

Genetic Legacy of Ancient Hunter-Gatherer Jomon in Japanese Populations

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This file contains all reviewer reports in order by version, followed by all author rebuttals in order by version.

Version 0:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

This paper by Yamamoto et al investigates the genetic legacy left by an ancestral component, the hunter gatherer Jomon, in a large Biobank cohort of modern Japanese and investigates its impact on phenotypic variation among Japanese people. The paper reports two main findings: First, they demonstrate that a previously proposed ancestry model involving three ancestral groups (Jomon, East Asia, and Northeast Asia) fits 90% of modern Japanese people. Second, they report an association of genome-wide Jomon ancestry with BMI or obesity. The paper is well written and I particularly appreciate the care taken in using multiple replication cohorts. My view is that this paper adds substantial understanding of the ancestry composition in a leading and widely used Biobank cohort, and presents original and new findings on ancestry associations with phenotypes in Japan, illustrating the potential impact of genetic ancestry on present-day phenotypic variation.

I have a few major comments:

- For the tripartite ancestry analysis, please clearly state how the source groups are defined (e.g. I couldn't find a sample list, although I might have missed this). My understanding is that the East Asian component is modelled using modern-day Han. Is there an ancient group you can substitute here? Also, how do you assess whether the model is 'fitting'? E.g. what is your p-value cutoff and it would be good to show p-values in Fig 1 d and e, as well as for the individual models.
- Impact of Jomon ancestry on phenotypic variation: It is a bit confusing that you correct for PCs and still observe an association between genome-wide Jomon proportions and phenotypes, because PCs are effectively measuring ancestry. You are mentioning that you are a bit worried about confounding. I am wondering if you tested if these associations still hold if you do the association separately for males and females (or ages), which could illustrate some robustness to environmental variables. Another approach to be robust is to run a regular GWAS for e.g. BMI with Jomon proportion as a covariate to get SNP betas that are uncorrelated with Jomon ancestry potentially tagging environmental factors (true betas should be a property of the variant and so uncorrelated with Jomon ancestry etc). You could then correlate genome-wide Jomon proportion with a PRS constructed with these betas.
- GWAS with Jomon ancestry as phenotype: Again I find it confusing what type of variants are picked up, and how we can interpret this. Assuming there is no unaccounted for stratification, then what type of variants do you expect to pick up? I am thinking it could either be variants tagging long Jomon haplotypes that have a marginal impact on genome-wide estimates, or variants that were at high frequency in Jomon, and low frequency elsewhere, indicating selection within Jomon. It would be good if this can be explored and interpreted in an evolutionary context, as I am struggling otherwise to understand why you are trying to identify these variants. Furthermore, to disentangle the two scenarios above (long haplotypes vs high frequency in Jomon), I think you can run the GWAS on genome-wide Jomon proportions inferred for all chromosomes, except the chromosome on which the test variant is found.
- The variance explained of genome-wide Jomon proportion is low for the 121 identified Jomon variants. Does this not mean they are not particularly useful to predict genome-wide Jomon proportions as stated in the paper? I also don't really understand when or why you would want to do this, if you could just run your ancestry models instead.

Reviewer #2

(Remarks to the Author)

The manuscript is on the study of the phenotypic impact of the Jomon admixture into the modern Japanese population using Biobank data along with phenotype data.

Below are my comments or questions:

86: since this tripartite model has been intensely used in the study, it would be helpful for readers to give more information and define what are Northeast Asian or East Asian ancestries and when are the Yayoi and Kofun periods.

168: Jomon may have closer relationships with Southeast Asian and Oceanian populations rather than the current East Asian populations. To answer this question, 1KG data have too little diversity to test. I don't think this question has been resolved, even a small proportion of the Jomon ancestry is detected in EAS, which is a vague and broad category of populations.

202: Yayoi looks much closer to Jomon rather than the present-day populations in Supp Fig 3a.

217: Only PC1 has a strong correlation with the Jomon proportion, not PC2.

221: I am not sure about this statement. The strong correlation with PC1 indicates the divergence between the Jomon ancestry and other ancestries. In terms of the amount/proportion of the Jomon ancestry in Japanese populations, the impact is obvious only in Okinawa and Hokkaido.

309: need a citation.

323: The citation should be Supp tables 7 and 8 in the Excel file (it seems the table numbering is all shifted). And this legend is not enough to understand the table.

339: Show the correlation stats. PC2 can be more correlated with EA ancestry.

382: What is the frequency of rs7572509 in UKB EAS and EG6?

435: do you mean the mainland population already possessed the tripartite ancestor? The sentence is not clear.

454: If you already have identified the genetic variant associated with BMI or obesity, such as rs7572509, you can directly predict the risk without the Jomon ancestry proportion. As you mentioned the Jomon ancestry-related variants can include several traits.

529: The method description for Admixture modeling and the fitness of ancestry model of tripartite and dual-structure is not clear.

Supp Fig 3a: Kohun or Kofun?

Other questions and suggestions:

The obesity-related Jomon variant was potentially positively selected before and have maintained in the Japanese population?

Can you estimate the age of admixture to shape the tripartite population structure or it is already known? If you know the age of Jomon admixture, it would be easier to understand why the Japanese have maintained the BMI-related variant during the transition of lifestyle.

At last, I believe the authors described well but still need to carefully describe the results of this study to distinguish from the previous studies(14 and 15), which support the same results in this study.

Reviewer #3

(Remarks to the Author)

In this paper by Yamamoto et al., the authors study the genetic legacy of the Jomon group of hunter-gatherers in modern Japanese populations: this includes the heterogeneous distribution of Jomon ancestry geographically within Japan, the strong link between Jomon ancestry and genetic variation, and the association of Jomon ancestry with high BMI/obesity. They also identify genetic variants which are indicative of high Jomon ancestry. These findings are validated in several independent cohorts, including a population living in the UK, from the UK Biobank.

Overall, this is a very well performed study, and I found the writing generally clear and concise. I believe the main conclusions of the study are backed up by strong statistical evidence. This represents an important advance into the study of the genetic legacy of ancient populations outside of a European context, where research in this area has until recently largely been focussed. The results are novel, and may help explain the higher levels of obesity in Okinawa compared to mainland Japan.

My comments below largely address small questions or errors in the text. My largest concern is the not small number of cases where the tripartite model does not fit, which questions the claim that the tripartite model presents the best fit across all regions. Ideally, I would like an explanation for why this is the case, given that after several thousands of years you would expect all individuals in a population to have ancestry from each admixing group. It might be helpful to break down the ~16,000 non-tripartite individuals by region, for example?

I was also surprised by the very high variability in the levels of Jomon ancestry across individuals. I wondered to what extent this might be an artefact of the small numbers of ancient samples, creating stochasticity in the inferred ancestry proportions. To this end, it would be useful to know the variation in the Han and NE Asian components, as these have smaller sample sizes still.

Finally, I would like to hear more about the utility of a GWAS for Jomon ancestry. It is not immediately obvious why it might be helpful to predict the Jomon ancestry proportion when you can simply re-run qpADMIX or another tool.

Minor comments:

73/74: "the insular region of East Eurasia" -> "an insular region of East Eurasia"

100/101: The transition from hunting-gathering to farming resulted in a reduction in genetic diversity⁴. Is there evidence for this outside of Europe/Near East? The reason for this was a population bottleneck, not an inherent result of the transition, as you seem to be implying. Modify the language accordingly.

101-104: "The lifestyle changes could have outpaced DNA evolution over the course of approximately 10,000 years, leaving the legacy of ancient hunter-gatherer ancestry to continue playing a crucial role in shaping phenotypic variation today".

Unclear what you mean by 'outpaced' in this context. Either expand on this idea (i.e. genetic adaptations no longer optimal for modern environment/lifestyles), or remove. Either way, there will still be some phenotypic impact of this diverse ancestry if it is present in modern populations.

107-108: "persist present-day populations" -> "persist in present-day populations"

137: State here how clustering of PCA results was done. How did you name them? Why did you not perform a more data-driven clustering, e.g. density-based clustering based on first X PCs? Visual selection based on PCA is fine however, I do not require you to change this.

204-213: You show that the tripartite model is the best fit model for 154,393 out of 171,287 individuals in BBJ. Is it possible that some of the remaining individuals are or are descended from recent immigrants? This result, that three-way admixture is not the best model, needs an explanation if you are going to justify the main claim of the paper that the tripartite model is the best fit. After 3kya you would expect all Japanese to inherit ancestry from each group, if the model is to be believed.

217: calculate and provide p values for correlation?

241: Does excluding individuals where the tripartite model does not fit in any way bias these results? I think you need to be able to justify excluding these samples.

242: Is this 95% CI? Please state.

Association between Jomon and 80 traits: lots of power is lost by controlling for PCs, which they have shown are strongly correlated with Jomon ancestry. You could consider controlling for PCs which are not significantly associated with Jomon ancestry? I suspect height would remain statistically significantly associated.

265 and throughout: is it justified to equate BMI with obesity? Obesity is merely a description of a subset of the BMI range.

308-313: need references for the association of rs7572509 with the traits listed. Assume the association data is coming from BBJ? Promising that it replicates in UKB. Also need to reference where the eQTL data is coming from (or reference Methods).

339: How have you derived the five clusters again? Is this projected onto the PCA from BBJ 1st cohort? Or have you just drawn the lines onto the second PCA?

382: You should also report effect of Jomon ancestry on obesity in the 669 individuals found to have Jomon ancestry.

436: State whether you consider this signal to be driven by Ainu likely. Does this impact your claims about Jomon ancestry?

Methods:

489: how did you exclude individuals based on PCA? More detail needed.

508: "previously piled up and integrated" -> typo?

535: unclear what other ancestry models were used

543: how did you selected the 80 trait analysed?

544-545: why did you use a cut-off of 20yo for smoking and drinking?

572: I can't see any trait specific covariates listed in ST4

675-676: again, need to state what other models were tested

679: here, you take an average of measurements whereas for BBJ you take the measurement at first visit to the recruitment centre. Why do you take different approaches?

Tables/Supplementary Information:

S.Table 1 and S.Table 2 are in separate file from other supplementary tables. This is confusing!

Supplementary Fig. 3.: "threes" -> "three"

S.Table 5: no P for BMI; no SE values

Version 1:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

I appreciate the care taken in revising the manuscript and thank the authors for their detailed responses. I think this paper is a significant contribution for understanding the genetic ancestry of the Biobank Japan cohort and links these insights in a very interesting and innovative way to phenotypic variation.

- The description of ancestry modelling using a previously proposed tripartite ancestry model is now much clearer.
- The authors have incorporated new analysis to address my concerns about residual confounding driving their signal of Jomon ancestry being associated with BMI. Following careful analysis, it appears as though the association has reduced by quite a bit and is now only marginally significant, which is a bit concerning. As now discussed by the authors in the main text, Jomon ancestry is acting as a weak confounder on the GWAS and inflates the PGS. How can we be sure that all confounding has now been removed? E.g. it could be that your inferred Jomon proportions are a bit noisy and so there is still some leftover confounding.
- You mention that the top hits for BMI are unchanged even when correcting for Jomon ancestry. This is certainly reassuring and suggests that if you compute the PGS with more stringent p-value cutoff than this should already reduce association in your original analysis as well. Could you check this?
- I. 312: Please report the updated beta and p-value of association after correcting for Jomon ancestry.
- It is interesting that 13% of the variance in Jomon ancestry is explained by the Jomon variants that were identified. I agree with the authors that this is a notable proportion and appreciate the added analyses to emphasise this.
- It does pose the question of why this is the case. As in my original review, I think the current manuscript is a bit weak in providing evolutionary interpretations on this phenomenon. Presumably this happens because there is strong drift in Jomon, and/or lots of unique mutations in Jomon that tag Jomon haplotypes. This must be unusual though, typically if ancestry is defined by small differences in allele frequency genome-wide than this type of approach should not work so well. Some simple summary statistics, such as the global frequencies of these Jomon variants, would be helpful in interpreting this phenomenon.

Reviewer #2

(Remarks to the Author)

Thank you for your sincere responses and additional analyses in addressing my comments and questions. The manuscript now presents a clearer message regarding the contribution of Jomon ancestry to a particular phenotype related to BMI. It is particularly noteworthy to detect extended haplotypes for Jomon-related variants, supporting the potential for positive selection.

1. Among the 132 independent Jomon-related variants, how many exhibit significantly longer haplotypes compared to non-Jomon-related variants? Additionally, among these longer haplotypes, are any specifically associated with BMI? This examination is crucial for understanding the potential positive selection on this phenotype introduced by Jomon admixture into the contemporary Japanese population.
2. Without clear evidence, it is challenging to comprehend why only BMI is significantly associated with Jomon ancestry among other traits (as shown in Fig 4), considering that Jomon admixture would occur randomly across the genome. This point also pertains to the conclusion that increasing BMI in the Japanese population is introduced by Jomon ancestry.
3. In modern populations, a high BMI is generally considered a risk factor. While this knowledge can positively impact the interpretation of GWAS results, it may also lead to negative perceptions of Jomon admixture among the public. Therefore, it becomes important to reveal the evolutionary story of this trait's adaptation, not only for the Jomon but also for the Yayoi populations. Alternatively, the authors could describe the potential positive influences of high BMI in certain contexts.

By addressing these points, the manuscript will emphasize novel finding and provide a more comprehensive understanding of the evolutionary implications and public health perspectives of BMI-related Jomon ancestry in the contemporary Japanese population.

Reviewer #3

(Remarks to the Author)

I thank the authors for their comprehensive response to my comments. They have fully addressed all my points, and updated the manuscript and supplementary information accordingly. In my view this paper now reaches the standards expected by the journal, and provides an important contribution to the field.

Version 2:

Reviewer comments:

Reviewer #1

(Remarks to the Author)

Thank you for your responses and overall I am happy with all the revisions made and think this is an important contribution.

I very much appreciate all the robustness checks done for the association between Jomon ancestry and BMI.

It is encouraging that the signal is robust to excluding people from Okinawa, and age/sex effects. Some association between PGS and Jomon ancestry remains after correcting for Jomon ancestry in the GWAS and so overall I would say I believe your result. But I am still confused by your interpretation of the result when correcting for Jomon proportions and perhaps you can include a clarifying sentence around the paragraph starting in line 311:

In my understanding, ideally you want the *true effect sizes* for BMI at every SNP, and then compute PGS and correlate to Jomon ancestry. Now we of course don't have true effect sizes and so have to estimate these using GWAS. You get very slightly different effect sizes depending on whether you incorporate Jomon ancestry as a covariate, and as you mention in the main text and your response you say

"Jomon ancestry acts as a confounding factor in estimating effect sizes for BMI".

In fact you say "Jomon ancestry works as a confounder in BMI GWAS, supporting the validity of the association between Jomon ancestry and BMI". That doesn't make sense to me, you could make the same argument for any of the covariates but it would usually be false? Or at least it wouldn't be a genetic association. Are you saying there are real interactions between global Jomon ancestry and the true effect size? I'm not sure that seems plausible.. More plausibly, the pre-Jomon corrected effect sizes are somewhat biased due to some residual confounding.

Reviewer #2

(Remarks to the Author)

Thank you so much for the authors' sincere responses.

I appreciate the revision.

The authors made a great point in the rebuttal rather than the revised text in the discussion: "Our study provides evidence of how the past action of natural selection has shaped present-day disease risks, primarily due to rapid changes in diet and societal lifestyle."

I believe the statement can be in the main text.

I have no more comments on the manuscript.

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Response to REVIEWERS' COMMENTS

We thank all three reviewers for their careful reading of our manuscript and valuable comments. We have carefully considered their comments and addressed them in the revised manuscript. We provide our detailed response to each comment below.

Reviewer #1 (Remarks to the Author):

This paper by Yamamoto et al investigates the genetic legacy left by an ancestral component, the hunter gatherer Jomon, in a large Biobank cohort of modern Japanese and investigates its impact on phenotypic variation among Japanese people. The paper reports two main findings: First, they demonstrate that a previously proposed ancestry model involving three ancestral groups (Jomon, East Asia, and Northeast Asia) fits 90% of modern Japanese people. Second, they report an association of genome-wide Jomon ancestry with BMI or obesity. The paper is well written and I particularly appreciate the care taken in using multiple replication cohorts. My view is that this paper adds substantial understanding of the ancestry composition in a leading and widely used Biobank cohort, and presents original and new findings on ancestry associations with phenotypes in Japan, illustrating the potential impact of genetic ancestry on present-day phenotypic variation.

