of our manuscript to highlight the connections among these regions better and clarify how our findings bridge past and current perspectives. We remain committed to providing a clear and concise overview of how ancient DNA research in East Asia can inform*

ongoing debates on the detailed population genetic history and fine-scale genetic structure of spatiotemporally different ancient and modern East Asians, evolutionary biology, anthropology, and related fields of ancient and modern people. We appreciate your thorough review and believe these revisions strengthen our manuscript's overall clarity and contribution.

Major concerns.

My main concern is that this review is too long. It will take a lot of patience for reviewers and readers to read this paper. So, I suggest simplifying the article and some sentences.

***Our response**: Thanks for your comments and for highlighting your concerns regarding the length of the manuscript. We agree that conciseness will improve readability and reviewer engagement. To address this, we have streamlined the article by removing redundant information and refining lengthy passages. We will focus on presenting the recent core findings and discussions clearly and succinctly, ensuring that the paper remains both accessible and rigorous for all readers. As you can see from our presented contents of recent advances in East Asia, which is increasing rapidly, it is better to present the complete landscape of ancient East Asia. We presented our framework of this work at the end of the INTRODUCTION to present a more straightforward way to get the main and important information about this work.*

This review offers new perspectives on the historical evolution and ancient evolutionary adaptations of individuals from ancient East Asia, spanning the Pleistocene to Holocene periods, structured around seven key themes:

***(Ⅰ)** Archaic introgression from the Neanderthal and Denisovan populations: This section explores the contributions of archaic lineages to the gene pool of East Asians, with an emphasis on introgression events from Neanderthals and Denisovans.*

***(Ⅱ)** Initial peopling of East Asia and the basal Asian ancestry: This review highlights basic East Asian-related ancient individuals during the Pleistocene, shedding light on the early peopling of East Asia.*

***(III)** North-to-South differentiation of millet and rice farmers and their population dynamics: The genetic differentiation between ancient northern East Asian (ANEA) and ancient southern East Asian (ASEA) populations during the Paleolithic period is examined, alongside an admixture event resulting from the Neolithic expansion of millet-farming populations in the YRB and rice-farming populations in the YZRB.*

***(Ⅳ)** Genetic profile of ancient highlanders: The genetic makeup of ancient TP populations and the genetic relationships of ancient southwestern Chinese groups involved in mixed farming are analyzed.*

***(Ⅴ)** Genetic interaction between ancient farmer-related and herder-related populations in trans-Eurasian regions: This section discusses the genetic continuity of ancient northwestern Chinese populations and shifts in human populations associated with subsistence changes in northeastern China.*

***(VI)** Biological adaptation inferred from aDNA and its influence on human health and disease: This section summarizes the evolutionary and genetic determinants of human health and disease, with a focus on allele trajectory changes across different periods and spatial regions in ancient hominins.*

***(Ⅶ)** aDNA-related technological innovations and complex social organization reconstruction: This paper discusses prospects for future aDNA-related technological advancements and their applications in reconstructing ancient human social organizations.*

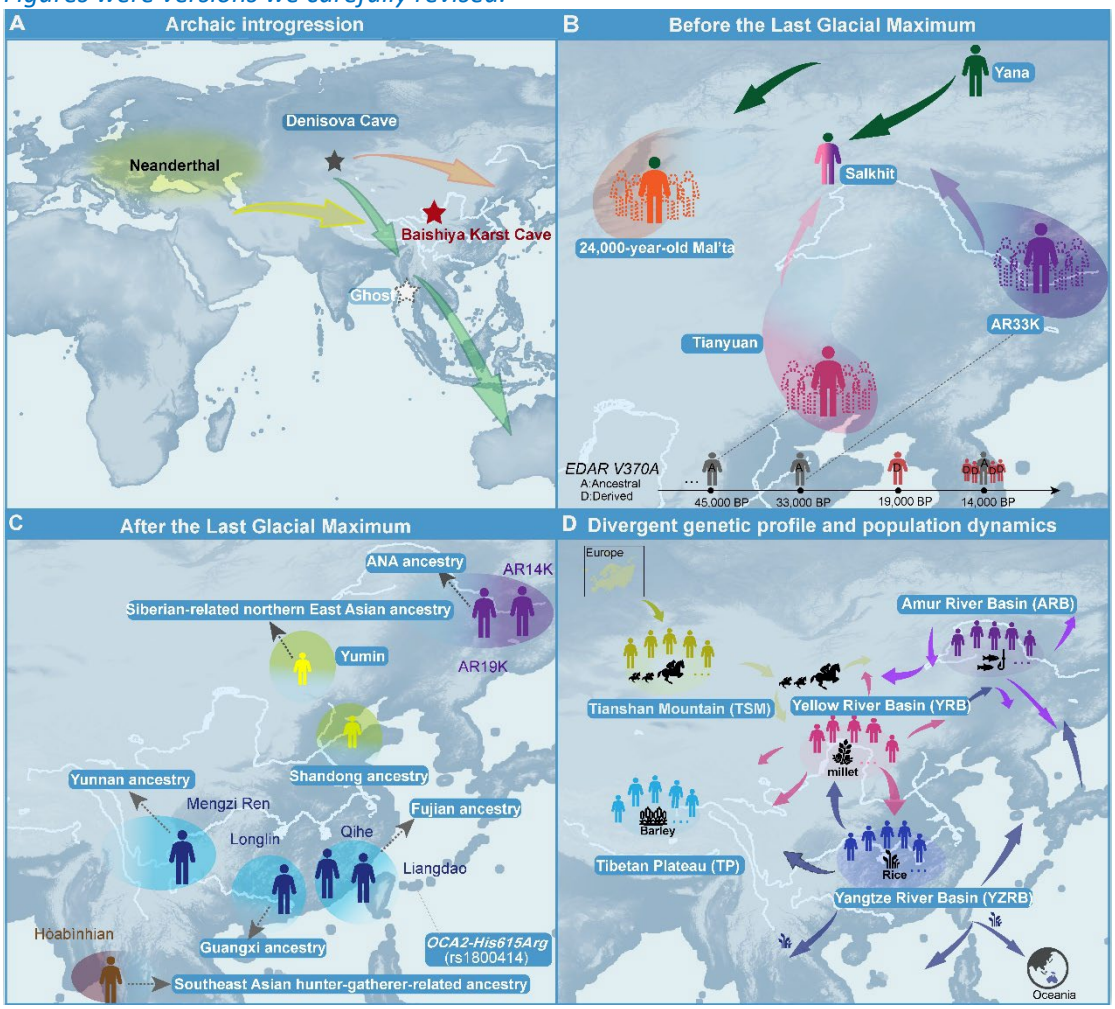
Finally, we also presented our designed framework in the Figrues, especially in the graphic Abtract figure.

Another concern is the abbreviations; there are too many abbreviations, so consider only using abbreviations for the most important descriptions.

***Our response**: Thanks for your suggestions and highlighting the overuse of abbreviations in our manuscript. We have carefully reviewed all abbreviations and removed those that are not essential to understanding our findings. For clarity, we now retain abbreviations only for terms that appear frequently or are standard within the field. We also presented the abbreviations used as the glossary at the end of the manuscript. We believe these changes will enhance readability and ensure that our manuscript remains accessible to a broad scientific audience. (aDNA Ancient DNA, ANE Ancient Northern Eurasians, ANA Ancient Northeast Asian, ANS Ancient Northern Siberians, AADR Allen Ancient DNA Resource, YRB Yellow River Basin, YZRB Yangtze River Basin, TP Tibetan Plateau, TYC Tibetan–Yi Corridor, TSM Tianshan Mountains, MP Mongolian Plateau, ARB Amur River Basin, WLRB West Liao River Basin, ANEA Ancient Northern East Asian, ASEA Ancient Southern East Asian, BKC Baishiya Karst Cave, NETP Northeastern regions of the Tibetan Plateau, LGM Last Glacial Maximum, MZR Mengzi Ren, PCA Principal component, HXC Hexi Corridor, BP Before present, BMAC Bactria-Margiana Archaeological Complex, IAMC Inner Asian Mountain Corridor)*

All figures should be concise and informative.

***Our response**: Thanks. We revised each figure to ensure clarity and brevity. First, we removed superfluous details and focus on the most relevant data points. We also simplified figure captions by using direct language that guides readers to the central findings. Then, we applied consistent formatting across all figures to maintain a cohesive presentation, allowing readers to grasp essential information without distraction quickly. Finally, we carefully polished the figures, and the revised version of the figures is improved to be of high quality. The following Figures were versions we carefully revised.*



Graphic abstract: He et al. compiled an integrated ancient genomic database encompassing spatiotemporally diverse populations across East Asia. This ancient genomic source provides a comprehensive framework for understanding population introgression, turnover, migration, and admixture among Paleolithic archaic humans, pre-LGM and post-LGM hunter-gatherers, and Holocene farmers and herders. ANA: Ancient Northeast ancients. LGM: Last Glacial Maximum. AR: Amur River.

