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Northeastern Asian and Jomon-related genetic structure in the Three Kingdoms period of Gimhae, Korea

Highlights

- Eight 1,700-year-old ancient Korean genomes were sequenced to the depths of 0.7×–6×
- The genetic diversity in the Gaya Kingdom region is linked to Jomon-related ancestry
- The genetic diversity of the eight genomes is not linked to social status
- There is substantial genetic continuity in Korea since the TK period

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In brief

Gelabert et al. report the first paleogenomic data from the Korean Three Kingdoms period. All individuals are modeled as an admixture between a northern-China Bronze Age genetic source and a Jomon-related ancestry. Phenotypically relevant SNPs show genetic continuity with modern Koreans.

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Northeastern Asian and Jomon-related genetic structure in the Three Kingdoms period of Gimhae, Korea

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SUMMARY

The genetic history of prehistoric and protohistoric Korean populations is not well understood because only a small number of ancient genomes are available. Here, we report the first paleogenomic data from the Korean Three Kingdoms period, a crucial point in the cultural and historic formation of Korea. These data comprise eight shotgun-sequenced genomes from ancient Korea (0.7×–6.1× coverage). They were derived from two archeological sites in Gimhae: the Yuha-ri shell mound and the Daesung-dong tumuli, the latter being the most important funerary complex of the Gaya confederacy. All individuals are from between the 4th and 5th century CE and are best modeled as an admixture between a northern China Bronze Age genetic source and a source of Jomon-related ancestry that shares similarities with the present-day genomes from Japan. The observed substructure and proportion of Jomon-related ancestry suggest the presence of two genetic groups within the population and diversity among the Gaya population. We could not correlate the genomic differences between these two groups with either social status or sex. All the ancient individuals' genomic profiles, including phenotypically relevant SNPs associated with hair and eye color, facial morphology, and myopia, imply strong genetic and phenotypic continuity with modern Koreans for the last 1,700 years.

INTRODUCTION

Recent studies reveal that East Asian populations have genetic continuity that can be traced back to the Neolithic.[1–8] Present-day Koreans are an East Asian group with distinct genetic and linguistic traits. The Korean language is often classified as an isolate, having no other closely related modern language,[9] and might have been introduced to the Korean peninsula by millet farmers during the Neolithic.[10] A recent study of the genomes of 1,094 present-day Koreans indicates that they are a genetically homogeneous group[11] due to genetic drift resulting from possible isolation during the last centuries. However, Korean maternal and paternal markers suggest that present-day Korean genetic structure was formed through a process that involved several past admixture events,[11,12] with research showing that modern Korean genomes can be modeled as a combination of ancestries belonging to ancient populations from the West Liao River (China) and the Japanese archipelago or, alternatively, from the admixture between Miaozigou_MN-Japan_Jomon.[1] Several recent low coverage captured genomes

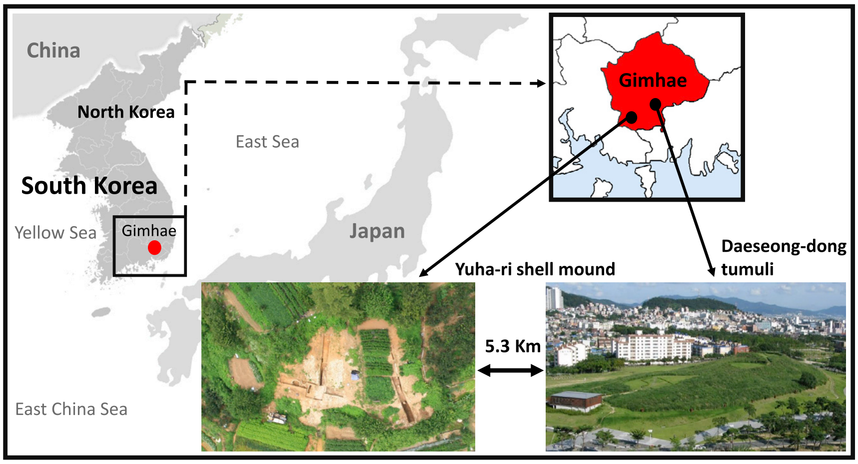

Figure 1. The sites of the Three Kingdoms period analyzed in this study

Geographical location and images of the sites of Daeseong-dong and Yuha-ri sites. The image of the Yuha-ri shell mound aerial view and the panoramic view of Daeseong-dong tumuli show a general perspective of the archeological sites.

See also Figure S1 and Data S1.

from Neolithic and Bronze Age Korea[10] suggest a significant level of dilution of this Jomon-related ancestry, which happened in Korea during the Bronze Age, akin to what is observed in the Kofun people from Japan, who are contemporary with the Three Kingdoms (TK) period (57 BCE–668 CE) in Korea.[13] However, a major knowledge gap remains due to the lack of ancient DNA studies on Koreans from the TK period, when the Korean ethnicity and culture were formed through the unification of all TK (Goguryeo, Baekje, and Silla) by the Silla Kingdom. This was then succeeded by the Korea Kingdom, which endowed the present-day Korean national identity.

Agriculturally complex societies emerged in Korea during the Bronze Age (1,400–300 BCE).[14] Both the genetic study of rice[14] and archeological fieldwork[10,15] suggest that rice and various legumes were introduced from the Liao River region with their cultivation subsequently intensifying in the Korean peninsula around 1,300–800 BCE.[10,15] From Korea, rice spread to the Japanese archipelago[10,16] together with the genetic ancestries related to Bronze Age northeast Asian populations.[13,16] In parallel, iron production arrived in Korea from China during the 4th century BCE[17,18] and then expanded to Japan from Korea during Japan's Yayoi period (10th century BCE–3rd century CE).[19] However, it is unclear whether the spread of iron technology in the Korean peninsula was accompanied by human migration or was transferred only via trade and cultural diffusion (STAR Methods). Recently analyzed ancient genomes from the Neolithic and Bronze Age periods from Korea[10] and the Japanese Kofun period[13] suggest a notable dilution of Jomon-related ancestry in both regions during the 4th–7th centuries CE.

The TK period of Korea saw the extensive development of iron technology and trade with neighboring populations during which time the Korean peninsula was ruled by the aforementioned TK.[17] The Gaya confederacy, which was the last independent territory, lasted until the 6th century CE before being assimilated by neighboring Silla.[20] Gaya had the most developed iron production and trading infrastructure during the early Korean TK period, which included the exchange of goods, and possibly also the movement of people, such as the Wa (Wae) inhabitants of the Japanese archipelago and northern China.[21] This suggests that Gimhae, the political center of Gaya, was an important trading center, and as such its populace may have included a level of cosmopolitanism, similar to that found in a recent genetic study of the ancient inhabitants of Rome.[22]

At the end of the 3rd century CE, archeological sites from the Gimhae area exhibit changes in funerary rituals that include the replacement of stone-lined pit tombs and jar coffin tombs with wooden-chambered burials.[23] Also introduced during this period was a mortuary practice that involved human sacrifices (STAR Methods), the inclusion of grave goods such as weapons, mirrors, and other items made of bronze and iron, and severed heads of livestock that accompanied the deceased.[21] The most important excavated center of the Gaya culture is a massive 3,700 m^2 burial complex of rulers in Daeseong-dong in Gimhae, dated to the 1st–5th centuries CE. It consists of 219 tombs, 69 of which are complexes with multiple burials that include human sacrifices and various mortuary goods such as pottery, iron armors, and items related to archery.[24,25] More modest burials are found throughout the TK period in shell mounds in the same Gyungsangnamdo province, including areas such as Busan and Gimhae. None of these shell mounds include human sacrifices or a family grave rather a single burial.[26]

RESULTS

We screened 27 petrous bones or teeth obtained from 22 individuals from two archeological sites in Gimhae City, Gyeongsangnam-do: Daeseong-dong and Yuha-ri, both dated to the 4^{th}–5^{th} centuries CE (Figure 1; Figures S1A–S1D).[27] The genomic libraries of eight individuals had over 7% of sequencing reads that aligned to the human reference genome (range from 7.3% to 68.9%; Data S1A), with whole-genome coverage depths between 0.7× and 6.1× (Table 1). All seven Daeseong-dong individuals were associated with specific mortuary practices linked to social status, specifically, main burials (grave owners) and human sacrifices (STAR Methods). Only the single grave of a child (AKG_3420) from the Yuha-ri shell mound (Table 1; Data S1A; Figures S1B and S1C) could not be assigned any social status due to the lack of mortuary elements. All eight genomes exhibited typical deamination patterns[28] at read ends, with deamination rates ranging between 12% and 33% (Data S1A). Five of the individuals were identified as females and three as males based on sex chromosomes and autosome depth coverage ratios (Data S1A). We generated two base-calling sets with these genomes: first, we called pseudo haploid positions of the 1,240k dataset[29] (a standard call set that is used in most downstream analyses involving ancient samples), and second, we generated imputed diploid calls that were used to identify

Table 1. Main descriptive information of the eight Korean Three Kingdoms period individuals

Individual	Cluster	mtDNA haplogroup	Y- haplogroup	Date	Typology	Cover age
AKG_10203 (♂)	Korea-TK_2	D4e2	D1a2a1	350–500 CE	Daeseong-dong -burial	1.58×
AKG_10204 (♂)	Korea-TK_1	D4e2a	O1b2a1a2a1b1	350–500 CE	Daeseong-dong -sacrifice	0.79×
AKG_10207 (♀)	Korea-TK_2	B4c1a1a1a	–	350–500 CE	Daeseong-dong -sacrifice	6.11×
AKG_10209 (♀)	Korea-TK_1	M10a1b	–	350–500 CE	Daeseong-dong -sacrifice	1.78×
AKG_10210 (♀)	Korea-TK_1	F1b1a1a1	–	350–500 CE	Daeseong-dong -sacrifice	2.72×
AKG_10218 (♂)	Korea-TK_1	D4a1	O	300–500 CE	Daeseong-dong -burial	0.64×
AKG_3420 (♀)	Korea-TK_1	D4a1	–	300–500 CE	Yuha-ri shell mound	3.20×
AKG_3421 (♀)	Korea-TK_1	D5a2a1a1	–	300–500 CE	Daeseong-dong -sacrifice	0.74×

See also Data S1.

haplotype-based connections with present-day populations, following previously described methodologies.[30]

There were no identifiable close familial relationships among the sequenced individuals, given the normalized proportion of non-matching alleles (P0) being greater than 0.906, indicating no relationship as close as second degree.[31] Two applied runs of homozygosity (ROH)-calling methods indicate that the ancient Korean Gimhae genomes (AKGs) show only a small fraction of the genome being homozygous (Figures S2A and S2B). All the AKG individuals show values located within the diversity of present-day populations. From the Korean individuals, AKG_10207 shows the highest amount of long ROH, pointing to more recent inbreeding.