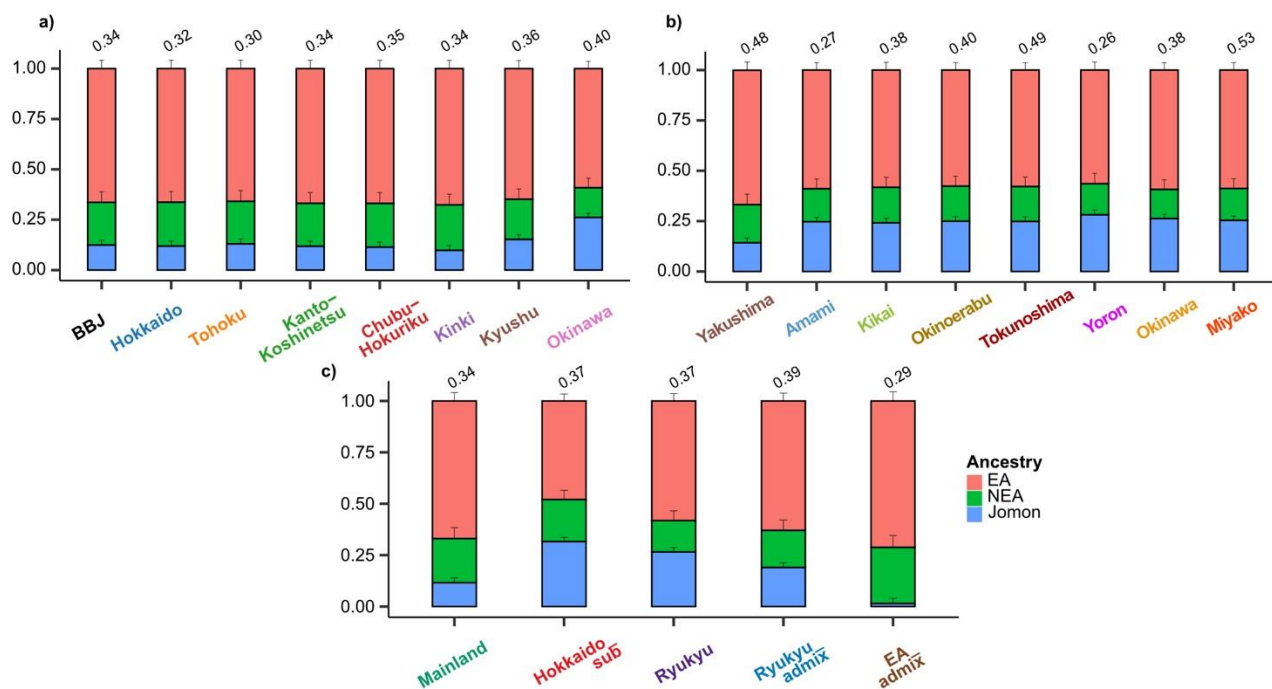
Response: We are grateful for your recognition of the value of our study. We have carefully considered the comments and addressed them step by step in the revised manuscript.

I have a few major comments:

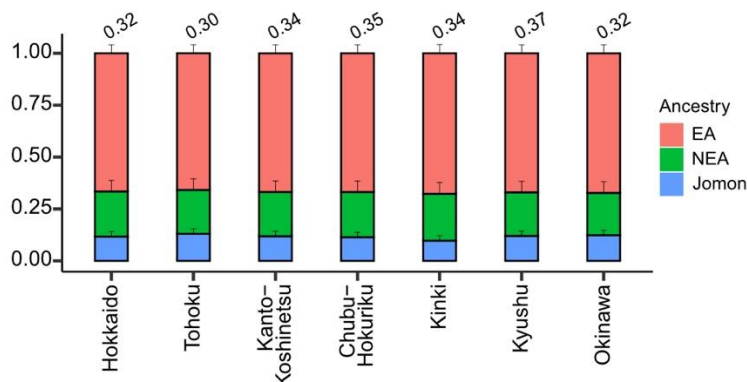
Comment: For the tripartite ancestry analysis, please clearly state how the source groups are defined (e.g. I couldn't find a sample list, although I might have missed this). My understanding is that the East Asian component is modelled using modern-day Han. Is there an ancient group you can substitute here? Also, how do you assess whether the model is 'fitting'? E.g. what is your p-value cutoff and it would be good to show p-values in Fig 1 d and e, as well as for the individual models.

Response: We included the details of source populations in admixture modeling in the "Admixture modeling and f_4 tests" section of **Methods**. Briefly, our source population dataset comprises 12 Jomon individuals, characterizing ancient hunter-gatherer ancestry. Two individuals from the West Liao River basin represent Northeast Asian ancestry, reflecting the migration to Japan during the agrarian Yayoi period. As the reviewer commented, present-day Han were used to represent widespread East Asian ancestry, which was introduced to Japan during the state-formation phase of the Kofun period. A previous study (Cooke *et al.* **Sci Adv.** 2021) comprehensively tested a diverse set of ancient groups, including those from the Yellow River basin and Southern China, as well as present-day populations to elucidate additional ancestry that is absent in Yayoi individuals but evident in Kofun individuals. This analysis demonstrated that present-day Han were the sole population supporting this admixture scenario. Therefore, we adhered to the findings of the previous study in defining the source populations.

With regard to assessing model fittings, we used two different metrics: one is a tail probability, and the other is a p -value for a nested model. Both cut-offs were set at 5%. A tail probability below 5% indicates that an admixture model fits the data considerably worse than a full rank model, *i.e.*, a model perfectly fitting the data. Meanwhile, the p -value for a nested model evaluates whether a small set of source populations can adequately explain a target population. In our analysis, we considered the tripartite model (*i.e.*, three ancestors; Jomon, Northeast Asia, and East Asia) to be in a good fit if the tail probability is greater than 5% and to fit the data significantly better than dual ancestral models (*i.e.*, any pairs of the three ancestors) if the nested p -value was below 5%.



(new) Fig. 2. Variation in tripartite ancestry structures across groups of Biobank Japan participants. Bar plots show the proportions of three distinct ancestors. **a)** Populations are defined either as a group of the entire BBJ samples or by the regions where participants were registered. **b)** Individuals recruited from the Ryukyu Islands are grouped by their specific islands. **c)** The BBJ samples are split into five different insular populations based on their PCA clusters shown in **Fig. 1**. The error bars represent standard errors, and the values at the top of each bar indicate the tail probabilities of the tripartite model for each group. NEA, Northeast Asian; EA, East Asian.



Supplementary Fig. 1. Variation in tripartite ancestry structures across groups of individuals in the Mainland cluster based on PCA.

Bar plots show the proportions of three distinct ancestors. Individuals within the Mainland cluster of BBJ ($n = 159,642$) are split into seven different regions based on their registered location (Hokkaido: $n = 7,829$, Tohoku: $n = 10,991$, Kanto-Koshinetsu: $n = 93,827$, Chubu-Hokuriku: $n = 9,403$, Kinki: $n = 24,365$, Kyushu: $n = 12,068$, Okinawa: $n = 284$). The error bars represent standard errors and the values at the top of each bar indicate the tail probabilities of the tripartite model for each group. EA, East Asian; NEA, Northeast Asian.

(new) Supplementary Table 2. List of source populations used in admixture modeling.

Source population	Sample ID	Data range (cal B.P.)	Median (cal B.P.)	Reference
Jomon	JpKa6904	8964-8991	8819	Cooke <i>et al.</i> (2021)

	JpOd274	6119-6289	6204	
	JpOd6	5934-6179	6057	
	JpOd181	5751-5917	5834	
	JpOd282	5737-5902	5820	
	JpFu1	5478-5590	5534	
	JpKo2	4294-4514	4404	
	JpKo13	3847-3978	3913	
	JpHo1	3685-3850	3768	
	F23	3550-3960	2569	
	F5	-	-	
	IK002	2418-2720	2569	
Northeast Asia	WLR_BA_o	3000-2300	2650	Ning <i>et al.</i> (2020)
	HMMH_MN	5500-5000	5250	
East Asia	Present-day Han	-	-	Mallick <i>et al.</i> (2016)

Modification: In this revised manuscript, we have compiled a list of individuals used to define each of three distinct ancestral sources, which is provided in **Supplemental Table 2**. For the model fitting, we have added tail probabilities of the tripartite models in different insular groups of Biobank Japan participants within the Ryukyu Islands to **Fig. 2** and **Supplementary Fig. 1**. We found a most elevated level of Jomon ancestry on Yoron Island. Furthermore, we have tested whether the tripartite structure significantly better explains the genetic makeup of present-day populations compared to all possible dual structure models, which are all summarized in **Supplementary Table 3**.

We have also modified the sentences related to these changes as follows:

- **Results** (Page 6, Line 158): We subsequently applied qpAdm in AdmixTools^{30,31}, based on a set of source populations defined in a previous study¹⁴ (see **Supplemental Table 2**), to evaluate the fit of admixture models and to estimate admixture proportions at both population and individual levels in the biobank.
- **Results** (Page 6, Line 165): This analysis demonstrates that the tripartite structure provides a better fit for all populations, both at broad and local scales, when compared to all possible dual-ancestral structure models (**Supplementary Table 3**). The only exception is EA_admix, where the population can be sufficiently explained by a two-way admixture between Northeast Asian and East Asian ancestry.
- **Results** (Page 6, Line 173): Within the Ryukyu Islands, there is an elevated level of Jomon

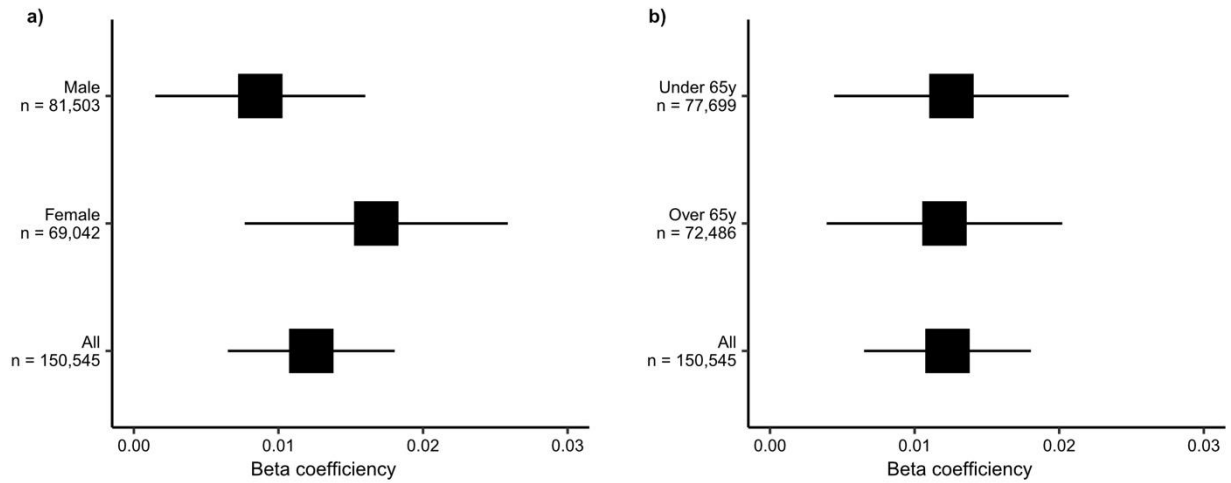
ancestry, with the highest proportion observed on Yoron Island (**Fig. 2b**).

- **Methods** (Page 26, Line 615): We evaluated whether the tripartite structure provides a better fit to the data than any other possible scenarios at population levels based on p -values for nested models with a cut-off of 0.05. These alternative models include dual structure models involving any combination of Jomon, Northeast Asian, and East Asian ancestry, as well as single ancestry models. When conducting admixture modeling at individual levels, we considered the tripartite model to be a good fit if (i) a tail probability was >0.05 and/or (ii) estimates of admixture fractions were feasible (*i.e.*, >0.0 and <1.0).

Comment: Impact of Jomon ancestry on phenotypic variation: It is a bit confusing that you correct for PCs and still observe an association between genome-wide Jomon proportions and phenotypes, because PCs are effectively measuring ancestry. You are mentioning that you are a bit worried about confounding. I am wondering if you tested if these associations still hold if you do the association separately for males and females (or ages), which could illustrate some robustness to environmental variables. Another approach to be robust is to run a regular GWAS for e.g. BMI with Jomon proportion as a covariate to get SNP betas that are uncorrelated with Jomon ancestry potentially tagging environmental factors (true betas should be a property of the variant and so uncorrelated with Jomon ancestry etc). You could then correlate genome-wide Jomon proportion with a PRS constructed with these betas.

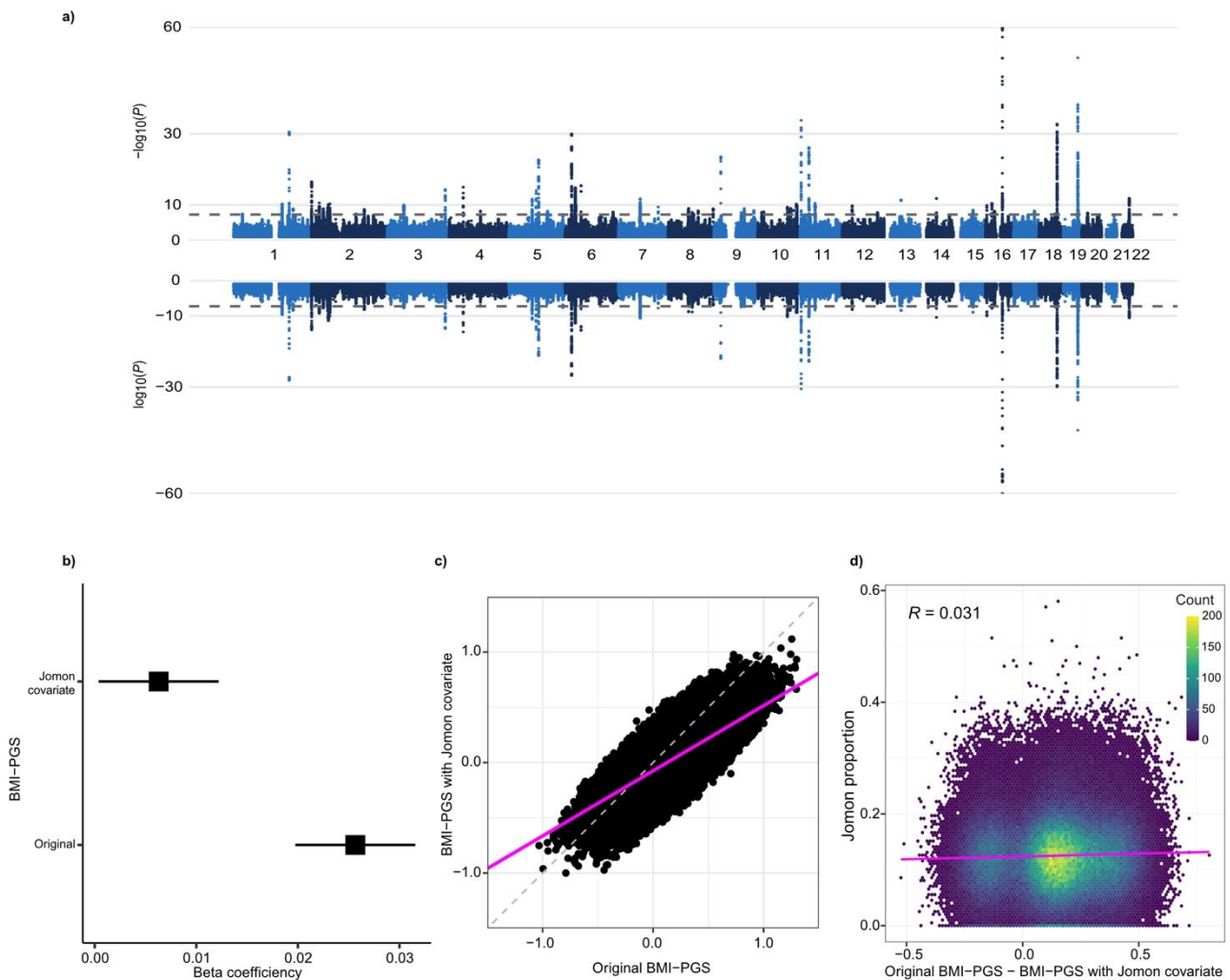
Response: We thank the reviewer for these suggestions. Population stratification is evident across various geographic regions within the Japanese archipelago, such as between Ryukyu and Mainland or even within the Ryukyu Islands, as demonstrated by principal components (PCs) in **Fig. 1**. Our primary concern thus revolved around the potential inflation of association signals between Jomon ancestry and phenotypes if we did not properly account for PCs as covariates. However, we found that the association with BMI remained robust after controlling for the population stratification by incorporating PCs as covariates; this robustness is still evident even when analyzing individuals solely from the Mainland cluster (see **Fig. 3**).

