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MtDNA ANALYSIS OF EARLY-MEDIEVAL HUMAN REMAINS FROM THE CEMETERY IN GRODOWICE (PL)

The genetic composition of the medieval populations of Central Europe, Poland in particular, has been poorly investigated to date. Although a few DNA datasets from Poland have been published recently, no large-scale ancient DNA study on medieval populations has hitherto been reported. This paper reports the study of mitochondrial DNA (mtDNA) and presents the first population-level human DNA study from Lesser Poland by establishing mitochondrial DNA profiles for 13 samples from the Grodowice cemetery dated to the Medieval Period (11th to mid-13th century). The medieval sequences encompass almost the entire range of Western Eurasian macro-haplogroups: H, J, U. Interestingly, there is one sample which belongs to the Asian haplogroup G. aDNA sequences were compared with a dataset of 35,203 present-day sequences of the HVR I region of mtDNA including European, Near Eastern, and Asian populations, as well as 775 ancient sequences. Analyses of population genetics were performed, including genetic distances (F_{ST}), multidimensional scaling (MDS), principal component analysis (PCA) and shared haplotype analysis (SHA). The shared haplotype analysis (SHA) showed that the medieval population from Grodowice shares the majority of haplotypes with the medieval populations from the contact-zones of today's Slovakia and Croatia (53.85%) as well as with Hungarian conquerors (46.15%).

KEY WORDS: ancient DNA, mitochondrial DNA, medieval period, Lesser Poland

INTRODUCTION

There has been a remarkable increase in the number of ancient human DNA studies in recent years. As a result, a substantial record of mitochondrial sequences from many prehistoric sites – including medieval ones from across Western Eurasia – is now available ((Brandt *et al.* 2013, Bollognino *et al.* 2013; Juras *et al*. 2014; Kozłowski *et al.* 2014; Płoszaj *et al.* 2016; Płoszaj *et al.* 2017; Rudbeck *et al.* 2005; Stolarek *et al*. 2018). Although ancient human DNA datasets regarding Europe are numerous, those for modern-day Poland (medieval Lesser Poland, in particular) remain uninvestigated. To reconstruct the ethnic history of different peoples, modern molecular genetic approaches are widely used. The most appropriate tool which allows for the characterization of gene pools and for tracking maternal gene flow seems to be analysis of maternally inherited mitochondrial DNA (mtDNA).

Over the centuries the populations of Western Eurasia were undoubtedly subject to various episodes of expansion, population replacement, admixtures between divergent groups, as well as the formations of new medieval states and ethnicities

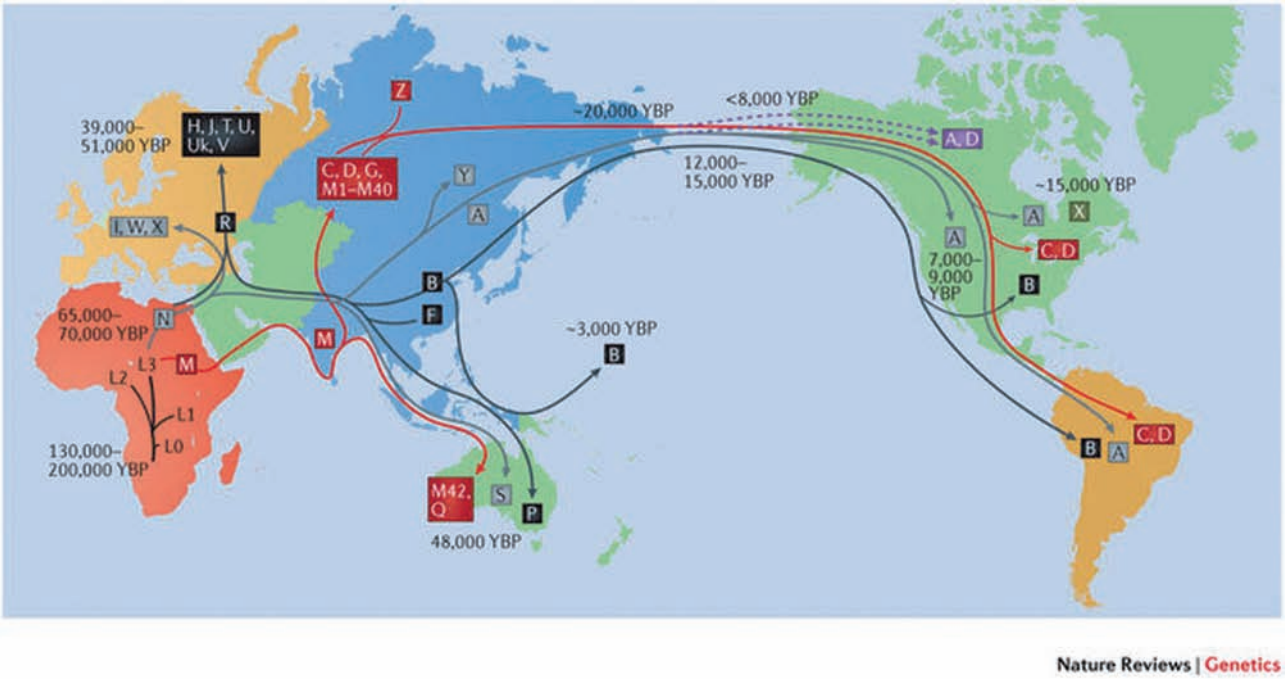

Fig. 1. Migration of human populations based on major mitochondrial haplogroups (Stewart & Chinnery 2015)

– and this is visible in present-day human genomes. Recent DNA studies suggest that most medieval populations were heterogeneous and shared common Western Eurasian mtDNA haplogroups, self-contained in their geographical locations and origin (Csákyova *et al.* 2016; Lazaridis *et al.* 2014; Meyer *et al.* 2012).

At present, the vast territories of East-Central and South-Eastern Europe are inhabited by Slavic populations. One should mention, however, that no large-scale ancient DNA study on the alleged ancestral populations of modern-day Slavs has been carried out to date. However, there are a few DNA datasets from Poland (Bogdanowicz, Grzybowski, Buś 2015; 2016; Juras *et al*. 2014; Juras *et al.* 2016; Kozłowski *et al.* 2014; Płoszaj *et al.* 2016; Płoszaj *et al.* 2017) and the regions geographically adjacent to present-day Poland: medieval Slovakia (Csákyová *et al.* 2015; 2016; Nagy *et al.* 2012; 2016), modern Czechia (Malyarchuk *et al.* 2006), Russia (Grzybowski *et al.* 2007), and 10th century Hungary (Tömöry *et al*. 2007; Csősz *et al*. 2016).

Mitochondrial DNA is often used in archaeogenetic research due to its smaller size compared to nuclear DNA, the lack of repetitive sequences, compact structure, and higher resistance to physical damage affecting it over the centuries. Mitochondrial genomes are located in the energy-generating organelles called mitochondria that are in each eukaryotic cell, where their amount per cell (about 8000 copies) depend on the type of organism and type of cells (Brown and Brown 2011). Human mtDNA is a double-stranded closed circular molecule of 16.6 kb in length consisting of a coding and non-coding region. The non-coding region (control region) of 1.1 kb in length contains two hypervariable regions (HVR I and HVR II). These regions are characterized by mutation rates ten times higher than for the coding region (Howel *et al.* 2007). Sequence variation of mtDNA has been generated by the sequential accumulation of new mutations along maternal lineages. Accordingly, human mtDNA contains a molecular recording of genealogical history and of the migrations of women who transmitted mitochondria through the generations (Torroni *et al.* 2006). Based on these mutations we can determine haplogroups, as was mentioned above, whose place of origin and extension path are already known (Fig. 1). Accordingly, there are Sub-Saharan (African) haplogroups: L0, L1, L2, L3, L4, L5, L6, L7, Western Eurasian haplogroups: H, T, U, V, X, K, I, J, W, Eastern Eurasian haplogroups: A, B, C, D, E, F, G, haplogroups of Native Americans: A, B, C, D, X, and the haplogroups of the South Pacific: P, Q, S (van Oven and Kayser 2009).

The universal standard for mtDNA nomenclature is derived from the first published complete mtDNA sequence "Cambridge Reference

Sequence" (CRS) (Anderson *et al.* 1981). A modified version of the CRS, in which 11 sequencing errors were corrected, was renamed "revised Cambridge Reference Sequence" (rCRS) and published in 1999 (Andrews *et al.* 1999). Nowadays, another reference sequence is used too – namely, the RSRS (Revised Sapience Reference Sequence) which contains a completely revised version of the rCRS (Behar *et al.* 2012).

The aim of this study is to determine matrilineal genetic structure and to present the first population-level human DNA study from Lesser Poland by establishing mitochondrial DNA profiles for 13 samples from the Grodowice cemetery dated to the Medieval Period (11th to mid-13th century)[1].

MATERIALS AND METHODS

Sample information, ancient DNA extraction, and amplification

The human skeletal remains (bones and teeth) used in this study were provided by the Institute of Archaeology, Jagiellonian University in Kraków (Poland). Thirteen bone specimens were collected from the Grodowice cemetery (Poland). Two bone fragments – one tooth and one compact bone fragment of a femur – were collected from each individual (see Table S1[2]). All stages of the work were performed under sterile conditions in a dedicated ancient DNA (aDNA) laboratory (Laboratory of Archaeogenetics at the Institute of Archaeology, Research Centre for the Humanities, Hungarian Academy of Sciences) following well-established aDNA workflow protocols.

The specimens were prepared following the protocols described by Kalmár *et al.* 2000; Shapiro and Hofreiter 2012. The bone and tooth samples were irradiated with UV-C light (1.0 J/cm^2, 25 min). The surfaces of tooth samples were cleaned by sandblasting (Bego, EasyBlast), while the surfaces of bone samples were removed with a fresh drilling bit at slow speed, followed by UV exposure for 20 min on each side. Bone and tooth fragments were mechanically ground into fine powder in a sterile mixer mill (Retsch MM301). Standard DNA extraction methods were used as described by Csákyová *et al.* (2016). First the samples (250 mg of bone powder) were washed with 8 ml EDTA (0.5 M, pH = 7.5) overnight at room temperature with continuous vertical rotation. After centrifugation, the supernatant was discarded and the remaining samples were suspended in 1.6 ml digestion mix (0.1 MEDTA, 20% N-lauryl sarcosine and 20 mg/ml proteinase K) and incubated overnight at 37°C with continuous vertical rotation. On the next day the samples were centrifuged at 13,000 rpm for 10 min, 350 µl supernatant was transferred to a fresh tube, 350 µl NH4-acetate (4 M) and 700 µl absolute ethanol were added, and samples were incubated overnight at −20°C. The DNeasy Tissue Kit (Qiagen) was used for further purification of the aDNA extract following the manufacturer's instructions: the mixture was transferred into a DNeasy Mini spin column and centrifuged at 6500 rpm for 1 min. The column was washed twice, DNA was eluted in a final volume of 70 µl and subsequently stored in the pre-PCR lab at −20°C. Several fragments of mtDNA hypervariable region I (HVR I) and the coding region were amplified in a total volume of 40 µl reaction mix, containing 6 µl of DNA extract, 20.4 µl H_2O, 1 × AmpliTaq Gold buffer, 0.8 mM dNTP mix, 0.9 mM MgCl2, 0.16 mg/ml BSA, 0.625 µM primers and 1.5 U AmpliTaq Gold DNA polymerase. The HVR I region of mtDNA was amplified in two overlapping fragments with two sets of primers, and an additional four primer pairs were used to amplify haplogroup diagnostic nucleotide positions in the coding region (see Table S2). The PCR reactions were performed in 38 amplification cycles consisting of three steps (denaturation at 94°C for 30 s, annealing at 55°C for 1 min and extension at 72°C for 30 s) with an initial denaturing step at 95°C for 10 min and final elongation at 72°C for 5 min. PCR products were checked on 8% native polyacrylamide gel. The purification of PCR products was performed using a QIAquick1PCR Purification Kit (Qiagen) following the manufacturer's protocol. Sequencing reactions were performed using the ABI PRISM BigDye1Terminator v3.1 Cycle Sequencing Ready Reaction Kit (Applied Biosystems) and sequencing products were purified

[1] Interdisciplinary research programme of materials from Grodowice was financed by the Ministry of Science and Higher Education in Poland (the *Diamond Grant*, project nr 0053/DIA/2012/41).