Uniparental markers

All individuals had typical East Asian mitochondrial DNA (mtDNA) haplogroups (Table 1; Data S1A), D (n = 5), B, F, and M, when we determined the haplogroups from the consensus. All of these prevail among present-day Koreans,[9,12] with the most common being haplogroup D4, which we identified in four of the eight AKGs. This haplogroup was also common among ancient Japanese Yayoi farmers but was absent in the Jomon.[32] Out of the three male individuals, we successfully called Y chromosome haplogroups (Table 1; Data S1A) for two: AKG_10203 (D1a2a1) and AKG_10204 (O1b2a1a2a1b1). The third male, AKG_10218, due to his lower coverage, was assigned to the major haplogroup O (Table 1; Data S1A). Haplogroup O is the most common Y haplogroup in present-day Korea and is shared by more than 73% of Korean males,[9] whereas haplogroup D is more common in the present-day Japanese population.[33]

Population genetics

A principal component analysis (PCA) was carried out using modern East Asian individuals restricted to positions from the human origins (HOs)[30] panel of SNPs (Data S1B). The eight Gaya individuals were projected onto their principal components (PCs) alongside other previously published ancient individuals.[1–8,10,13,34–36] A list of the individuals used for population genetic analysis and their groupings and labels can be found in Data S1B. The results showed the presence of four differentiated genetic groups in ancient East Asia: (1) Amur River, (2) northern China, (3) southern China and Taiwan, and (4) Jomon-related populations (Figure 2A; Data S1B). The eight individuals from the Korean TK period are positioned within the diversity of East Asian individuals, particularly close to present-day Koreans and Japanese, ancient Kofun from Japan, and several Neolithic Koreans (Figure 2A). However, two individuals (AKG_10203 and AKG_10207) clustered rather closely with present-day Japanese and closer to Japanese Jomon individuals (Figure 2A), a pattern that distinguishes them from the other six individuals. Next, we further explored the ancestral compositions of the eight individuals using a pairwise qpWave analysis[37] to formally identify individuals who might not form a clade with the others. When using a set of ten outgroup populations described in the STAR Methods section, which included the ancient Japanese Jomon, most individuals formed a clade with each other. However, AKG_10203 and AKG_10207 did not form a clade with any of the remaining individuals. Therefore, we separated the eight TK individuals into two groups: (1) the two outliers (Korea-TK_2) and (2) the remaining six individuals (Korea-TK_1) (Figure S2C). One individual from the Korea-TK_1 cluster (AKG_3421) is positioned slightly toward Korea-TK_2 in the PCA and does not form a clade with the five other individuals from Korea-TK_1 (Figure 2A). This individual is nevertheless grouped as part of Korea-TK_1, as all statistics f_4(Mbuti, Japan_Jomon; AKG_3421, Korea-TK_1_individual) for each individual within Korea-TK_1 have a Z score below |2.45|. The same pattern is observed when comparing this individual with Yellow_River_LBIA. None of the comparisons show a Z score higher than |2.59| (Data S1C). Lastly, the observed clustering pattern could not be linked to mortuary status, as there are individuals within both Korea-TK_1 and Korea-TK_2 that were part of either grave owners or sacrificial burials (Table 1).

Next, we assessed the genetic structure of the Gimhae Korean individuals with ADMIXTURE,[38] which included 2,283 present-day and ancient individuals (Figure S3), and a pruned SNP set

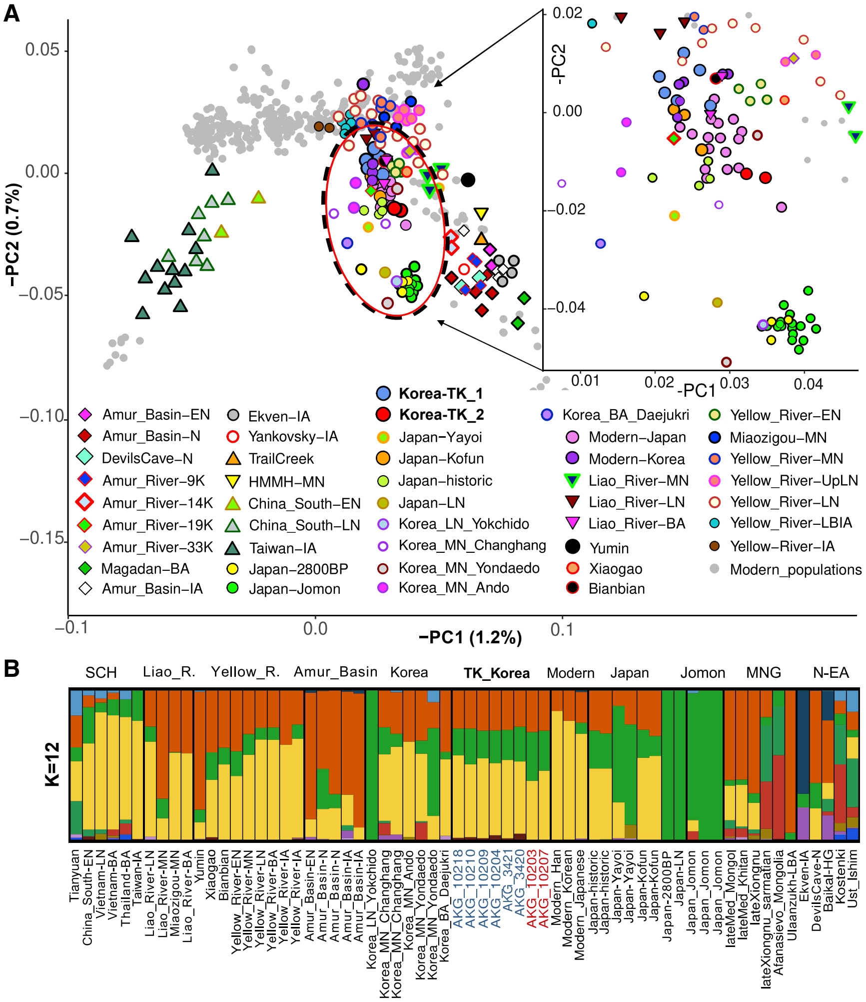

Figure 2. Three Kingdoms period Korean genomic context

(A) Principal components analysis performed with present-day East Asian populations and 96 projected ancient individuals, including eight TK period Korean samples. We used principal components, PC1 and PC2, that account for (1.2%) and (0.7%) variation, respectively.

(B) ADMIXTURE plot (K = 12) of the most representative populations used in the analyses show a shared genomic profile across ancient and modern East Asians, including TK Koreans and present-day Koreans.

See also Figures S2 and S3 and Data S1.

of the HO dataset (extended methods). At K = 12, the Korea-TK individuals are best represented by three main genetic components: two shared with the majority of other ancient and modern East Asian populations, and the third represented mostly as the Japanese Jomon (Figure 2B; Figure S3). The analysis also suggests that the Korea-TK_2 individuals, AKG_10203 and AKG_10207, may have more Jomon-related ancestry than the other six TK individuals. This component is absent in present-day Koreans, but it is present among present-day Japanese.

Differential genetic affinities of the Three-Kingdoms-period Koreans

We computed outgroup f_3 statistics that measure the amount of shared genetic similarity between populations by comparing Korea-TK_1 and Korea-TK_2 with ancient (Data S1D) and present-day populations (Figures 3A and 3B; Data S1D). The results indicate that Korea-TK_1 shares the highest affinity to modern Korean, Japanese, and present-day Han populations and ancient populations from the Liao and Yellow Rivers, as well as Kofun. Korea-TK_2, however, appears to be closer to the present-day Japanese, the ancient Kofun, and other ancient Japanese populations with Jomon ancestry (Figures 3A and 3B; Data S1D).