In this revision, we have further confirmed the significance of this association regardless of sex or age (see **Supplementary Fig. 7**). Furthermore, we conducted GWAS for BMI, incorporating Jomon ancestry as a covariate. While nearly all the lead SNPs remain consistent (**Supplementary Fig. 9a**), we observed a reduction in the effect of Jomon ancestry on the polygenic scores (PGS) of BMI (**Supplementary Fig. 9b**). It is worth noting that this effect persists to some extent, indicating a functional correlation between Jomon ancestry and BMI-PGS. Still, our finding suggests that Jomon ancestry acts as a confounding factor for estimates of effect size in BMI. Indeed, the PGS tends to be inflated when Jomon ancestry is not accounted for in GWAS (**Supplementary Fig. 9c**); the degree of inflation weakly correlates with the proportion of Jomon ancestry (**Supplementary Fig. 9d**).



(new) Supplementary Fig. 7. Associations between BMI and Jomon proportions, stratified by sex and age groups.

Forest plots represent the effect size of Jomon ancestry on BMI, stratified by **a)** sex (male or female) or **b)** age groups (older or younger than 65 years old, which is a mean age among the BBJ participants). Error bars indicate 95% confidence intervals.



(new) Supplementary Fig. 9. Assessing impacts of Jomon ancestry on GWAS for BMI (BMI-GWAS) and

polygenic scores of BMI (BMI-PGS).

a) A Miami-plot of the original BMI-GWAS (top) and the BMI-GWAS including Jomon proportions as a covariate (bottom). **b)** Forest plots contrast the effect sizes of Jomon ancestry on the original BMI-PGS (labeled as "Original") with the effect on BMI-PGS after correction for Jomon ancestry ("Jomon covariate"). Error bars indicate 95% confidence intervals. **c)** Scatter plots compare the values of the original BMI-PGS (i.e., GWAS results without accounting for Jomon ancestry) with those PGS derived from GWAS that incorporate Jomon proportions as a covariate. A dashed line coloured by gray represents $y = x$. **d)** Plots show the association of the difference in BMI-PGS between GWAS with and without using Jomon ancestry as a covariate with the Jomon proportions. A correlation coefficient ($R = 0.031$; $P = 8.5 \times 10^{-37}$) is measured by Pearson's method.

Modification: We have added these results to **Supplementary Figs. 7 and 9** and revised corresponding texts as follows:

- Results regarding **Supplementary Fig. 7**

Results (Page 12, Line 298): We further confirmed the significance of the association with BMI regardless of sex or age (see **Supplementary Fig. 7**).

Methods (Page 27, Line 666): For BMI, we further tested the association with Jomon ancestry, stratified by sex and age groups, with the age threshold set at 65 years old, which reflects a mean age among the BBJ participants.

- Results regarding **Supplementary Fig. 9**

Results (Page 12, Line 311): To assess the impact of Jomon ancestry on BMI, we incorporated the proportion of Jomon ancestry as a covariate in our genome-wide association study (GWAS) for BMI (**Supplementary Fig. 9a**). While the majority of lead SNPs remain consistent, we observed a reduction in the effect of Jomon ancestry on polygenic scores (PGS) of BMI (**Supplementary Fig. 9b**). It is worth noting that this effect persists to some extent, indicating a functional correlation between Jomon ancestry and BMI-PGS. Still, our finding suggests that Jomon ancestry acts as a confounding factor in estimating effect sizes for BMI. Indeed, the PGS tends to be inflated when Jomon ancestry is not accounted for in GWAS (**Supplementary Fig. 9c**), with the degree of inflation weakly correlating with the proportion of Jomon ancestry (**Supplementary Fig. 9d**).

Methods (Page 27, Line 677): We investigated the influence of the Jomon proportions on GWAS for BMI and the accuracy of BMI polygenic scores (PGS). We performed BMI GWAS on all BBJ participants using a generalized mixed linear model (MLM) approach of GCTA-fastGWA⁶². We employed a sparse genetic relationship matrix (GRM) constructed with variants subject to minimal LD pruning. Covariates in the original BMI GWAS included sex, age, age squared, the top 20 PCs, and 45 disease statuses. To evaluate the impact of Jomon ancestry, we introduced Jomon proportions as an additional covariate and compared the results with those obtained from the original GWAS.

To compute PGS, we adopted a five-fold leave-one-group-out GWAS method due to the absence of independent external reference for GWASs or genotype data with Japanese ancestry^{19,63}. In brief, we initially split the BBJ individuals randomly into five subsets. We then performed BMI GWAS on samples, excluding the subset under investigation (i.e., target subset), using GCTA-fastGWA. To estimate the posterior effects of SNPs from GWAS summary data, we utilized PRS-CS-auto (version Jun 4, 2021) with the HapMap3 LD reference panel of 1KG EAS^{64,65}. These posteriors of effect sizes were estimated both from the original GWAS and from the GWAS with the covariate of Jomon ancestry. Finally, we applied the PLINK2 score function to compute PGS for individuals within the target subset, comparing those scores with and without the Jomon ancestry covariate.

Comment: GWAS with Jomon ancestry as phenotype: Again I find it confusing what type of variants are picked up, and how we can interpret this. Assuming there is no unaccounted for stratification, then what type of variants do you expect to pick up? I am thinking it could either be variants tagging long Jomon haplotypes that have a marginal impact on genome-wide estimates, or variants that were at high frequency in Jomon, and low frequency elsewhere, indicating selection within Jomon. It would be good if this can be explored and interpreted in an evolutionary context, as I am struggling otherwise to understand why you are trying to identify these variants. Furthermore, to disentangle the two scenarios above (long haplotypes vs high frequency in Jomon), I think you can run the GWAS on genome-wide Jomon proportions inferred for all chromosomes, except the chromosome on which the test variant is found.

Response: Thank you for this insightful comment. Our GWAS approach aims to identify genetic variants that can explain variation in Jomon ancestry across different individuals. In this revision, in response to the suggestions from the other reviewers, we have expanded our analysis by including individuals who exhibit better fittings to alternative scenarios (e.g., dual ancestry models) than the tripartite model ($n = 163,243$; see **Supplementary Fid. 6**). Consequently, we have identified more Jomon-related variants (132 in total) than the number (121 variants) reported in the original manuscript. We examined haplotype structures of the genomic regions containing each of 132 Jomon-related variants ($p < 5 \times 10^{-8}$), contrasting them with those of allele frequency-matched non-Jomon-related variants ($p > 0.05$) by comparing the lengths of linkage disequilibrium blocks ($r^2 > 0.8$) (see **Fig. 5**). The Jomon-related variants exhibit significantly longer haplotypes than non-Jomon-related variants ($p = 3.1 \times 10^{-32}$). Given the inherent strong linkage disequilibrium throughout the Jomon genome, attributed to the high genetic homogeneity and small effective population size ($\sim 1,000$) within the Jomon population—as demonstrated by Cooke *et al.* **Sci Adv.** 2021 and Cooke *et al.* **iScience** 2024—this result strongly suggests a Jomon origin for these extended haplotypes. Furthermore, the reason why these Jomon haplotypes are still extremely long in the contemporary populations can be due to the relatively recent admixture with the continental ancestors, coupled with insufficient time for recombination to break apart the haplotypes. Leveraging selection scan results based on singleton density score (SDS), which detects selection over approximately the past 100 generations, from Okada *et al.* **Nat Comm** 2018, we further observe an enrichment of selection signals in the Jomon-derived haplotypes ($p = 0.008$). These results support the idea that the Jomon-related variants we identified can serve as markers for quantifying Jomon ancestry, tag Jomon-derived haplotypes, and potentially have been subject to recent selective pressures.

We also employed a leave-one-chromosome-arm-out approach on a randomly selected sample of 10,000 individuals, in which we excluded all sites on one chromosome arm, measured Jomon proportions using the remaining sites, and computed effect sizes of the variants located within the removed chromosome region. This approach confirms the consistency of effect size estimates ($R = 0.9$).

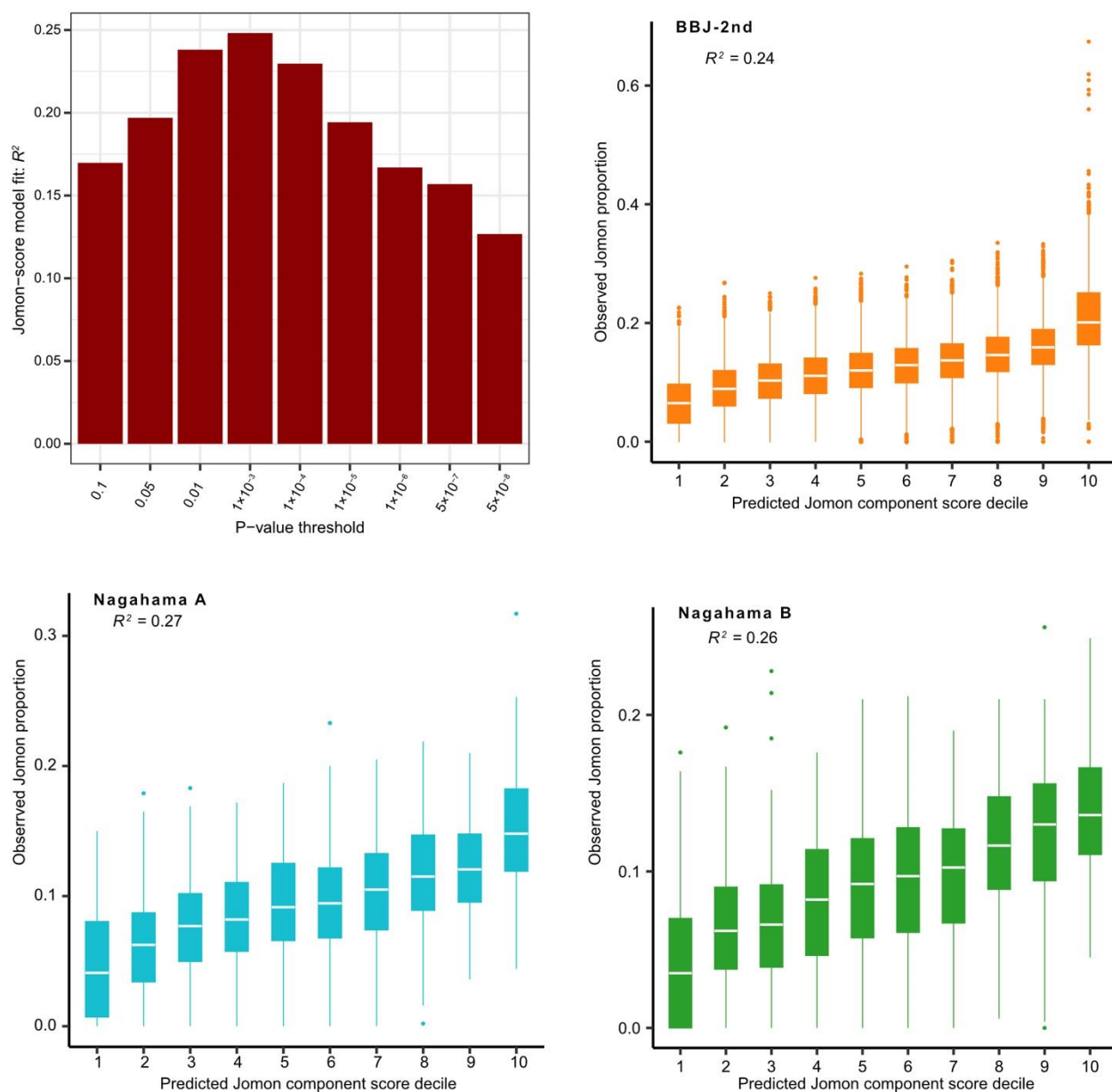
variants (i.e., variants with $P > 0.05$), we find that the Jomon-related variants exhibit significantly longer haplotypes than non-Jomon-related variants ($P = 3.1 \times 10^{-32}$) (**Figs. 5b and 5c**). This strongly suggests a Jomon origin for these extended haplotypes, supported by strong linkage disequilibrium throughout the genome possibly due to the high genetic homogeneity and small effective population size ($\sim 1,000$) within the Jomon population^{14,37}. Furthermore, the persistence of these long Jomon-derived haplotypes in the contemporary populations can be attributed to the relatively recent admixture with the continental ancestors¹⁴, coupled with insufficient time for recombination to break apart the haplotypes. Leveraging selection scan results based on singleton density score (SDS)¹⁷, which detects selection over approximately the past 100 generations³⁸, we further observe an enrichment of selection signals in the Jomon-derived haplotypes ($P = 0.008$). These results support the idea that the Jomon-related variants can serve as markers for quantifying Jomon ancestry, tag Jomon-derived haplotypes, and potentially have been subject to recent selective pressures.

- **Methods** (Page 28, Line 714): To identify independent variants, we performed LD clumping on those variants with positive Z scores using BBJ1K and 1KG EAS as reference populations, with the following PLINK parameters: $p1=1$, $p2=1$, $r2=0.01$, $kb=2000$. Jomon-related variants (total 132 variants) were defined as those that are independent, not located in the HLA loci, and satisfy a genome-wide significance ($P < 5.0 \times 10^{-8}$).
- **Methods** (Page 28, Line 719): Variants with p -values greater than 0.05 were classified as non-Jomon-related. We then identified 132 non-Jomon-related variants matching allele frequencies of the Jomon-related variants using a nearest neighbor matching method. making a comparison of haplotypic lengths with LD greater than 0.8 between the Jomon-related and non-Jomon-related variants. The enrichment of selection signals in the Jomon-related variants was tested based on Z-scores of singleton density scores (SDS) estimated in a previous study¹⁷. The sum of squared values of rank-based normalized Z-scores were compared to a chi-squared distribution with the degree of freedom equal to the number of available variants.

Comment: The variance explained of genome-wide Jomon proportion is low for the 121 identified Jomon variants. Does this not mean they are not particularly useful to predict genome-wide Jomon proportions as stated in the paper? I also don't really understand when or why you would want to do this, if you could just run your ancestry models instead.

Response: Thank you for highlighting this point. The set of markers selected with a p -value $< 5 \times 10^{-8}$ accounts for approximately 13% of the variance in Jomon ancestry. It's worth emphasizing that while this proportion may seem modest, it holds notable significance when compared to many other genome-wide association studies on complex traits, such as height or type II diabetes (Sakaue *et al. Nat Med* 2020), where similar proportions of variance explained by trait-associated variants are commonly reported. In this revision, we assessed how this prediction improves across different p -value cut-offs for SNP selection, ranging from the order of 5×10^{-8} to that of 10^{-1} using the BBJ-2nd cohort as an independent dataset from the discovery cohort. We observed a significant increase in predictive power, reaching 24.3% at a p -value cut-off of 10^{-3} , a finding further confirmed with our additional independent cohorts of Nagahama A (27%) and Nagahama B (26%) (**Supplementary Fig. 14**). However, it is crucial to note that this clumping and thresholding method has been demonstrated to potentially overfit the data, particularly when lowering the p -value cut-offs (Choi *et al. Nat Protoc.* 2020). As shown in the original manuscript, we successfully validated the efficacy of Jomon-related variants meeting a genome-wide significance of 5×10^{-8} in identifying individuals with Jomon ancestry from the UK BioBank. Therefore, our p -value cut-off remains 5×10^{-8} in the revised manuscript. For the second question, we posit that if one can readily determine the presence or absence of Jomon ancestry using only a small set of genetic variants like ancestral informative markers, as opposed to

whole-genome or genome-wide data, it will markedly reduce costs and burdens required to construct, handle, process, and analyze such complicated data.



(new) Supplementary Fig. 14. Predictive power of polygenic scores for Jomon ancestry.

Bar plots show the variances in Jomon ancestry explained by different sets of variants selected from GWAS on Jomon proportions using nine different p-value thresholds. A set of variants at a p-value cut-off of 10^{-3} shows the highest variance of 24.3%, which is validated with three independent cohorts: BBJ-2nd, Nagahama A, and Nagahama B.

Modification: We have newly created **Supplementary Fig. 14** to clarify the predictive power of Jomon-related variants and also modified the sentences as follows:

- **Results** (Page 19, Line 426): The predictive power of Jomon-related variants shows an increase when the p-value cut-off is relaxed from 5×10^{-8} to 1×10^{-3} , resulting in the variance of 0.27 (Nagahama A), 0.26 (Nagahama B), and 0.24 (BBJ-2nd) respectively for Jomon ancestry explained by genetic variants (**Supplementary Fig. 14**). However, it is crucial to note that this

clumping and thresholding method has been demonstrated to potentially overfit the data, particularly when relaxing the p -value cut-offs⁴³. Therefore, we adhere to a p -value cut-off of 5×10^{-8} for predicting Jomon ancestry in subsequent analysis.

- **Methods** (Page 29, Line 772): To evaluate the predictive power of Jomon component prediction score, we employed the clumping and thresholding approach. We built the Jomon component score for BBJ-2nd individuals using genetic variants meeting the following p -value thresholds: 5×10^{-8} , 5×10^{-7} , 1×10^{-6} , 1×10^{-5} , 1×10^{-4} , 1×10^{-3} , 0.01, 0.05, 0.1. To assess the proportion of variance explained by the Jomon component score, we calculated the adjusted R^2 from a full model that included the score and all covariates, compared to a null model without the score.