[2] All tables are included on the CD attached to this volume of *Przegląd Archeologiczny*.

by ethanol precipitation. The sequences were determined on an ABI PRISM 3100 (PE Applied Biosystems) in cooperation with BIOMI (Budapest, Hungary). The sequences were evaluated with Chromas Lite 2.4.1 and GeneDoc (Nicholas *et al.* 1996). The sequence polymorphisms in mtDNA (the range of HVR I is nucleotide positions (np) 16053–16420) were compared with the revised Cambridge Reference Sequence (rCRS) (Andrews *et al.* 1999) as well as the Reconstructed Sapiens Reference Sequence (RSRS, www.mtdnacommunity.org) (Behar *et al.* 2012). Haplogroup determination was carried out according to the mtDNA phylogeny of PhyloTree build 17, accessed on 18 February 2016 (www.phylotree.org) considering polymorphic positions of HVR I and four haplogroup- diagnostic position of the coding region (van Oven, Kayser 2009).

Reference population data

aDNA sequences were compared with a dataset of 35,203 present-day mtDNA sequences from the HVR I region including European, Near Eastern and Asian populations, as well as 775 ancient sequences. The sequences were collected from published data and are listed in Tables S7-S9. Only those modern mtDNA sequences which were reported for the same sequence range as our ancient HVR I data were included in the comparison.

Population genetics analysis

Population comparison was calculated using Arlequin 3.5.1 (Excoffier, Lischer 2010). Pairwise genetic distances (F_{ST}) were calculated based on HVR I sequences (np 16050–16383) assuming a Tamura & Nei substitution model (Tamura, Nei 1993) with a gamma value of 0.325. Significant variations in F_{ST} values were tested by 10,000 permutations between 52 modern and 23 medieval populations and particularly between the medieval populations alone (Tables S7 and S8).

Multidimensional scaling (MDS) of medieval and modern populations was applied on the matrix of linearized Slatkin F_{ST} values (Slatkin 1995) (Table S7 and S8) and visualized in a two-dimensional space (Fig. 2 and 3) using the metaMDS function based on Euclidean distances implemented in the Vega library of R 3.1.3 (http://www.r-project.org/).

Principal component analysis (PCA) was performed, based on mtDNA haplogroup frequencies of 22 medieval and 53 modern-day populations (Table S5 and S6). In the PCA of medieval populations, we considered 31 mtDNA haplogroups (A, B, C, D, G, F, H, HV, I, J, K, M, N, N1a, N1b, R/R0, T, T1, T2, U, U2, U3, U4, U5a, U5b, U7, U8, V, W, X, YZ), whereas in PCA with modern day populations 36 mtDNA haplogroups (A, B, C, D, G, F, H, HV, HV0, I, J, K, M, N, R/R0, T, T1, T2, U, U1, U2, U3, U4, U5, U5a, U5b, U6, U7, U8, U9, V, W, X, Y, Z, Other – all remaining haplogroups) were considered (Figs. 4-7). All PCA was performed using the prcomp function for categorical PCA, implemented in R 3.1.3 and plotted in a two-dimensional space, displaying the first two or the second and third principal components (PCs), respectively.

Shared haplotype analysis (SHA) was performed in order to detect and compare the mtDNA haplotypes shared between 22 ancient populations. Twelve populations from Europe and ten from Asia were studied, and the absolute and relative shared haplotypes were counted (Table S4).

RESULTS

We obtained thirteen mtDNA sequences spanning the range of np 16053-16420. The DNA of investigated samples were extracted and amplified at least twice per individual from different elements, the HVR I fragments were reproduced in several PCR products. The results of these replicates along with the negative control results suggest the haplotypes to be authentic.

The medieval sequences encompass almost the entire range of Western Eurasian macro-haplogroups: H, J, U. Interestingly, there is one sample which belongs to an Asian haplogroup G (Table S3). We detected identical haplotypes by individuals G2 and G4 (belonged to haplogroup U5a1), G44 and G51 (haplogroup J2a1a1). Furthermore, there were three individuals with rCRS haplotype (haplogroups H2a2a1).

Pairwise genetic distances were calculated between 23 medieval populations (Table S7) and between the same medieval populations and a set of modern-day populations from Eurasia (Table S8). Following populations of ancient population-pool

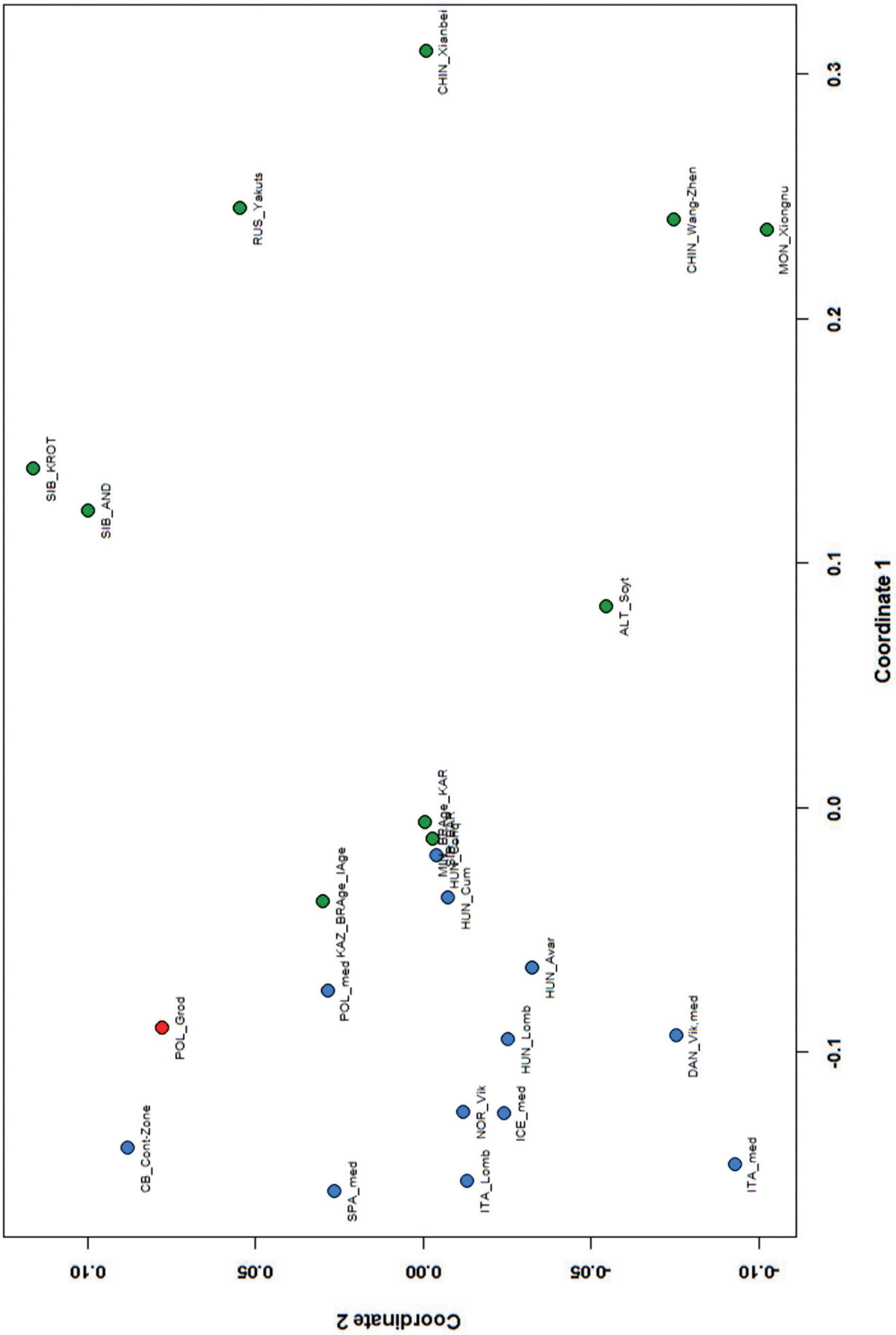

Fig. 2. MDS plot of ancient populations: The MDS plot was performed with linearized F_{ST} values of 13 European populations (blue dots; the medieval population from Grodowice is marked with a red dot) and 10 Asian populations (green dots). (For F_{ST} values, abbreviations and reference see Tables S7 and S9)