In order to observe differential affinities between Korea-TK_1 and Korea-TK_2, we calculated the statistic f_4(Mbuti,Pop A; Korea-TK_1, Korea-TK_2), generating 30 combinations with the highest |value| (Figure 3C; Data S1C). We observed a closer relationship of Korea-TK_1 to the populations from the Neolithic and Bronze Age Liao and Yellow rivers in northern China and other historical populations from Nepal, Vietnam, and China. We identified allelic share between Korea-TK_2 and ancient populations from Korea, Japan, and the Amur River basin. However, the test f_4(Mbuti, Korea-TK_1; Yellow-River_LBIA, Liao-River_BA) yielded statistically nonsignificant results ($Z < |3|$), and, therefore, we cannot detect a clear source for the north Asian Bronze Age ancestry, which follows the same pattern of f_4(Mbuti, X; Yellow-River_LBIA, Liao-River_BA), where X are the different Korean Neolithic individuals from Robbeets et al.[10] (Data S1C), and with no excessive allele sharing toward these two populations ($Z < |3|$). Although Korea-TK_2 has significantly more Japan_Jomon-related ancestry than Korea-TK_1 (f_4(Mbuti, Japan_Jomon; Korea-TK_1, Korea-TK_2), Z = 14.31), the nonsignificant statistic f_4(Mbuti, Korea-TK_2; Korea-TK_1, Japan_Jomon) (Z = 1.40) suggests that Korea-TK_2 does not share statistically more derived alleles with Korea-TK_1 or Japan_Jomon. Additionally, we observe significantly more gene flow between Korea-TK_1 and Liao_River-BA/Yellow_River-LBIA over Korea_MN (Z = |3.38| and |4.37|, respectively). Neither test is significant for Korea-TK_2 (|1.29| and |0.97| respectively) (Data S1C). Taking into consideration the low coverage of the ancient Korean individuals from Robbeets et al.,[10] we merged three Middle Neolithic individuals with the highest sequence coverage from the sites of Yeondaedo and Gadeokdo (Changhang) islands with up to 20% Jomon-related ancestry (GDI002, GDI008, and TYD007) as Korea_MN to reduce bias related to data sparsity. We also included individual TYJ001 from Korea_LN_Yokchido, which has more Jomon ancestry in Robbeets et al. analyses.[10] The remaining samples from this study[10] were not included, as they all have low coverage. Furthermore, Korea-TK_1 is more closely related to Korea_MN

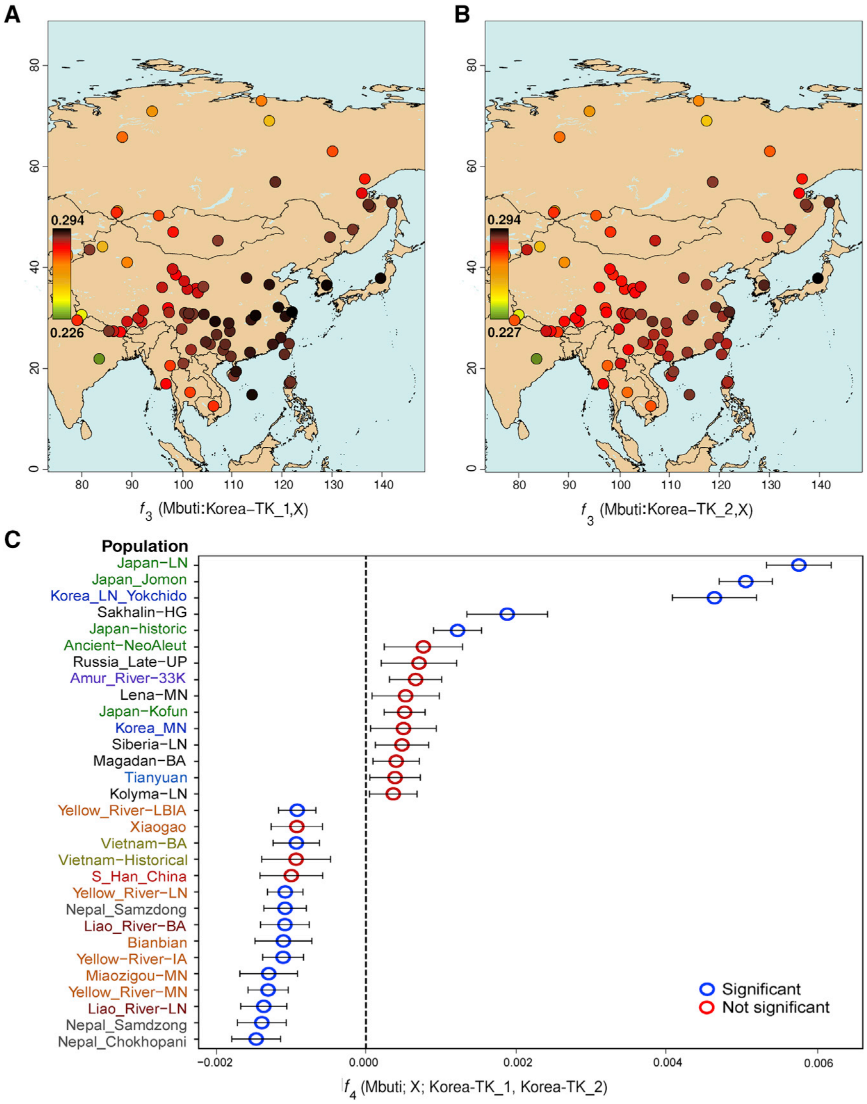

Figure 3. *f*-statistics

(A and B) f_3-statistics of present-day Asian populations with Korea-TK populations Korea-TK_1 (A) and Korea-TK_2 (B) in the combination f_3(Mbuti; Korea-TK,X). Korea-TK_2 has more affinity to the Japanese than to Koreans. Korea-TK_1 is associated with populations from Korea, Japan, and eastern China, whereas Korea-TK_2 has the closest affinity to the Japanese.

(C) f_4-statistics emphasize differential genetic affinities of Korea-TK_1 and Korea-TK_2 using the static *f_4(Mbuti, X; Korea-TK_2, Korea-TK_1)*. Korea-TK_2 has the closest affinity to the ancient Japanese populations with substantial Jomon ancestry, and Korea-TK_1 shows affinity to Liao and Yellow River Bronze Age populations. The colors in the sample name denote the region as follows: dark red, Liao River; orange, Yellow River; red, Southern China and Taiwan; purple, Amur Basin; gray, Nepal; black, Russia (Russian Far East, Siberia); light brown, Vietnam; and green, Japan.

than to Jomon-related ancestry (f_4(Mbuti, Korea-TK_1; Korea_MN, Japan_Jomon), Z = −8.10), whereas Korea-TK_2 has the inverse correlation (Z = 2.30). When compared with Korea_LN_Yokchido, both groups have more shared derived SNPs with Korea_LN_Yokchido, without being significant (−0.000960, Z = −2.50 for Korea-TK_1 and −0.000347, Z = −0.76 for Korea-TK_2). The little difference in this case is probably linked with the larger Jomon affinity of Korea_LN_Yokchido.

Lineage analyses based on ancestry composition

We applied qpAdm[37] to investigate the overall ancestry composition of the two groups and to confirm the observed Jomon-related ancestry. We used a total of ten possible sources that were close to Korea-TK, both geographically and temporally. We also included Korea_MN from Robbeets et al.[10] as the only source of pre-TK Korean ancestry due to the fact that most ancient Korean genomic data from Robbeets et al. do not pass our qpAdm threshold of 150,000 SNPs; therefore, they were excluded from this analysis to ensure unbiased and more confident admixture models. Applying a two-step model competition approach,[39] Korea-TK_1 could only be modeled as composed of 93% ± 6% ancestry related to Yellow_River_LBIA and 7% ± 6% related to Japan_Jomon (p = 0.42), whereas Korea-TK_2 could be modeled using a single model as Korea_MN (p = 0.06) (Figure 4; Data S1E). To quantify the amount of Jomon-related ancestry in Korea-TK_2 and how it compares with Korea_MN, we modeled both using the same sources as above, but this time with Korea_MN as one of the test populations as well. The results show that Korea-TK_2 and Korea_MN could be modeled with Liao_River-BA as the proxy for northern China ancestry (66% ± 7% and 70% ± 8%, respectively), with the remaining ancestry from a Jomon-related source (34% ± 7% and 30% ± 8%, respectively) (Data S1E). The amount of Jomon-related ancestry in Korea_MN is, therefore, about twice as much as the percentage reported in the original publication (average of Yeondaedo and Changhang individuals ∼15%[10]) but is arguably a more powerful estimate due to the substantial increase in data used as the result of merging these individuals into a single population. In order to compare our genomes with the previously reported analyses on Japanese ancient individuals,[13] we repeated the qpAdm analysis using the same right and left set (Data S1E), which indicates that Korea-TK_1 can be modeled as 28% NEasia, 63% Han, and 8% Jomon. Korea-TK_2 can be modeled as 32% NEasia, 43% Han, and 25% Japan Jomon. In comparison with the contemporary Japan_Kofun, Korea-TK_2 shows more Jomon ancestry than Japan_Kofun, which is compensated by less Han ancestry.[13]

Based on the evidence that Korea-TK_2 could be modeled as a single source as Korea_MN, suggesting genetic continuity in

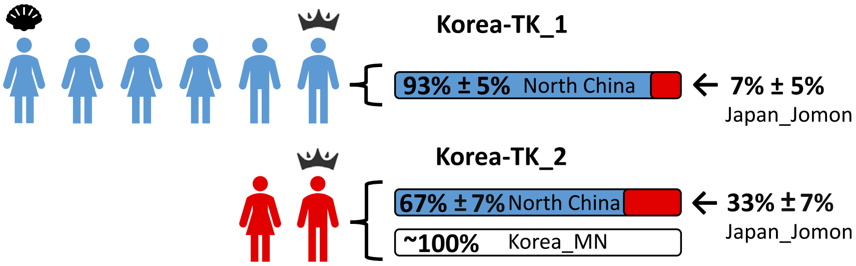

Figure 4. qpAdm analysis

Results of the qpAdm analyses at a group level (TK_1 and TK_2). We represent the ancestral components of the eight TK individuals by their minimum number of source populations. The complete results are displayed in Data S1E. The shell represents the individual from the shell mound, whereas crowns represent the grave owners.

the Korean peninsula (Data S1E), we tested the possibility of Korea-TK_2 representing the source population for Korea-TK_1, in addition to other north-China related sources. This led to the qpAdm result of Korea-TK_1 modeled as 87.5% Yellow_River-LBIA and 12.5% Korea-TK_2. These proportions are very similar to the proportions when Korea_MN is used as a source (Data S1E). However, we cannot discard the scenario in which Korea-TK_2 represents a recent admixture between Korea-TK_1 or a population with similar ancestry and a Jomon-related source.