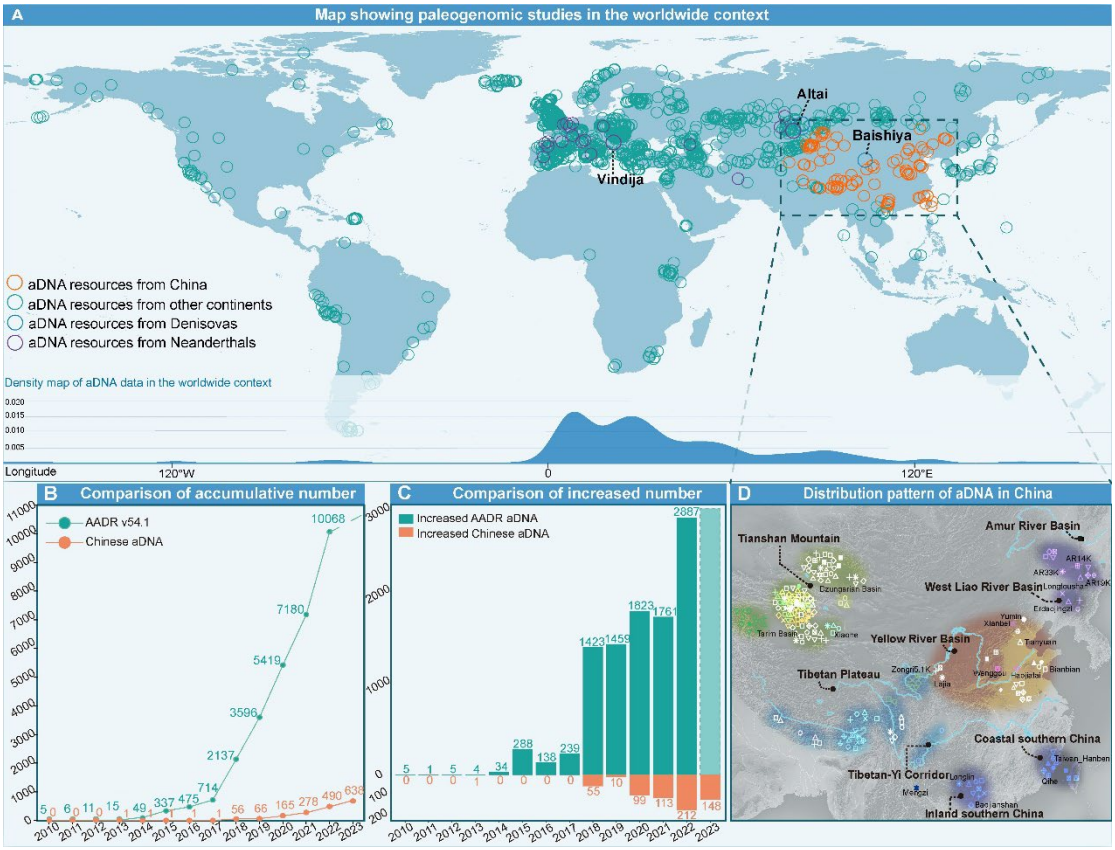


Fig. 1. Geographical distribution patterns and the number of ancient DNA (aDNA) data published worldwide over the past two decades. (A) *Global distribution of released aDNA. Orange and green circles represent aDNA from China and other continents, respectively. Blue and purple ones indicate Denisovan and Neanderthal genomes. The blue curve shows the density of aDNA samples by longitude.* ***(B)*** *Annual totals of worldwide and Chinese ancient genomes. The green solid line in AADR v54.1 indicates published aDNA statistics updated as of Nov. 16, 2022. Because updates were unavailable in 2023, the green dashed line illustrates the projected trend. The orange line indicates published aDNA data points from China.* ***(C)*** *The pattern of the newly increased aDNA data. The green columns show the cumulative aDNA in AADR by the corresponding year on the x-axis, and the orange columns reflect the annual growth of Chinese aDNA.* ***(D)*** *Geographical distribution of ancient Chinese populations. Different colors represent distinct cultural sites or regions. Detailed information on data sources and methodologies is provided in the Supplementary Materials.*

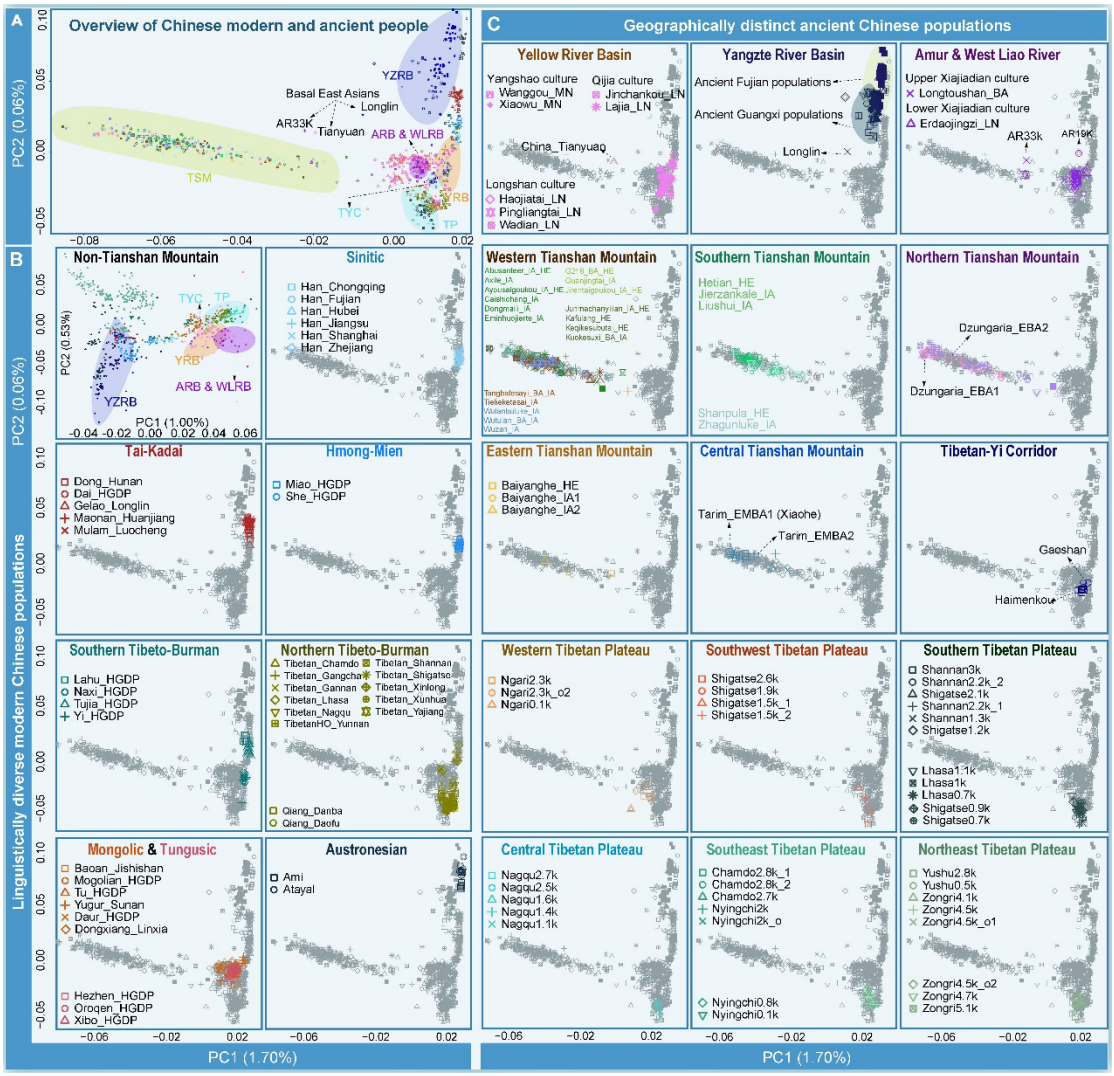


Fig. 2 The patterns of the genetic structure of spatiotemporally diverse ancient and contemporary populations. (A) *Overview of genetic structure inferred from principal component analyses of modern and ancient Chinese populations, with ancient individuals projected.* ***(B)*** *Genetic substructure among linguistically distinct modern Chinese populations. Language families delineated subgroups of present-day populations, with the primary component reflecting general patterns of eastern Eurasians and excluding individuals with apparent western Eurasian ancestry, such as the Tianshan mountain groups.* ***(C)*** *Clustering patterns of spatiotemporally diverse ancient populations. Colors in the legend correspond to regions: YZRB, Yangtze River Basin; ARB, Amur River Basin; WLRB, West Liao River Basin; YRB, Yellow River Basin; TP, Tibetan Plateau; TYC, Tibetan-Yi Corridor; TSM, Tianshan Mountain. Detailed information on data sources and methodologies is provided in the Supplementary Materials.*