showed a non-significant difference from the medieval Grodowice (POL_Grod): medieval population of Conquest period from Hungary (HUN_Conq; F_{ST} = 0.02553 and p = 0.06504±0.0025), medieval population from present-day Slovakia and Croatia (CB_Cont-Zone; F_{ST} = 0.02743 and p = 0.12415±0.0038), medieval Cumanians from Hungary (HUN_Cum; F_{ST} = 0.02904 and p = 0.19018±0.0038), Lombards from Szólád – Hungary (HUN_Lomb; F_{ST} = 0.02398 and p = 0.11543±0.0032), Lombards from Italy (ITA_Lomb; F_{ST} = 0.02488 and p = 0.14117±0.0032), medieval Spaniards (SPA_med; F_{ST} = 0.02600 and p = 0.12444±0.0034), medieval Slavs from Poland (POL_med; F_{ST} = 0.00602 and p = 0.32799±0.0049), Vikings from Norway (NOR_Vik; F_{ST} = 0.00029 and p = 0.41026±0.0055), medieval Danes and Vikings (DAN_Vik,med; F_{ST} = 0.03358 and p = 0.05207±0.0023), early medieval Icelander (ICE_med; F_{ST} = 0.01977 and p = 0.12197±-0.0033), south western Siberian Baraba Late Bronze Age culture (SIB_Bar; F_{ST} = 0.03615 and p = 0.06584±0.0025), Bronze Age population from south central Siberian Minusinsk Hollow (MIN_BRAge_KAR; F_{ST} = 0.02477 and p = 0.12355±0.0034) and Bronze and Iron Age populations in present-day Kazakhstan (KAZ_BRAge_IAge; F_{ST} = 0.02767 and p = 0.08286±0.0029) (Table S6 and Fig. 2). Pairwise F_{ST} values of modern-day populations indicated non-significant difference (p > 0.05) between Grodowice and populations with Slavic languages: Russian (RUS; F_{ST} = 0.02506 and p = 0.06217±0.0024), Poles (POL; F_{ST} = 0.02358 and p = 0.06732±0.0027), Czechs (CZE; F_{ST} = 0.01095 and p = 0.19186±0.0041), Slovaks (SVK; F_{ST} = 0.02273 and p = 0.06772±0.0025), Ukrainians (UKR; F_{ST} = 0.02044 and p = 0.08108±0.0026), Slovenians (SLO; F_{ST} = 0.02622 and p = 0.05891 ±0.0027), Belarusians (BEL; F_{ST} = 0.01802 and p = 0.10850±0.0029), furthermore with Khants (KHAN; F_{ST} = -0.00082 and p = 0.44015±0.0047), Armenians (ARM; F_{ST} = 0.01407 and p = 0.12821±0.0034), Iranians (IRN; F_{ST} = 0.00872 and p = 0.21077±0.0037), Iraqi (IRQ; F_{ST} = 0.01120 and p = 0.17978±0.0040), Turks (TUR; F_{ST} = 0.01322 and p = 0.14454±0.0036), Austrians (AUS; F_{ST} = 0.02566 and p = 0.06267±0.0024), Germans (GER; F_{ST} = 0.02392 and p = 0.06584±0.0024), Hungarians (HUN; F_{ST} = 0.02435 and p = 0.06296±0.0024), Szeklers from Romania (ROM_Sek; F_{ST} = 0.01845 and p = 0.10157±0.0032), Albanians (ALB; F_{ST} = 0.02368 and p = 0.07871±0.0028), Bulgarians (BUL; F_{ST} = 0.02453 and p = 0.05455±0.0022), Greeks (GRE; F_{ST} = 0.02124 and p = 0.06752±0.0024), Estonians (EST; F_{ST} = 0.00683 and p = 0.26393 ±0.0038), Danes (DAN; F_{ST} = 0.01237 and p = 0.17790±0.0037), Norwegians (NOR; F_{ST} = 0.01718 and p = 0.12335±0.0033), Icelanders (ICE; F_{ST} = 0.02318 and p = 0.08870±0.0027) and Lithuanians (LIT; F_{ST} = 0.02670 and p = 0.05386±0.0021) (Table S7 and Fig. 3).

The multi-dimensional scaling (MDS) plot based on linearized Slatkin F_{ST} values of ancient populations (stress value = 0.0945, Fig. 2) contains segregation of west and east Eurasian populations along the coordinate 1. The nearest population to our investigated Grodowice is that of the medieval Contact-Zone population from Slovakia and Croatia.

In order to visualize the genetic distances, the values of linearized Slatkin F_{ST} of 23 ancient and 52 modern populations were displayed on the MDS plot (stress value = 0.0969, Fig. 3). The plot contains aggregation of the European population along the coordinates 1 and 2. The Asian populations are separated along the coordinate 1. Between the European and Asian populations the Near-Eastern are situated. The ancient populations are separated on this European-Asian lineage along coordinate 1, too. The medieval population from Grodowice is between the modern European and Asian populations near to modern Near-Eastern and ancient European populations, but along coordinate 2 it is separated from these European populations as well as the medieval contact zone population from Slovakia and Croatia.

PCAs were calculated based on mtDNA haplogroup frequencies of medieval and modern populations (Tables S5 and S6).

The PCA plots showed a marked difference between ancient European and Asian populations. Furthermore, along the PC1 and PC2 components (values of displayed variance: PC1 = 20.4%, PC2 = 11.2%) the plot shows a clustering of populations of the Bronze and Iron Ages from Kazakhstan and Avars and conquerors from Hungary, South European populations from Italy and Spain (Fig. 4). The medieval population from Grodowice is situated between European and Asian populations along the PC1, which could be caused by the presence of Asian haplogroup G; along PC2 it is separated from most of the populations, whilst along the PC3 (10.5% of total variance) this phenomenon is not presented (Fig. 5).

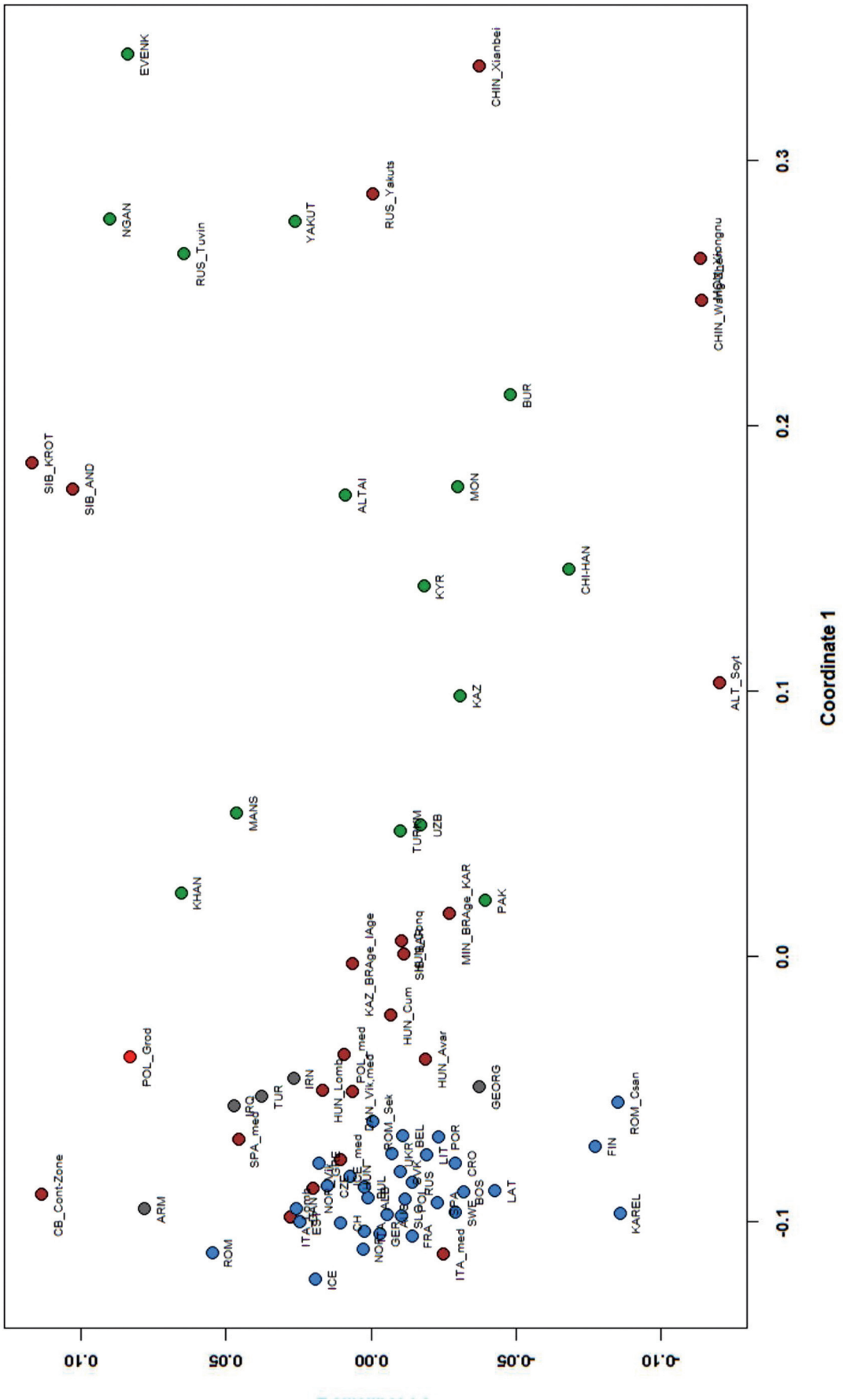

Fig. 3. MDS plot of ancient and modern populations: The MDS plot was performed with linearized F_{ST} values of 52 modern European (blue dots), Near Eastern (grey dots), Asian (green dots) and 23 ancient (brown dots; the medieval population from Grodowice is marked with a red dot) populations. (For F_{ST} values, abbreviations and reference see Tables S8 and S9)

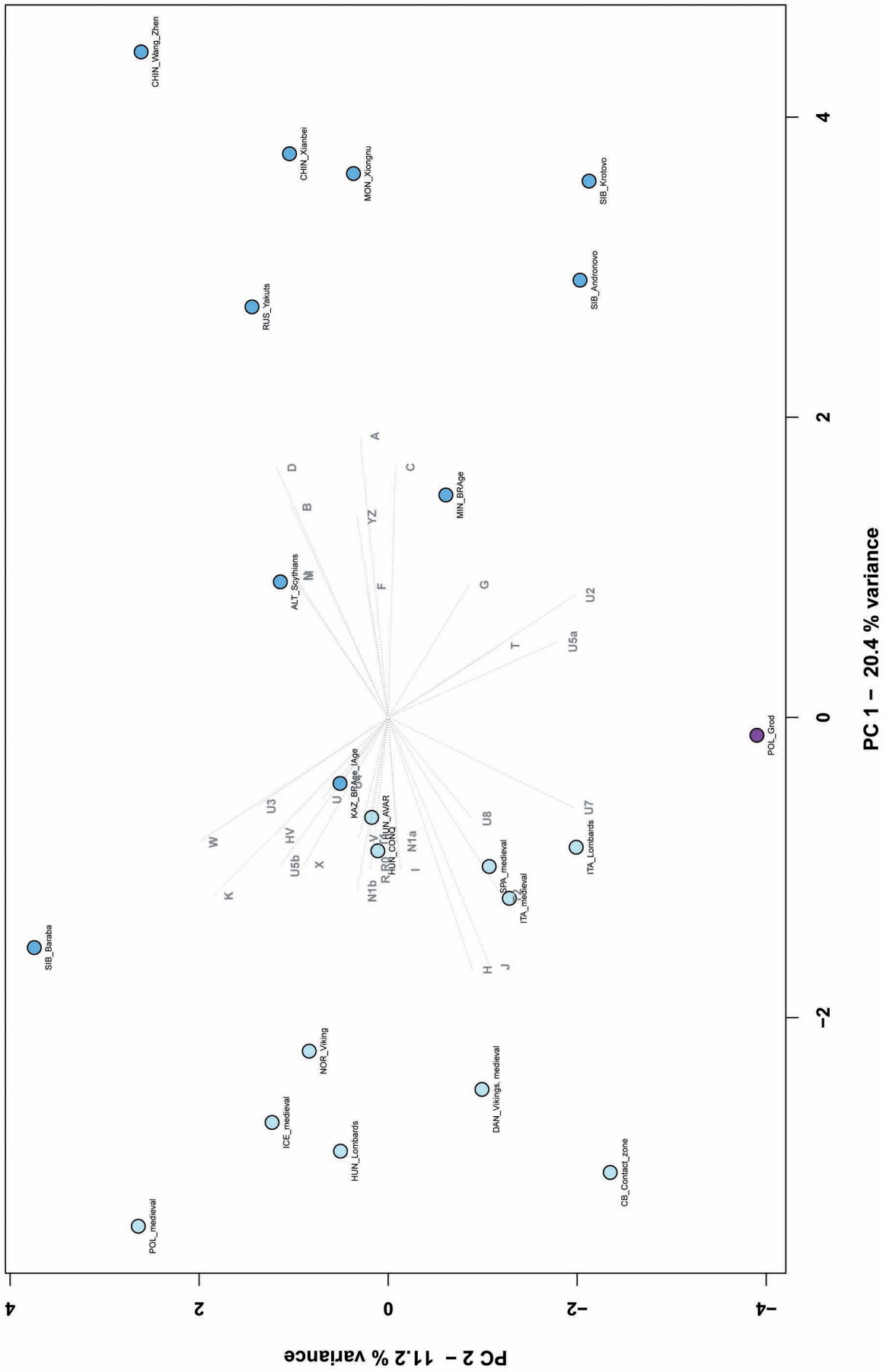

Fig. 4. PCA of ancient populations. The PCA is based on mtDNA haplogroup frequencies of the medieval population from Poland (marked with a violet dot), 11 ancient European (light blue dots) and 10 ancient Asian populations (blue dots), and shows PC1 and PC2. The haplogroup frequencies and the population information are shown in Table S5