When we performed the same qpAdm modeling on an individual basis, we found that four of the six individuals in the Korea-TK_1 cluster could be modeled with a single source, as Yellow_River-BA or Liao_River-BA, whereas individuals AKG_10218 and AKG_4321 could be modeled with a single source as Korea_MN/Japan_Kofun and Korea_MN, respectively (Data S1F). These models, however, might be overrepresenting the Jomon-related ancestry in these individuals, as the statistics f_4(Mbuti, Japan_Jomon; AKG_3421/AKG_10218, Other_Korea-TK_1_Individual) suggest nonsignificant affinities to Japan Jomon when compared with the other Korea-TK_1 individuals ($Z < |2.4|$) (Data S1C). Additionally, we did not observe a clear genetic affiliation of Korea-TK_1 to either the Yellow_River-BA or Liao_River-BA population as a close Chinese source.

The findings of Robbeets et al.[10] restrict the Jomon presence in Korea to the Neolithic. Furthermore, given the very low coverage of the Bronze Age individual from Taejungni (9,747 SNPs), it is possible that the qpAdm analyses presented in Robbeets et al.[10] lacked the statistical power for simpler one-way models to be rejected and that this individual did, in fact, have Jomon-related (or other) ancestry.[39] Indeed, we obtained biased results by randomly subsampling our Korea-TK_2 individual AKG_10203 to four independent sets of 10,000 SNPs and modeling it with both allsnps: YES/NO options. We found that for each replicate, between five and eight populations worked as one-way models with p values above 0.05 ($0.064 < p < 0.924$; Data S1F), whereas AKG_10203's full SNP set models required substantial amounts of Jomon-related ancestry above 30% (Data S1F). It is also worth noting that all models with subsampled data using two or three source populations failed due to their having standard errors higher than the ancestry amounts (higher than 1 or p value < 0.05).

We also investigated with ancestry modeling the similarity between the Korea-TK and the contemporaneous Japan Kofun genomes. When not considering either of the two TK groups as possible sources, the Japan Kofun individuals, dated to 600–700 CE, can be modeled as requiring a source of northern China ancestry related to Liao_River-BA (78% ± 7%) and another source carrying Jomon-related ancestry (22% ± 7%) (Data S1E), revealing that these individuals had less Jomon ancestry than Korea-TK_2 but more than Korea-TK_1. The statistic f_4(Mbuti, Japan_Jomon; Japan_Kofun, Korea-TK_2) supports this finding ($Z = 4.01$). When using Korea-TK as possible sources, Japan Kofun could be modeled as 71% ± 10% Korea-TK_2 and 29% ± 10% Yellow-River_LBIA (Data S1E).

Lastly, we applied qpAdm to model present-day Koreans using the same ancestry sources that were used for modeling the ancient Koreans, including the two TK groups as separate possible sources. We found that the simplest working models were with a source related to Yellow_River-LBIA ($p = 0.75$) (Data S1E). However, when present-day Koreans were modeled using each Korea-TK period individual as a source, at least three out of the six individuals from Korea-TK_1 were sufficient as a single source, just like Yellow_River-LBIA ($0.15 < p < 0.69$) (Data S1E).

We estimated the dates of the admixture events identified through the qpAdm analyses using DATES.[40] No combinations yielded significant results for Korea-TK_2, and only Korea-TK_1 modeled as the admixture of Yellow_River-LBIA and Japan Jomon yielded significant results (Data S1G), modeled as 47.93 generations with a CI of 13.7 generations. Assuming 28 years per generation, this corresponds to an admixture that happened 1,400–600 BCE.[40]

Genetic and phenotypic continuity

The results obtained with fineSTRUCTURE and identity-by-descent (IBD) analyses, which were based on the imputed genotypes dataset (STAR Methods), support a clear separation between the Korea-TK_1 and Korea-TK_2 groups. FineSTRUCTURE suggested that the Korea-TK_1 individuals were related to present-day Koreans and Han Chinese (Figure 5A). On the other hand, Korea-TK_2 individuals had a closer genetic affinity to present-day Japanese, Japan Kofun, and the Japan Jomon representatives (Figures 5A and 5B; Data S1H) than modern Korean or Chinese. The shared IBD between the Korea-TK_1 and Korea-TK_2 individuals with modern and ancient populations show clearly differentiated trends. The normalized length of the shared IBD between the Korea-TK_2 and the Japan Jomon is 3.37 on average, which is 5.16 times higher than that of the Korea-TK_1 (0.65). When compared with present-day populations, we observe a similar trend. Korea-TK_2 and modern Japanese share 2.52 per individual, while Korea-TK_1 shares 0.51. The shared average length with modern Koreans is more similar between both groups: 0.67 for Korea-TK_2 and 0.38 for Korea-TK_1, which suggests that the Jomon-related ancestry explains the observed differences,

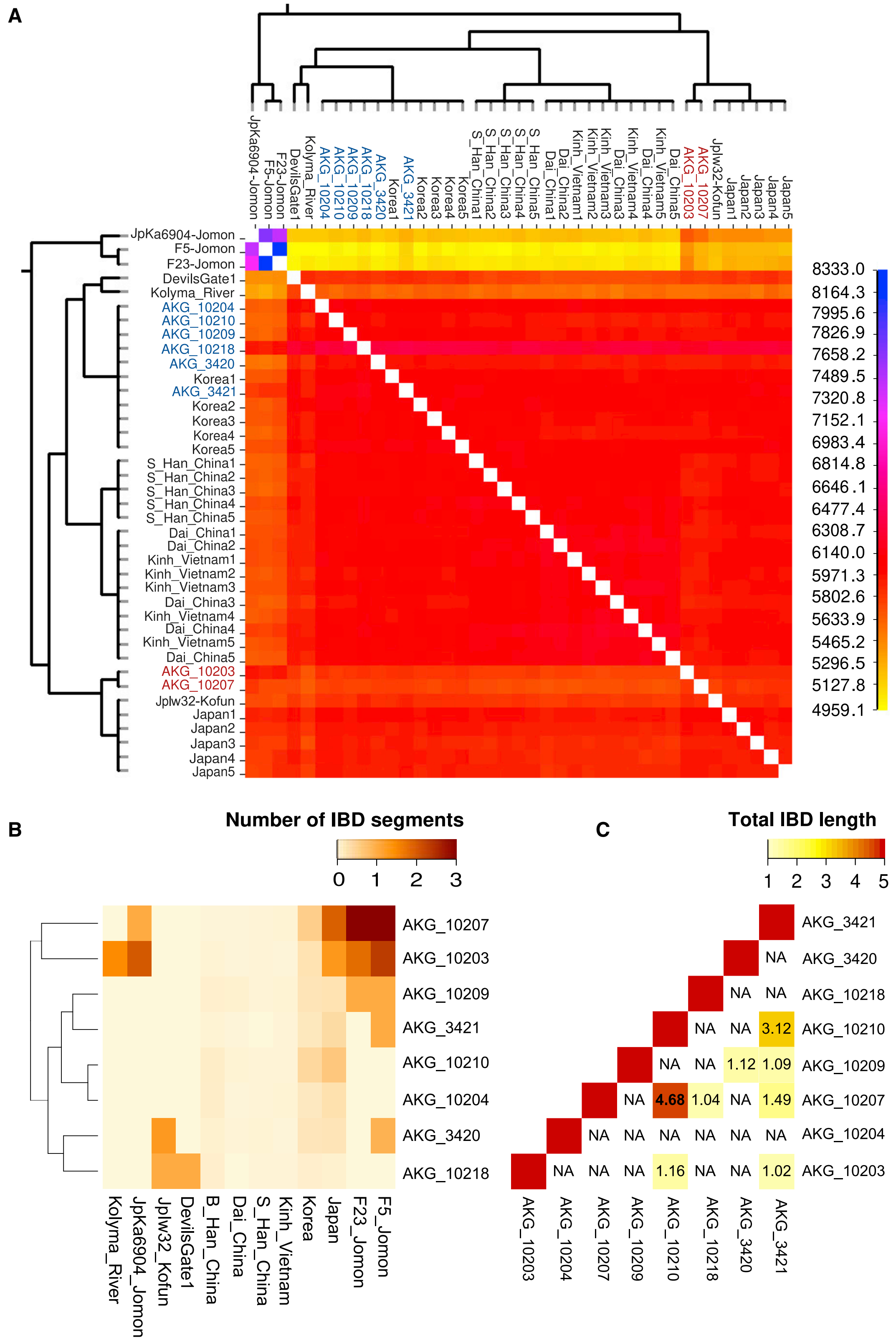

Figure 5. Imputed genotypes analyses

(A) fineSTRUCTURE. The plot shows that Korea-TK_2 clusters with modern Japanese and JpIw32 individuals, whereas Korea-TK_1 shows connections with Han Chinese and Korean individuals.

(legend continued on next page)

showing more connections between Japan Jomon and Korea-TK_2. Very few IBD blocks have been observed between TK individuals (Figure 5C; Data S1H). This can be explained by the low coverage of our individuals, which does not allow for the assessment of clear tendencies.

In order to use our data to explore possible differences from present-day Koreans, we selected 160 relevant variants that reflect morphology as well as other characteristics from the literature (Data S1I) to estimate whether Koreans changed phenotypically in the last 1,700 years. The small sample size did not enable us to draw any statistically significant inferences; thus, we limited our analysis to the presence/absence of the relevant variants. Based on our eight individuals, we conclude that all the relevant variants present in the modern Korean population were already established in the Korean TK period at least 1,700 years ago, showing deep genetic continuity (Data S1I). Some East Asian traits were detected among all the analyzed individuals, including dry earwax, no body odor (SNP -rs17822931),[41,42] and no homozygous alleles associated with excess sweating.[43] Seven of the eight individuals carried the canonical ectodysplasin A receptor (*EDAR*) gene variant (rs3827760) associated with Asian hair straightness/thickness[44] (except AKG_10207), which is homozygous in six of them (Data S1I). The HirisPlex system,[45] a phenotype prediction protocol, predicted that all the individuals had brown eyes, most likely black hair, and a variation in skin tone ranging from intermediate to darker (Data S1H), a result which is also in agreement with the phenotypes of present-day Koreans.