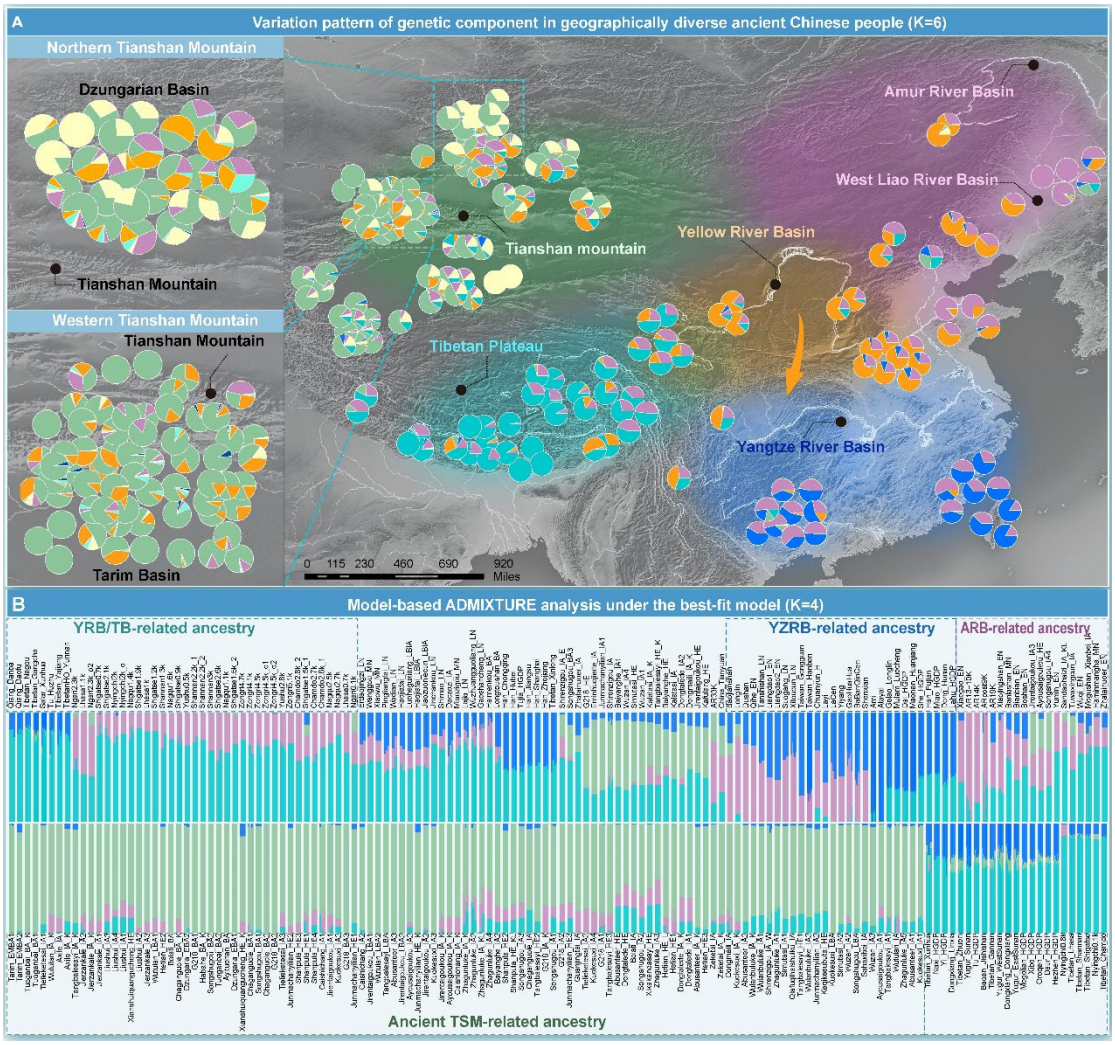


Fig. 3 Potentially differentiated admixture components contributing to the genetic differentiation of ancient Chinese populations inferred from the model-based ADMIXTURE models. *(**A**) A model-based admixture model was used to investigate the fundamental patterns of admixture sources and proportions. Distinct ancient Chinese-specific ancestral components were identified among geographically diverse groups at K = 6, revealing multiple ancestral compositions, including TSM-related ancestry (green and cream), northern Chinese ancestry represented by YRB-related individuals (orange) and ARB- and WLRB-related groups (purple), highlander-related ancestry represented by TP populations (light blue), and southern Chinese ancestry represented by YZRB rice farmers (dark blue). (**B**) The best-fitting admixture model (K = 4) among geographically diverse ancient Chinese populations included ancestral components of Sino-Tibetan-related groups (light blue), ARB-related groups (purple), YZRB-related speakers (dark blue), and TSM-related ancients (green). Detailed information on data sources and methodologies is provided in the Supplementary Materials.*

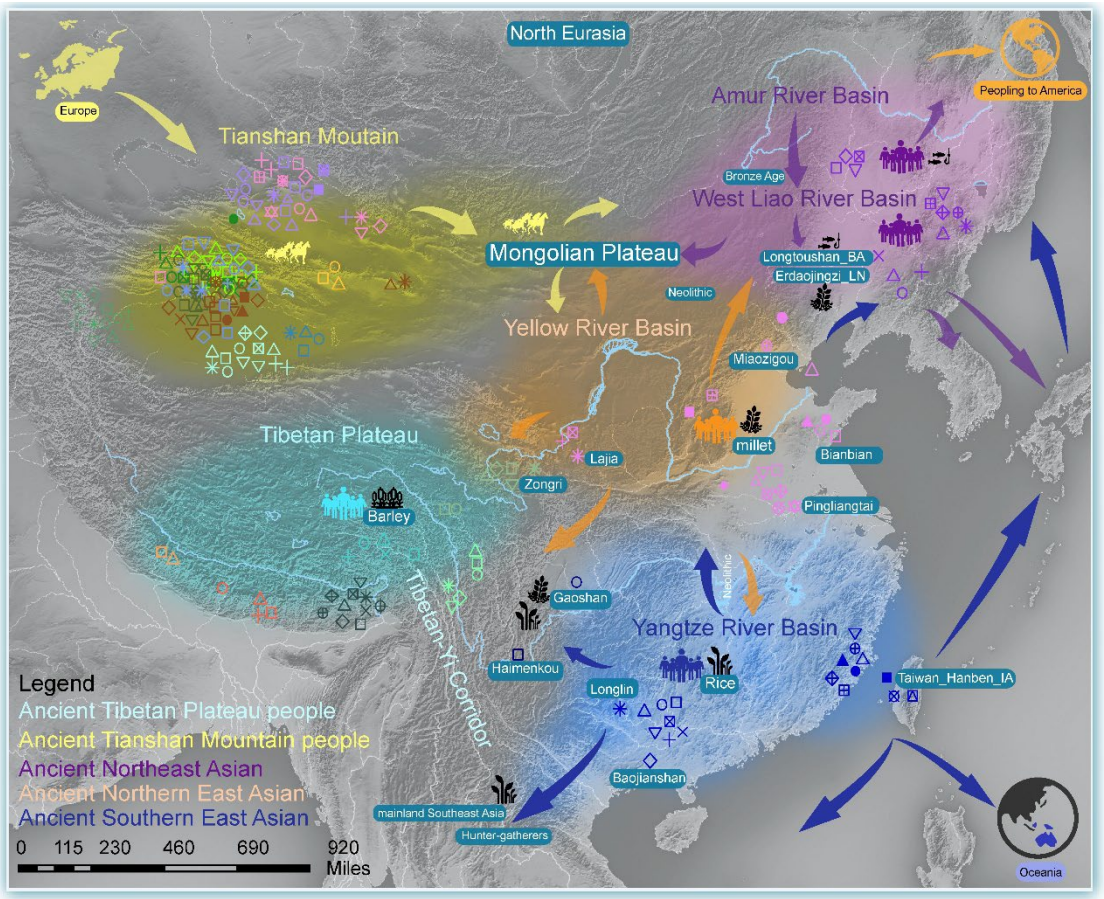


Fig. 4 Human evolutionary process and potential migrations in East Asia. *Symbols of different colors and shapes are used to represent individuals with distinct archeological backgrounds and genetic connections. Colored outlines delineate various regions: yellow for TSM, purple for ARB and WLRB, light yellow for YRB, dark blue for YZRB, and light blue for TP and TYC. The individual labels were consistent with the shapes and colors used in Fig. 2. Arrows indicate directions of past population spread or diffusion, with arrow colors corresponding to specific regions.*

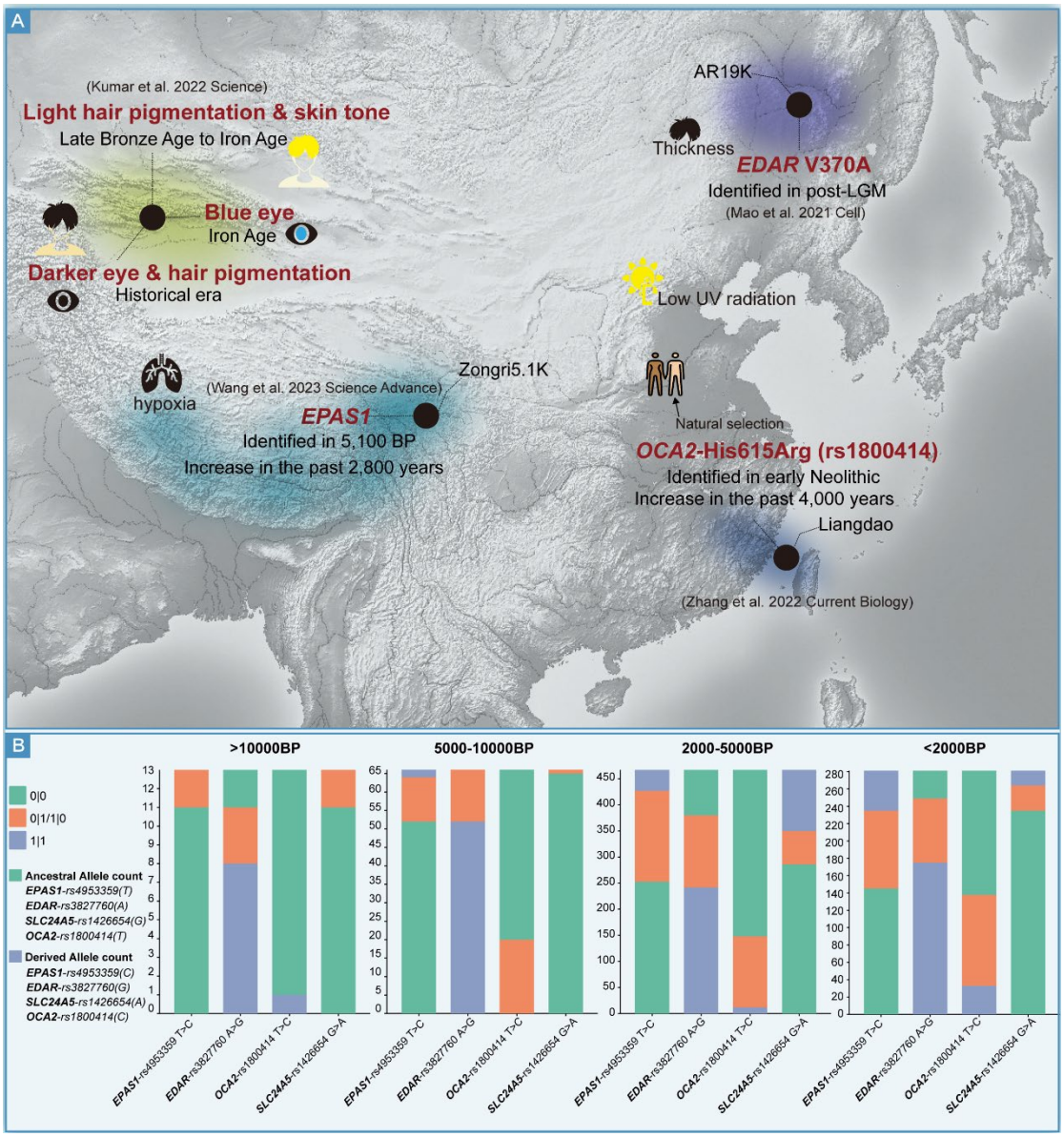


Fig. 5 Evolutionary trajectory of adaptive genetic variants. *(**A**) The genetic architecture of biological adaptive signatures at geographic and temporal resolutions is shown, including the timing of their emergence and frequency. (**B**) The evolutionary trajectories of the East Asian-specific EDAR V370A, SLC24A5, OCA2-His615Arg, and EPAS1 variants of all ancient East Asian populations are illustrated based on our integrative data. The height of each bar indicates the total sample size of ancient individuals at the corresponding spatiotemporal resolution. Green and blue denote the counts of derived and ancestral homozygotes, respectively, while red indicates the number of samples with heterozygotes. All the data were estimated via our high-quality imputed diploid ancient genomes via high-quality and population-specific Huaxi reference panels, which should be confirmed via high-coverage ancient genomes. Detailed information on data sources and methodologies is provided in the Supplementary Materials.*