For the second question, we modified the sentences as follows:

- **Discussion** (Page 24, Line 536): The set of genetic variants associated with the proportion of Jomon ancestry could potentially serve as **ancestral informative markers (AIMs)**, offering a convenient way to predict an individual's Jomon ancestry.

Reviewer #2 (Remarks to the Author):

The manuscript is on the study of the phenotypic impact of the Jomon admixture into the modern Japanese population using Biobank data along with phenotype data.

Below are my comments or questions:

[We would like to thank the reviewer for many insightful comments. Please find our response to each comment below.](#)

Comment: 86: since this tripartite model has been intensely used in the study, it would be helpful for readers to give more information and define what are Northeast Asian or East Asian ancestries and when are the Yayoi and Kofun periods.

Response and Modification: Thank you for the comment. As responded to the first major comment from Reviewer 1, we have included a list of source populations defining the tripartite ancestral structure in **Supplementary Table 2**.

(new) Supplementary Table 2. List of source populations used in admixture modelling.

Source population	Sample ID	Data range (cal B.P.)	Median (cal B.P.)	Reference
Jomon	JpKa6904	8964-8991	8819	Cooke <i>et al.</i> (2021)
	JpOd274	6119-6289	6204	
	JpOd6	5934-6179	6057	
	JpOd181	5751-5917	5834	
	JpOd282	5737-5902	5820	
	JpFu1	5478-5590	5534	
	JpKo2	4294-4514	4404	
	JpKo13	3847-3978	3913	
	JpHo1	3685-3850	3768	
	F23	3550-3960	2569	Kanzawa-Kiriyama <i>et al.</i> (2019)
	F5	-	-	
	IK002	2418-2720	2569	McColl <i>et al.</i> (2018)
Northeast Asia	WLR_BA_o	3000-2300	2650	Ning <i>et al.</i> (2020)
	HMMH_MN	5500-5000	5250	
East Asia	Present-day Han	-	-	Mallick <i>et al.</i> (2016)

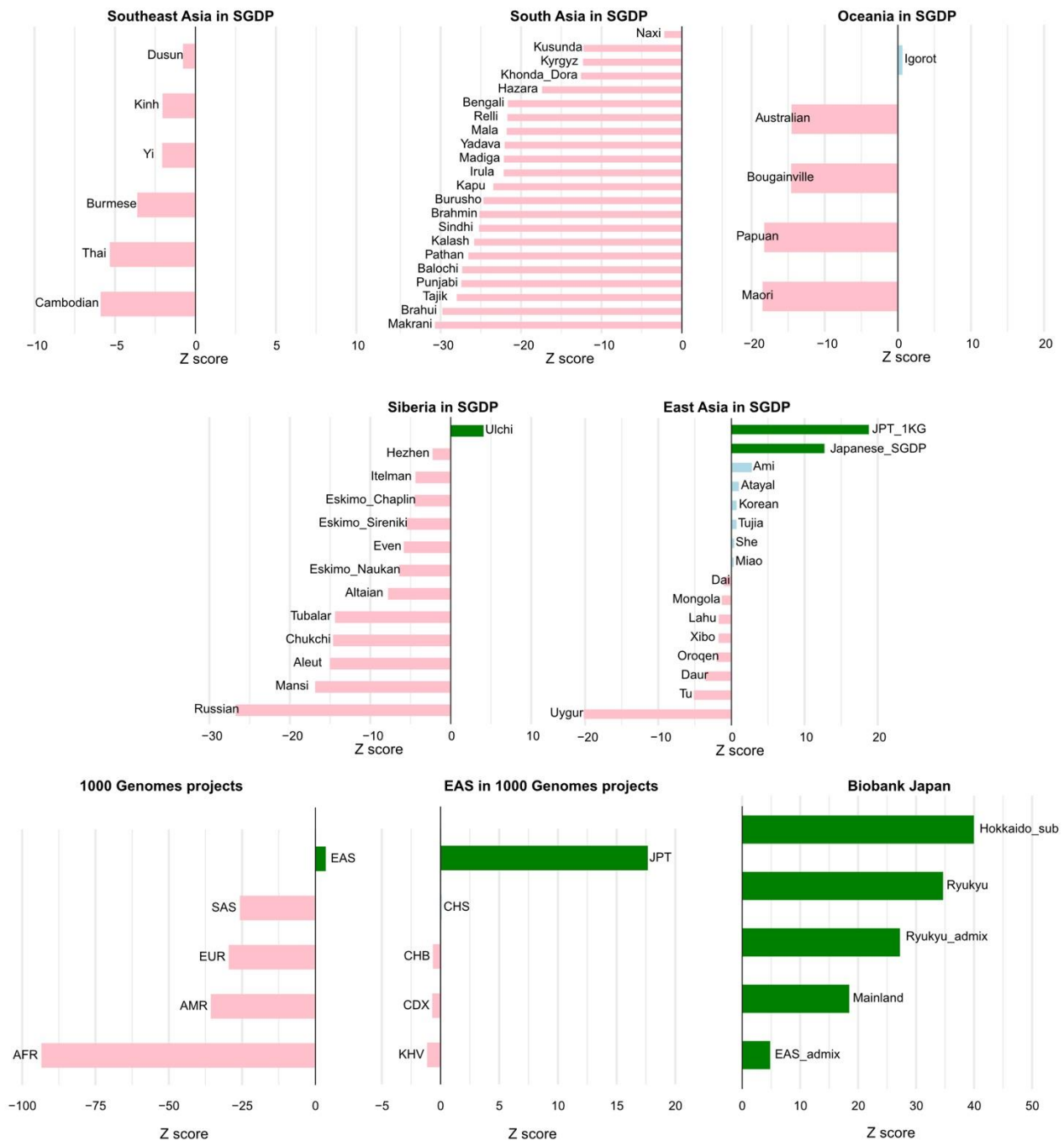
We have also revised the sentences to clarify the duration of each period as follows:

- **Introduction** (Page 4, Line 89): The Jomon period lasted until approximately 3 kya, when immigrants from the continent introduced rice cultivation during the Yayoi period **that spanned from 3 to 1.7 kya**. This agricultural revolution prompted sociopolitical developments, leading to the establishment of the Japanese state in the Kofun period, which began around 1.7 kya **and endured for 200-300 years¹¹**.

Comment: 168: Jomon may have closer relationships with Southeast Asian and Oceanian populations rather than the current East Asian populations. To answer this question, 1KG data have too little diversity to test. I don't think this question has been resolved, even a small proportion of the Jomon ancestry is detected in EAS, which is a vague and broad category of populations.

Response and modification: In revision, we additionally conducted f_4 -tests using the Simon Genome Diversity Project (SGDP) panel in the form of $f_4(\text{Mbuti, Jomon; Han, } X)$, where X is a target population tested for their genetic affinity to the Jomon. Contrary to the suggested potential link between Jomon and populations from Southeast Asia or Oceania, our analysis identifies the significant affinities ($Z > 3$) between Jomon and present-day Japanese or Ulchi (please see a revised **Supplementary Fig. 2**), which are consistent with findings from previous studies (Kanzawa-Kiriyama *et al.* **Anthropological Science** 2019; Gakuhari *et al.* **Comm Biol** 2020). It is important to note that while some genetic affinities have been reported between a 2800-years-old Jomon individual (*i.e.*, IK002) and ancient hunter-gatherers from Southeast Asia (McColl *et al.* **Science** 2018), this relationship has not been reproducible when tested with a temporarily and spatially diverse set of Jomon individuals as a population (Cooke *et al.* **Sci Adv.** 2021). We have modified the sentences as follows:

- **Results** (Page 7, Line 188): "Our target populations include those in **the Simon Genome Diversity Project (SGDP) panel**, the 1000 Genomes Project (1KG), and the subpopulations within BBJ. As shown in previous studies^{8,9,14}, there is no extra genetic affinity between the Jomon and any of the populations **tested**, except for **Ulchi in the SGDP or the East Asians (EAS) within the 1KG** (**Supplementary Fig. 2**).
- **Methods** (Page 26, Line 628): The f_4 -statistics were measured in the form of $f_4(\text{Mbuti, Jomon; Han, } X)$ using qpDstat with the f_4 mode in AdmixTools. We used the BBJ and the populations in the 1KG ($n = 2,504$) or **the SGDP as target populations** (X in the form).



Supplementary Fig. 2. Genetic affinities of Jomon with present-day populations in the SGDP panel, 1000 Genomes Project (1KG), and Biobank Japan.

Bar plots represent Z-scores from f_4 -tests with the form of $f_4(\text{Mbuti}, \text{Jomon}; \text{Han}, X)$, where X is a population from the SGDP dataset, 1KG, or sub-populations within the BBJ dataset. Positive or negative Z-scores are colored in right blue or pink. If the Z score is greater than 3, the bar is colored in green. The target populations are grouped by: Southeast Asia, South Asia, Oceania, Siberia, East Asia in SGDP; AFR, AMR, EUR, EAS, SAS in 1KG; JPT, CHS, CHB, CDX, and KHV in EAS in 1KG; Hokkaido_sub, Ryukyu, Ryukyu_admix, Mainland, and EAS_admix in Biobank Japan.

Comment: 202: Yayoi looks much closer to Jomon rather than the present-day populations in Supp Fig 3a.

Response: Two individuals of the Yayoi included in this study were initially considered Jomon based on their morphological characters (Kaifu *et al. Anthro Sci* 2017). However, a previous ancient

genomic study has clearly demonstrated that these Yayoi individuals are genetically admixed between Jomon and continental ancestry originated in Northeast Asia, with almost equal contributions from these two ancestors (Cooke *et al.* **Sci Adv.** 2021). This genetic admixture likely explains their proximity to Jomon individuals. It is important to note that, due to the limited availability of publicly accessible ancient genomes from the Yayoi period, the results from our analysis may not fully capture the broader patterns of genetic variation among ancient Japanese populations during this period.

Modification: We have modified the text related to this result as follows:

- **Results** (Page 10, Line 230): While the Kofun are included within variation in the present-day populations, the Jomon are clustered in a position extending from the Ryukyu and Hokkaido_sub subpopulations. The two individuals from the Yayoi period, who are morphologically considered as the Jomon³² but genetically admixed between the Jomon and continental ancestry¹⁴, are positioned between the Jomon and the present-day populations (**Supplementary Fig. 3a**).

Comment: 217: Only PC1 has a strong correlation with the Jomon proportion, not PC2.

Response and Modification: The PC2 indeed has a significant correlation with the Jomon proportion as well, with a p -value $< 1.0 \times 10^{-300}$ (please see a newly added **Supplementary Table 4**) We have summarized correlation coefficients and corresponding p -values for the relationships between each of three distinct ancestors and PCs (*i.e.*, PC1 and PC2) in **Supplementary Table 4**.

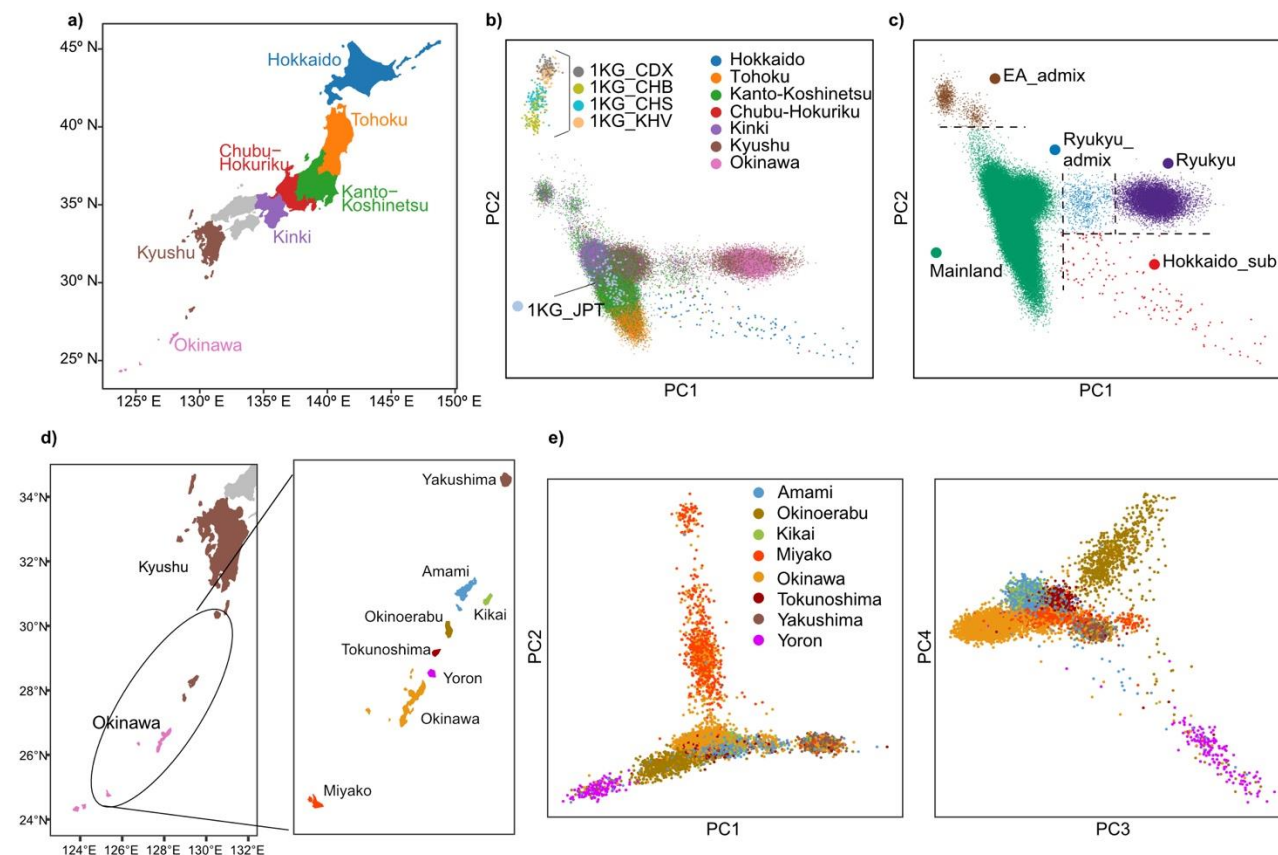
Dataset	Ancestry	Absolute correlation coefficient (P value)	
		PC1	PC2
BBJ-1st	Jomon	0.61 ($<1.0 \times 10^{-300}$)	0.09 ($<1.0 \times 10^{-300}$)
	NEA	0.14 ($<1.0 \times 10^{-300}$)	0.02 (1.3×10^{-12})
	EA	0.26 ($<1.0 \times 10^{-300}$)	0.04 (2.8×10^{-71})
BBJ-2nd	Jomon	0.53 ($<1.0 \times 10^{-300}$)	0.14 ($<1.0 \times 10^{-300}$)
	NEA	0.11 (1.1×10^{-193})	0.02 (9.7×10^{-6})
	EA	0.22 ($<1.0 \times 10^{-300}$)	0.08 (1.7×10^{-89})

(new) **Supplementary Table 4. Absolute value of correlations between each of three distinct ancestors and PCs in the BBJ-1st and BBJ-2nd cohorts.**

Comment: 221: I am not sure about this statement. The strong correlation with PC1 indicates the divergence between the Jomon ancestry and other ancestries. In terms of the amount/proportion of the Jomon ancestry in Japanese populations, the impact is obvious only in Okinawa and Hokkaido.