Surprisingly, the genetic makeup of the eight Gaya Korean individuals also included a common present-day Korean health phenotype: all individuals have multiple (four or more) ''susceptibility-to-myopia-risk'' alleles. Myopia is highly prevalent in modern Korea, reaching values of 95% in adult males from Seoul.[46] In several individuals we detected variants associated with alcohol flush reaction.[47,48] Also, all individuals had, on average, at least one homozygous balding risk allele or eight androgenic alopecia risk variants,[49] with individual AKG_10209 having five of these homozygous risk alleles. The eight genomes also captured the diversity of SNP profiles related to eyelid morphology, hair curliness, and other phenotypic features (Figure 6; Figure S4). The female individual, AKG_10207, from the Korea-TK_2 group, stood out with a reference allele ''A'' in the EDAR gene (rs3827760) (Data S1J), which is generally uncommon in East Asians, except for the Jomon in Japan. Although it is difficult to assess hair morphology, in addition to reference allele homozygosity in EDAR (rs3827760), AKG_10207 had multiple SNPs related to hair curliness, two of them homozygous, suggesting she may have had wavy hair, whereas the other individuals most likely had straight hair.

All the phenotypic features assessed by the HirisPlex phenotype prediction protocol and our selected forensically relevant facial-morphology-related SNPs were independently validated by the Parabon NanoLabs (https://snapshot.parabon-nanolabs.com/) (STAR Methods). Unfortunately, due to the poor preservation of all TK individuals' skeletal remains, the standard skull-based facial reconstruction was not possible at the time of this study. Therefore, to illustrate our analysis beyond the basic phenotypic features and to comprehensively reconstruct the facial characteristics of the studied individuals, we used Snapshot facial prediction based on the imputed calls, which, again, showed phenotypic diversity indistinguishable from the present-day Koreans (Figure 6; Figure S4).

DISCUSSION

Our study presents several novelties in comparison with the previous research on Korean genomes. First, we present samples with sufficient coverage to obtain confident statistics that permit precise comparison of the retrieved samples with the remaining available datasets. Second, we confirm the presence of Jomon ancestry in Korea until the 5th century CE. Third, we demonstrate with confident qpAdm results that present-day Koreans are closely related to the TK period individuals. Finally, we report the existence of two genetic groups that do not present the same genetic history, pointing toward population diversity in the past.

Several recent studies have reported genomic data of ancient individuals from China, Japan, Mongolia, Philippines, and Vietnam[1–4,6,8,10,50] and also ancient low coverage capture data from the southeastern Korean region close to Gimhae.[10] Here, we provide shotgun data from the Korean TK period, which allowed us to genetically characterize the population of Gimhae at that time, as well as to shed light on the genetic history of Korea. However, the lack of Neolithic and Bronze Age high-coverage Korean samples does not allow the in-depth assessment of the Jomon-related diversity dilution in the Korean peninsula. Therefore, future studies with more samples from these protohistoric periods will be interesting for understanding the Korean TK period genetic dynamics. Moreover, it should be noted that all the currently available ancient Korean genomes, including those presented in this study, are only from the southeastern part of Korea and may not reflect the general picture of the origin, migration, and admixture of ancient and modern Koreans.

The genetic variability seen among the Gaya TK period individuals points to population diversity in the Gimhae region during this time. It is interesting that some individuals are genetically close to previously reported Neolithic individuals from the Korean coastline,[10] while others do not share this affinity, pointing toward populations with different genetic histories. The individuals of the Korea-TK_1 cluster had comparable amounts of Jomon ancestry with Korean Middle Neolithic Ando population, but not Yeondaedo or Gadeokdo (Changhang). This can be explained by Yeondaedo and Gadeokdo (Changhang) islands being not only physically closer to Japan[10] but also genetically closer, as Korea-TK_2 affinity may suggest.. This suggests that the presence of Japan Jomon ancestry in Korea varied over time. Although in Gimhae we observed diversity within the same area, previous Neolithic data suggest geographic differences in Jomon-related ancestry.[10] Individuals of the Korea-TK_2 cluster share more Jomon-related ancestry than contemporary Japan Kofun-related ancestry, which

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(B) Shared IBD segments (>1 cM). Korea-TK_2 shows the highest number of shared IBD with Japan Jomon, whereas Korea-TK_1 shows very little IBD with the rest of the dataset.

(C) IBD (cM) shared among the pairs of TK individuals. Even though none of the pairs indicate familial relationship, the highest IBD sharing is between AKG_10207 and AKG_10210.

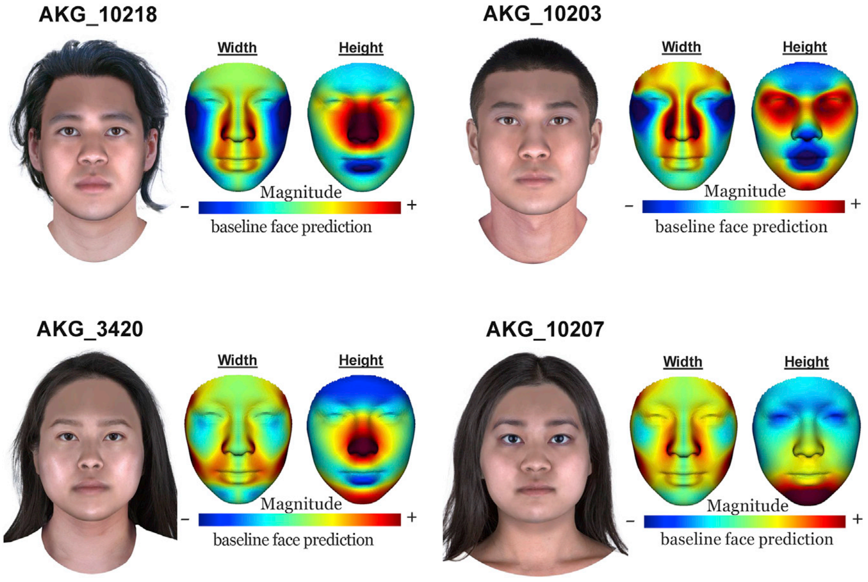

Figure 6. Facial predictions of Three Kingdoms period Korean individuals

Face reconstruction of the Korean TK period individuals AKG_10218, AKG_10203, AKG_3420, and AKG_10207 shows a diversity in phenotypic features commonly observed in present-day Koreans. Face morphology differences are emphasized relative to a baseline face prediction made using sex and ancestry. The facial reconstruction was performed with the imputed calls dataset.
See also Figure S4 and Data S1.

also evidences the existence of differential Jomon-related ancestry at a regional level. This would be primarily influenced by the amount of incoming northeastern China ancestry.

Similarly, we demonstrated that the reported lack of Jomon-related ancestry in the previously published single Bronze Age Korean genome may not be statistically supported with such low amounts of data[10] and with the analytical tools used (i.e., qpAdm). Nevertheless, our results show that Jomon-related ancestry persisted at high levels in South Korea until at least the TK period, around 500 CE. Moreover, the fact that Jomon has not been identified as a dominant cultural component in the Gimhae area at any time and that present-day Koreans show high genetic homogeneity with no Jomon-related ancestry implies that the Korea-TK populations bearing Jomon-related ancestry were probably completely absorbed by incoming populations originating from northern China who entered along the Korean peninsular coastlines, leading to the genetic homogeneity observed in present-day Koreans. A recent archeological report[51] on the southeastern islands (Yokji-do and Yeondaedo) of Korea confirms the presence of Jomon in Korean territory, citing finds like obsidian tools, which were traded before the Bronze and Iron Ages. These artifacts originated from Koshidake (obsidian source, Nagashaki in Japan) and indicate Gaya's cosmopolitanism and an established trade network with the Japanese archipelago up until that time.[51] The evidence of Jomon-related ancestry during the Neolithic suggests that this ancestry would be local in Korea and probably not associated with the Jomon culture from Japan. Therefore, pan-peninsular paleogenomics is required to precisely resolve the origin and extent of Jomon admixture in the whole Korean peninsula. With the current data, we are not able to point toward specific source populations in northern China. In the near future, larger sample sizes and more advanced informatic tools based on individual genetic histories may allow us to differentiate between these similar populations to uncover the origin of this ancestry.

Despite the genetic variability and the two genetic subgroups identified among the eight TK period Gaya individuals, we could not point out any consistent phenotypic differences between the groups. Instead, our phenotypic analysis suggests strong genetic continuity in modern Koreans, including important SNPs, such as an intronic variant rs1534480 in *PLB1* and the *ABCC11* G538A (rs17822931), which have already been found in Tianyuan man and suggest the genetic continuity of these variants in East Asia for the last 40,000 years.[52] The analysis of the diploid calls also evidences a connection between Korea-TK_1 and modern Koreans as well as Korea-TK_2 and modern Japanese. However, the relatively low coverage of our samples has proven insufficient for high-resolution IBD analyses.

Our results also suggest that the grave owners and the human sacrifices were not associated with distinct genetic substructures, as both the Korea-TK_1 and Korea-TK_2 groups included individuals associated with both burial types. However, the information inferred from the artifacts was limited due to tomb looting and poor grave preservation. For example, the male AKG_10203 was identified as a grave owner, possibly a warrior class or a lower-class noble, while the female AKG_10207 was possibly a human sacrifice. The remaining gilt bronze artifact and arrow quiver ornaments are insufficient to accurately determine the social status of AKG_10203. Due to these issues, we could not identify the owner of each grave, which is also the case for AKG_10207, who was a sacrifice for a possibly high-class noble, as indicated by the finding of an iron spear and bronze mirror.[25] Among the Korea-TK_1 group, AKG_10218 was the grave owner, while the rest were sacrifices for other grave owners. Our evidence suggests that the ruling class of the Gaya Confederacy of the 5th–6th Centuries CE are characterized by genetic diversity that may point toward a cosmopolitan society.