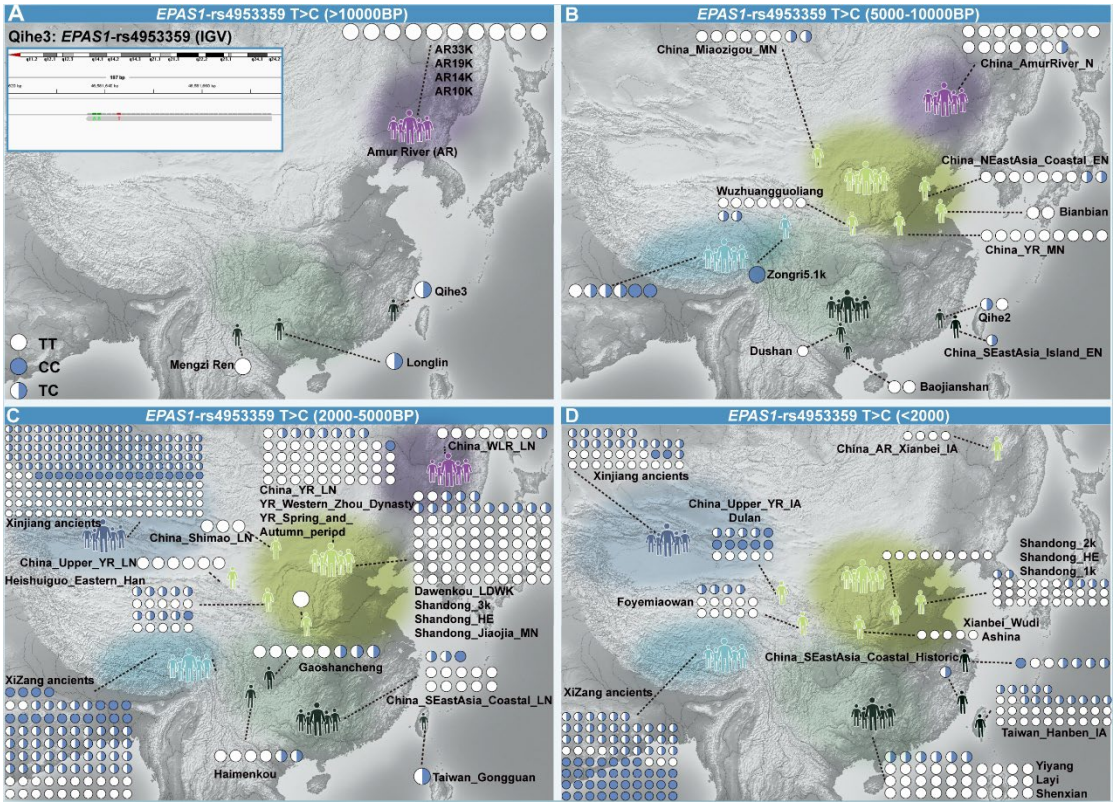


Fig. 6 The temporal and geographic distribution of EPAS1-rs4953359 alleles. *EPAS1 genotypes were determined for ancient East Asian individuals from high-quality imputed genomes, each represented by a circle. White circles indicate homozygosity for the nonadaptive EPAS1 variant, blue circles indicate homozygosity for the adaptive EPAS1 variant, and half-filled circles indicate heterozygosity. We labeled the major group names or famous archeologically-documented sites in each cluster. Further details on data sources and methodologies are provided in the Supplementary Materials.*

Some language errors need to be corrected.

Our response*: Thank you for highlighting the need to correct language errors in our manuscript. In the revised manuscript, we have thoroughly revised the text to address grammatical issues and improve overall clarity. We have also replaced passive constructions where possible to favor an active voice, thereby enhancing readability and aligning with standard American English usage. We believe these changes improve the manuscript's precision and flow. We appreciate your feedback and hope the revised version meets the journal's standards.*

Title

I think not only focus on Chinese history, should be East Asia.

Our response*: Thanks for your suggestions. We revised the title of this work following your suggestions. The new title is "**160,000-year population dynamics and biological adaptation of East Asia's past insights from ancient genome**s". Besides we also revised the corresponding contents in the revised manuscript,*

Introduction

There should be a description of the origin and migrant for humans, then introduce East Asians.

Our response*: Thanks for your insightful suggestion to include a description of human origins and migration patterns, followed by an introduction to East Asian populations. We have expanded and carefully revised the first three paragraphs, which focused on the human population origin and complex population movements and admixtures in the non-East Asians*

in the perspectives of ancient DNA. For example, at the end of the first paragraph, we highlighted the general patterns of human origin and migrations. Despite these achievements, existing studies have emphasized key milestones in human evolution, such as migration out of Africa, early settlement patterns, and subsequent migrations and admixture events in western Eurasia, Siberia, Oceania, and the Americas. However, this focus has been characterized by persistent overrepresentation of European populations, resulting in significant biases in the broader understanding of human evolution [5].

In the following two paragraphs, we provide a comprehensive overview of the origins of Homo sapiens, detailing the key migration routes out of Africa. Subsequently, we introduce East Asian populations, highlighting their complexity and rapid developments in recent years. These additions enhance the contextual framework of our study and offer a clearer understanding of the population dynamics of East Asians discussed.

Minor concerns

1. As mentioned in this manuscript, the farming-language-people codispersal model among ASEA farmers, changes in subsistence strategies among ANA, and the adoption of barley-based agriculture in ancient TP people are all factors that may have influenced the evolution of past populations. Previous studies have demonstrated that the domestication of animals and plants has enabled humans to effectively exploit grassland resources, leading to migration to new environments. In other words, the adaptational evolution of animals and plants to the environment, to some extent, also reflects past Chinese population dynamics. My first concern is that it is essential to appropriately expand discussions on the evolution of animals and plants to provide more comprehensive insights into China's past population dynamics and biological adaptations. This interdisciplinary approach will enrich the manuscript and contribute to a more comprehensive understanding of the complex environmental adaptation of human populations.

Our response**: Thanks. We appreciate the reviewer's insightful feedback regarding the integration of animal and plant evolution into our content of past population dynamics in China. In response, we have expanded Section [Necessity of multidisciplinary collaboration and indigenous engagement] to provide a more comprehensive analysis of how the domestication and adaptational evolution of animals and plants influenced human populations. Specifically, we have elaborated on the domestication processes of key species and their role in enabling ASEA farmers to exploit grassland resources more effectively. This, in turn, facilitated migrations to new environments and contributed to the codispersal of farming languages and people. **Interdisciplinary collaboration and effective communication are crucial for developing balanced narratives in aDNA research. Engaging archaeologists, geneticists, anthropologists, and specialists in animal and plant sciences has significantly advanced aDNA studies. By integrating recent insights into the origins and adaptations of ancient domesticated plants and animals within agricultural centers and surrounding regions, along with their biological adaptations, we can better understand the complex dynamics of human movements and biological evolution [43, 119, 120].

*Additionally, we also revised the Section [**Highland TP Peopling Processes and Genetic Connections to Neolithic ANEA and TYC Populations**] to include a detailed examination of barley-based agriculture adoption among ancient TP populations, highlighting its impact on subsistence strategies and societal development. **Archaeological evidence from the BKC site indicates that Denivovan-related populations inhabited the NETP at elevations of 3,280 m above sea level (masl), approximately 160 to 60 kya [28]. Nywa Devu hunter-gatherers are believed to have reached the central regions of the TP (4,600 masl) between 30–40 kya [87]. Permanent settlement of the TP may have occurred through the Chusang hunter-gatherers, who lived between 7.4 to 12.7 thousand years ago, or via later barley-based agricultural populations from the lowlands [88, 89].***

By incorporating these interdisciplinary perspectives, the revised manuscript offers a more nuanced understanding of the complex interactions between biological adaptations and human population dynamics in ancient China. We believe these enhancements enrich the overall analysis and provide a more holistic view of the environmental adaptations that shaped historical populations. Thank you for your valuable suggestion, which has significantly improved the depth and comprehensiveness of our study.

2. Another concern is that you have expounded on the genetic interaction between populations with trans-Eurasian herder-related ancestry and Chinese farmer-related ancestry populations, and previous studies have suggested that the rise of genetic risk for disease in modern European populations was associated with Yamnaya-related ancestry populations' migration approximately 5,000 years ago, such as multiple sclerosis (Barrie et al., 2024; Nature). Please clarify whether similar genetic admixture/introgression events occurred in ancient Chinese populations and investigate their potential implications for disease susceptibility and population dynamics, which would significantly advance our understanding of human evolutionary history.