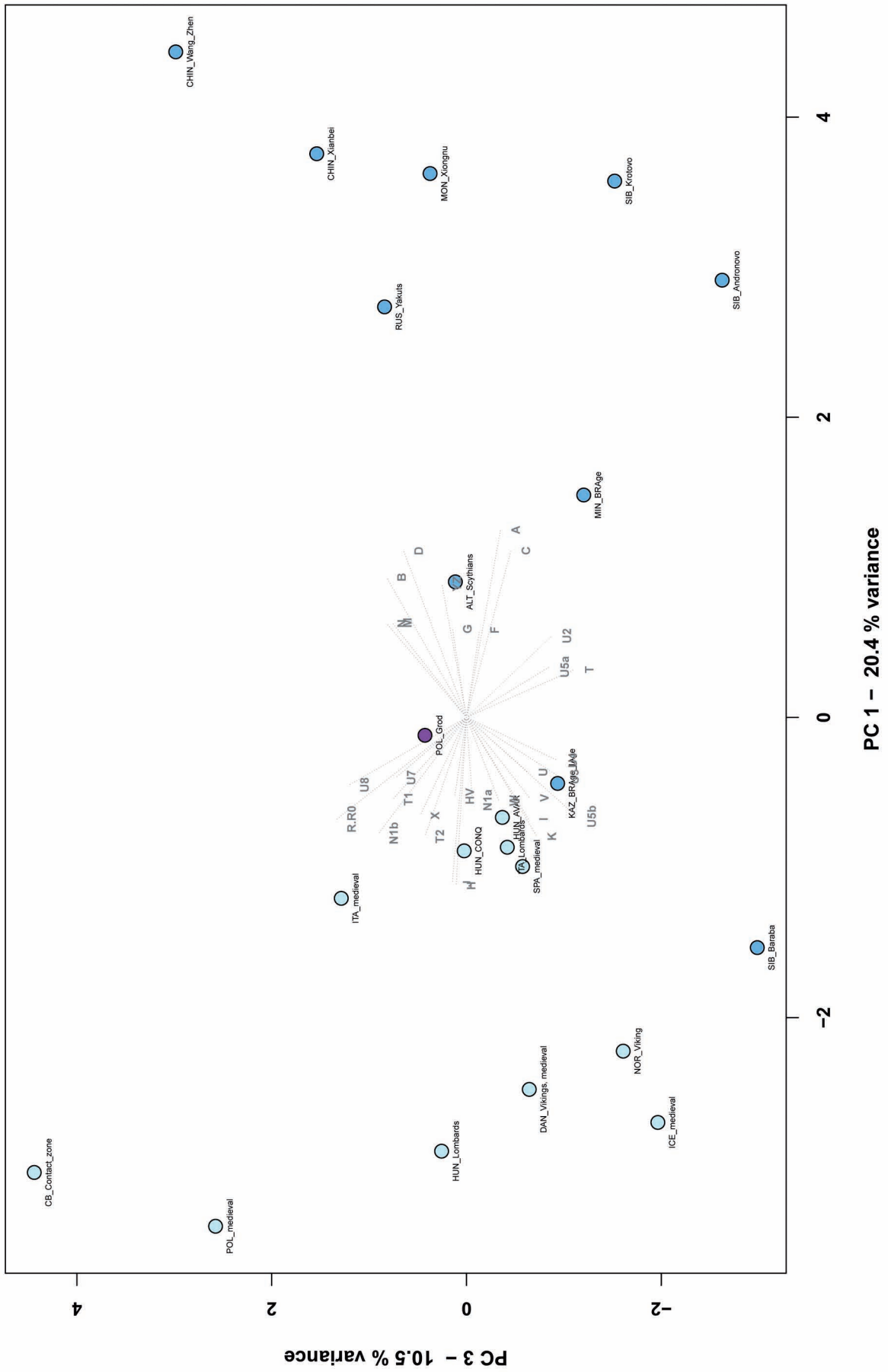

Fig. 5. PCA of ancient populations. The PCA is based on mtDNA haplogroup frequencies of the medieval population from Poland (marked with a violet dot), 11 ancient European (light blue dots) and 10 ancient Asian populations (blue dots), and shows PC1 and PC3. The haplogroup frequencies and the population information are shown in Table S5

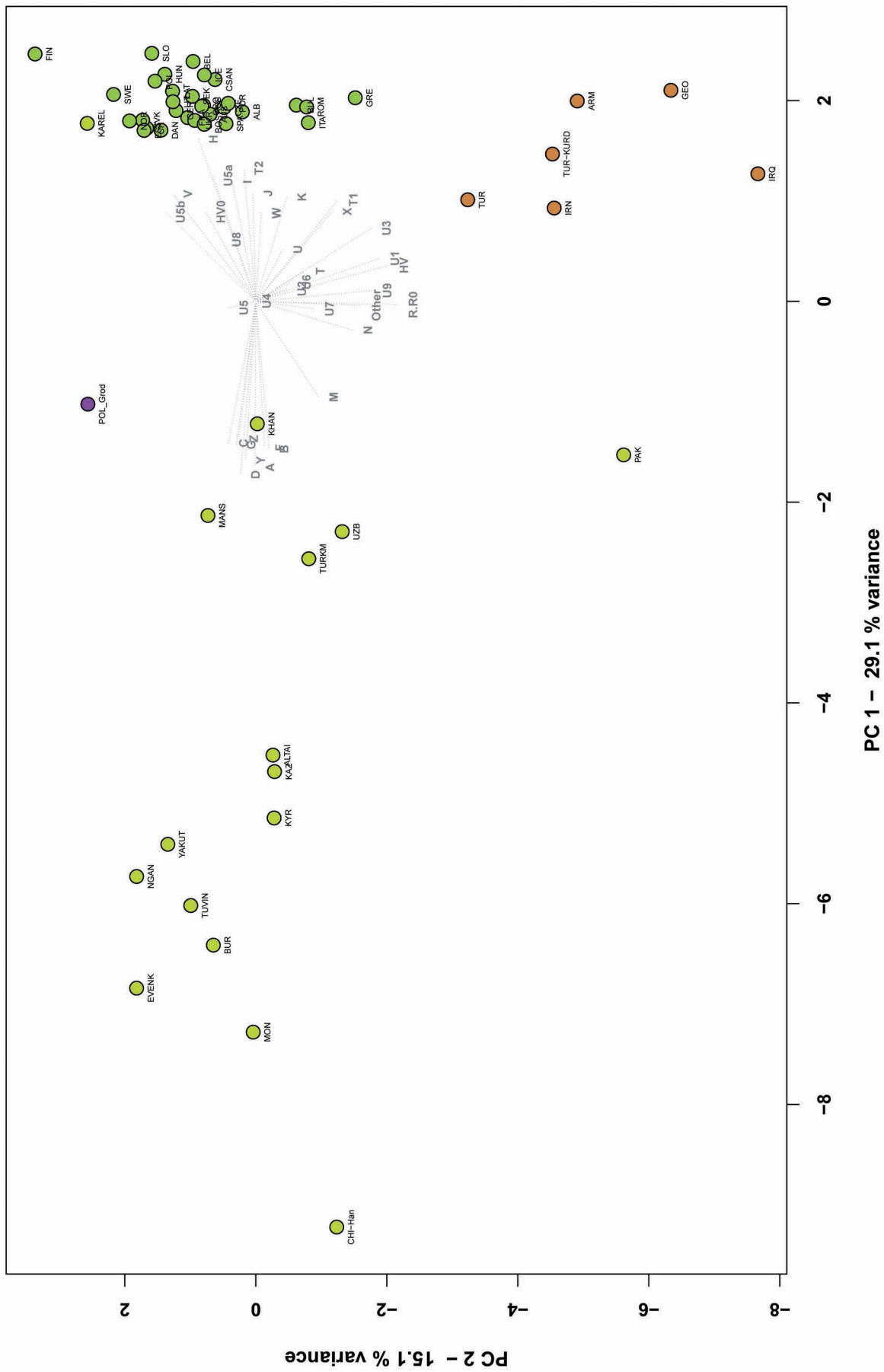

Fig. 6. PCA of the investigated medieval and modern-day populations. The PCA is based on mtDNA haplogroup frequencies of the medieval population from Grodowice (marked with a violet dot) and 53 modern-day populations from Asia (marked with yellow-green dots), Near East (brown dots) and Europe (green dots), and represents PC1 and PC2. For the frequencies and the list of abbreviations see Table S6