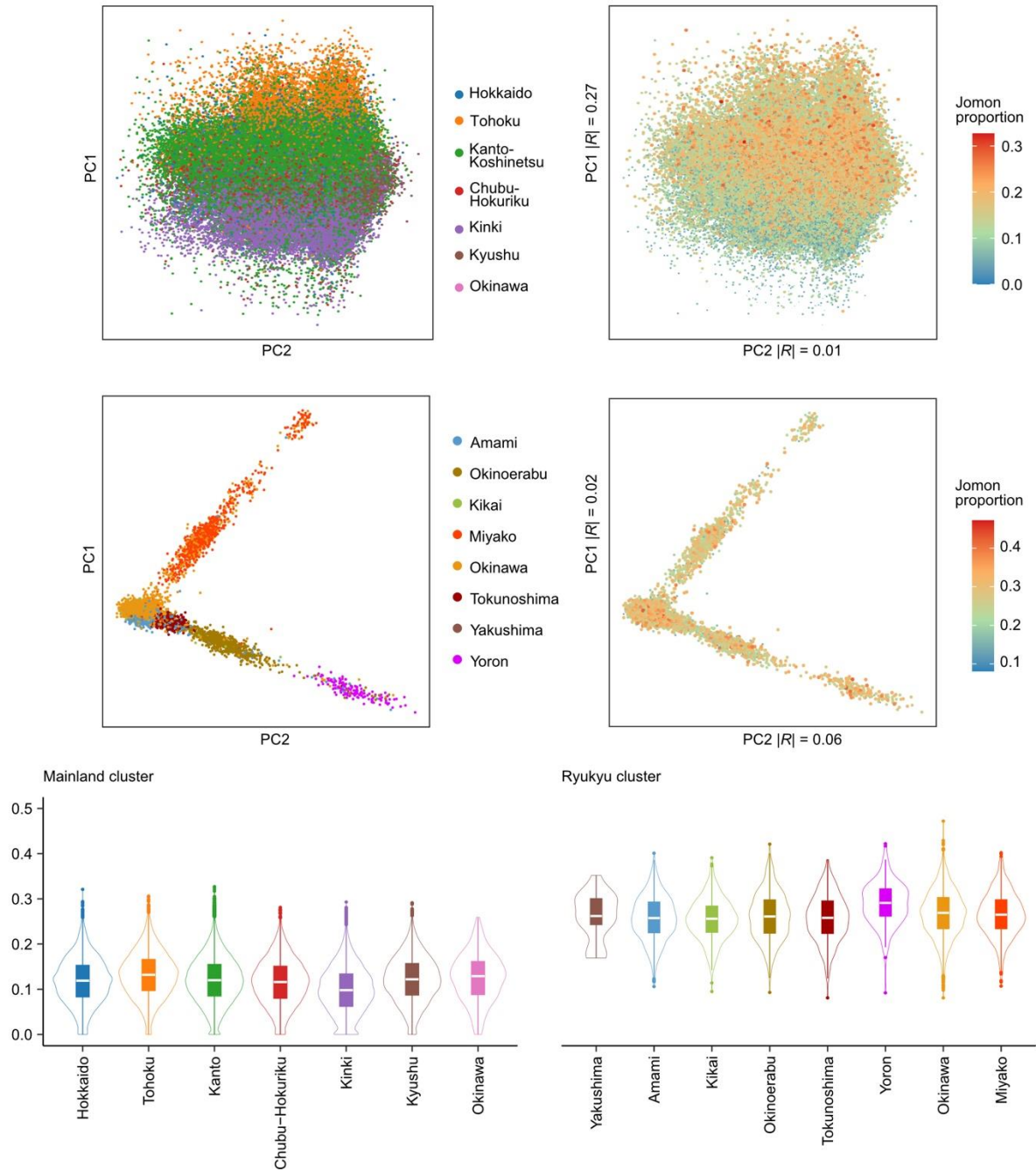
Response: Thank you for the comment. To better understand the extent to which Jomon ancestry underlies genomic variation among individuals, in revision, we have integrated more detailed insular regional information registered for the BBJ participants into our analysis. The PCA analysis clearly demonstrates that contemporary populations are stratified not only by different parts of the archipelago but also by the distinct islands of the Ryukyu Islands (**Fig. 1**). This is the population stratification projection of the Japanese population with the most detailed regional resolution to date. Even when focusing on individuals from the Mainland or Ryukyu clusters, strong correlations remain

evident between Jomon ancestry and PC1 or PC2 (see **Supplementary Fig. 5**). These findings underscore the impact of Jomon ancestry on population structure at both broad and local levels.



(new) Fig. 1. Population structure of Biobank Japan.

a) Seven geographic regions, each represented by a different color, indicate the locations where participants were registered at local hospitals. **b)** A scatter plot of PCA for BBJ participants with 1KG EAS samples. The colors of the BBJ participants correspond to those used in **a)**. **c)** Clustering results of BBJ participants based on PCA. **d)** Geographic locations of the Ryukyu Islands. **e)** Scatter plots of PCA for BBJ participants from the Ryukyu Islands. The individuals are colored differently according to the locations of their registered hospitals. In **a)** and **d)**, the map of Japan is drawn using the R package “jpndistrict” (<https://github.com/uribo/jpndistrict>). PC, principal component; 1KG, 1000 Genomes Project; JPT, Japanese in Tokyo; CDX, Chinese Dai in Xishuangbanna; CHB, Han Chinese in Beijing; CHS, Han Chinese South; KHV, Kinh in Ho Chi Minh City; NEA, Northeast Asian; EA, East Asian.



(new) Supplementary Fig. 5. Projection of the proportions of Jomon ancestry onto the PCA plots based on the Mainland or Ryukyu clusters within BBJ.

The proportion of Jomon ancestry is projected onto the PCA plots based on **(top)** individuals from the Mainland ($n = 152,148$) or **(middle)** individuals from the Ryukyu clusters ($n = 8,112$). $|R|$ represents the absolute values of correlation coefficients between the proportion of Jomon ancestry and PCs. **(bottom)** Boxplots represent variation in Jomon ancestry across individuals from different geographic contexts within the Mainland or Ryukyu clusters (Yakushima; $n = 19$, Amami; $n = 1,314$, Kikai; $n = 465$, Okinoerabu; $n = 726$, Tokunoshima; $n = 415$, Yoron; $n = 148$, Okinawa; $n = 4,311$, Miyako; $n = 714$). The boxes denote the interquartile range (IQR) and the median is shown as white horizontal bars; whiskers extend to 1.5 times the IQR; outliers are shown as individual points. PC, Principal component

Modification: In this revision, we provide the population stratification of the Japanese archipelago at

both broad and local levels in **Fig. 1** and **Supplementary Fig. 5**. Additionally, observed variation in the tripartite structures across populations is presented in **Fig. 2**. Corresponding text has been revised accordingly:

For new **Fig. 1**,

- **Results** (Page 6, Line 149): Population stratification is further evident even within the Ryukyu Islands, reflecting their geographic affinities in this local insular context (**Fig. 1 d and e**, Yakushima; $n = 431$, Amami; $n = 1,531$, Kikai; $n = 561$, Okinoerabu; $n = 845$, Tokunoshima; $n = 476$, Yoron; $n = 167$, Okinawa; $n = 4,795$, Miyako; $n = 827$).
- **Discussion** (Page 23, Line 521): Our biobank-scale analysis provides a refined picture of the genetic makeup of people throughout the Japanese archipelago **both at broad and local scales**.
- **Method** (Page 25, Line 574): **Based on where participants were registered, we** defined seven geographically-defined populations **for the Japanese archipelago** (i.e., Hokkaido, Tohoku, Kanto-Koshinetsu, Chubu-Hokuriku, Kinki, Kyushu, and Okinawa, which are ordered from northeast to southwest Japan) **and eight populations for the Ryukyu Islands** (i.e., Yakushima, Amami, Kikai, Okinoerabu, Tokunoshima, Yoron, Okinawa, and Miyako).

For **Supplementary Fig. 5**,

- **Results** (Page 10, Line 260): The correlation between Jomon ancestry and PCs is still evident even upon focusing solely on individuals from the Mainland or Ryukyu cluster (**Supplementary Fig. 5**). These findings strongly indicate that Jomon ancestry has a critical role in shaping genomic variation among Japanese individuals at the level of PCA.

Comment: 309: need a citation.

Response and Modification: We agree to your suggestions, and have included Sakaue *et al.* *Nat Genet.* 2021, The GTEx consortium. *Science* 2020, Moutjoy *et al.* *Nat Genet.* 2021, and Ghossaini *et al.* *Nucleic Acids Res.* 2020, respectively.

Comment: 323: The citation should be Supp tables 7 and 8 in the Excel file (it seems the table numbering is all shifted). And this legend is not enough to understand the table.

Response: Thank you for identifying the mistakes in numbering. We have corrected this and made the legend more readable.

Comment: 339: Show the correlation stats. PC2 can be more correlated with EA ancestry.

Response and Modification: We thank the reviewer for the suggestion. We have included correlation coefficients, together with p -values in BBJ-2nd. We have also tested a correlation between PC2 and EA ancestry. These results are listed in **Supplementary Table 4**.

Comment: 382: What is the frequency of rs7572509 in UKB EAS and EG6?

Response: In response to comments from Reviewer 3, we expanded our study by incorporating all individuals with Jomon ancestry into the GWAS on Jomon proportions. This addition increased our dataset by 8,904 individuals, resulting in a total of 154,339 individuals. The findings from this extended dataset are largely consistent with those reported in the original manuscript, identifying a total of 132 Jomon-related variants. Our LD clumping analysis has led to the selection of different SNP sites, including rs13017060 instead of rs7572509, which are in strong linkage disequilibrium with those reported in the original manuscript. Here, we report the frequency of rs13017060 as 16.8% and 21.7% in UKB EAS and Ethnic group 6.

Comment: 435: do you mean the mainland population already possessed the tripartite ancestor? The sentence is not clear.

Response and Modification: Yes. That is what we found from our previous study (Cooke *et al. Evol Hum Sci.* 2023). Briefly, the three distinct ancestors (i.e., Jomon, Northeast Asian, and East Asian ancestry) are also characterizing genetic makeup of people from the Ryukyu Islands. However, considering that there were no archaeological periods equivalent to the agrarian Yayoi or the state-formation Kofun in the area, immigrants from the continent did not come to the region directly. Instead, we found that those who already had the tripartite ancestors through admixture in the main islands of Japan migrated to the Ryukyu Islands and admixed with indigenous Jomon-like people around the 11th century AD, which coincided with the end of the prehistoric period and the emergence of the Gusuku period.

To clarify that this was an event in the past, rather than at the present, we have modified the sentence as follows:

- **Discussion** (Page 23, Line 509): The continental ancestor (i.e., Northeast Asian and East Asian) is **also** observable in individuals from Okinawa. However, it is important to note that these continental components were **not directly** introduced **from the continent but** by immigrants from the main islands of Japan, **who already possessed the tripartite ancestor¹⁵**. This migration has been **estimated to have occurred** around the eleventh century AD, marking the end of the prehistoric period in the area. Until this transition, it is widely accepted that people with Jomon-like genetic characteristics continued to inhabit the region for at least several thousand years⁴⁴. **Therefore, the** elevated levels of Jomon ancestry in Okinawa can be attributed to **this historical event**.

Comment: 454: If you already have identified the genetic variant associated with BMI or obesity, such as rs7572509, you can directly predict the risk without the Jomon ancestry proportion. As you mentioned the Jomon ancestry-related variants can include several traits.

Response: We thank the reviewer for this comment. We would like to emphasize that our findings do not support the idea that one can use a person's Jomon ancestry alone to predict their BMI or likelihood of being obese. When predicting BMI at the genetic level, it is advisable to utilize a set of variants identified from the large-scale existing GWAS results on BMI. As stated in **Discussion**, one potential application of genetic variants identified from our analysis is to estimate an individual's Jomon ancestry. Another significant finding from our study is the positive correlation between increased Jomon ancestry and higher BMI, aligning with epidemiological observations of higher BMI in Okinawa compared to other regions within Japan at the population level rather than individual level (Matsushita *et al. Int. J. Obes.* 2008). Furthermore, as demonstrated in our response to the second major comment from Reviewer 1, incorporating Jomon ancestry as a covariate in GWAS on BMI can help reduce overall inflation in effect size estimates. Therefore, the novelty of this study lies in the significance of understanding a person's genetic ancestry not only for tracing their genetic origins but also for minimizing confounding effects in GWAS.

Comment: 529: The method description for Admixture modeling and the fitness of ancestry model of tripartite and dual-structure is not clear.

Response: We have revised the sentences as follows:

- **Methods** (Page 26, Line 615): **We evaluated whether the tripartite structure provides a better fit to the data than any other possible scenarios at population levels based on p -values for nested models with a cut-off of 0.05. These alternative models include dual structure models involving any combination of Jomon, Northeast Asian, and East Asian ancestry, as well as single ancestry models. When conducting admixture modeling at individual levels, we considered the tripartite model to be a good fit if (i) a tail probability was >0.05 and/or (ii) estimates of admixture fractions were feasible (i.e., >0.0 and <1.0).**

Comment: Supp Fig 3a: Kohun or Kofun?

Response: Thank you for identifying the typo. We have corrected it accordingly.

Other questions and suggestions:

Comment: The obesity-related Jomon variant was potentially positively selected before and have maintained in the Japanese population?

Response: We thank the reviewer for this great suggestion. As responded to the third major comment from Reviewer 1, we found that:

- Jomon-related variants are linked with significantly longer haplotypes than those variants without any association with Jomon ancestry.
- Jomon-related variants are significantly enriched with selection signals based on SDS ($p = 0.008$), which is the most powerful to detect selection over approximately the past 100 generations.

These results indicate that the Jomon-related variants, including the ones associated with obesity, have undergone selection in Japanese populations.

longer haplotypes than non-Jomon-related variants ($P = 3.1 \times 10^{-32}$) (Figs. 5b and 5c). This strongly suggests a Jomon origin for these extended haplotypes, supported by strong linkage disequilibrium throughout the genome possibly due to the high genetic homogeneity and small effective population size ($\sim 1,000$) within the Jomon population^{14,37}. Furthermore, the persistence of these long Jomon-derived haplotypes in the contemporary populations can be attributed to the relatively recent admixture with the continental ancestors¹⁴, coupled with insufficient time for recombination to break apart the haplotypes. Leveraging selection scan results based on singleton density score (SDS)¹⁷, which detects selection over approximately the past 100 generations³⁸, we further observe an enrichment of selection signals in the Jomon-derived haplotypes ($P = 0.008$). These results support the idea that the Jomon-related variants can serve as markers for quantifying Jomon ancestry, tag Jomon-derived haplotypes, and potentially have been subject to recent selective pressures.

Comment: Can you estimate the age of admixture to shape the tripartite population structure or it is already known? If you know the age of Jomon admixture, it would be easier to understand why the Japanese have maintained the BMI-related variant during the transition of lifestyle.

Response: Yes, the timings of admixture events have been estimated in a previous study (Cooke *et al. Sci Adv* 2021). The admixture between Jomon and continental immigrants with Northeast Asian ancestry goes back to $3,448 \pm 825$ years before the present, while the second admixture with East Asian ancestry was dated to $1,748 \pm 175$ years B.P., which are roughly consistent with timings of culture transitions from Jomon to Yayoi or from Yayoi to Kofun.

Comment: At last, I believe the authors described well but still need to carefully describe the results of this study to distinguish from the previous studies(14 and 15), which support the same results in this study.

Response: We thank the reviewer for this suggestion. Our study not only confirms the findings from these previous studies but also provides novel insights into the genetic and phenotypic legacy of ancient hunter-gatherer ancestry in Japanese populations. To the best of our knowledge, this represents the most comprehensive and large-scale exploration within an Asian context utilizing the nation-wide biobank resources.

Modification: We have provided clarification on the novelty of this study compared with those previous works in **Introduction** as follows, while also emphasizing our findings throughout **Discussion**.

- **Introduction** (Page 4, Line 102): Japanese populations are well characterized with their north-to-south gradient of genomic variation^{17–19}. However, the applicability of the tripartite model and variability of the three distinct ancestral components throughout the archipelago remains unknown due to limitations in samples and geographic representation of modern individuals used in previous studies^{14,15}. It is thus crucial to model this ancestral structure using comprehensive, population-level genomic data.
- **Introduction** (Page 4, Line 108): Recent advancements in paleogenomics have made it possible not only to identify diverse genetic ancestors^{20–23}, both locally and globally, but also to uncover their impact on health and disease in contemporary populations, such as the exacerbation and resistance of coronavirus disease 2019 through Neanderthal-introgressed genes^{24,25} or the genetic predisposition to multiple sclerosis brought by Steppe ancestry²⁶. However, the extent to which the human past has shaped phenotypic variation today remains poorly understood, especially in non-European contexts²⁷.