***Our response**: Thanks for the insightful comment regarding the genetic admixture between trans-Eurasian herder-related and Chinese farmer-related ancestry populations. In our revised manuscript, we have clarified that similar admixture events did occur in ancient Chinese populations, but the admixture proportion is different in different populations. We detailedly presented these pieces of information in the Sections [**Dynamic Population Histories in the Crossroad Regions of Northern China and the Eastern Eurasian Steppe Since the Neolithic**] and sub-section [**Intense Admixture of Ancient TSM Populations**]. The admixture from Yamnaya-related migrations was observed in ancient Xinjiang people,*

*This is important for our understanding of the origin of human disease, but the deep research on ancient East Asians is limited. In the revised manuscript, we added a sub-section [**The influence of aDNA on tracing the genetic origin of human disease**] to summarize recent advances in this field and illuminate the implications of these genetic interactions for disease susceptibility and population dynamics within ancient populations. The evolutionary determinants of human diseases and complex traits have been shaped by diverse forces, including extreme environments on the TP, pathogen exposure (e.g., malaria in South China), and shifts in prehistoric subsistence strategies. These determinants have been inferred through advanced statistical and computational techniques based on patterns of genomic variations traditionally [102]. Ancient genetic analysis with comprehensive spatiotemporal coverage has provided direct insight into previously undisclosed population dynamics underlying human disease susceptibility, elucidating the evolutionary foundations of adaptive signatures and suggesting why diseases emerge in modern environments [103-106]. aDNA has yielded valuable perspectives in evolutionary medicine by revealing how beneficial variants in past environments have influenced modern human health and disease susceptibility.*

Ancestral gene flows have been examined to determine how trait evolution relevant to health was shaped, as noted in previous genetic research focused on the possible genetic origin of multiple sclerosis (MS) in modern and ancient Europeans [103]. Another promising aspect of aDNA research for human disease involves spatiotemporal windows to test evolutionary hypotheses, including the thrifty gene hypothesis, hygiene hypothesis, antagonistic pleiotropy hypothesis, and others [104, 107]. By capitalizing on the time-series nature of aDNA, the genetic trajectories of past adaptations have been traced to identify changes critical for survival under distinct environmental pressures, such as disease resistance, dietary tolerance, or adaptation to climate stressors. Akbari et al. reported numerous genome-wide significant signals and strong directional selection signatures related to celiac disease, blood type B, tuberculosis risk, rheumatoid arthritis, and 31 other aDNA-attested selection-related disease traits [104]. This large-scale aDNA research has also illuminated complex directional selection

that reshaped dozens of complex human traits. Such investigations are beneficial for understanding complex conditions (e.g., diabetes, cardiovascular disease, and cancer), which may carry an evolutionary component driven by ancestral gene flows.

The genetic legacy of ancient populations has also been characterized more accurately through aDNA, indicating how ancestral gene flows have shaped the contemporary human gene pool [108]. Marnetto et al. used combined genomic admixture modeling of ancient and modern Europeans and elucidated differentiated ancient European ancestral sources that contributed to varying effective sizes of anthropometric, pigmentation, and metabolic traits, such as the distinct influences of local hunter-gatherer and Yamnaya steppe ancestry on cholesterol levels [108]. In addition, genes transmitted during archaic human migrations, including those involving early Homo sapiens or Neanderthals, have clarified the genetic underpinnings of disease susceptibility and resistance [6-8]. The introgression of Neanderthal DNA, for instance, has been associated with immune system responses necessary for coping with infections.

Genetic changes associated with the agricultural revolution spanning thousands of years during the Holocene have been documented through aDNA, particularly those linked to immune-related host-pathogen co-evolution-related chronic diseases, aging, or metabolic disorders [103, 105, 107]. These findings indicate how genetic variants tied to disease risk have evolved, as variants once protective against ancient pathogens continue to shape modern disease susceptibility. By examining these variants, evolutionary medicine can identify pathways that influence present-day health and disease. Kerner et al. recently reported that the Holocene host-pathogen interaction landscape illuminated genetic adaptation to increased infectious disease, which also affected the susceptibility of inflammatory disorders [105].

*A more comprehensive picture of human evolution and its bearing on health was also promoted via integrating aDNA with modern genomic data. If ancient populations are found to carry immune-related genetic variants conferring resistance to human disease, comparisons with modern groups possessing similar variants can clarify how those traits affect current disease outcomes [105]. This approach can inform precision medicine and genetic counseling. Barrie et al. combined the risk of MS among modern northern and southern Europeans with ancient human migration and admixture date [103]. A strong association was observed between elevated genetic risk of MS and the ancestry of steppe pastoralist populations [103]. However, recent large-scale analyses of modern and ancient European genomes suggested that elevated MS risk in northern Europe was not attributable to selection by steppe pastoralists, despite the positive selection of HLA-DRB1*15:01 increasing to 18% between ~6000 and ~2000 years ago [104].*

*Besides, the advances and effects of ancient East Asian genomes on the dissection of biological adaptations of East Asians were carefully revised, which can provide some insights into genetic determinants of diseases in the revised Section [**Evolutionary trajectory reconstruction among East Asians through the lens of aDNA**].*

3. Line 61: “evolution” should be “evolutionary”.
***Our response**: Thanks. We fixed it.*

4. Lines 103-106: “The aDNA data revolutionized our previous understanding of European genetic diversity that three ancestral sources related to the Near East farmers, Yamnaya-related pastoralists, and local hunter-gatherers generally contributed to the formation of the gene pool of modern Europeans [13]”. Recent studies using a large ancient genome dataset highlighted the Neolithic period’s and Bronze Age’s critical importance as determinants of immune responses for modern Europeans. Specifically, Yamnaya-related migration has introduced a high genetic risk of multiple sclerosis into Europe. My concern is that since you have comprehensively discussed population evolution and biological adaptation, it is

imperative to elucidate the impact of past population admixture/migration events on the genetic basis of biological adaptation.

***Our response**: Thanks for sharing these suggestions. We appreciate the insightful feedback regarding the impact of past population admixture and migration events on the genetic basis of biological adaptation. In response to your suggestion, we have expanded our discussion to elucidate how these historical demographic processes have shaped the adaptive landscape of modern East Asians and present the detailed answer to question 2.*

*Specifically, we have incorporated recent findings from large ancient genome datasets that underscore the significance of the Neolithic and Bronze Age periods in determining immune response profiles among ancient and contemporary European populations. For instance, the migration of Yamnaya-related pastoralists not only contributed to the genetic diversity of Europe but also introduced alleles associated with an increased risk of multiple sclerosis. This example illustrates how admixture events can have lasting effects on the genetic architecture related to disease susceptibility and other adaptive traits. We also **presented the advances of the evolutionary events inferred from ancient DNA on human disease and health**. By integrating these perspectives, we provide a more comprehensive understanding of how population movements and genetic admixture have driven biological adaptation and genetic determinants of complex determinants. These additions enhance the manuscript by linking historical demographic events to present-day genetic and health-related outcomes, thereby addressing the interplay between population evolution and biological adaptation. We added a sub-section [**The influence of aDNA on tracing the genetic origin of human disease**] and carefully revised the advances and effects of ancient East Asian genomes on the dissection of biological adaptations of East Asians, which can provide some insights into genetic determinants of diseases in the revised Section [**Evolutionary trajectory reconstruction among East Asians through the lens of aDNA**]. We believe these revisions meet the academic standards of Genome Biolog and significantly improve the clarity and depth of our analysis.*

5. Lines 180-183: Change “The following investigations on archaic DNA introgression have supported the genetic interaction between archaic and modern humans and the acquisition of beneficial variants, which improved survival in new environments from archaic humans” to “The following investigations on archaic DNA introgression have supported the genetic interaction between archaic and modern humans, and the acquisition of beneficial variants from archaic humans improved the survival of modern humans in new environments” for clarity.

***Our response**: Thanks. We fixed it. We carefully polished it further, “Further investigations into archaic introgression have substantiated genetic interactions between archaic and modern humans, highlighting the acquisition of beneficial variants that increase survival in new environments [30].”*

6. Line 185: Clarify the “separation” of specific groups.

***Our response**: Thanks. We revised it. “One wave of Neanderthal gene flow occurred before periods of separation of non-African individuals [13]”*

7. Lines 268-271: The language of the paper has been polished well, but long sentences will affect the understanding of the content, such as “Sagart et al. also reconstructed the phylogeny topology with estimated divergence times, which confirmed the direct connection between the Sino-Tibetan language and late Cishan and the early Yangshao millet farmers in northern China from 7,200 before present (BP) [36]”.

***Our response**: Thank you for your insightful feedback regarding the sentence structure; we carefully revised and polished it. We believe these changes improve the overall comprehension and academic quality of the manuscript.*

8. Lines 286-287: The sentence “mixed” is repeated with “with each other”.
***Our response**: Thanks. We fixed it. “Long-range spatiotemporal migrations of ancient Near Easterners from Anatolia, the Levant, and the Iran Zagros Mountains have been shown to involve genetically differentiated early farmers who first intermixed and later spread beyond the Near East, significantly influencing the genetic makeup of East Africa, the Eurasian Steppe, Europe, and South Asia [47].”*

9. Lines 336-339: Clarify who is the “Austronesian-related ancients”.
***Our response**: Thanks. We revised it. “These proto-Austronesian-related ancient populations, such as Iron Age Hanben people, share significant genetic alleles with present-day Tai-Kadai speakers in southern China (Fig. 2b-c) [45].”*

10. Lines 378-383: Change “The Paleolithic separation between western Eurasian Steppe and eastern MP hunter-gatherer and Bronze Age large-scale westward migrations of Yamanaya, Afanasievo, Chemurchek, Sintashta and even involving Bactria-Margiana Archaeological Complex (BMAC)-related barley farmers, and of post-Bronze Age westward migrations of the nomadic regime of Xiongnu, Xianbei, Rouran, Türk, Mongolian and others were the major themes across Eurasian Steppe” to “The Eurasian Steppe is characterized by population separation between western Eurasian Steppe and eastern MP hunter-gatherer during the Paleolithic period. Bidirectional migration within the Eurasian Steppe has mainly occurred after the start of the Bronze Age, including Bronze Age large-scale eastward migrations of Yamanaya, Afanasievo, Chemurchek, Sintashta and even involving Bactria-Margiana Archaeological Complex (BMAC)-related barley farmers, and post-Bronze Age westward migrations of the nomadic regime of Xiongnu, Xianbei, Rouran, Türk, Mongolian and others".
***Our response**: Thanks. We carefully revised it and polished it using shortened sentences following your suggestions. “The Paleolithic separation between the western Eurasian Steppe and eastern MP hunter-gatherer populations, along with the large-scale westward migrations during the Bronze Age, are central themes in the history of the Eurasian Steppe [63]. These migrations included the movements of the Yamnaya, Afanasievo, Chemurchek, and Sintashta cultures and, to a lesser extent, Bactria-Margiana Archaeological Complex (BMAC)-related barley farmers. Post-Bronze Age migrations, such as those of the Xiongnu, Xianbei, Rouran, Türk, and Mongolian nomadic regimes, further shaped the demographic landscape of the Eurasian Steppe. ”*