It is also notable that we could not ascertain the relationship between sex and social status in the Daeseong-dong tumuli. Even though the two grave owners were males, the human sacrifices in our study were associated with both sexes.

STAR★METHODS

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.cub.2022.06.004.

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AUTHOR CONTRIBUTIONS

D.M.F. and R.P. collected the samples. J.G.H., J.Y., Y.K., and S.Y. provided the samples and archeological and historical context. D.M.F., O.C., K.T.Ö., S.S., and V.O. performed the lab work. P.G., A.B., S.J., H.C., and D.M.F. analyzed the data. E.M.G. organized face predictions for ancient genomes. P.G., A.B., D.M.F., J.B., and R.P. wrote the paper with input from all co-authors. Y.C. and K.B. organized sampling and archeological data.

DECLARATION OF INTERESTS

Clinomics paid for the eight TK period sample's face prediction to the Parabon NanoLabs.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Biological samples		
Ancient Korean Genome	Gimhae National Museum	AKG_10203
Ancient Korean Genome	Gimhae National Museum	AKG_10204
Ancient Korean Genome	Gimhae National Museum	AKG_10207
Ancient Korean Genome	Gimhae National Museum	AKG_10208
Ancient Korean Genome	Gimhae National Museum	AKG_10209
Ancient Korean Genome	Gimhae National Museum	AKG_10202
Ancient Korean Genome	Gimhae National Museum	AKG_10210
Ancient Korean Genome	Gimhae National Museum	AKG_10212
Ancient Korean Genome	Gimhae National Museum	AKG_10213
Ancient Korean Genome	Gimhae National Museum	AKG_10214
Ancient Korean Genome	Gimhae National Museum	AKG_10215
Ancient Korean Genome	Gimhae National Museum	AKG_10216
Ancient Korean Genome	Gimhae National Museum	AKG_10217
Ancient Korean Genome	Gimhae National Museum	AKG_10218
Ancient Korean Genome	Gimhae National Museum	AKG_10219
Ancient Korean Genome	Gimhae National Museum	AKG_10220
Ancient Korean Genome	Gimhae National Museum	AKG_10221
Ancient Korean Genome	Gimhae National Museum	AKG_10206
Ancient Korean Genome	Gimhae National Museum	AKG_10211
Ancient Korean Genome	Gimhae National Museum	AKG_3420
Ancient Korean Genome	Gimhae National Museum	AKG_3421
Deposited data		
Whole genome data of eight Korean genomes from the TK period	This paper	PRJEB45573
Software and algorithms		
Cutadapt 1.2.1	Martin[53]	https://cutadapt.readthedocs.io/en/stable/
BWA 0.7.5	Li and Durbin[54]	http://bio-bwa.sourceforge/
Picard Tools 2.21.4	Picard-Tools[55]	https://broadinstitute.github.io/picard/
Qualimap-2.2.1	Okonechnikov et al.[56]	http://qualimap.conesalab/
MapDamage-2.0.9	Jónsson et al.[57]	https://ginolhac.github.io/mapDamage/
sequenceTools	Schiffels et al.[58]	https://github.com/stschiff/sequenceTools
Schmutzi	Renaud et al.[59]	https://github.com/grenaud/schmutzi
ANGSD 0.91.8	Korneliussen et al.[60]	http://www.popgen.dk/angsd/index.php/ANGSD
FASTQC 0.11.8	Andrews[61]	https://www.bioinformatics.babraham.ac.uk/projects/fastqc/
READ v3	Monroy Kuhn et al.[31]	https://bitbucket.org/tguenther/read
Yleaf	Ralf et al.[62]	https://github.com/genid/Yleaf
Eigensoft 7.2.1	Alexander et al.[63]	https://guix.gnu.org/packages/eigensoft-7.2.1/
R 4.1.1	R Core Team[64]	https://cran.r-project.org/
ADMIXTURE 1.3.0	Alexander et al.[63]	https://dalexander.github.io/admixture/download.html
PONG 1.4.9	Behr et al.[65]	https://pypi.org/project/pong/1.4.9/
admixtools 5.1	Patterson et al.[37]	https://bioweb.pasteur.fr/packages/pack@AdmixTools@5.1
DATES 753	Patterson et al.[37]	https://github.com/priyamoorjani/DATES
GATK 3.7	McKenna et al.[66]	https://gatk.broadinstitute.org/
samtools 1.9	Li and Durbin[54]	http://www.htslib.org/doc/1.9/samtools.html

(*Continued on next page*)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
bcftools 1.6	Li and Durbin[54]	https://github.com/samtools/bcftools/releases/
Beagle 4.0	Browning et al.[67]	https://faculty.washington.edu/browning/beagle/b4_0.html
Plink 1.9b6.13	Purcell et al.[68]	https://www.cog-genomics.org/plink/
vcftools 0.1.13	Danecek et al.[69]	http://vcftools.sourceforge.net/
KING v.2.2.4	Manichaikul et al.[70]	https://bioweb.pasteur.fr/packages/pack@king@2.2.4
ChromoPainterv2	Lawson et al.[71]	https://github.com/sahwa/ChromoPainterv2
Refined IBD version “17Jan20.102	Browning and Browning[72]	https://faculty.washington.edu/browning/refined-ibd.html

RESOURCE AVAILABILITY

Lead contact

Questions regarding further information on materials, datasets, and protocols should be directed to and will be fulfilled by the lead contact, Pere Gelabert (pere.gelabert@univie.ac.at).

Materials availability

The raw genomic data used in all the analyses can be accessed at the European Nucleotide Archive (ENA) under the accession number: PRJEB45573.

Data and code availability

Sequencing data and the filtered sequences are available at the European Nucleotide Archive (ENA) under the accession number: PRJEB45573. All code used in this study and other previously published genomic data is available at the sources referenced in the key resources table.

EXPERIMENTAL MODEL AND SUBJECT DETAILS

We studied several genomic sequences from ancient Korean individuals from the Three-Kingdoms period.

Archeological context

The practice of human sacrifices

Human sacrifice was a common practice in several ancient societies, and is usually interpreted as evidence for the existence of a wealthy class that sought political benefits at the expense of victims belonging to the general population (i.e., the non-wealthy).[73,74] The practice of human sacrifice was known as sunjang in the southern part of Korea during the first half of the first millennium CE. It has been hypothesized that this practice lasted for centuries during the Three Kingdoms period. In Silla, these practices lasted until the 4th to 5th centuries CE and are connected to the appearance of the stone-piled wooden chamber burials. In these graves there is a main burial chamber which housed the grave owner, and an auxiliary chamber with numerous grave goods; in both chambers the bodies of human sacrifices are also found.[75] There are controversies regarding the status of the human sacrifices; several authors have hypothesized that some of the sacrificed individuals may have enjoyed relatively high status in Silla society based on the numerous ornaments found with their bodies, although this evidence is indirect, and the written records do not clarify their status.[76,77]

Identification of human sacrifices and grave owners in Daeseong-dong funerary complex

The Daesong-dong samples were excavated during the second (AKG-10203, AKG-10204, AKG-3421; 03 Sep. 1990 – 14 Apr. 1991) and the third excavation (AKG-10207, AKG10209, AKG-10204, AKG-10218; 08 Oct. 1991 – 08 Mar. 1991) in the Gimhae tumuli area. The determination of grave owners was carried out by the excavation team and advisory committees. The excavation teams were composed of ten experts in Korean archaeology during the second excavation, and seven during the third excavation (researchers and professors in the Museums, Universities, and Cultural Heritage Administration). In both cases, the advisory committees were composed of five experts in Korean archaeology (current and former Korean national cultural heritage research fellows or archaeology professors). The advisory committee members were different in each of the excavations.

A common belief in Korean archaeology is that the formation of social hierarchy stretches back to the Bronze Age. During the Three Kingdoms period of Korea, the hierarchy became increasingly evident. The human sacrifices found in the tombs of nobility demonstrate the existence of social and economic hierarchies. The most important factor in determining whether the samples were human sacrifice was the location where they were excavated within the tomb. A commonly accepted view among archeologists and the advisory committee in Korea, is that main burials, in our manuscript referred to as “grave owners”, are buried in the center of a tomb or in a separate special room in a tomb (Figure S1D). As a side note, even though there is a possibility of two individuals being buried side-by-side and owning the same tomb (husband and wife), there were no such cases in Daesong-dong. Therefore, if more than one person other than the owner of a tomb were buried together, they were regarded as human sacrifices.[25,26,78,79]

Daeseong-dong

The archeological site of Daeseong-dong, Gimhae in Gyungsangnamdo province, is situated on the hill facing the southeastern coastline of South Korea between the Gujibong Peak and Bonghwangdae, and east of the Surowangneung Tomb (Tomb of King Suro). The tombs of this site were built during the Geumgwan Gaya period (42–532 CE). The burial complex of Daeseong-dong, together with the complex of Bokcheon-dong, are the most well-known sites of the Gaya Royal Class. The funerary complex covers 3,700 m^2. Since fieldwork started in 1990, 219 individuals have been recovered,[27] 69 of which are buried in large tombs made of wooden coffins that are believed to belong to the ruling classes. Most of these burials include human sacrifices and grave goods.

During the Korean Three Kingdoms period, Gimhae was a major trading port, as is evident from the multiple Chinese and Japanese artifacts recovered from the tombs (Figure S1A). One example is the mortuary finds from Tomb 91, dating to the early 4th century CE, which included a bronze vessel made in northeast China, a gilt bronze harness made from Conidae shells imported from Japan, bronze horse bells, a chamfron, and cylindrical bronze implements from Japan. Substantial Japanese bronze ornaments were also unearthed from Tomb 88 which dates to the early 5th century CE. These two tombs are the only ones with absolute dates. For Tomb 88, there are two dates: (KRKD-1:*Oxcal v4.4/IntCal 20;1700±20 330-415calCE (78.8%) (from Ottchil)) and (KRKD-2: *Oxcal v4.4/IntCal 20;1610±20 415-540calCE (95.4%) (from bone)). There are also two dates for Tomb 91 (*Oxcal v4.4/IntCal 20;1770±20 275-350calCE (72.4%) (from Ottchil)) and *Oxcal v4.4/IntCal 20;1740±20 245-385calCE (95.4%) (from bone)).[80] Based on matching relative chronology records and artifacts it is clear that the remaining burials are dated between the 4th–5th centuries CE.