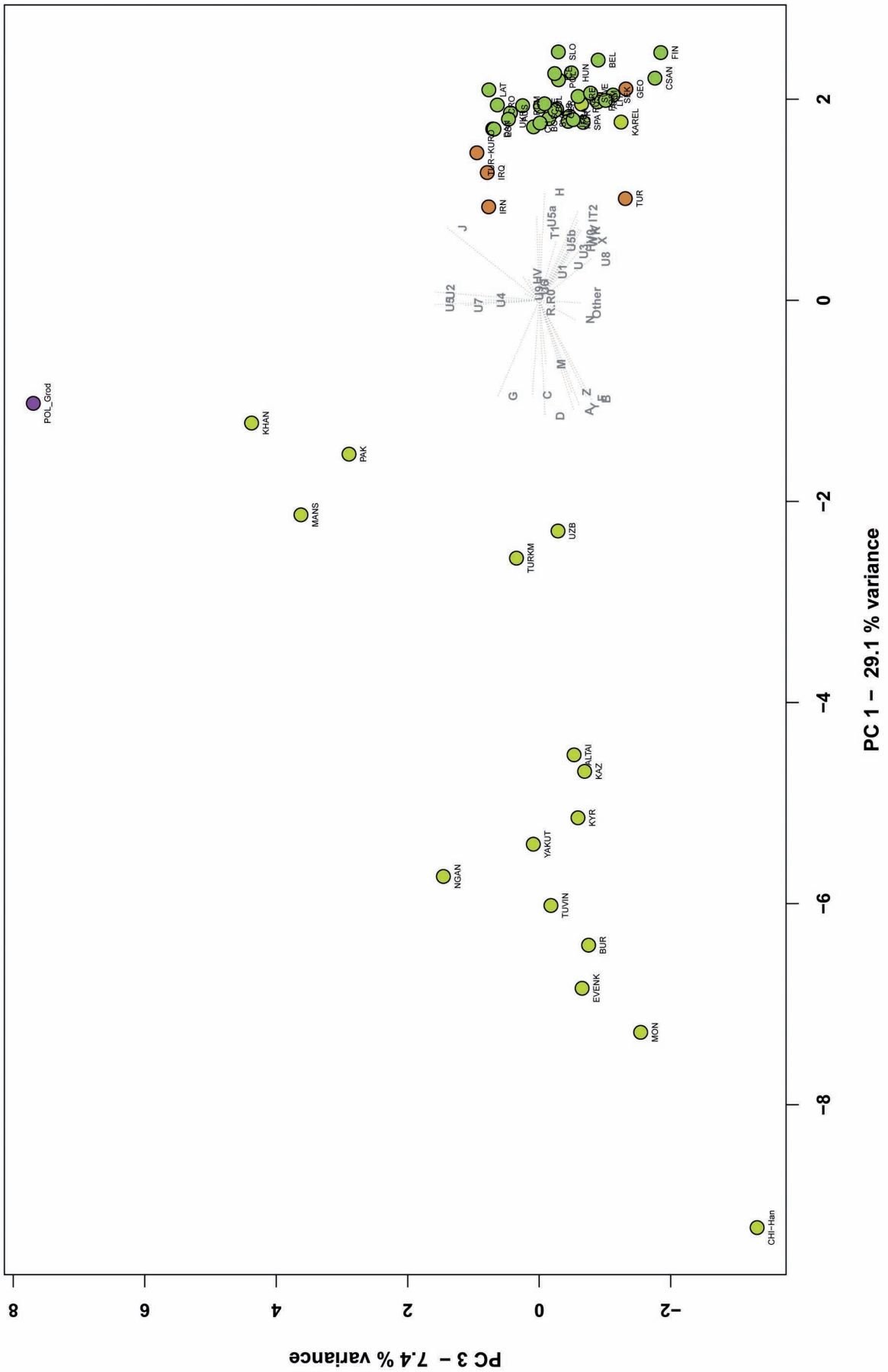

Fig. 7. PCA of the investigated medieval and modern-day populations. The PCA is based on mtDNA haplogroup frequencies of the medieval population from Grodowice (marked with a violet dot) and 53 modern-day populations from Asia (marked with yellow-green dots), Near East (brown dots) and Europe (green dots), and represents PC1 and PC3. For the frequencies and the list of abbreviations see Table S6.

The PCA plots of the modern Eurasian population indicate an alignment of the European populations and separated Asian population on PC1 and PC2 components (variance: PC1 = 29.1%, PC2 = 15.1%). The Near-Eastern populations are clustered together along PC2, but they are closer to the European population along component PC3 (7.4% of total variance). The investigated medieval population is again between the European and Asian populations along these three components (Figs. 6 and 7).

The shared haplotype analysis (SHA) shows that the medieval population from Grodowice shares the majority of haplotypes with medieval populations from Contact-Zones of today´s Slovakia and Croatia (53.85%) as well as with Hungarian conquerors (46.15%) (Table S4).

DISCUSSION

In this study we investigated a medieval population from the cemetery in Grodowice, Poland. Using well established protocols for aDNA analysis we have obtained the mtDNA dataset of thirteen samples, concerning the genetic variation within the HVR I region and four nucleotide polymorphisms in the coding region of mtDNA. These markers enabled us to assign the mtDNA haplogroup of the samples. The representative haplogroups of the investigated medieval population belong to common modern European (West Eurasian) haplogroups except in the case of 1 sample, namely G26, which belongs to the typical Asian haplogroup G2a5. The age of this haplogroup was estimated by (Behar *et al.* 2012): 1,199.6 ± 1,670.4; CI=95%. The haplogroup G2 is characteristic for east and central Asian population based on the empop haplogroup browser (empop.org) (Röck *et al.* 2013). Relatively high presence of G2a was detected by Buryats from Russia (Derenko *et al.* 2013; 2018), Kazakhs (Comas *et al.* 2004; Gokcumen *et al.* 2008; Irwin *et al.* 2010), Kyrgyz (Comas *et al.* 1998; Irwin *et al.* 2010), populations from China (Yao *et al.* 2000; 2002a; 2002b; 2003; 2004; Qian *et al.* 2001; Oota *et al.* 2002; Kong *et al.* 2003; Wen *et al.* 2004; Li *et al.* 2007; Liu *et al.* 2011), Japanese (Horai *et al.* 1996; Imaizumi *et al.* 2002; Maruyama *et al.* 2003; Tajima *et al.* 2004; Tanaka *et al.* 2004; Mabuchi *et al.* 2007;) , Koreans (Lee *et al.* 2006; Derenko *et al.* 2007; Lee *et al.* 2006), Khants and Mansi (Derbeneva *et al.* 2002; Pimenoff *et al.* 2008) and Yakuts (Derenko *et al.* 2007; Fedorova *et al.* 2003; Pakendorf *et al.* 2003; 2006; Puzyrev 2003). The frequencies of European haplogroups are quite different from present-day populations: the detected frequency of haplogroup H at medieval Grodowice was a little bit lower (38.5%) than it is in European modern-day populations, where this most frequent haplogroup has a frequency of 45.4% (Torroni *et al.* 2006; Brotherton *et al.* 2013). Haplogroup H, the most common haplogroup in Europe, was also detected in all typed individuals in the Late Viking Age cemetery in Bodzia (central Poland) (Bogdanowicz, Grzybowski, Buś 2015; 2016). The most ancient European haplogroup U occurs in higher frequency in the medieval population (30.8%). Haplogroups J (23.1%) occurred nearly two times more frequently than in present-day Europe (Soares *et al.* 2010; Behar *et al.* 2012).

The investigated population from Grodowice evinces a European character based on population genetic analysis, but is situated mostly between Asian and European populations, which is caused by the presence of a haplotype belonging to the haplogroup G. DNA studies for other medieval Polish populations from Cedynia and Lednica indicate that the investigated samples share their more rare haplotypes with East-Central and South-East European populations (Juras *et al.* 2014, 1). Archaeological data suggest that during the Middle Ages, Asian populations, including Altaic tribes (Huns, Avars, and Mongols) were engaged in wars on the European continent (Curta 2001). This could in consequence leave traces in the form of Asian haplotypes in European populations. The existence of a haplotype representing, among other examples, clade G2a in Polish populations could further reflect the influx of Asian haplotypes during medieval wars in which Altaic tribes were engaged (Mielnik-Sikorska *et al.* 2013, 7). The medieval population from the Contact-Zone of Slovakia and Croatia (Csákyová *et al.* 2016; Csősz *et al.* 2016) is relatively near to the investigated population based on haplotype analyses like MDS and SHA (Fig. 2).

CONCLUSION

It seems that further research of more samples, along with analysis of the whole mitochondrial genome and nuclear DNA markers, are necessary in the investigation of the genetic connections between medieval and modern populations in order to arrive at more concrete conclusions. Further analyses of ancient samples of the region will probably result in a more accurate estimation of the medieval populations' affinity.

Taking all of the above data into account, it is relatively difficult to pinpoint any specific region as the place of origin of the examined individuals. The results could be biased due to the small sample size, and genetic drift could also unnoticeably influence our results. It is worth noting that analyses of haplogroup frequencies can be very sensitive to small sample sizes that increase the randomness of the identified genetic affinities. Furthermore, we assessed population interactions and migrations only along the maternal lineages; the paternal side of the medieval population history remained unexamined in this study. Moreover, as has been proved elsewhere, mtDNA analyses of control region sequences alone, do not allow identification of any specific features which would clearly distinguish Slavs from the other European populations. Thus, only large-scale complete mitochondrial genome sequences studies may point out mtDNA lineages, which could have been involved in the formation of Slavs. Nevertheless, the study presented here of the first successful description of medieval mtDNA variability at the Grodowice cemetery contributes an important dataset to help reveal the genetic diversity of medieval Europe.

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S1: Samples from Grodowice (Poland) used for genetic analysis

Grave Number	Tooth, Bones	Samples	Sex	Age
G2	T	M3 (Lower, R)	M	Adultus/Maturus
	B	Femur R		
G4	T	M3 (Lower, R)	M	Adultus/Maturus
	B	Femur R		
G6	T	M3 (Upper, L)	M	Adultus
	B	Femur R		
G9	T	M1 (Upper)	F	Adultus
	B	Femur L		
G10	T	M2 (Lower, R)	F	Adultus
	B	Femur R		
G20	T	M1 (Upper, L)	M	Adultus
	B	Femur L		
G26	T	M3 (Lower, L)	M	Maturus
	B	Femur		
G44	T	M1 (Upper, L)	F	Adultus
	B	Femur R		
G49	T	M3 (Lower, R)	M	Adultus
	B	Femur R		
G50	T	M3 (Lower, R)	F	Adultus/Maturus
	B	Femur R		
G51	T	M2 (Lower, L)	F	Adultus
	B	Femur L		
G102	T	M3 (Lower, L)	M	Maturus
	B	Femur L		
G104	T	M1 (Upper, L)	M	Maturus
	B	Femur L		

S2: Performed PCRs of 2 overlapped HVI sequences and sequences of coding region contained pointed SNPs and primer pairs used for amplification

Samples	Tooth, Femur	HVI region		Coding region			
		16020-16259	16182-16401	4833	7028	12308	12705
G2	T	✓	✓			✓	
	B	✓	✓				
G4	T	✓	✓			✓	
	B	✓	✓				
G6	T	✓	✓		✓		
	B	✓	✓				
G9	T	✓	✓				✓
	B	✓	✓				
G10	T	✓	✓		✓		
	B	✓	✓				
G20	T	✓	✓		✓		
	B	✓	✓				
G26	T	✓	✓	✓	✓	✓	
	B	✓	✓	✓			
G44	T	✓	✓				✓
	B	✓	✓				
G49	T	✓	✓			✓	
	B	✓	✓				
G50	T	✓	✓			✓	
	B	✓	✓				
G51	T	✓	✓				✓
	B	✓	✓				
G102	T	✓	✓		✓		✓
	B	✓	✓				
G104	T	✓	✓		✓		
	B	✓	✓				

	Name of primers	Sequences	Reference	Reported as
HVR I	L-16020	5'-TCT GTT CTT TCA TGG GGA AG-3'	Tömöry et al. 2007	L16040
	H-16259	5'-GTG GCT TTG GAG TTG CAG TT-3'		H16239
	L-16182	5'-AAC CCC CTC CCC ATG CTT AC-3'	Tömöry et al. 2007	L16201
	H-16401	5'-TGA TTT CAC GGA GGA TGG TG-3'		H16400
SNPs of coding region	L-4833	5'-CTATCCTCTTCAACAATATACTCT-3'	Xie et al. 2007	L4701
	H-4833	5'-ATGTGAGAAGAAGCAGGC-3'		H4848
	7028-F	5'-TTT TCA CCG TAG GTG GCC TG-3'	Tömöry et al. 2007	L6962
	7028-R	5'-TGA AAT GGA TTT TGG CGT AGG-3'		H7126
	12308m-F	5'-ACA GAG GCT TAC GAC CCC TTA-3'	Csákyová et al. 2016	12308m-F
	12308m-R	5'-TTT ATT TGG AGT TGC ACC AG-3'		12308m-R
	12705-F	5'-CAT CCC TGT AGC ATT GTT CG-3'	Tömöry et al. 2007	L12622
	12705-R	5'-AAT TCC TAC GCC CTC TCA GC-3'		H12764