11. Lines 551 and 552: The title needs to be revised according to the context.
***Our response**: Thanks. We revised it as “Complex admixture of ancient TSM populations”*

12. Lines 571-576: Although ancient people in the plateau region have relatively high genetic continuity, some ancient individuals on the plateau are closely related to ancient people outside the plateau. For example, individuals in the 5th century AD and the 12th century AD in Shigatse on the southern plateau showed signals of interaction with ancient groups in Central Asia. Therefore, the complex communication history of people inside and outside the plateau should be described in detail.
***Our response**: Thanks. We revised it. We added the depth discussed in the section [**Paleolithic Occupation and Neolithic Expansion of the Ancient TP Groups**]. “This study also highlighted substantial gene flow between the TP and regions of East and Southeast Asia, further shaping the genetic composition of present-day Tibetans (Fig. 3) [93]. Bai et al. performed in-depth genetic modeling to investigate the population dynamics of ancient western Tibetans from Naru Prefecture [95]. These findings revealed 3,500 years of regional genetic continuity, multiple westward expansions from the southern Plateau, and gene flow between the Guge*

kingdom and Central and South Asian populations [95]. Similarly, Yang et al. analyzed ancient genomes from the Mabu Co site, one of the highest-elevation sedentary settlements globally [96]. They reported that indigenous foragers of the TP primarily carried southern Plateau ancestry. Additionally, historical populations have undergone extensive genetic exchange with other Eurasian populations. Zhu et al. reported 10 Tubo-related samples from the Dulan site dating to 1,308–1,130 BP, which indicated that the Tubo Empire influenced the NETP through both demic and cultural diffusion. This influence affected core Tibetan populations and Eurasian Steppe-related ancestry groups during the historical period [97].”

13. Line 719: “the gene and variant spectrum of immunity genes” needs to be improved for readability.

***Our response**: Thanks. We fixed it. “The signatures of natural selection in post-Neolithic Europe suggest that genetic adaptation to disease pathogens reshaped immunity-related genes, increasing the risk of inflammatory disorders [105]”*

14. Line 767: “benefit” should be deleted.

***Our response**: Thanks. We fixed it. “Childebayeva et al. highlighted the computational workflows involved in analyzing low-coverage NGS data, addressing the limitations and decision-making processes at each stage of analysis, which is crucial for the progress of both aDNA and forensic studies [121].”*

15. The abbreviations in the text are quite numerous. It would be helpful to present them appropriately to avoid confusion for the readers.

***Our response**: Thanks. We also presented the abbreviations used as the glossary at the end of the manuscript. (aDNA Ancient DNA, ANE Ancient Northern Eurasians, ANA Ancient Northeast Asian, ANS Ancient Northern Siberians, AADR Allen Ancient DNA Resource, YRB Yellow River Basin, YZRB Yangtze River Basin, TP Tibetan Plateau, TYC Tibetan–Yi Corridor, TSM Tianshan Mountains, MP Mongolian Plateau, ARB Amur River Basin, WLRB West Liao River Basin, ANEA Ancient Northern East Asian, ASEA Ancient Southern East Asian, BKC Baishiya Karst Cave, NETP Northeastern regions of the Tibetan Plateau, LGM Last Glacial Maximum, MZR Mengzi Ren, PCA Principal component, HXC Hexi Corridor, BP Before present, BMAC Bactria-Margiana Archaeological Complex, IAMC Inner Asian Mountain Corridor)*

Figures

16. Fig. 1: I appreciate the thorough insights that the manuscript delineated the genetic introgression from archaic humans during the Paleolithic and the admixture-introduced population dynamics from the Neolithic to the historical period. I think depicting these two distinct patterns in Fig. 1a using different styles for ancient/archaic human genomic resources would significantly enhance the clarity and visual representation.

***Our response**: Thanks. We revised it.*

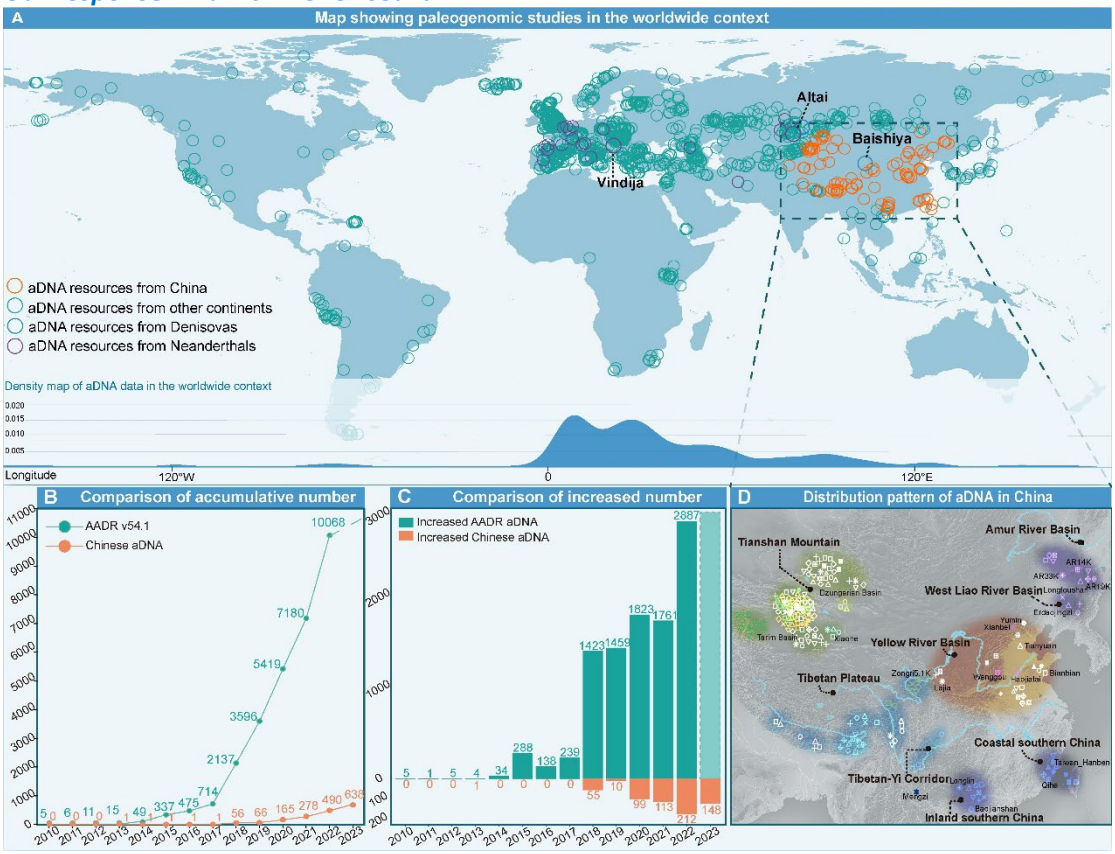


***Fig. 1. Geographical distribution patterns and the number of ancient DNA (aDNA) data published worldwide over the past two decades. (A)** Global distribution of released aDNA. Orange and green circles represent aDNA from China and other continents, respectively. Blue and purple ones indicate Denisovan and Neanderthal genomes. The blue curve shows the density of aDNA samples by longitude. **(B)** Annual totals of worldwide and Chinese ancient genomes. The green solid line in AADR v54.1 indicates published aDNA statistics updated as of Nov. 16, 2022. Because updates were unavailable in 2023, the green dashed line illustrates the projected trend. The orange line indicates published aDNA data points from China. **(C)** The pattern of the newly increased aDNA data. The green columns show the cumulative aDNA in AADR by the corresponding year on the x-axis, and the orange columns reflect the annual growth of Chinese aDNA. **(D)** Geographical distribution of ancient Chinese populations. Different colors represent distinct cultural sites or regions. Detailed information on data sources and methodologies is provided in the Supplementary Materials.*

17. Fig. 2: Based solely on the legends and the picture, I find it difficult to distinguish between parts a, b, and c.

***Our response**: Thanks. We fixed it based on your suggestions.*

18. I noticed that you have performed the genetic analyses, which include PCA and ADMIXTURE analyses, and this manuscript lacked detailed information regarding the sources of the data used in the abovementioned analyses. Thus, providing comprehensive information about the data sources in the figure legends is essential.

***Our response**: Thanks. We revised it, and we also provided detailed methods and materials in the supplementary materials. Detailed information was presented in the aforementioned information focused on the Figure revision.*

Reviewer 2

He and colleagues performed a review of human demographic history in East Asia based on ancient DNA evidence. They base their interpretations on existing literature as well as a basic analysis of publicly available ancient DNA data (i.e. PCA, admixture plots, allele frequencies). Notwithstanding my previous comment, I commend the authors for trying to summarise ancient DNA research performed in East Asia to date. However, I consider that some major changes are needed before accepting the manuscript for publication.

***Our response**: Thank you for your constructive feedback on our manuscript. We appreciate your recognition of our effort to summarize ancient DNA research in East Asia and acknowledge your concerns regarding the need for major revisions. We incorporated more comprehensive analyses and revisions. We refined our discussions in each section to address any discrepancies or limitations in the current literature. By expanding our analysis and carefully revising it, we aim to enhance the reliability of our interpretations of East Asia's demographic history from the ancient DNA perspectives. We value your insightful comments and look forward to strengthening our manuscript accordingly.*

I strongly suggest avoiding a focus on China in the title, and rather considering East Asia. Indeed, the population history starts long before China, and includes archaeogenomic evidence and several episodes of gene flow from many Eurasian sources.