Reviewer #3 (Remarks to the Author):

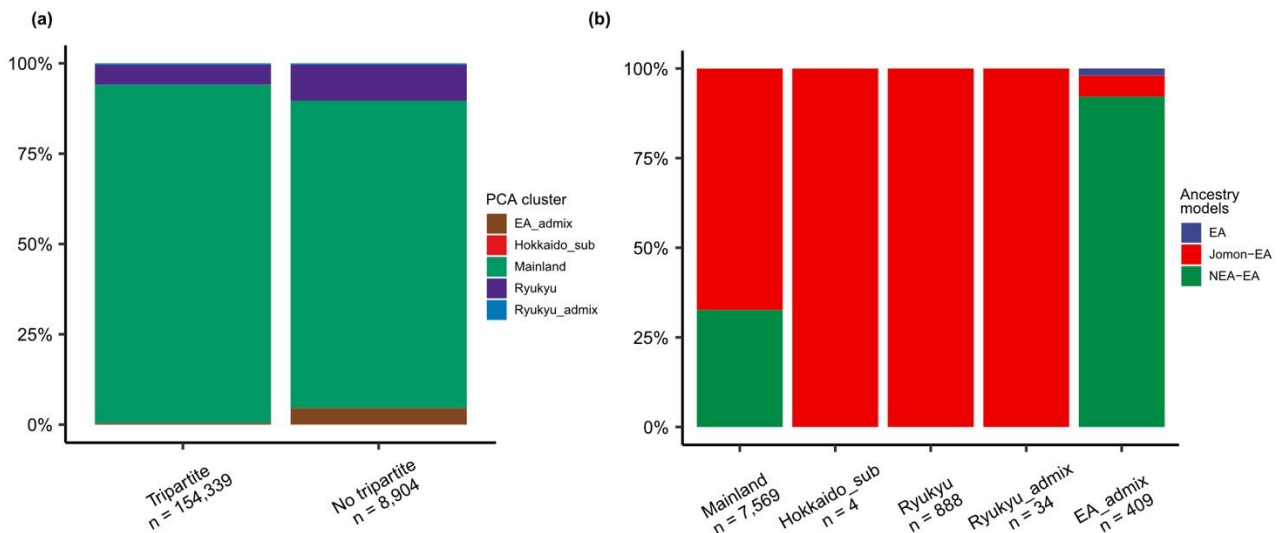
In this paper by Yamamoto et al., the authors study the genetic legacy of the Jomon group of hunter-gatherers in modern Japanese populations: this includes the heterogeneous distribution of Jomon ancestry geographically within Japan, the strong link between Jomon ancestry and genetic variation, and the association of Jomon ancestry with high BMI/obesity. They also identify genetic variants which are indicative of high Jomon ancestry. These findings are validated in several independent cohorts, including a population living in the UK, from the UK Biobank.

Overall, this is a very well performed study, and I found the writing generally clear and concise. I believe the main conclusions of the study are backed up by strong statistical evidence. This represents an important advance into the study of the genetic legacy of ancient populations outside of a European context, where research in this area has until recently largely been focussed. The results are novel, and may help explain the higher levels of obesity in Okinawa compared to mainland Japan.

We are grateful for your recognition of the value of our manuscript. Please find our response to each comment below.

Comment: My comments below largely address small questions or errors in the text. My largest concern is the not small number of cases where the tripartite model does not fit, which questions the claim that the tripartite model presents the best fit across all regions. Ideally, I would like an explanation for why this is the case, given that after several thousands of years you would expect all individuals in a population to have ancestry from each admixing group. It might be helpful to break down the ~16,000 non-tripartite individuals by region, for example?

Response: We thank the reviewer for the comment. It is important to emphasize that the tail probability works in a way that a certain proportion of individuals is inevitably expected to deviate from fitting a given model, due to its statistical nature. We set a threshold of 5% for this purpose. Consequently, 7,962 individuals out of 16,894, constituting approximately 5% of all BBJ participants ($n = 171,287$), were excluded from our subsequent analysis. Additionally, 28 individuals were excluded due to a contradiction: the tripartite model was favored over the dual structure models, despite the tail probabilities of the tripartite model being below 5%. We then examined each of the remaining 8,904 cases (**Supplementary Fig. 4a**). In terms of the PCA-based groups (i.e., genetic clusters), 85.01% ($n = 7,569$) of those individuals belong to the Mainland group, followed by the Ryukyu (9.97%; $n = 888$), EAS_admix (4.60%; $n = 409$), Ryukyu_admix (0.38%; $n = 34$), and Hokkaido_sub groups (0.04%; $n = 4$). Upon further investigation into the breakdown of models supported in these cases (**Supplementary Fig. 4b**), we observed that the majority are explained by a dual structure of Jomon and East Asian ancestry. However, the EAS_admix group is predominantly characterized by the other type of dual structure between Northeast Asian and East Asian ancestry, aligning with our hypothesis that individuals from this group likely represent recent immigrants from the continent. In this revision, we have included all individuals whose tail probabilities are greater than 5%, regardless of their plausible models being the tripartite or the others, in subsequent analyses to comprehensively capture variation in Jomon ancestry within our datasets (see also **Supplementary Fig. 6**).



Supplementary Fig. 4. Comparisons of admixture scenarios in different geographic contexts.

a) Bar plots show the composition of PCA clusters within individuals supporting either the tripartite model (154,339 individuals labeled as “Tripartite”) or alternative scenarios (8,904 individuals labeled as “No tripartite”). In the tripartite model, each proportion of the five clusters (EA_admix, Hokkaido_sub, Mainland, Ryukyu, and Ryukyu_admix) is as follows: 0.4%, 0.08%, 93.7%, 5.5%, and 0.4%. The proportions in the non-tripartite models are 4.6%, 0.04%, 85.0%, 10.0%, and 0%. **b)** Bar plots represent a detailed breakdown of “No tripartite” individuals by their PCA-based groups, together with the composition of admixture models (*i.e.*, dual ancestors: Jomon and East Asia or Northeast Asia and East Asia; single ancestor: East Asia).

Modification: We have modified **Supplementary Fig.4** and the sentences as follows:

- **Results** (Page 10, Line 242): Approximately 5% of the individuals did not conform to the tripartite model (8,932 individuals), showing a better fit for a two-way admixture involving either Jomon and East Asian ancestry or Northeast Asian and East Asian ancestry. However, 28 individuals were excluded from subsequent analysis due to insufficient support for the dual ancestry models over the tripartite model, as indicated by nested p -values <0.05 . There was also a minor exception, where a model of East Asian ancestry alone, rather than any dual structure models, was preferred in ten individuals (**Supplementary Fig. 4**). It's important to note that none of the models could adequately fit the remaining individuals, comprising nearly 5% (7,962 individuals). This is likely due to the use of a tail probability cut-off of 5%, which accounts for the inherent variability in the data, as a defined proportion of all tests would be expected to deviate from the models tested.
- **Methods** (Page 26, Line 622): For individuals who did not support the tripartite model due to the tail probability being below 5%, we sought to identify an alternative dual ancestry model with the highest tail probability, subsequently confirmed through nested p -values >0.05 by comparing the tripartite and dual structure models. If a single ancestry model was plausible, we further assessed whether a specific ancestor alone can adequately explain the individual using the nested p -values.

Comment: I was also surprised by the very high variability in the levels of Jomon ancestry across individuals. I wondered to what extent this might be an artefact of the small numbers of ancient samples, creating stochasticity in the inferred ancestry proportions. To this end, it would be useful to know the variation in the Han and NE Asian components, as these have smaller sample sizes still.

Response: We have included an Early Neolithic hunter-gatherer population from Lake Baikal

(Lokomotiv_EN; $n = 4$), which has been previously shown to share a strong genetic affinity with the Yayoi (Cooke *et al.* **Sci Adv** 2021), as part of Northeast Asian ancestry originally defined by two individuals from the West Liao River basin. Comparing the proportions of all three ancestors between the source populations with and without these additional hunter-gatherer individuals, we observed strong correlations across the ancestors (Jomon: $R = 0.96$; Northeast Asia: $R = 0.94$; East Asia: $R = 0.95$). These results suggest that the effect of small sample size in the source populations can be marginal.

Comment: Finally, I would like to hear more about the utility of a GWAS for Jomon ancestry. It is not immediately obvious why it might be helpful to predict the Jomon ancestry proportion when you can simply re-run qpADMIX or another tool.

Response: Thank you for the comment. In response to the third major comment from Reviewer 1, we posit that if one can readily determine the presence or absence of Jomon ancestry using only a small set of genetic variants like ancestry informative markers, as opposed to whole-genome or genome-wide data, it will markedly reduce costs and burdens required to construct, handle, process, and analyze such complicated data. It is crucial to acknowledge the substantial knowledge and workload required for processing ancient genomes, underscoring the importance of streamlined approaches.

Minor comments:

Comment: 73/74: “the insular region of East Eurasia” -> “an insular region of East Eurasia”

Response and Modification: We would like to thank the reviewer for this grammatical error. We have corrected it accordingly.

Comment: 100/101: The transition from hunting-gathering to farming resulted in a reduction in genetic diversity⁴. Is there evidence for this outside of Europe/Near East? The reason for this was a population bottleneck, not an inherent result of the transition, as you seem to be implying. Modify the language accordingly.

Response and Modification: Thank you for the comment. We fully agree that the transition from hunting-gathering to farming did not necessarily lead to a reduction in genetic diversity, unless other factors, such as population bottlenecks, intervened. To avoid any potential confusion, we have removed this sentence.

Comment: 101-104: “The lifestyle changes could have outpaced DNA evolution over the course of approximately 10,000 years, leaving the legacy of ancient hunter-gatherer ancestry to continue playing a crucial role in shaping phenotypic variation today”. Unclear what you mean by ‘outpaced’ in this context. Either expand on this idea (i.e. genetic adaptations no longer optimal for modern environment/lifestyles), or remove. Either way, there will still be some phenotypic impact of this diverse ancestry if it is present in modern populations.

Response and Modification: We have removed these sentences.

Comment: 107-108: “persist present-day populations” -> “persist in present-day populations”

Response and Modification: We agree and have revised it accordingly.

Comment: 137: State here how clustering of PCA results was done. How did you name them? Why did you not perform a more data-driven clustering, e.g. density-based clustering based on first X PCs? Visual selection based on PCA is fine however, I do not require you to change this.

Response: Thank you for the comment. Geographic clusters in Japanese populations have been

defined through visual inspection based on PCA, particularly for the Mainland and Ryukyu clusters (Yamaguchi-Kabata *et al. Am J Hum Genet.* 2008 and Okada *et al. Nat Commun* 2018). Since our PCA results confirm the population structure previously reported in these studies, we have adapted a similar approach.

Comment: 204-213: You show that the tripartite model is the best fit model for 154,393 out of 171,287 individuals in BBJ. Is it possible that some of the remaining individuals are or are descended from recent immigrants? This result, that three-way admixture is not the best model, needs an explanation if you are going to justify the main claim of the paper that the tripartite model is the best fit. After 3kya you would expect all Japanese to inherit ancestry from each group, if the model is to be believed.

Response and Modification: Thank you for the comment. As explained in our response to the first major comment, approximately 5% of our samples (7,962 out of 171,287 individuals) were expected to be excluded due to the use of a 5% threshold for the tail probability. Among the remaining 8,904 individuals, who exhibit better fittings to dual or single ancestry models than the tripartite model, we find that the genetic makeup of 2,860 individuals is explained by continental ancestors (either a dual structure of Northeast Asia and East Asia or East Asian ancestry only). Given that Jomon ancestry is exclusively present in the Japanese archipelago (see **Supplementary Fig. 2**), these individuals are likely immigrants from the continent. We have revised the terminology regarding “the best fit model” throughout the manuscript.

Comment: 217: calculate and provide p values for correlation?

Response: We have added the *p*-values to **Supplementary Table 4**.

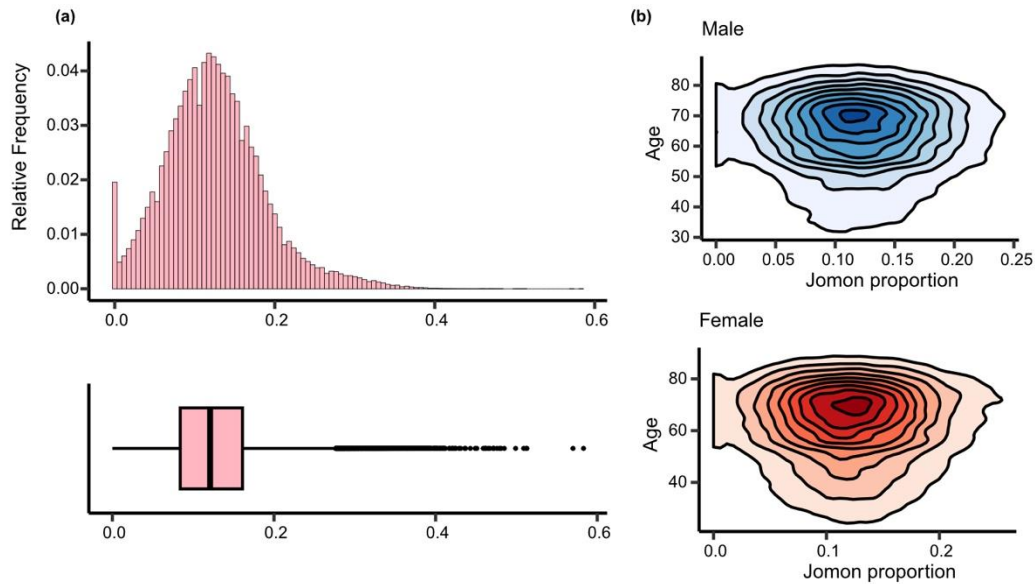
Dataset	Ancestry	Absolute correlation coefficient (<i>P</i> value)	
		PC1	PC2
BBJ-1st	Jomon	0.61 ($<1.0 \times 10^{-300}$)	0.09 ($<1.0 \times 10^{-300}$)
	NEA	0.14 ($<1.0 \times 10^{-300}$)	0.02 (1.3×10^{-12})
	EA	0.26 ($<1.0 \times 10^{-300}$)	0.04 (2.8×10^{-71})
BBJ-2nd	Jomon	0.53 ($<1.0 \times 10^{-300}$)	0.14 ($<1.0 \times 10^{-300}$)
	NEA	0.11 (1.1×10^{-193})	0.02 (9.7×10^{-6})
	EA	0.22 ($<1.0 \times 10^{-300}$)	0.08 (1.7×10^{-89})

(new) **Supplementary Table 4. Absolute value of correlations between each of three distinct ancestors and PCs in the BBJ-1st and BBJ-2nd cohorts.**

Comment: 241: Does excluding individuals where the tripartite model does not fit in any way bias these results? I think you need to be able to justify excluding these samples.

Response: Thank you for the suggestion. In this revision, we have incorporated all individuals into subsequent analysis (*i.e.*, phenotypic associations of Jomon ancestry and GWAS on Jomon ancestry), as long as they are in a good fit with one of the models tested (*i.e.*, the tail probability is >0.05 and/or the estimates of admixture fractions are feasible). This expanded the dataset to include 163,243 individuals (**Supplementary Fig. 6**), compared to the 154,339 individuals used in our original analysis. BMI remains the most significant in the association with Jomon ancestry after multiple testing corrections, regardless of using all BBJ participants or only those from the Mainland cluster (see a

revised **Fig. 4**). Additionally, other traits are nominally significant, including adult height, alcohol habit, body weight, glaucoma, E/A ratio, red blood cell count, mean corpuscular hemoglobin, and LDL cholesterol. Using this expanded dataset, we have identified 132 Jomon-related variants, which include 121 variants reported in our original manuscript. These results support the idea that while there was no obvious bias in the results from the original manuscript, the inclusion of additional individuals has enhanced the statistical power of our analysis.



Supplementary Fig. 6. Variation in the proportion of Jomon ancestry among BBJ participants.

a) The distribution of Jomon ancestry in all participants ($n = 163,243$). b) The density plot between the Jomon proportion and age for male and female.

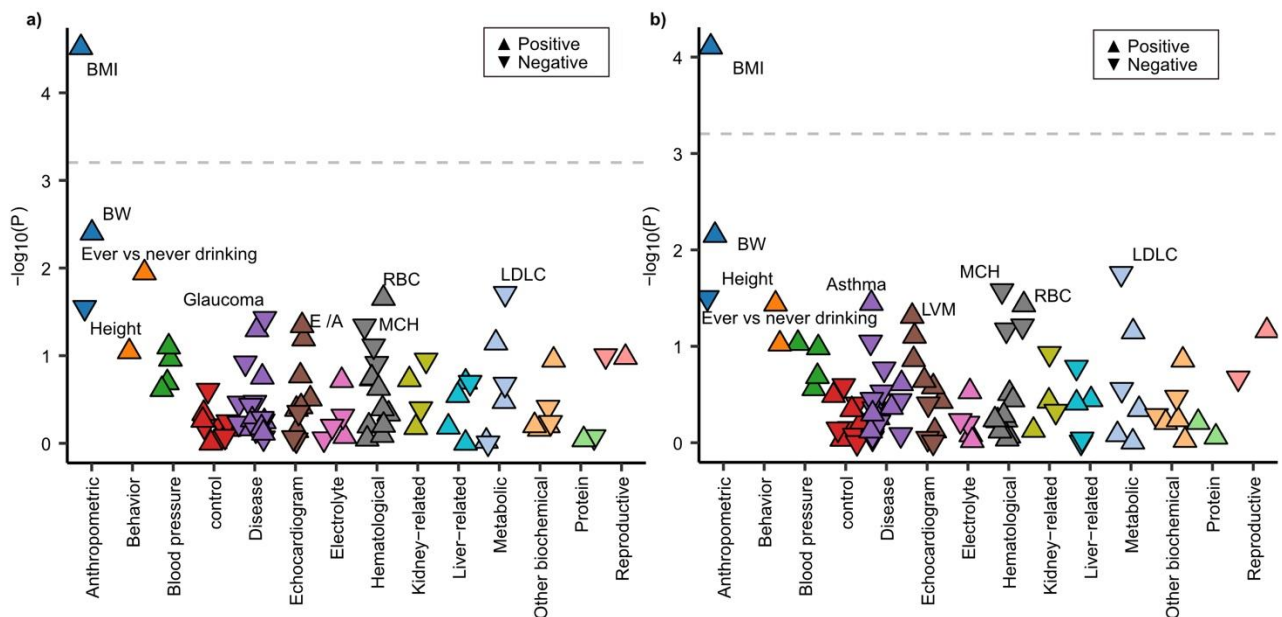


Fig. 4. Associations of Jomon ancestry with 80 complex traits.