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S3: Obtained mtDNA haplogroups and haplotypes

Samples	HVI region based on rCRS (1)	HVI region based on RSRS (2)	Coding region	Haplogroup (Phylotree 17 – Haplogrep)	Overall_Rank
G2	16192T 16256T 16270T 16291T 16399G	16129G 16187C 16189T 16192T 16223C 16230A 16256T 16270T 16278C 16311T 16399G	12308G	U5a1	1.0000
G4	16192T 16256T 16270T 16291T 16399G	16129G 16187C 16189T 16192T 16223C 16230A 16256T 16270T 16278C 16311T 16399G	12308G	U5a1	1.0000
G6	rCRS	16129G 16187C 16189T 16223C 16230A 16278C 16311T	7028C	H2a2a1	0.5000
G9	16069T 16126C	16069T 16126C 16129G 16187C 16189T 16223C 16230A 16278C 16311T	12705C	J	1.0000
G10	rCRS	16129G 16187C 16189T 16223C 16230A 16278C 16311T	7028C	H2a2a1	0.5000
G20	rCRS	16129G 16187C 16189T 16223C 16230A 16278C 16311T	7028C	H2a2a1	0.5000
G26	16223T 16227G 16234T 16278T 16362C	16129G 16187C 16189T 16227G 16230A 16234T 16311T 16362C	4833G 7028T 12308A	G2a5	0.8836
G44	16069T 16126C 16145A 16231C 16261T	16069T 16126C 16129G 16145A 16187C 16189T 16223C 16230A 16231C 16261T 16278C 16311T	12705C	J2a1a1	1.0000
G49	16129c 16183A 16193.1C 16362C	16129c 16183A 16187C 16189T 16193.1C 16223C 16230A 16278C 16311T 16362C	12308G	U2e1h	1.0000
G50	16318T	16129G 16187C 16189T 16223C 16230A 16278C 16311T 16318T	12308G	U7	0.8607
G51	16069T 16126C 16145A 16231C 16261T	16069T 16126C 16129G 16145A 16187C 16189T 16223C 16230A 16231C 16261T 16278C 16311T	12705C	J2a1a1	1.0000
G102	16209C 16261T	16129G 16187C 16189T 16209C 16223C 16230A 16261T 16278C 16311T	7028C 12705C	H107	1.0000
G104	16189C 16288C 16362C	16129G 16187C 16223C 16230A 16278C 16288C 16311T 16362C	7028C	H8	1.0000

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14. Shared haplotype analysis with 11 ancient populations

| | | |

S5: Haplogroup frequencies used for PCA with 22 ancient populations

PC (Components)	% Variance
1	20,435
2	11,166
3	10,496

Region	Country	Population	Abbreviation	Centuries	Culture/Period	N	Haplogroup frequencies																														Reference
							A	B	C	D	G	F	HV	I	J	K	M	N	N1a	N1b	R/R0	T	T1	T2	U	U2	U3	U4	U5a	U5b	U7	U8	V	W	X	YZ	
	Poland					13	0	0	0	0	7,692307692	0	38,46153846	0	0	23,07692308	0	0	0	0	0	0	0	0	7,692307692	0	0	15,38461538	0	7,692307692	0	0	0	0	0	Thu study (13)
Europe_east	Hungary	medieval populations of Conquest period from Hungary	HUN_CONQ	10th – 12th AD	medieval	101	6,930693069	1,980198022	2,97029703	5,940594059	1,980198022	0,990099901	21,78217822	1,980198022	1,980198022	4,950495055	1,980198022	1,980198022	0	0	1,980198022	3,96039604	1,980198022	5,940594059	1,980198022	1,980198022	0	4,950495055	7,920792079	0,990099901	0	1,980198022	1,980198022	1,980198022	3,96039604	0	Csáky et al. 2016 (75), Tömöry et al. 2007 (26)
Europe_east	Hungary	Avars	HUN_AVAR	6th – 9th AD	medieval	26	0	0	3,846153846	3,846153846	0	3,846153846	38,46153846	3,846153846	0	0	0	3,846153846	3,846153846	0	0	3,846153846	3,846153846	11,53846154	0	3,846153846	3,846153846	3,846153846	0	0	0	0	0	0	3,846153846	0	Csáky et al. 2016 (26)
Europe_east	Hungary	medieval populations from present-day Slovakia (Nitra-Šindolka, Čakajovce) and Croatia (Zvonimirovo)	CB_Contact_zone	10th – 11th AD	medieval	23	0	0	0	0	0	0	21,73913043	0	0	17,39130435	0	0	0	4,347826087	4,347826087	0	13,04347826	13,04347826	0	0	4,347826087	8,695652174	0	4,347826087	8,695652174	0	4,347826087	8,695652174	0	„Csáky et al. 2016 (3); Csákyová et al. 2016 (18)"
Europe_east	Hungary	Lombards from Hungary: Szolád	HUN_Lombards	6th AD	Lombard	28	0	0	0	0	0	0	32,14285714	7,142857143	7,142857143	14,28571429	7,142857143	0	3,571428571	7,142857143	0	0	0	7,142857143	3,571428571	0	0	10,71428571	0	0	0	0	0	0	0	0	Alt et al. 2014 (28)
Europe_south	Italy - Piedmont	„Lombards from Italy: Rivoli, Corso Levi (Turin); Rivoli, La Perosa (Turin); Mombello Monferrato (Alessandria); Collegno (Turin); Centallo, San Gervasio (Cuneo)"	ITA_Lombards	6th – 8th AD	Lombard	28	0	0	0	0	0	0	50	0	3,571428571	17,85714286	0	0	0	0	3,571428571	0	7,142857143	0	3,571428571	3,571428571	0	3,571428571	10,71428571	0	0	0	0	0	0	0	Vai et al. 2015 (28)
Europe_south	Italy - Tuscany	„medieval Italiane: Firenze, Piazza Signoria; Pisa, Vicopisano; Livorno, Castello Donoratico; Massa Carrara, Fivizzano; Siena, Santa Maria della Scala; Grosseto, San Quirico"	ITA_medieval	10th – 15th AD	Medieval	27	0	0	3,703703704	0	0	0	66,66666667	0	0	3,703703704	3,703703704	3,703703704	0	0	0	0	0	0	0	0	0	0	0	0	3,703703704	0	0	0	0	0	Gaimarano et al. 2009 (27)
Europe_south	Spain	medieval Spaniards: Basque, Aldasta	SPA_medieval	6th – 7th AD	Medieval	56	0	0	0	0	0	0	60,71428571	0	0	14,28571429	1,785714286	0	0	0	0	1,785714286	0	3,571428571	5,357142857	7,142857143	0	0	0	0	0	1,785714286	0	0	0	Alnualde et al. 2006 (56)
Europe_east	Poland	medieval Slavs from Poland: Cedynia (West Pomerania Province), Ostrów Lednicki (Greater Poland Province)	POL_medieval	11th – 15th AD	Medieval	20	0	0	0	0	0	0	40	10	0	10	10	0	0	5	5	0	5	0	0	0	0	0	0	0	0	0	5	10	0	Juras et al. 2015 (20)
Europe_north	Iceland	early medieval Icelander	ICE_medieval	10th – 12th AD	Medieval	73	0	0	0	0	0	0	32,87671233	1,369863014	6,849315068	15,06849315	13,69863014	0	0	0	0	0	0	2,739726027	0	6,849315068	0	1,369863014	4,109589041	4,109589041	4,109589041	0	0	2,739726027	2,739726027	1,369863014	0	Helgason et al. 2009 (73)
Europe_north	Denmark	medieval Danes with Vikings: Galgedil, Kongemarken, Risby	DAN_Vikings, medieval	8th – 16th AD	Viking, Medieval	29	0	0	0	0	0	0	34,48275862	0	13,79310345	13,79310345	6,896551724	0	0	3,448275862	0	0	0	6,896551724	0	0	0	0	0	0	0	0	0	0	3,448275862	Melchior et al. 2010 (29)
Europe_north	Norway	Vikings from Norway: Nord-Trøndelag, Nordland, Sør-Trøndelag, Telemark, Troms, Oppland, Hedmark	NOR_Viking	6th – 11th AD	Viking	56	1,785714286	0	0	0	0	0	39,28571429	7,142857143	3,571428571	17,85714286	7,142857143	0	0	0	0	0	0	3,571428571	0	0	1,785714286	1,785714286	7,142857143	7,142857143	0	1,785714286	0	0	0	Krzewińska et al. 2015 (56)
Asia, central	Mongolia	Xiongnu period: Hsiung-nu	MON_Xiongnu	3rd BC – 2nd AD	Xiongnu	46	17,39130435	2,173913043	10,86956522	43,47826087	2,173913043	8,695652174	0	0	2,173913043	0	4,347826087	0	0	0	0	0	0	0	0	4,347826087	0	0	0	0	0	0	0	0	0	„Keyser-Tracqui (2003) 43; Kim et al. 2010 (3)"
Asia, central	China-Inner Mongolia	Tuoba Xianbei	CHIN_Xianbei	4th – 5th AD	Xianbei	16	6,25	12,5	31,25	43,75	6,25	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	Changchun et al. 2006 (16)
Asia, central	China-Inner Mongolia	Wanggu, Upper Capital - Zhenzishan	CHIN_Wang_Zhen	12th – 14th AD	Jin-Yuan period	33	12,12121212	6,060606061	3,03030303	24,24242424	0	0	3,03030303	0	0	0	0	0	27,27272727	18,18181818	0	0	0	0	0	0	0	0	0	0	0	0	0	0	6,060606061	„Fu et al. 2007 (12); Fu et al. 2009 (21)"
Asia, north	Russia - Eastern Siberia	Yakuts	RUS_Yakuts	15th – 19th AD	15-19th	57	3,508771193	5,263157895	36,84210526	38,59649123	0	3,508771193	1,754385965	0	0	3,508771193	1,754385965	1,754385965	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1,754385965	1,754385965	Crubézy et al. 2010 (37)
Asia, north	Russia	„Sofonovoarmaia: Tataria; Ozat-Abakanets; Bogratskiy; Afontova Gora, south central Siberia"	MIN_BRAge_Karasuk	14th – 9th BC	Bronze Age	20	5	0	5	5	0	10	10	0	0	5	0	0	0	0	0	0	5	5	0	5	0	15	20	0	0	0	0	0	5	„Keyser et al. 2009 (11); Allentoft et al. 2015 (9)"
Asia, central	Kazakhstan, Russia	„Ak-Mustafa; Izmaylovka; Ob-Zhaylau III; Vodokhranilische; Zevakinskiy; Birlik; Molaly, Shubarat; Kok-Mardan; Solyanka; Bulanovo; Stepnoe VII; Bol'shekaraganskii; Kapova cave; Tatsbergen II"	KAZ_BRAge_IAge	23rd – 4th BC	Bronze Age-Iron Age	34	8,823529412	0	0	0	2,941176471	0	20,58823529	5,882352941	5,882352941	8,823529412	0	8,823529412	0	0	2,941176471	0	5,882352941	5,882352941	0	8,823529412	5,882352941	0	0	2,941176471	2,941176471	0	0	0	2,941176471	0	„Lalueza-Fox et al. 2004 (27); Allentoft et al. 2015 (8)"
Asia, central	Altai Republic, Mongolia	Scytho-Siberian	ALT_Scythians	5th – 3rd BC	Iron Age	22	4,545454545	0	9,090909091	27,27272727	4,545454545	4,545454545	0	13,63636364	0	4,545454545	13,63636364	0	0	0	0	0	0	4,545454545	0	0	0	0	0	0	0	0	0	0	0	„Rikunt et al. 2004 (2); Gonzalez-Ruiz et al. 2012 (16); Allentoft et al. 2015 (1); Pilipenko et al. 2010 (5)"
Asia, north	Russia	Early-Late Krotovo	SIB_Krotovo	23rd – 17th BC	Early Bronze Age	26	15,38461538	0	34,61538462	3,846153846	3,846153846	0	0	0	0	0	0	0	0	0	7,692307692	0	3,846153846	0	7,692307692	0	0	19,23076923	0	0	0	0	0	0	3,846153846	Mednikova 2012 (26)
Asia, north	Russia	Baraba, Late Bronze Age	SIB_Baraba	13th – 7th BC	Late Bronze Age	19	5,263157895	0	10,52631579	5,263157895	0	0	10,52631579	0	0	5,263157895	15,78947368	0	0	0	0	0	5,263157895	0	5,263157895	0	5,263157895	15,78947368	5,263157895	5,263157895	0	0	0	5,263157895	0	Mednikova 2012 (19)
Asia, north	Russia	Andronovo	SIB_Andronovo	20th – 13th BC	Middle Bronze Age	24	12,5	0	41,66666667	0	0	0	0	0	0	0	0	0	0	0	12,5	0	0	0	0	8,333333333	0	8,333333333	16,66666667	0	0	0	0	0	0	Mednikova 2012 (20), Allentoft 2016 (4)