***Our response**: Thank you for highlighting the importance of framing our study within a broader East Asian context. We agree that the population history predates the emergence of modern China and involves multiple waves of gene flow from diverse Eurasian sources. In line with your recommendation, we revised our title to reflect a wider geographical scope and emphasize the deep temporal and spatial dimensions of these population movements. We appreciated your insights and integrated them into the manuscript to ensure a clearer, more comprehensive perspective on East Asian population history. We revised our title following your suggestions. "1**60,000-year population dynamics and biological adaptation of East Asia's past insights from ancient genomes**"*

The manuscript looks and reads like a review, but the authors also performed some basic population genetics analyses (PCA, admixture, allele counts) to summarise the data. However, they provide no details about data or methods used to build Figure 2, 3, and 5, and I strongly suggest including a material and methods section in the main text or as supplementary material.

***Our response**: Thank you for your insightful feedback. We acknowledge that while our manuscript primarily presents a review, it also includes essential population genetics analyses such as PCA, admixture, and allele counts to synthesize the data. To address your concern, we have incorporated a comprehensive Materials and Methods section detailing the data sources, analytical procedures, and methodologies employed to generate Figures 2, 3, and 5 and submitted it as supplementary material that provides further methodological specifics to ensure the clarity and reproducibility of our analyses. We believe these additions significantly enhance the transparency and robustness of our study. As the following contents:*

Materials and methods

Population samples and genomic datasets

Ancient genomic resources from Allen Ancient DNA Resource (AADR) (https://reich.hms.harvard.edu/allen-ancient-dna-resource-aadr-downloadable-genotypes-present-day-and-ancient-dna-data) were merged with recently reported ancient DNA from spatiotemporally diverse ancient and modern populations in East Asia [1-14]. Patterns of ancient DNA were also summarized based on these resources. The final merged dataset, used for population structure analysis, comprised 929 individuals and 593,050 SNPs. This study was approved by the Medical Ethics Committee of West China Hospital of Sichuan University (2023-

306), and all procedures were performed in accordance with the Helsinki Declaration of 2013 [15].

Model-based ADMIXTURE analysis

SNPs in strong linkage disequilibrium were pruned from the merged dataset using PLINK v1.90 with the parameters --indep-pairwise 200 25 0.4 [16]. Subsequently, unsupervised ADMIXTURE analyses were performed with ADMIXTURE v1.3.0 to elucidate overall genetic patterns and investigate the contribution of differentiated admixture components to the genetic structure of ancient Chinese populations, considering ancestral components (K) ranging from 2 to 20. To determine the optimal K value with the lowest cross-validation (CV) error, we conducted 100 bootstrap replicates (-B100), and 10-fold cross-validation (--cv=10) was applied [17]. A model with four ancestral sources was identified as the best-fitting admixture model. However, at K = 6, geographically distinct groups were clearly distinguished, revealing that ancient Chinese populations possessed multiple ancestral components.

Principal component analysis

To investigate the genetic relationships between spatiotemporal-resolution ancients and contemporary populations, we applied the SmartPCA algorithm implemented in EIGENSOFT [18] (numoutlieriter = 0, lsqproject = YES) to conduct PCA clustering and project ancient populations onto modern Chinese populations. To clarify the genetic substructure at the East Asian scale, we presented clustering patterns based on linguistically distinct modern Chinese populations and spatiotemporally differentiated ancient populations. To reveal the broader genetic patterns of Eastern Eurasians, we removed individuals with apparent Western Eurasian ancestry (e.g., TSM groups), and a second analysis was performed using the same methodology.

Evolutionary trajectory of the East Asian population-specific genetic variant

Raw sequenced BAM files of ancient genomes were obtained from published datasets [5, 6, 10, 11, 19-38] [24]. Post-mortem DNA damage patterns were assessed using mapDamage v2.2.1 [39] prior to extracting a subset of the 1240K dataset from East Asia. To minimize the impact of such damage on genotyping, we utilizedBamUtil v1.0.15 [40] to trim five base pairs from each end of the reads. Genotype imputation was conducted with GLIMPSE2 using the GSRD reference panel to enhance the resolution of the ancient genomes. Only biallelic sites were retained, and Picard LiftoverVCF v1.18.11 (https://gatk.broadinstitute.org/hc/en-us/articles/360037060932-LiftoverVcf-Picard-) was employed to convert genomic coordinates from build 38 to hg19.

The imputation procedure adhered to previously published protocols [41]. Ancient genomes were imputed using GLIMPSE v2.0. Chromosomes were first divided into chunks of 1–2 Mb with a 400 kb buffer region flanking each chunk via GLIMPSE_chunk. Subsequently, imputation was performed on these chunks using GLIMPSE_phase with parameters --burn 5, --main 15, and --pbwt-depth 12, utilizing the GSRD reference panel. Only high-quality genomic alignments were retained based on the parameters --mapg 30 and --baseq 20. The imputed chunks were then merged using GLIMPSE_ligate, and the analysis was restricted to SNPs with a genome-wide average imputation probability (GP) of ≥0.98 and a minor allele frequency (MAF) of ≥0.05. The imputed dataset was analyzed using bcftools to calculate counts of derived and ancestral alleles, facilitating the exploration of population-specific evolutionary trajectories in East Asian populations.

The use of many acronyms makes reading the manuscript quite challenging, even if these acronyms are explicitly explained in the introduction. The structure of many sentences and the sometimes poor choice of words also make reading the manuscript very challenging. In many

places, I think I could understand what the authors meant, but I am concerned that I had to interpret the text in light of my own knowledge of the literature and many readers may not be able to do so. I acknowledge that none of the authors may be native English speakers, and I know it is unfair for non-English speakers to have to publish in English, but I am afraid the services of a professional translator will be needed to improve readability.

***Our response**: Thank you for your insightful feedback on the readability of our manuscript. We recognize that the extensive use of acronyms and certain word choices may pose challenges for readers, particularly those less familiar with our research context. We have taken the following steps to address your concerns. Firstly, we reduced acronyms. We minimized the use of acronyms throughout the manuscript. When acronyms are necessary, we ensure they are clearly defined and used sparingly to maintain clarity. We also presented the abbreviations used as the glossary at the end of the manuscript. (aDNA Ancient DNA, ANE Ancient Northern Eurasians, ANA Ancient Northeast Asian, ANS Ancient Northern Siberians, AADR Allen Ancient DNA Resource, YRB Yellow River Basin, YZRB Yangtze River Basin, TP Tibetan Plateau, TYC Tibetan–Yi Corridor, TSM Tianshan Mountains, MP Mongolian Plateau, ARB Amur River Basin, WLRB West Liao River Basin, ANEA Ancient Northern East Asian, ASEA Ancient Southern East Asian, BKC Baishiya Karst Cave, NETP Northeastern regions of the Tibetan Plateau, LGM Last Glacial Maximum, MZR Mengzi Ren, PCA Principal component, HXC Hexi Corridor, BP Before present, BMAC Bactria-Margiana Archaeological Complex, IAMC Inner Asian Mountain Corridor).*

Secondly, we improved sentence structure and word choice by using native English speakers and a professional translator style. We revisied the manuscript to employ a more direct, active voice. This involved reworking sentences to ensure concise phrasing and selecting more precise terminology to convey our ideas effectively. Thirdly, we focused on professional language editing, and in light of your comments about readability, we sought the assistance of a professional language editor to review our revisions. This helped ensure that the manuscript meets high standards of clarity and linguistic quality, making it accessible to a broad readership. We greatly appreciate your feedback and will incorporate these revisions to enhance the manuscript's overall readability and impact.

Some of the conclusions seem superfluous or just a repeat of what has been shown or suggested, without showing relevance to the demographic history of East Asia. For example, how would haplotype-based methods improve research? What would be the benefits of studying large pedigrees in historical burial sites in the context of Chinese dynasties? It also seems that the authors suggest several times that current databases and methods are not adequate to answer questions related to the ancestry of people currently living in China. It is a valuable point, and something that would need to be consolidated in the manuscript and discussed more comprehensively.

***Our response**: Thanks for their thoughtful comments and for highlighting areas where our manuscript can be strengthened. In response to the concern that some conclusions appear superfluous or repetitive, we have streamlined our concluding sections to focus on the direct implications of our findings for understanding the demographic history of East Asia. We separated the sections of [conclusion] and [Challenges and future directions]. In the sections of [**Challenges and future directions**], we now emphasize how haplotype-based methods can refine population genetic analyses by uncovering deeper ancestral connections and identifying subtle gene flow events that traditional marker-based approaches might overlook. In addition, we have clarified how large pedigrees from historical burial sites could yield novel insights into lineage continuity and population structure across different Chinese dynasties, ultimately enhancing our understanding of long-term demographic patterns. We also acknowledge the reviewer's point regarding the limitations of current databases and methods when investigating the ancestry of individuals living in East Asia today. To address this concern, we*

have consolidated our discussion of these methodological constraints and proposed potential strategies for improvement, including the integration of more extensive genomic datasets and the development of specialized analytical tools.

*In the section of [**conclusion**], we comprehensively summarized the significant findings of this work. This work systematically summarizes the population history of deeply diverged East Asian hunter-gatherer ancestry, including AR19K, Longlin, Qihe, and MZR, which represent ANA, Guangxi, Fujian, and Yunnan ancestries, respectively. It also provides a comprehensive overview of Holocene eastern Eurasian population dynamics linked to millet and rice farmers and herders, indicating that complex movements and admixture have shaped the genomic diversity of modern and ancient East Asians. The Central Plain, particularly the YRB, is regarded as the origin of the Sino-Tibetan language family and an early agricultural center, whose hunter-gatherers or cultivators, in concert with rice farmers from the YZRB, contributed the primary ancestries of populations across other East Asian regions. Challenges associated with the preservation of aDNA in hot, humid environments, especially among upper and middle YZRB rice farmers in South China, have constrained the recovery of critical spatiotemporal sequences. The relative scarcity of genomic resources from geographically distinct Holocene East Asian populations underscores the need for a high-quality, high-coverage, and spatiotemporally resolved ancient genomic database. Such a database would advance understanding of the evolutionary origins of human disease and biological adaptations in Paleolithic hunter-gatherers, Neolithic farmers, Bronze Age pastoralists, and contemporary East Asian populations. Although recent advances in aDNA research in East Asia have refined our view of human evolutionary history in the eastern Eurasian continent, the spatiotemporal gap in aDNA data continues to impede a complete understanding of the genetic profiles of rice- and millet farmers and their ancestors and decedents. Establishing a robust genomic record from a spatiotemporally high coverage remains vital for illuminating the origins and evolutionary trajectories of East Asian populations.*

By incorporating these revisions, we believe our manuscript now presents a more straightforward, more focused argument for how emerging techniques and improved data resources can advance the study of East Asian demographic history in the section of [Challenges and future directions] and made a concise conclusion at the end of the manuscript. We appreciate the reviewer's guidance and trust these changes sufficiently address their comments.