The associations are tested using a generalized linear model in a) the entire BBJ participants ($n = 163,243$) and b) the participants only in the Mainland cluster ($n = 152,148$). The quantitative traits are modeled by linear

regression, while the binary traits are analyzed with logistic regression. The direction of the triangle corresponds to the sign of the beta coefficient for each trait. The gray dashed line represents the statistical significance based on the Bonferroni correction ($P < 0.05/80 = 6.3 \times 10^{-4}$). Control indicates 10 dummy phenotypes. All traits labeled on the plots have nominal significance with $P < 0.05$. BMI, Body mass index; BW, Body weight; LVM, Left ventricular mass; E/A, E/A ratio; RBC, Red blood cell count; LDLC, low-density lipoprotein cholesterol.

Modification: We have modified **Fig. 4, Table 1, Supplementary Fig. 6 and 10, and Supplementary Table 6-10**. We have also revised the text as follows:

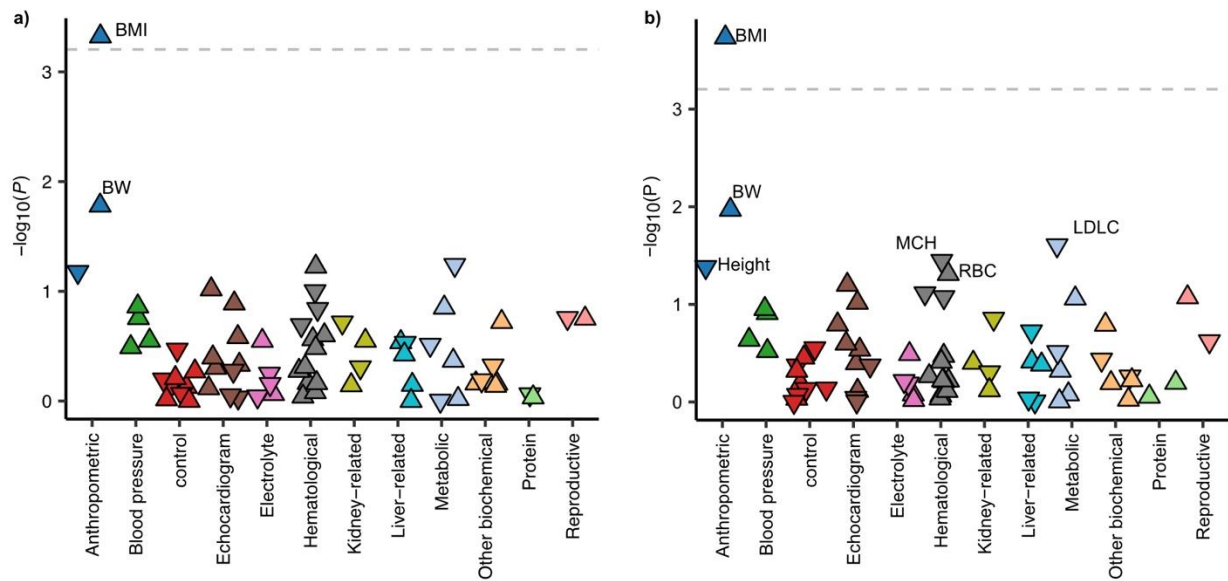
- **Results** (Page 12, Line 281): Among 163,243 individuals from BBJ, who were successfully modeled for their genetic ancestry, the average proportion of the Jomon component is $12.5 \pm 6.3\%$ (mean $\pm$ SD). There is no substantial difference in the Jomon proportion between ages or between genders (**Supplementary Fig. 6**).
- **Methods** (Page 26, Line 622): For individuals who did not support the tripartite model due to the tail probability being below 5%, we sought to identify an alternative dual ancestry model with the highest tail probability, subsequently confirmed through nested p -values >0.05 by comparing the tripartite and dual structure models. If a single ancestry model was plausible, we further assessed whether a specific ancestor alone can adequately explain the individual using the nested p -values.

Comment: 242: Is this 95% CI? Please state.

Response and Modification: Thank you for the comment. This is the mean $\pm$ SD. We have modified the sentence “ $12.5 \pm 6.3\%$ (mean $\pm$ SD)” in **Results**.

Comment: Association between Jomon and 80 traits: lots of power is lost by controlling for PCs, which they have shown are strongly correlated with Jomon ancestry. You could consider controlling for PCs which are not significantly associated with Jomon ancestry? I suspect height would remain statistically significantly associated.

Response: We appreciate the reviewer's suggestion. While it may be argued that our approach is conservative, we prioritized addressing any potential inflation of association signals stemming from population stratification (Berg *et al.* *eLife*. 2019). Therefore, it is crucial to account for its effect by incorporating PCs as covariates, despite the stratification originating from variation in Jomon ancestry across individuals to some degree. To mitigate this issue, we robustly employed an alternative methodology where phenotypes underwent correction by regressing their measures with all covariates, including PCs, before being tested for associations with Jomon ancestry. We observed a significant association in BMI, while none of the other traits reached statistical significance (see **Supplementary Fig. 8**). Taking all results together, our analyses clearly demonstrate the robustness of the BMI signals, regardless of the method used for correcting population stratification (see **Fig. 4** and **Supplementary Fig. 8**). In contrast, height is significant only when PCs are not included as covariates (see **Supplementary Fig. 6**). As the association with height reached nominal significance when including all individuals with Jomon ancestry, it suggests that the effect of Jomon ancestry on height might not be strong enough to be reliably detected with the current sample size.



(new) Supplementary Fig. 8. Associations of Jomon ancestry with phenotypes (80 complex traits) corrected for covariates.

Quantitative measures for the traits are regressed out by covariates including PCs before being tested for associations with Jomon ancestry based on a generalized linear model in **a)** the entire BBJ participants ($n = 163,243$) and **b)** Mainland participants ($n = 152,148$). A gray dashed line represents the statistical significance based on the Bonferroni correction ($p < 0.05/80 = 6.3 \times 10^{-4}$). The traits labeled on the plots are nominally significant in their association with Jomon ancestry at $p < 0.05$. BMI, Body mass index; BW, Body weight; MCH, mean corpuscular hemoglobin; RBC, Red blood cell; LDLC, low-density lipoprotein cholesterol.

Modification: We have added **Supplementary Fig. 8** and modified the sentences as follows:

- **Results** (Page 12, Line 300): While it may be argued that our approach is conservative, we prioritize addressing any potential inflation of association signals stemming from population stratification³⁴. Therefore, it is crucial to account for its effect by incorporating PCs as covariates, despite the stratification originating from variation in Jomon ancestry across individuals to some degree. To mitigate this issue, we also employed an alternative methodology where phenotypes underwent correction by regressing their measures with all covariates, including PCs, before being tested for associations with Jomon ancestry. We observed a significant association in BMI, while none of the other traits reached statistical significance (**Supplementary Fig. 8**, and **Supplementary Table 8** and **9**). Taking all results together, our analyses consistently demonstrate the robustness of the BMI signals, regardless of the methods used for correcting population stratification (see **Fig. 3** and **Supplementary Fig. 8**).
- **Methods** (Page 27, Line 669): To address potential multicollinearity issues between Jomon ancestry and PCs, we employed an alternative approach. Initially, we conducted regression analysis of the quantitative trait measurements on all covariates, including PCs. Subsequently, we tested the associations between Jomon ancestry and the residuals derived from this regression using a linear regression model.

Comment: 265 and throughout: is it justified to equate BMI with obesity? Obesity is merely a description of a subset of the BMI range.

Response and Modification: We agree with the comment and have revised the sentence as follows:

- **Results** (Page 13, Line 325): Overall, these results suggest that the genetic legacy of the ancient hunter-gatherer Jomon significantly influences BMI across populations today, regardless of geographic differences, which may consequently contribute to an increased risk

of obesity.

- **Abstract** (Page 3, Line 64): We also find that the genetic legacy of Jomon ancestry underlies an elevated body mass index (BMI).

Comment: 308-313: need references for the association of rs7572509 with the traits listed. Assume the association data is coming from BBJ? Promising that it replicates in UKB. Also need to reference where the eQTL data is coming from (or reference Methods).

Response and Modification: Thank you for the comment. We have added the references to the sentences.

Ref.29: Sakaue S et al. A cross-population atlas of genetic associations for 220 human phenotypes *Nat. Genet.* **53**:1415-1425 (2021).

Ref. 39: The GTEx Consortium. The GTEx Consortium atlas of genetic regulatory effects across human tissues. *Science* **369**, 1318–1330 (2020).

Ref. 40: Mountjoy, E. et al. An open approach to systematically prioritize causal variants and genes at all published human GWAS trait-associated loci. *Nat. Genet.* **53**, 1527–1533 (2021).

Ref. 41: Ghoussaini, M. et al. Open Targets Genetics: systematic identification of trait-associated genes using large-scale genetics and functional genomics. *Nucleic Acids Res.* **49**, D1311-D1320 (2020).

Comment: 339: How have you derived the five clusters again? Is this projected onto the PCA from BBJ 1st cohort? Or have you just drawn the lines onto the second PCA?

Response and Modification: This is the result from PCA based on 68,632 individuals within the BBJ-2nd cohort, rather than those individuals projected onto the plots based on the BBJ cohort. As explained in our response to the earlier comment, we defined the geographic clusters by visual inspection. However, we are not using those groups from BBJ-2nd for any analysis and have decided to remove the labels of geographic clusters from the PCA plots.

Comment: 382: You should also report effect of Jomon ancestry on obesity in the 669 individuals found to have Jomon ancestry.

Response and Modification: We apologize for the mistake in the phrasing of this sentence and would like to clarify that our analysis focused on examining the effect of Jomon ancestry on BMI, not obesity. We have corrected the sentence as follows:

- **Results** (Page 21, Line 465): Finally, we tested the effect of Jomon ancestry on BMI in UKB EAS.

Comment: 436: State whether you consider this signal to be driven by Ainu likely. Does this impact your claims about Jomon ancestry?

Response and Modification: Thank you for the comments. We admit that we do not have any information on ancestral self-identification of the BBJ participants. To avoid any potential speculation on this, we have removed this sentence. We do not consider this revision will have any impact on the main findings of this study.

Methods:

Comment: 489: how did you exclude individuals based on PCA? More detail needed.

Response and Modification: Based on visual inspection, individuals were excluded if they were positioned far from the Japanese cluster defined within the 1000 Genomes Project (1KG) dataset. We have clarified this point as follows:

- **Methods** (Page 25, Line 572): individuals were excluded if they were positioned far from the

Japanese cluster defined within the 1000 Genomes Project (1KG) dataset in PCA plots using PLINK2 (v2.00a2.3).

Comment: 508: “previously piled up and integrated” -> typo?

Response and Modification: We have modified the sentence as follows:

- **Methods** (Page 25, Line 592): We used ancient genomes from the Japanese archipelago and East Eurasian continent, which had been previously compiled and integrated with the Simons Genome Diversity Project (SGDP) panel (SGDP_Ancient).

Comment: 535: unclear what other ancestry models were used

Response and Modification: Thank you for the comment. In this context, the term “other ancestry models” covers any possible scenarios beyond the tripartite model, which include dual-structure models between all pairs of the three ancestors (*i.e.*, Jomon, Northeast Asian, and East Asian ancestry), as well as single-ancestry models. To make this clearer, we have revised this entire paragraph as follows:

- **Methods** (Page 26, Line 615): We evaluated whether the tripartite structure provides a better fit to the data than any other possible scenarios at population levels based on *p*-values for nested models with a cut-off of 0.05. These alternative models include dual structure models involving any combination of Jomon, Northeast Asian, and East Asian ancestry, as well as single ancestry models. When conducting admixture modeling at individual levels, we considered the tripartite model to be a good fit if (i) a tail probability was >0.05 and/or (ii) estimates of admixture fractions were feasible (*i.e.*, >0.0 and <1.0). The correlation of admixture proportions of the three distinct ancestors was calculated using Pearson’s method. For individuals who did not support the tripartite model, we sought to identify an alternative dual ancestry model with the highest tail probability, subsequently confirmed through nested *p*-values >0.05 by comparing the tripartite and dual structure models. If a single ancestry model was plausible, we further assessed whether a specific ancestor alone can adequately explain the individual using the nested *p*-values.

Comment: 543: how did you selected the 80 trait analysed?

Response: We focused on the traits that have been studied in previous GWAS, with sufficient sample sizes.

Comment: 544-545: why did you use a cut-off of 20yo for smoking and drinking?

Response: Biobank Japan recruited participants from a wide range of ages. Given that Japanese law permits smoking and drinking from age 20, we used this age as the cutoff for analyzing these two habits.

Comment: 572: I can’t see any trait specific covariates listed in ST4

Response and Modification: We apologize for this mistake. We have listed trait-specific covariates in **Supplementary Table 5**.

Comment: 675-676: again, need to state what other models were tested

Response and Modification: We have revised this sentence as follows:

- **Methods** (Page 30, Line 799): We estimated the Jomon proportion for individuals in each cohort by using qpAdm. In cases where individuals did not support the tripartite ancestry structure, we applied the same procedure used for BBJ to identify a plausible model with a two-way admixture, where Jomon served as one of the source ancestors. Jomon component

prediction scores based on 132 independent Jomon-related variants were calculated using PLINK2.

Comment: 679: here, you take an average of measurements whereas for BBJ you take the measurement at first visit to the recruitment centre. Why do you take different approaches?

Response: Thank you for the comment. While UK Biobank has BMI measurements from multiple visits, Biobank Japan has the data only from the first visit. Therefore, we took different approaches to represent BMI at individual levels between these two biobanks.

Tables/Supplementary Information:

Comment: S.Table 1 and S.Table 2 are in separate file from other supplementary tables. This is confusing!

Response and Modification: Thank you for the comment. We have included Supplementary Table 1 and 2 in the same spreadsheet.

Comment: Supplementary Fig. 3.: “threes” -> “three”

Response and Modification: We have revised it accordingly.

Comment: S.Table 5: no P for BMI; no SE values

Response and Modification: We have revised it accordingly.

Response to REVIEWERS' COMMENTS

Response: We thank all three reviewers for their positive feedback and insightful comments. We have carefully addressed each of the additional comments below.

Reviewer #1 (Remarks to the Author):

I appreciate the care taken in revising the manuscript and thank the authors for their detailed responses. I think this paper is a significant contribution for understanding the genetic ancestry of the Biobank Japan cohort and links these insights in a very interesting and innovative way to phenotypic variation.

Response: We sincerely appreciate your recognition of the values of our study.

- The description of ancestry modelling using a previously proposed tripartite ancestry model is now much clearer.

Response: We are glad to hear that the methodological sections are now more accessible.

- The authors have incorporated new analysis to address my concerns about residual confounding driving their signal of Jomon ancestry being associated with BMI. Following careful analysis, it appears as though the association has reduced by quite a bit and is now only marginally significant, which is a bit concerning. As now discussed by the authors in the main text, Jomon ancestry is acting as a weak confounder on the GWAS and inflates the PGS. How can we be sure that all confounding has now been removed? E.g. it could be that your inferred Jomon proportions are a bit noisy and so there is still some leftover confounding.

Response: We would like to take this opportunity to re-emphasize that the association between Jomon ancestry and BMI is robustly significant, rather than marginally so, and that polygenic BMI scores are deflated, rather than inflated when Jomon ancestry is accounted for as a covariate in BMI GWAS.

The robustness of the statistical significance is consistently evident, even after Bonferroni correction, regardless of sex, age (**Supplementary Fig. 7**), regional disparities (**Fig. 4**), and methodological approaches (**Supplementary Fig. 8**). Importantly, we validated this association with UK Biobank.

Furthermore, we demonstrated that Jomon ancestry works as a confounder in BMI GWAS, supporting the validity of the association between Jomon ancestry and BMI. This is evidenced by the deflation of PGS (**Supplementary Fig. 9c**), when comparing individuals' BMI PGS from GWAS with and without correcting for the effect of Jomon ancestry. Therefore, understanding the genetic ancestry of present-day individuals is critical for minimizing confounding effects in GWAS.