S6: Haplogroup frequencies used for PCA with 53 modern and the ancient population of Gioshevice

G_Georgian	S_Svan
C_Chechen	I_Ingush
D_Dargin	L_Lezgin

| Region | Country | Population | Abbreviation | N | Haplogroup frequencies | | |

S7: FST values, p values and Slatkin FST matrix of 23 ancient populations.
Np 16050-16383 were considered at the FST computation in Arlequin software. P values are presented in the upper diagonal of the first table.
FST values are presented in the lower diagonal of the first table. Significant Fst values are indicated with p values (<0.05).

pairwise FST values/p values

Region	Country	Population	Abbreviation	N		POL_Grod	HUN_Conq	HUN_Avar	CB_Cont-Zone	HUN_Cum	HUN_Lomb	ITA_Lomb	ITA_med	SPA_med	POL_med	NOR_Vik	DAN_Vik.med	ICE_med	RUS_Yakuts	MON_Xiongnu	CHIN_Wang-Zhen	CHIN_Xianbei	SIB_KROT	SIB_AND	SIB_BAR	MIN_BRAge_KAR	KAZ_BRAge_IAge	ALT_Scyt	References
	Poland		POL_Grod	13	POL_Grod	*	0.06504+-0.0025	0.03772+-0.0018	0.12415+-0.0038	0.19018+-0.0038	0.11543+-0.0032	0.14117+-0.0032	0.00564+-0.0007	0.12444+-0.0034	0.32799+-0.0049	0.41026+-0.0055	0.05207+-0.0023	0.12197+-0.0033	0.00010+-0.0001	0.00010+-0.0001	0.00000+-0.0000	0.00000+-0.0000	0.00277+-0.0005	0.00960+-0.0010	0.06584+-0.0025	0.12355+-0.0034	0.08286+-0.0029	0.01812+-0.0015	This study (13)
Europe, east	Hungary	medieval populations of Conquest period from Hungary	HUN_CONQ	101	HUN_Conq	0.02553	*	0.32036+-0.0041	0.00347+-0.0006	0.63865+-0.0043	0.14652+-0.0036	0.01297+-0.0012	0.01911+-0.0014	0.00337+-0.0006	0.42748+-0.0048	0.00059+-0.0003	0.00812+-0.0009	0.00109+-0.0003	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00020+-0.0001	0.82952+-0.0042	0.51302+-0.0046	0.53460+-0.0050	0.00406+-0.0006	Csósz et al. 2016 (75), Tömöry et al. 2007 (26)
Europe, east	Hungary	Avars	HUN_AVAR	26	HUN_Avar	0.03987	0.00233	*	0.12108+-0.0032	0.41847+-0.0055	0.08108+-0.0027	0.07851+-0.0032	0.57796+-0.0048	0.03554+-0.0018	0.27354+-0.0045	0.05643+-0.0020	0.11029+-0.0031	0.09851+-0.0032	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00010+-0.0001	0.00238+-0.0005	0.37947+-0.0044	0.58549+-0.0048	0.42006+-0.0051	0.01287+-0.0011	Csósz et al. 2016 (26)
Europe, east	Slovakia, Croatia	medieval populations from present-day Slovakia (Nitra-Šindolka, Čakajovce) and Croatia (Zvonimirovo)	CB_Contact_zone	23	CB_Cont-Zone	0.02743	0.03777	0.01634	*	0.03158+-0.0018	0.01346+-0.0012	0.09732+-0.0028	0.01544+-0.0015	0.00248+-0.0004	0.19147+-0.0033	0.01158+-0.0010	0.01257+-0.0012	0.00644+-0.0009	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00020+-0.0001	0.01940+-0.0012	0.01267+-0.0012	0.05891+-0.0020	0.00020+-0.0001	„Csósz et al. 2016 (4); Csákyová et al. 2016 (19)"
Europe, east	Hungary	medieval Cumanians	HUN_Cum	11	HUN_Cum	0.02904	-0.00895	0.00030	0.05467	*	0.33779+-0.0047	0.09237+-0.0025	0.06069+-0.0021	0.33759+-0.0042	0.31116+-0.0045	0.30908+-0.0046	0.09365+-0.0028	0.40560+-0.0044	0.00277+-0.0006	0.00889+-0.0003	0.00020+-0.0001	0.00020+-0.0001	0.01346+-0.0012	0.05960+-0.0027	0.95426+-0.0020	0.68924+-0.0052	0.19473+-0.0036	0.10771+-0.0031	Bogácsi-Szabó et al. 2005 (11)
Europe, east	Hungary	Lombards from Hungary: Szólád	HUN_Lomb	28	HUN_Lomb	0.02398	0.00762	0.01676	0.04086	0.00583	*	0.20760+-0.0038	0.06603+-0.0023	0.02000+-0.0015	0.59836+-0.0041	0.21226+-0.0039	0.22008+-0.0043	0.79378+-0.0036	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00010+-0.0001	0.00020+-0.0001	0.19186+-0.0047	0.04267+-0.0019	0.07148+-0.0027	0.00010+-0.0001	Alt et al. 2014 (28)
Europe, south	Italy - Piedmont	„Lombards from Italy: Rivoli, Corso Levi (Turin); Rivoli, La Perosa (Turin); Mombello Monferrato (Alessandria); Collegno (Turin); Centallo, San Gervasio (Cuneo)"	ITA_Lomb	28	ITA_Lomb	0.02488	0.02216	0.01893	0.02121	0.03698	0.00863	*	0.02327+-0.0014	0.02505+-0.0015	0.05683+-0.0021	0.24602+-0.0044	0.06356+-0.0029	0.49322+-0.0044	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00010+-0.0001	0.06128+-0.0023	0.05683+-0.0022	0.01525+-0.0012	0.00000+-0.0000	Vai et al. 2015 (28)
Europe, south	Italy - Tuscans	„medieval Italians: Firenze, Piazza Signoria; Pisa, Vicopisano; Livorno, Castello Donoratico; Massa Carrara, Fivizzano; Siena, Santa Maria della Scala; Grosseto, San Quirico"	ITA_med	27	ITA_med	0.08406	0.02220	-0.00630	0.04787	0.05385	0.02188	0.03746	*	0.00475+-0.0007	0.02109+-0.0012	0.08069+-0.0026	0.09851+-0.0028	0.12326+-0.0035	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.01238+-0.0011	0.01931+-0.0012	0.00594+-0.0008	0.00020+-0.0001	Guimaraes et al. 2009 (27)
Europe, south	Spain	medieval Spaniards: Basque, Aldaieta	SPA_med	56	SPA_med	0.02600	0.01991	0.02365	0.05358	0.00439	0.02840	0.02833	0.04931	*	0.06207+-0.0024	0.03584+-0.0017	0.00406+-0.0006	0.03257+-0.0017	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.04089+-0.0019	0.02218+-0.0016	0.05386+-0.0021	0.00000+-0.0000	Alzualde et al. 2006 (56)
Europe, east	Poland	medieval Slavs from Poland: Cedynia (West Pomerania Province), Ostrów Lednicki (Greater Poland Province)	POL_med	20	POL_med	0.00602	-0.00018	0.00642	0.01282	0.00776	-0.00592	0.02651	0.03882	0.02353	*	0.32729+-0.0042	0.09415+-0.0031	0.27661+-0.0047	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00050+-0.0002	0.00099+-0.0003	0.39254+-0.0048	0.13870+-0.0031	0.39461+-0.0046	0.00267+-0.0006	Juras et al. 2015 (20)
Europe, north	Norway	Vikings from Norvay: Nord-Trøndelag, Nordland, Sør-Trøndelag, Telemark, Troms, Oppland, Hedmark	NOR_Vik	56	NOR_Vik	0.00029	0.02549	0.01653	0.03194	0.00552	0.00587	0.00485	0.01461	0.01473	0.00278	*	0.06673+-0.0027	0.83190+-0.0036	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.03960+-0.0019	0.05861+-0.0023	0.00822+-0.0010	0.00030+-0.0002	Krzewińska et al. 2015 (56)
Europe, north	Denmark	medieval Danes and Vikings: Galgedil, Kongemarken, Risby	DAN_Vik.med	29	DAN_Vik.med	0.03358	0.02415	0.01475	0.03819	0.02633	0.00761	0.01973	0.01693	0.04019	0.01724	0.01476	*	0.12969+-0.0035	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.01297+-0.0011	0.02841+-0.0015	0.05108+-0.0023	0.00099+-0.0003	Melchior et al. 2010 (29)
Europe, north	Iceland	early medieval Icelander	ICE_med	73	ICE_med	0.01977	0.01768	0.01245	0.03700	0.00072	-0.00764	-0.00177	0.01071	0.01375	0.00458	-0.00533	0.00959	*	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.00000+-0.0000	0.13038+-0.0033	0.03891+-0.0018	0.00960+-0.0008	0.00000+-0.0000	Helgason et al. 2009 (73)
Asia, north	Russia - Eastern Siberia	Yakuts	RUS_Yakuts	57	RUS_Yakuts	0.16168	0.10337	0.13531	0.18807	0.10484	0.15236	0.19450	0.19790	0.18479	0.12798	0.19010	0.16460	0.18955	*	0.00149+-0.0004	0.00109+-0.0004	0.09989+-0.0030	0.00168+-0.0004	0.00317+-0.0005	0.00030+-0.0002	0.00010+-0.0001	0.00000+-0.0000	0.00010+-0.0001	Crubézy et al. 2010 (57)
Asia, central	Mongolia	Xiongnu period: Hsiung-nu	MON_Xion	46	MON_Xiongnu	0.16426	0.09454	0.14305	0.21200	0.11067	0.17532	0.20933	0.22341	0.18924	0.14496	0.19583	0.18380	0.19580	0.04243	*	0.05534+-0.0021	0.10672+-0.0031	0.00000+-0.0000	0.00010+-0.0001	0.00050+-0.0002	0.00030+-0.0002	0.00000+-0.0000	0.00267+-0.0005	„Keyser-Tracqui (2003: 4); Kim et al. 2010 (3)"
Asia, central	China-Inner Mongolia	Wanggus, Upper Capital - Zhenzishan	CHIN_Wang_Zhen	33	CHIN_Wang-Zhen	0.16614	0.08464	0.14997	0.21978	0.13249	0.16508	0.21776	0.24031	0.20957	0.14690	0.18975	0.16897	0.18806	0.04720	0.01635	*	0.00505+-0.0007	0.00000+-0.0000	0.00010+-0.0001	0.00000+-0.0000	0.00020+-0.0001	0.00000+-0.0000	0.00079+-0.0003	„Fu et al. 2007 (12); Fu et al. 2009 (21)"
Asia, central	China-Inner Mongolia	Tuoba Xianbei	CHIN_Xian	16	CHIN_Xianbei	0.21648	0.13933	0.18248	0.24425	0.16935	0.22223	0.27241	0.31859	0.29434	0.18985	0.24674	0.21861	0.25411	0.02274	0.02039	0.05758	*	0.00406+-0.0006	0.00634+-0.0007	0.00010+-0.0001	0.00050+-0.0002	0.00000+-0.0000	0.00317+-0.0005	Changchun et al. 2006 (16)
Asia, north	Russia	Early-Late Krotovo	SIB_KROT	26	SIB_KROT	0.11010	0.06330	0.09116	0.13509	0.08569	0.11542	0.13901	0.16261	0.17177	0.10027	0.11783	0.11124	0.13188	0.06559	0.10113	0.08109	0.08707	*	0.99287+-0.0009	0.01851+-0.0013	0.01881+-0.0014	0.00020+-0.0001	0.00564+-0.0006	Molodin 2012 (26)
Asia, north	Russia	Andronovo	SIB_AND	24	SIB_AND	0.09514	0.05683	0.07684	0.11530	0.05785	0.09785	0.12014	0.14174	0.15051	0.08519	0.10738	0.10580	0.11923	0.05846	0.09552	0.08514	0.07855	-0.02985	*	0.05405+-0.0024	0.04366+-0.0019	0.00020+-0.0001	0.00881+-0.0008	Molodin 2012 (20), Allentoft 2015 (4)
Asia, north	Russia	Baraba, Late-Bronze Age	SIB_BAR	19	SIB_BAR	0.03615	-0.01003	0.00230	0.04504	-0.03374	0.01097	0.02762	0.04762	0.02874	0.00249	0.02447	0.03600	0.01271	0.08864	0.08924	0.09416	0.11973	0.05527	0.03746	*	0.90308+-0.0032	0.28839+-0.0046	0.13731+-0.0035	Molodin 2012 (19)
Asia, north	Russia	„Solenoozernaia; Tatarka; Oust-Abakansty; Bogratsky; Afontova Gora"	MIN_BRAge	20	MIN_BRAge_KAR	0.02477	-0.00208	-0.00544	0.04840	-0.01420	0.02491	0.02719	0.03796	0.03413	0.01679	0.01987	0.02883	0.02247	0.09708	0.08777	0.08980	0.11698	0.04882	0.03892	-0.02128	*	0.24532+-0.0042	0.33630+-0.0045	„Keyser et al. 2009 (11); Allentoft et al. 2015 (9)"
Asia, central	Kazakhstan, Russia	„Ak-Mustafa; Izmaylovka; Oi-Zhaylau-III; Vodokhranilische; Zevakinskiy; Birlik; Molaly, Shubarat; Kok-Mardan; Solyanka; Bulanovo; Stepnoe VII; Bofshekaragankii; Kapova cave; Tanabergen II"	KAZ_BRAge_IAge	34	KAZ_BRAge_IAge	0.02767	-0.00164	0.00014	0.02347	0.01595	0.01661	0.03113	0.04092	0.01715	0.00126	0.02570	0.01871	0.02285	0.11049	0.11870	0.10911	0.16846	0.08099	0.07433	0.00579	0.00757	*	0.00228+-0.0005	„Lalueza-Fox et al. 2004 (26); Allentoft et al. 2015 (8)"
Asia, central	Altai Republic, Mongolia	Scytho-Siberian	ALT_Scyt	22	ALT_Scyt	0.06618	0.03696	0.04427	0.11103	0.03496	0.07947	0.09597	0.10988	0.10984	0.06636	0.07707	0.06983	0.07804	0.09277	0.06195	0.07155	0.07648	0.06760	0.06315	0.01968	0.00463	0.05278	*	„Ricaut et al. 2004 (2); Gonzalez-Ruiz et al. 2012 (16); Allentoft et al. 2015 (1); Pilipenko et al. 2010 (3)"