I am not sure the authors make a convincing argument when they summarise EPAS1, EDAR, SLC42A5 and OCA2 allele frequencies to demonstrate that aDNA evidence is important to identify or characterise determinants of health and disease in the present-day Chinese population (of note, Figure 5 is rather confusing given that the time periods are different in panels b-e). I understand that the authors argue for a more comprehensive spatiotemporal coverage of genetic diversity in East Asia, but they may need to demonstrate better why and how aDNA could actually lead to evolutionary medicine insights given the many ancestral gene flows described in previous sections.

***Our response**: Thanks. We appreciate the reviewer's concern regarding the clarity and persuasiveness of our discussion on EPAS1, EDAR, SLC42A5, and OCA2 allele frequencies and the layout of Figure 5. Our intent was to illustrate how ancient DNA studies complement modern genomics by revealing temporal changes in allele frequencies of key adaptative evolutionary determinants that may help pinpoint the origins and adaptive significance of variants relevant to contemporary health and disease.*

In the revised period, we have revised Figure 5 to ensure the time frames in panels b–e are clearly labeled and consistent across subfigures. Besides, we used the more high-confidence SNP variants to present the general patterns in Figure 5 and deep insights into one EPAS-related mutation in Figure 6. This revision highlights each temporal window so that

readers can distinguish changes in allele frequencies over different periods. By clarifying these temporal intervals, we underscore how aDNA offers insights into when specific variants likely arose or rose to prominence, thus laying the groundwork for understanding their physiological and clinical relevance in modern populations.

*Additionally, we expanded our discussion section in [**Ancient Biological Adaptation among East Asian Populations**] and sub-section of [**The influence of aDNA on tracing the genetic origin of human disease**] to explain more explicitly how aDNA aids evolutionary medicine by tracing the interplay between gene flow, local adaptation, and disease susceptibility. The detailed information as the following contents: The influence of aDNA on tracing the genetic origin of human disease*

The evolutionary determinants of human diseases and complex traits have been shaped by diverse forces, including extreme environments on the TP, pathogen exposure (e.g., malaria in South China), and shifts in prehistoric subsistence strategies. These determinants have been inferred through advanced statistical and computational techniques based on patterns of genomic variations traditionally [102]. Ancient genetic analysis with comprehensive spatiotemporal coverage has provided direct insight into previously undisclosed population dynamics underlying human disease susceptibility, elucidating the evolutionary foundations of adaptive signatures and suggesting why diseases emerge in modern environments [103-106]. aDNA has yielded valuable perspectives in evolutionary medicine by revealing how beneficial variants in past environments have influenced modern human health and disease susceptibility.

Ancestral gene flows have been examined to determine how trait evolution relevant to health was shaped, as noted in previous genetic research focused on the possible genetic origin of multiple sclerosis (MS) in modern and ancient Europeans [103]. Another promising aspect of aDNA research for human disease involves spatiotemporal windows to test evolutionary hypotheses, including the thrifty gene hypothesis, hygiene hypothesis, antagonistic pleiotropy hypothesis, and others [104, 107]. By capitalizing on the time-series nature of aDNA, the genetic trajectories of past adaptations have been traced to identify changes critical for survival under distinct environmental pressures, such as disease resistance, dietary tolerance, or adaptation to climate stressors. Akbari et al. reported numerous genome-wide significant signals and strong directional selection signatures related to celiac disease, blood type B, tuberculosis risk, rheumatoid arthritis, and 31 other aDNA-attested selection-related disease traits [104]. This large-scale aDNA research has also illuminated complex directional selection that reshaped dozens of complex human traits. Such investigations are beneficial for understanding complex conditions (e.g., diabetes, cardiovascular disease, and cancer), which may carry an evolutionary component driven by ancestral gene flows.

The genetic legacy of ancient populations has also been characterized more accurately through aDNA, indicating how ancestral gene flows have shaped the contemporary human gene pool [108]. Marnetto et al. used combined genomic admixture modeling of ancient and modern Europeans and elucidated differentiated ancient European ancestral sources that contributed to varying effective sizes of anthropometric, pigmentation, and metabolic traits, such as the distinct influences of local hunter-gatherer and Yamnaya steppe ancestry on cholesterol levels [108]. In addition, genes transmitted during archaic human migrations, including those involving early Homo sapiens or Neanderthals, have clarified the genetic underpinnings of disease susceptibility and resistance [6-8]. The introgression of Neanderthal DNA, for instance, has been associated with immune system responses necessary for coping with infections.

Genetic changes associated with the agricultural revolution spanning thousands of years during the Holocene have been documented through aDNA, particularly those linked to immune-related host-pathogen co-evolution-related chronic diseases, aging, or metabolic disorders [103, 105, 107]. These findings indicate how genetic variants tied to disease risk have evolved, as variants once protective against ancient pathogens continue to shape modern

disease susceptibility. By examining these variants, evolutionary medicine can identify pathways that influence present-day health and disease. Kerner et al. recently reported that the Holocene host-pathogen interaction landscape illuminated genetic adaptation to increased infectious disease, which also affected the susceptibility of inflammatory disorders [105].

*A more comprehensive picture of human evolution and its bearing on health was also promoted via integrating aDNA with modern genomic data. If ancient populations are found to carry immune-related genetic variants conferring resistance to human disease, comparisons with modern groups possessing similar variants can clarify how those traits affect current disease outcomes [105]. This approach can inform precision medicine and genetic counseling. Barrie et al. combined the risk of MS among modern northern and southern Europeans with ancient human migration and admixture date [103]. A strong association was observed between elevated genetic risk of MS and the ancestry of steppe pastoralist populations [103]. However, recent large-scale analyses of modern and ancient European genomes suggested that elevated MS risk in northern Europe was not attributable to selection by steppe pastoralists, despite the positive selection of HLA-DRB1*15:01 increasing to 18% between ~6000 and ~2000 years ago [104].*

*The section on [**Evolutionary trajectory reconstruction among East Asians through the lens of aDNA**] was revised to illuminate more ancient DNA from East Asia. It was the chance to illustrate the trajectory of key adaptative mutations in East Asians. Because East Asia has experienced multiple complex migrations and admixture events, aDNA enables us to disentangle these processes and identify selective pressures that shaped genetic diversity. Through more comprehensive spatiotemporal sampling, we aim to reveal how past events contributed to present-day health disparities, facilitating a more targeted investigation of gene-environment interactions in the region.*

2nd round

Editor

Please could you make your imputed dataset available? We ask that this is included in a DOI-issuing repository such as FigShare or Zenodo. The DOI should be included in the reference list and then this reference should be cited. Please include more detail of how the data for the figures were generated from this. If this involved custom code please also include this in the repository. Our data Reference format is: Last, First. [all authors] Title. Datasets. Repository. hyperlink/doi (year).

Dear editors and reviewers,

Thanks for giving us one chance to submit our revised manuscript and for your constructive feedback and suggestions. All recommendations have been carefully considered, and the manuscript has been revised accordingly. Please find our response below (in blue Italics).

Editor

Please could you make your imputed dataset available? We ask that this is included in a DOI-issuing repository such as FigShare or Zenodo. The DOI should be included in the reference list and then this reference should be cited. Please include more detail of how the data for the figures were generated from this. If this involved custom code please also include this in the repository. Our data Reference format is: Last, First. [all authors] Title. Datasets. Repository. hyperlink/doi (year).

***Answerer**: Thanks for these suggestions. We have now made the full imputed dataset publicly available via Zenodo, a DOI-issuing repository, in accordance with your data sharing policy. The deposited materials include: The high-quality imputed diploid VCFs used across all analyses and corresponding metadata (archaeological context, coordinates, temporal bins).*

All R custom codes, including figure generation scripts, variant imputation pipelines were also made the full imputed dataset publicly available via Zenodo, a DOI-issuing repository, in accordance with your data sharing policy. We also added more detailed information for data analysis and visualization in the Additional file 1.

Availability of data and materials

All data and codes are available at Zenodo. The repository includes the imputed ancient genomic datasets generated in this study, detailed information and references for the ancient genomes analyzed, and scripts used for data processing and analysis. Imputed genomes can be found at Zenodo: [https://zenodo.org/records/17270846] [149]. Code and detailed information for analysis can be found at Zenodo: [https://zenodo.org/records/17270850] [150]. The code for visualization, including PCA and ADMIXTURE, is also available at Zenodo: [https://doi.org/10.5281/zenodo.17271668] [151]. All the other data generated in this study are included in the article and the Additional files.

149. He G, Sun Y, Duan S, Luo L, Sun Q, Li B, Yun L, Liu C, Wang M: Data for research article "Ancient genomes give insight into 160,000 years of East Asian population dynamics and biological adaptation". Zenodo 2025. https://zenodo.org/records/17270846

150. He G, Sun Y, Duan S, Luo L, Sun Q, Li B, Yun L, Liu C, Wang M: Code for research article "Ancient genomes give insight into 160,000 years of East Asian population dynamics and biological adaptation". Zenodo 2025. https://zenodo.org/records/17270850

151. He G, Sun Y, Duan S, Luo L, Sun Q, Li B, Yun L, Liu C, Wang M: Code for review "Ancient genomes give insight into 160,000 years of East Asian population dynamics and biological adaptation". Zenodo 2025. https://doi.org/10.5281/zenodo.17271668