- You mention that the top hits for BMI are unchanged even when correcting for Jomon ancestry. This is certainly reassuring and suggests that if you compute the PGS with more stringent p-value cutoff than this should already reduce association in your original analysis as well. Could you check this?

Response: The approach we took to measure polygenic scores, PRS-CS-auto, does not rely on a *p*-value cutoff. Instead, it estimates posterior effect sizes of HapMap3 SNPs using GWAS summaries. Therefore, our comparison of BMI PGS shown in **Supplementary Fig. 9c** accurately represents the differences in estimated effect sizes between GWAS with and without correcting for Jomon ancestry.

- I. 312: Please report the updated beta and p-value of association after correcting for Jomon ancestry.

Response and Modification: Thank you for the suggestion. We have provided a list of the *p*-values, beta values, and standard errors for 94 SNPs that are significant in BMI-GWAS with or without the correction of Jomon ancestry (**Supplementary Table 11**).

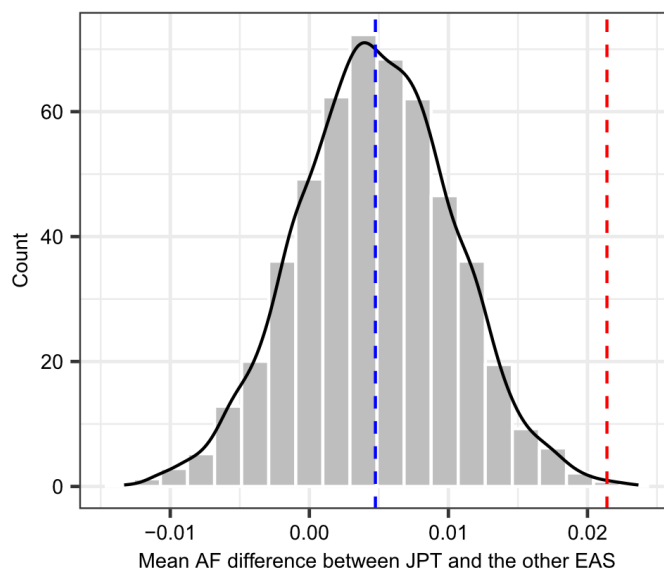
- It is interesting that 13% of the variance in Jomon ancestry is explained by the Jomon variants that were identified. I agree with the authors that this is a notable proportion and appreciate the added analyses to emphasise this.

Response: Thank you for the positive feedback. We appreciate your recognition of the significance of this finding.

- It does pose the question of why this is the case. As in my original review, I think the current manuscript is a bit weak in providing evolutionary interpretations on this phenomenon. Presumably this happens because there is strong drift in Jomon, and/or lots of unique mutations in Jomon that tag Jomon haplotypes. This must be unusual though, typically if ancestry is defined by small differences in allele frequency genome-wide than this type of approach should not work so well. Some simple summary statistics, such as the global frequencies of these Jomon variants, would be helpful in interpreting this phenomenon.

Response: We thank the reviewer for the insightful comment. Allele frequencies of the Jomon-related variants are presented in **Table 1** and **Supplementary Table 12**. In this revision, we have demonstrated that the frequencies of Jomon-related variants are systematically shifted towards higher frequencies in the Japanese population compared with those in the continental East Asian populations ($p = 0.001$; **Supplementary Fig. 11** attached below). Our previous studies have shown that the very small population size of the Jomon (~1,000), coupled with many thousands of years of insular isolation, resulted in strong linkage disequilibrium throughout their genomes due to strong genetic drift (Cooke *et al.* 2021 **Science Advances**; Cook *et al.* 2024 **iScience**). This history explains why the Jomon-related variants exhibit significantly longer haplotypes than those linked with non-Jomon-related variants (**Fig. 5b**). Overall, the patterns of allele frequencies and haplotypes provide strong evidence that the Jomon-related variants likely tag genomic segments descended from the Jomon.

These Jomon-derived segments are significantly enriched with active functions of skeletal muscle cells (**Fig. 5a**), supporting the idea that the Jomon experienced strong selective pressures due to the high physical activity essential for hunting and gathering.



Supplementary Fig 11. Mean allele frequency difference of Jomon-related and non-Jomon related variants between JPT and the other East Asian populations within the 1000 Genome dataset.

A histogram shows the distribution of mean frequency difference for 5,000 sets of randomly sampled 132 non-Jomon-related SNPs, matched by the frequencies of Jomon-related SNPs. A blue dashed line represents the average of the distribution, while a red line indicates the mean AF difference observed in the Jomon-related variants.

Modification: We added **Supplementary Fig. 11** and modified the sentences as follows:

- **Results (Page 15, Line X):** These Jomon-related variants are significantly more frequent in JPT compared to the other East Asian populations within the 1KG dataset (**Table1**, **Supplementary Fig. 11**, and **Supplementary Table 12**). Even within the BBJ population, the Ryukyu group exhibits higher frequencies of these variants than the mainland group, supporting their strong linkage with the Jomon-derived segments.
- **Discussion (Page 24, Line):** Given that the set of genetic variants associated with the proportion of Jomon ancestry likely tags genomic segments descended from the Jomon, they could serve as ancestral informative markers (AIMs), offering a convenient way to predict an individual's Jomon ancestry. We demonstrate the predictive power of these markers by leveraging three additional validation cohorts. Furthermore, the Jomon-related variants are significantly enriched with active functions in skeletal muscle cells, some of which are also associated with increased BMI, body weight, and height. These findings suggest potential adaptations related to the high physical activity required for hunting and gathering lifestyles⁴⁷,

as supported by selection scans in the Jomon showing that the top variants are associated with increased bone mineral density³⁷. However, the genetic legacy of this selection may now pose a risk factor for elevated levels of BMI through the interaction with modern environments. It is important to note that further analysis with post-Jomon genomes (e.g. Yayoi or Kofun genomes) is crucial to uncover how Jomon segments have been inherited by descendants as lifestyles transformed from hunting and gathering to farming.

- **Methods (Page 28, Line):** We then identified 132 non-Jomon-related variants matching allele frequencies of the Jomon-related variants using a nearest neighbor matching method, contrasting the Jomon-related and non-Jomon-related variants by examining their frequency differences between the Japanese and continental East Asian populations, as well as their haplotypic lengths with LD greater than 0.8.

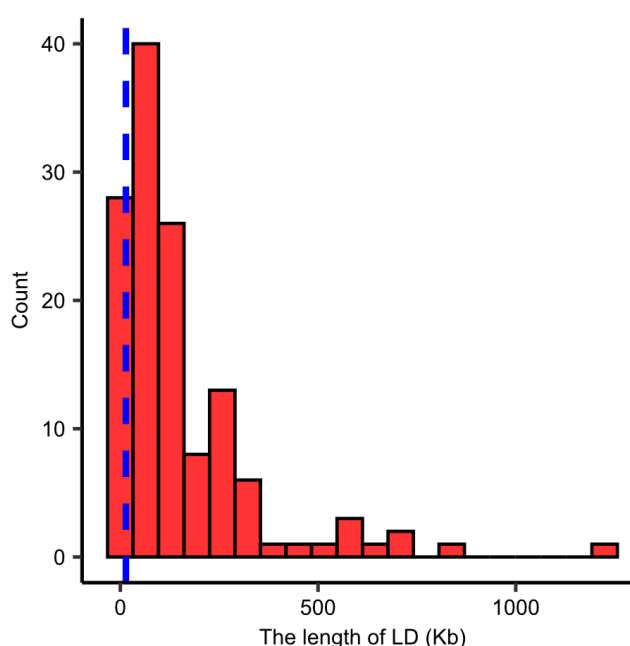
Reviewer #2 (Remarks to the Author):

Thank you for your sincere responses and additional analyses in addressing my comments and questions. The manuscript now presents a clearer message regarding the contribution of Jomon ancestry to a particular phenotype related to BMI. It is particularly noteworthy to detect extended haplotypes for Jomon-related variants, supporting the potential for positive selection.

Response: We appreciate the reviewer's thoughtful comments. Please find our responses to the additional comments below.

1. Among the 132 independent Jomon-related variants, how many exhibit significantly longer haplotypes compared to non-Jomon-related variants? Additionally, among these longer haplotypes, are any specifically associated with BMI? This examination is crucial for understanding the potential positive selection on this phenotype introduced by Jomon admixture into the contemporary Japanese population.

Response: Thank you for the comment. We have plotted a histogram of Jomon-related variants along with their haplotype lengths, defined by $R^2 > 0.8$ (see below **Response Figure**). 123 out of 132 Jomon-related variants (>90%) exhibit longer haplotypes compared with the mean haplotype length of non-Jomon-related variants (14 kb). The information on the haplotype lengths of the Jomon-related variants has been added to **Supplementary Table 12**. With regard to the second question, as shown in **Supplementary Table 12**, five Jomon-related variants are associated with BMI or Body weight.



Response Figure. A length distribution of haplotypes tagged by the 132 Jomon-related variants.

Haplotypes are defined by linkage disequilibrium with $R^2 > 0.8$. A blue dashed line represents the mean of haplotype lengths across 132 non-Jomon-related variants, matched by the frequencies of the Jomon-related variants.

2. Without clear evidence, it is challenging to comprehend why only BMI is significantly associated with Jomon ancestry among other traits (as shown in Fig 4), considering that Jomon admixture would occur randomly across the genome. This point also pertains to the conclusion that increasing BMI in the Japanese population is introduced by Jomon ancestry.

Response: Thank you for the comment. To address this question, it is important to understand selective pressures imposed on the Jomon. Given that continental immigrants began arriving in Japan only ~3,000 years ago, it is not surprising that Jomon ancestry remains evident in the genomes of present-day Japanese populations. Our stratified linkage disequilibrium score regression shows that Jomon-related variants are significantly enriched with active functions in skeletal muscle cells (Fig. 5a), some of which are also associated with BMI (Table 1; Supplementary Table 12). The reason BMI stands out in our analysis is primarily because the Jomon were exposed to strong selective pressures driven by the high level of physical activity that was required for their hunting and gathering lifestyle. However, under modern environments, the genetic legacy of this selection may now pose a risk factor for elevated levels of BMI.

3. In modern populations, a high BMI is generally considered a risk factor. While this knowledge can positively impact the interpretation of GWAS results, it may also lead to negative perceptions of Jomon admixture among the public. Therefore, it becomes important to reveal the evolutionary story of this trait's adaptation, not only for the Jomon but also for the Yayoi populations. Alternatively, the authors could describe the potential positive influences of high BMI in certain contexts.

Response: Thank you for the suggestions. As detailed in our response to the previous comment, we consider that this association highlights the critical role of skeletal muscle functions for the Jomon due to their hunting-gathering lifestyle. This is further supported by our recent work on selection scans in the Jomon, which has identified selection signatures from variants associated with the increase of bone mineral density (Cooke *et al.* 2024 *iScience*). Therefore, our study provides evidence of how the past action of natural selection has shaped present-day disease risks, primarily due to rapid changes in diet and societal lifestyle. To reveal this trait adaptation in more detail, variable ancestral analyses including Yayoi-derived variants are warranted in the future study.

Modification: To reflect the comment, we modified the sentences as follows

- **Discussion (Page 24, Line):** Given that the set of genetic variants associated with the proportion of Jomon ancestry likely tags genomic segments descended from the Jomon, they could serve as ancestral informative markers (AIMs), offering a convenient way to predict an individual's Jomon ancestry. We demonstrate the predictive power of these markers by leveraging three additional validation cohorts. Furthermore, the Jomon-related variants are significantly enriched with active functions in skeletal muscle cells, some of which are also associated with increased BMI, body weight, and height. These findings suggest potential adaptations related to the high physical activity required for hunting and gathering lifestyles⁴⁷, as supported by selection scans in the Jomon showing that the top variants are associated

with increased bone mineral density³⁷. However, the genetic legacy of this selection may now pose a risk factor for elevated levels of BMI through the interaction with modern environments. It is important to note that further analysis with post-Jomon genomes (e.g. Yayoi or Kofun genomes) is crucial to uncover how Jomon segments have been inherited by descendants as lifestyles transformed from hunting and gathering to farming.

By addressing these points, the manuscript will emphasize novel finding and provide a more comprehensive understanding of the evolutionary implications and public health perspectives of BMI-related Jomon ancestry in the contemporary Japanese population.

Reviewer #3 (Remarks to the Author):

I thank the authors for their comprehensive response to my comments. They have fully addressed all my points, and updated the manuscript and supplementary information accordingly. In my view this paper now reaches the standards expected by the journal, and provides an important contribution to the field.

Response: Thank you very much for your kind words and for taking the time to review our manuscript. We are delighted to hear that our responses to your comments have addressed your concerns and that the updated manuscript meets the journal's standards. We greatly appreciate your recognition of our work and your acknowledgment of its contribution to the field.

Response to REVIEWERS' COMMENTS

Response: We thank both reviewers for their careful reading of our revised manuscript. We have addressed the additional comments and revised the manuscript accordingly. Below, we provide our response to each comment.

Reviewer #1 (Remarks to the Author):

Thank you for your responses and overall I am happy with all the revisions made and think this is an important contribution.

I very much appreciate all the robustness checks done for the association between Jomon ancestry and BMI.

It is encouraging that the signal is robust to excluding people from Okinawa, and age/sex effects. Some association between PGS and Jomon ancestry remains after correcting for Jomon ancestry in the GWAS and so overall I would say I believe your result. But I am still confused by your interpretation of the result when correcting for Jomon proportions and perhaps you can include a clarifying sentence around the paragraph starting in line 311:

In my understanding, ideally you want the *true effect sizes* for BMI at every SNP, and then compute PGS and correlate to Jomon ancestry. Now we of course don't have true effect sizes and so have to estimate these using GWAS. You get very slightly different effect sizes depending on whether you incorporate Jomon ancestry as a covariate, and as you mention in the main text and your response you say

"Jomon ancestry acts as a confounding factor in estimating effect sizes for BMI".

In fact you say "Jomon ancestry works as a confounder in BMI GWAS, supporting the validity of the association between Jomon ancestry and BMI". That doesn't make sense to me, you could make the same argument for any of the covariates but it would usually be false? Or at least it wouldn't be a genetic association. Are you saying there are real interactions between global Jomon ancestry and the true effect size? I'm not sure that seems plausible. More plausibly, the pre-Jomon corrected effect sizes are somewhat biased due to some residual confounding.

Response: We would like to thank the reviewer's suggestion and have revised the manuscript accordingly.

Modification (Lines 311-320):

To assess the impact of Jomon ancestry on BMI, we incorporated the proportion of Jomon ancestry as a covariate in our genome-wide association study (GWAS) for BMI (**Supplementary Fig. 9a** and **Supplementary Table 11**). While the majority of lead SNPs remain consistent, we observed a reduction in the effect of Jomon ancestry on polygenic scores (PGS) of BMI (**Supplementary Fig. 9b**). It is worth noting that this effect persists to some extent, indicating a functional correlation between Jomon ancestry and BMI-PGS. Still, the PGS tends to be inflated when Jomon ancestry is not accounted for in GWAS (**Supplementary Fig. 9c**), with the degree of inflation weakly correlating with the proportion of Jomon ancestry (**Supplementary Fig. 9d**). **These findings suggest that the**

effect sizes estimated from GWAS without including Jomon ancestry as a covariate may be biased due to residual confounding.

Reviewer #2 (Remarks to the Author):

Thank you so much for the authors' sincere responses.

I appreciate the revision.

The authors made a great point in the rebuttal rather than the revised text in the discussion: "Our study provides evidence of how the past action of natural selection has shaped present-day disease risks, primarily due to rapid changes in diet and societal lifestyle."

I believe the statement can be in the main text.

I have no more comments on the manuscript.

Response: Thank you for your positive feedback on our responses. We have incorporated the sentence in the Discussion section of the revised manuscript.

Modification (Lines 538-551):

Given that the set of genetic variants associated with the proportion of Jomon ancestry likely tags genomic segments descended from the Jomon, they could serve as ancestry informative markers (AIMs), offering a convenient way to predict an individual's Jomon ancestry. We demonstrate the predictive power of these markers by leveraging three additional validation cohorts. Furthermore, the Jomon-related variants are significantly enriched with active functions in skeletal muscle cells, some of which are also associated with increased BMI, body weight, and height. These findings suggest potential adaptations related to the high physical activity required for hunting and gathering lifestyles⁴⁷, as supported by selection scans in the Jomon showing that the top variants are associated with increased bone mineral density³⁷. However, the genetic legacy of this selection may now pose a risk factor for elevated levels of BMI through the interaction with modern environments. **Our study provides evidence of how the past action of natural selection has shaped present-day disease risks, primarily due to rapid changes in diet and societal lifestyle.** It is important to note that further analysis with post-Jomon genomes (e.g. Yayoi or Kofun genomes) is crucial to uncover how Jomon segments have been inherited by descendants as lifestyles transformed from hunting and gathering to farming.