Iron artifacts, including armors, helmets, and coins, were very common in most tombs. Some of the most remarkable finds were the grave goods from Tomb 29. The owner of Tomb 29 was buried inside a large wooden coffin built in the mid 3rd century CE that lies over more than 100 iron ingots. Additionally, this tomb consisted of multiple pieces of pottery and bronze pots which are stylistically typical of a northern-style culture. This culture was introduced to the Gimhae area at the end of the 3rd century CE, probably from the northern nomadic steppe tribes.[81,82] The high-status burials of this culture are characterized by Gimhae-type wooden-chambered burials, Dojil pottery, Ordos-type bronze pots, and iron armors and trappings for horse riding typical of nomadic tribes. This has been understood as a signal of intensive trade between the northern East Asian region and Gimhae area. However, it is unclear whether this could also indicate the migration of northern tribes, especially from the Buyeo Tribe (an ancient Korean Kingdom located in Middle Manchuria),[83] as no anthropological or genetic studies have been carried out to test this hypothesis. The appearance of these non-local objects also corresponds to the timing of the appearance of human sacrifices in the complex at the end of the 3rd century CE, and to the burial division starting from the 4th–5th centuries CE, as indicated by the appearance of Gimhae-type wooden-chambered burials. The majority of the burials and objects associated with ruling class tombs date to the 4th and 5th centuries. The tombs of the rulers were placed on the upper parts of the hills.[84]

The ritual of human sacrifice as a funeral practice first appeared in the Gyeongsang province, particularly in Gimhae. No evidence of sacrificial rituals was reported from other Korean regions. The first human sacrifices appeared in the Gimhae region at the end of the 3rd century CE and became prominent during the 4th century, and then rapidly disappeared in the Gimhae area during the late 5th century. The sacrificed persons in the Daeseong-dong tumuli were previously considered slaves, but recent studies suggest they were warehouse keepers, concubines, horsemen, and guards related to the grave owner. It is not possible to discern the relationship between the owner of a tomb and the associated sacrificial victims. Anthropological data suggest that human sacrifices in Gaya were mostly in their 20s and 40s. Sacrifices of women are a bit more common than those of men, a pattern which may relate to division of labor.[26,79,81]

Individuals from Daeseong-dong included in the study

Individual AKG_3421, Burial 11. Individual AKG_3421 (Ancient Korean Gimhae Genome 3421) is a female sacrifice found in Tomb 11. Individual AKG_3421 was found together with the grave owner. The main buried individual was found in a single wooden coffin. The tomb is indirectly dated to the early 5th century CE based on the coffin characteristics.

Individual AKG_10207, Burial 23. Burial 23 is a single wooden coffin with four human sacrifices dated to the late 4th century CE based on the burial style. Individual AKG_10207 is female sacrifice B of this burial. Japanese and Chinese style relics were discovered in the tomb. The funerary objects include a bronze mirror and iron spear suggesting that the burial owner was a high-class individual.

Individual AKG_10203, Burial 12. Individual AKG_10203 is the grave owner. He was found inside a wooden coffin together with gold and bronze artifacts from the early 5th century CE. This individual likely belongs to a warrior class or to the low nobility, as the tomb is not one of the richest. He is therefore classified as a grave owner individual.

Individual AKG_10204, Burial 12. Male sacrifice of Tomb 12 representing a non-grave owner individual.

Individual AKG_10209, Burial 24. Individual AKG_10209 is the sacrifice B of Burial 24, of a non-grave owner female. The owner of Burial 24 is buried in a wooden coffin together with three sacrifices dating to the early 5th century CE. One spindle was found in the burial, which is a rare object for the Deesong-dong site.

Individual AKG_10210, Burial 24. Individual AKG_12010 is the sacrifice C of Burial 24.

Individual AKG_10218, Burial 62. Individual AKG_10218 is the grave owner of Burial 62. The relics found in the burial date to the mid 4th century CE. The objects found in the tomb suggest a grave owner. This individual is a male.

Yuha-ri, Gimhae

The Yuha-ri site, also known as Monument Gyeongsangnam-do No. 45, is located on a slope about 15 m above the sea level in the city of Gimhae (Figure 1). Multiple artifacts have been documented from this site, including Joseon Dynasty artifacts found in Trenches 1 and 2 that include porcelain, pottery, and tiles.

Trench 3, situated along the southern walls of the complex, is 17 m long, 2 m wide and 60 cm deep. A shell-rich layer at the eastern section of the trench, at a depth of 20 cm, yielded (in addition to the numerous shells) Korean Three Kingdoms period vessels and soft earthenware. A five-year-old child (individual AKG_3420) was excavated at the western part of the trench; a Joseon dynasty tomb was identified about 30 cm below the topsoil (Figure S1C). The ceramics and other artifacts unearthed surrounding the body indicate that the burial dates to the 4th century CE (Three Kingdoms period). No other tombs were found at this site.[85]

An anthropological study was carried out in 2017 in the Bioanthropology Lab (Department of Anthropology, Seoul National University) using standardized anthropological methods.[86] Based on the length of the long bones the age estimate is 3–6 years old.[87] All the deciduous teeth were erupted, with the dental analysis suggesting the child is at least 3 years old (±1 year). Considering the growth status of the crowns of the permanent dentition the subject is estimated to be 4 years old (±1 year).

METHOD DETAILS

Sampling

All individuals were sampled with the permission of the local authorities at the Gimhae National Museum following preventive measures to avoid contamination. Six teeth were collected and twenty petrous bones were drilled using the protocol described in Sirak et al.[88] Tooth roots were cleaned and cut using a sandblaster and later pulverized using a mixer mill (Retsch Mixer Mill 400).[89] For this study we sampled all the available petrous bones and/or teeth.

DNA extraction, library preparation, and sequencing

All experimental work was performed in the dedicated ancient DNA laboratory of the University of Vienna. Personnel and facilities followed all the standard measures to guarantee the authenticity of the data. We included extraction of negative controls (no powder), library, and PCR negative controls (the extract was supplemented with water) in every batch of samples processed and carried them through the entire wet laboratory processing to test for reagent contamination. DNA was extracted from ~75 mg of powder using an optimized DNA extraction protocol.[89] Illumina sequencing libraries were constructed using 12.5–25 μl of extract and amplified using Accuprime Pfx Supermix (Thermo Fisher Scientific), following Gamba et al.[90]; a protocol adapted from.[91] Quality assessment of the amplified library was performed on an Agilent 2100 Bioanalyzer and a Qubit 2.0 Fluorometer. All the amplified libraries were initially screened using NovaSeq platform and further sequenced with NovaSeq platform in the Vienna Biocentre.

QUANTIFICATION AND STATISTICAL ANALYSIS

Bioinformatic analysis

Sequencing reads were trimmed using cutadapt (version. 1.2.1) with a minimum length of 30 and removing polyA tails,[53] and aligned to the human reference genome (GRCh37), with the revised Cambridge reference sequence (rCRS) mitochondrial genome using BWA[54] (Version 0.7.5) disabling seeding and setting a gap penalty open of 2. Duplicate mapped reads were removed using Picard Tools 2.21.4[55] with default parameters. Reads with mapping qualities below 30 were also removed. Unique and filtered reads were analyzed with Qualimap-2.2.1[56] to assess whole genome coverage depths. MapDamage-2.0.9[57] was used to estimate the level of deamination and the authenticity of the data. Reads used for the genotype-calling were trimmed again, 5 nucleotides in both ends, to minimize the genotyping errors in the downstream analyses.

Pseudo-haploid genotypes were called with a pileup caller of sequenceTools,[58] filtering out bases with a base-quality score below 30. At this stage we called the positions of the 1240k dataset.[29] These calls were merged with individuals from[1,2,4,36,92–95] and the rest of present-day genomes available from David Reich's group website (reich.hms.harvard.edu). The full list of samples is available in Data S1D. The final dataset is composed of 645 present-day Eurasian[1,96] samples and 237 ancient individuals.

Facial prediction and ancestry analysis

Facial prediction and ancestry analysis were conducted by Parabon NanoLabs, Inc. based on genotyping data from 851,274 SNPs. Facial prediction is based on autosomal SNPs and sex information to infer skin, eye, and hair color, as well as facial morphology using the Snapshot™ DNA Phenotyping System (https://snapshot.parabon-nanolabs.com/). The ancestry analysis is based on the PCA using Eigensoft v. 6.0.1 and Admixture v. 1.23, both run on a background dataset with approximately 13,000 individuals from more than 150 different genetic/ethnic backgrounds (denoted here: https://snapshot.parabon-nanolabs.com/ancestry). Regarding East Asians relevant to our analysis, the background population dataset included 163 Koreans and 341 Japanese that are divided into either "Japanese" (292 individuals) or "Ryukyuan" (49 individuals) ancestry. The map of all populations in the background dataset is available online (https://snapshot.parabon-nanolabs.com/img/populations-1.png).

Data authenticity

Contamination level was estimated from the mtDNA sequences using Schmutzi[59] and by estimating the heterozygous content of the male X chromosomes using ANGSD 0.91.8.[60] The estimates of both analyses are presented in Data S1A. Mapping qualities and read length distribution were also examined with FASTQC 0.11.8,[61] and the average genomic and mitochondrial depths were obtained using Qualimap 2.2.11.[56]

Uniparental markers

Reads aligned to mitochondria were processed with Schmutzi[59] to generate a consensus sequence of the mitochondrial genomes. We called the mitochondrial haplogroups of the mitochondrial consensus sequences using Haplogrep 2.0[97] and version 17 of Phylotree. Y chromosome haplogroups were estimated using Yleaf.[62]

We used READ[31] to explore the presence of kinship relationships between the samples analyzed. We filtered the SNPs present in the 1,240k dataset with an (Minor allele frequency) MAF of 0.01. There are four possible outcomes when running READ: unrelated (normalized P0 $\geq$ 0.90625), second degree (0.90625 $\geq$ normalized P0 $\geq$ 0.8125), first degree (0.8125 $\geq$ normalized P0 $\geq$ 0.625).

Population genetic tools

All the subsequent analyses were performed with the 1,240K dataset. Principal component analysis was conducted using 597,573 SNPs and 645 present-day genomes using smartpca from Eigensoft package 7.2.1[37] with numoutlieriter: 0 and 10 eigenvectors. The resulting data were plotted using R 4.1.1.[64] Ancient samples were projected onto the PCA that was based on present-day samples using the option ''lsqproject''. Two rounds of outlier removal were used. Results with ancient samples were plotted using R. We excluded first-degree relatives from the population genetics analyses, and when founding it, we chose the one with higher coverage according to the labeling publicly available.

An unsupervised ADMIXTURE analysis was performed with ADMIXTURE 1.3.0[63] with the modern genotypes from the publicly available Human Origins (HO) panel[37] together with the eight 1,700-year-old Korean TK individuals, restricting the analysis to the 597,573 SNPs of the HO dataset.[96] These SNPs were filtered for MAF < 0.05 and Missing sites > 0.05. Filtered SNPs were pruned for linkage-disequilibrium (LD) using PLINK 1.9[68] flag –indep-pairwise with a window size of 200 SNPs, advanced by 50 SNPs and establishing an r2 threshold of 0.4. A total of 282,896 SNPs were used in the analysis. The final ADMIXTURE was run with K ranging from 2 to 15 and 100 bootstrap replications. The ADMIXTURE result was plotted with PONG 1.4.9 and in R.[65]

f_3-statistics were run using admixtools 5.1[37] in the form f_3(X, Test; Mbuti) using all the populations of the dataset, both present-day and ancient. f_4-statistics were also run using the same package. We used the form f_4(X, Test; PopA, PopB) using all the multiple combinations. Only results with $|Z| > 3$ and supported by more than 100,000 SNPs were considered.

qpWave and qpAdm

We used the software qpWave[98] from admixtools 5.1[37] with parameter ''allsnps: NO'' to investigate the homogeneity of the ancient individuals and if any would require additional sources of ancestry to explain their genomes. All the samples included in our study have sufficient SNPs to perform the qpAdm modeling (at least 50% of the 1240k set) without using the ''allsnps: YES'' option. Therefore, we prefer to avoid the bias that the option may introduce.[39] To capture a wide range of distal ancestries we used the following base ''Right'' outgroup set of populations (''R10''): Mbuti.DG, Ami.DG, ONG.SG, Mixe.DG, Tianyuan, Iran_GanjDareh_N, DevilsCave_N, Bianbian, Liangdao2, Yumin. We used a threshold of p=0.01.

In admixture modeling we used qpAdm,[29] again with parameter ''allsnps: NO'', to find populations and potential admixture events from which the two ancient Korean subgroups could have descended. As the outgroup ''Right'' set we used the base R10. As sources we used a set of populations based on the top results from the outgroup-f_3 statistics of the form f_3(X, Test, Mbuti), for Korea-TK_1 and Korea-TK_2: Amur_Basin-IA, Ekven_IA, Japan_Jomon, Japan_Kofun, LateXiongnu, LateXiongnu_Han, Yankovsky_IA, Yellow_River-BA, Taiwan-IA, Liao_River-BA, Korea_LN_Yokchido, and Korea_MN. We applied a more stringent threshold of significance when compared with *qpWave*, of p=0.05. As part of a two-step model competition approach, models passing this threshold were re-evaluated and only accepted if they remained significant after moving all unused source populations to the ''Right''.

Dates

We used DATES 753[99] to estimate the dates of different admixture events based on the results of qpAdm. We ran the program using parameters as follows: bin size: 0.001, maxdis: 1.0, runmode: 1, qbin: 10 and jackknife: YES, setting a generation time of 28 years.

Imputation

The eight TK period genomes, together with other ancient shotgun genomes (NEO240_DevilsGate2, Kolyma_River, F5, F23, JpKa6904, JpIw32) with a coverage greater than 1x have been imputed following the procedure described in Cassidy et al.[30,100] We first processed the filtered mapped reads with RealignerTargetCreator from GATK 3.7,[66] and realigned the indels from The Mills and 1000 Genomes Project gold standard indels.[101] Afterwards, we added MD tags to the bam reads using samtools 1.9 calmd.[54] SNPs overlapping the 1000 Genomes Project dataset version 3[101] were called in these reads using GATK UnifiedGenotyper, setting the options-out_mode EMIT_ALL_SITES and –genotyping_mode GENOTYPE_GIVEN_ALLELES. Option -glm SNP was also used to get the genotype likelihoods of the SNPs. Obtained calls were filtered to add equal likelihoods to both missing data and the sites possibly affected by deamination, which corresponds to the transitions. Resulting files were split into chromosomes with bcftools 1.6[102] and filtered for sites with MAF>0.05, and less than 10% of genotype missingness. Resulting files were split with splitvcf.jar from Beagle in 100,000 SNPs files with 20,000 SNPs of overlap. We used Beagle 4.0[67] to impute the genotypes setting ''gl'' option, ''gprobs=true'', ''window=15000'', ''impute=true'', providing genetic maps and reference files from Beagle. Imputed calls were filtered by Genotype probability > 0.99 and MAF > 0.05, excluding transitions. The resulting datasets consist of 1,574,651 filtered and imputed calls.

Homozygosity

We used two methods to call the ROH in our samples. First, we used the imputed transversions overlapping with the 1000 Genomes Project phase 3 database (402,250 SNPs) to detect Runs of Homozygosity (ROH) using Plink 1.9b6.13,[68] following the previous parameter described in Gamba et al.,[90] classifying ROH between long and short using a threshold of 1.6 Mb. using the –homozyg command and the following options: -homozyg –homozyg-density 50 –homozyg-gap 100 –homozyg-kb 500 –homozyg-snp 50 –homozyg-window-het 1 –homozyg-window-snp 50, –homozyg-window-kb 5000 and –homozyg-window-threshold 0.05. Second, we called ROH with the methodology described in Ringbauer et al.[103] optimized for the study of aDNA with the same dataset used for the population genetics analyses.

Chromosome painting analysis (fineSTRUCTURE)

We used vcftools 0.1.13 to merge our eight imputed samples with modern East Asian samples from the 1,000 Genome Project[101] (Han, Dai, Korean, Japanese, and Kihn_Vietnamese), a Korean set from Jeon et al.,[11] and six additional ancient shotgun genomes from east Asia at a high coverage (NEO240_DevilsGate2, Kolyma_River, F5, F23, JpKa6904, JpIw32).[4,13,35,36] The total number of SNPs after filtering out all the positions with genotype missingness "–max-missing 1", Hardy-Weinberg equilibrium "–hwe 0.000001", and minor allele frequencies "–maf 0.00000000001", to exclude fixed variants, was 402,250. Relatives were excluded from the present-day populations utilizing KING v.2.2.4[70] with options "–related" and "–degree 3". The representatives for each present-day population were randomly downsampled to leave a) 10; b) 5; c) 3 representatives per each group. The finalized datasets were converted to map and ped formats using plink v1.90 with option "–recode 12" to preserve the phasing information. We ran scripts "plink2chromopainter.pl" and "makeuniformrecfile.pl" from the FineSTRUCTURE pipeline "fs_4.1.1" to generate phasing and recombination information files. Effective population size (*Ne*) was estimated using ChromoPainter v2 with 10 random samples and settings: "-a -i 20 -in -iM". The average *Ne* was estimated using "neaverage.pl" (in="3250.16016988007" and iM="0.000779866001166277). We ran FineSTRUCTURE v.4.1.1 in linked mode with options "-x 1000000 -y 1000000 -z 10000" and "-y 10000 -m T". The results were visualized in FineSTRUCTURE GUI for Windows v.0.1.0 (https://people.maths.bris.ac.uk/∼madjl/finestructure/finestructureGUI.html).

IBD analyses

IBD blocks were identified using a Refined IBD version "17Jan20.102" with default settings and a recombination map provided with the program. As input, we merged our eight imputed ancient Korean genomes with East Asians from the 1,000 Genome Project,[101] a Korean set from Jeon et al.,[11] and six additional ancient shotgun genomes from East Asia and filtered them as described in the "Chromosome painting analysis" section above. Our eight imputed samples were generated and prefiltered as described in the "Imputed Calls and imputation" section. Additionally, all the sites with missing variants and fixed alleles (MAF>0) had been excluded, yielding 402,250 high-quality markers used as an input.

IBD segments with LOD>3 were analyzed excluding the few sporadic large-size segments (>39cM) that are most likely an artifact (shown in S19). We averaged the values of segment length and segment counts to reflect the pattern of genetic material shared between each ancient sample and present-day East Asian populations. The results were plotted using heatmap.2 in R.

Phenotypic variant analysis

We selected 160 variants with phenotypic implications from the imputed ancient genomes, retrieving the alleles and their "rs ID" from dbSNP. The calls included in Data S1J correspond to the genotype probabilities imputed with the Korean Genome project and 1000 Genomes Project data overlap-based merged panel (denoted in black font) or the 1000 Genomes Project data alone (denoted in blue font). Genotypes were filtered by genotype probabilities of 0.9. For reference, we included their allelic frequencies in East Asian populations.