Slatkin FST values

Region	Country	Population	Abbreviation	N		POL_Grod	HUN_Conq	HUN_Avar	CB_Cont-Zone	HUN_Cum	HUN_Lomb	ITA_Lomb	ITA_med	SPA_med	POL_med	NOR_Vik	DAN_Vik.med	ICE_med	RUS_Yakuts	MON_Xiongnu	CHIN_Wang-Zhen	CHIN_Xianbei	SIB_KROT	SIB_AND	SIB_BAR	MIN_BRAge_KAR	KAZ_BRAge_IAge	ALT_Scyt	References
	Poland		POL_Grod	13	POL_Grod	0.00000	0.02620	0.04153	0.02821	0.02991	0.02457	0.02552	0.09177	0.02669	0.00605	0.00029	0.03475	0.02017	0.19287	0.19654	0.19924	0.27629	0.12372	0.10514	0.03750	0.02540	0.02846	0.07087	This study (13)
Europe, east	Hungary	medieval populations of Conquest period from Hungary	HUN_CONQ	101	HUN_Conq	0.02620	0.00000	0.00233	0.03925	0	0.00768	0.02266	0.02271	0.02031	0	0.02615	0.02474	0.01799	0.11529	0.10441	0.09246	0.16189	0.06757	0.06025	0	0	0	0.03838	Csósz et al. 2016 (75), Tömöry et al. 2007 (26)
Europe, east	Hungary	Avars	HUN_AVAR	26	HUN_Avar	0.04153	0.00233	0.00000	0.01661	0.00030	0.01704	0.01930	0	0.02422	0.00647	0.01680	0.01497	0.01261	0.15648	0.16693	0.17642	0.22321	0.10031	0.08323	0.00231	0	0.00014	0.04632	Csósz et al. 2016 (26)
Europe, east	Slovakia, Croatia	medieval populations from present-day Slovakia (Nitra-Šindolka, Čakajovce) and Croatia (Zvonimirovo)	CB_Contact_zone	23	CB_Cont-Zone	0.02821	0.03925	0.01661	0.00000	0.05784	0.04260	0.02167	0.05028	0.05661	0.01299	0.03300	0.03971	0.03843	0.23164	0.26904	0.28168	0.32318	0.15619	0.13032	0.04717	0.05086	0.02404	0.12490	„Csósz et al. 2016 (4); Csákyová et al. 2016 (19)"
Europe, east	Hungary	medieval Cumanians	HUN_Cum	11	HUN_Cum	0.02991	0	0.00030	0.05784	0.00000	0.00586	0.03840	0.05691	0.00441	0.00782	0.00555	0.02704	0.00072	0.11712	0.12445	0.15272	0.20387	0.09372	0.06140	0	0	0.01621	0.03623	Bogácsi-Szabó et al. 2005 (11)
Europe, east	Hungary	Lombards from Hungary: Szólád	HUN_Lomb	28	HUN_Lomb	0.02457	0.00768	0.01704	0.04260	0.00586	0.00000	0.00870	0.02237	0.02923	0	0.00591	0.00767	0	0.17975	0.21258	0.19771	0.28572	0.13048	0.10847	0.01110	0.02554	0.01689	0.08633	Alt et al. 2014 (28)
Europe, south	Italy - Piedmont	„Lombards from Italy: Rivoli, Corso Levi (Turin); Rivoli, La Perosa (Turin); Mombello Monferrato (Alessandria); Collegno (Turin); Centallo, San Gervasio (Cuneo)"	ITA_Lomb	28	ITA_Lomb	0.02552	0.02266	0.01930	0.02167	0.03840	0.00870	0.00000	0.03892	0.02916	0.02723	0.00487	0.02013	0	0.24147	0.26476	0.27838	0.37441	0.16145	0.13655	0.02840	0.02795	0.03213	0.10615	Vai et al. 2015 (28)
Europe, south	Italy - Tuscans	„medieval Italians: Firenze, Piazza Signoria; Pisa, Vicopisano; Livorno, Castello Donoratico; Massa Carrara, Fivizzano; Siena, Santa Maria della Scala; Grosseto, San Quirico"	ITA_med	27	ITA_med	0.09177	0.02271	0	0.05028	0.05691	0.02237	0.03892	0.00000	0.05186	0.04039	0.01482	0.01722	0.01083	0.24673	0.28769	0.31633	0.46755	0.19418	0.16515	0.05000	0.03946	0.04266	0.12344	Guimaraes et al. 2009 (27)
Europe, south	Spain	medieval Spaniards: Basque, Aldaieta	SPA_med	56	SPA_med	0.02669	0.02031	0.02422	0.05661	0.00441	0.02923	0.02916	0.05186	0.00000	0.02410	0.01495	0.04188	0.01394	0.22667	0.23341	0.26514	0.41711	0.20739	0.17718	0.02959	0.03533	0.01745	0.12339	Alzualde et al. 2006 (56)
Europe, east	Poland	medieval Slavs from Poland: Cedynia (West Pomerania Province), Ostrów Lednicki (Greater Poland Province)	POL_med	20	POL_med	0.00605	0	0.00647	0.01299	0.00782	0	0.02723	0.04039	0.02410	0.00000	0.00279	0.01754	0.00460	0.14676	0.16954	0.17219	0.23434	0.11145	0.09312	0.00250	0.01707	0.00126	0.07108	Juras et al. 2015 (20)
Europe, north	Norway	Vikings from Norvay: Nord-Trøndelag, Nordland, Sør-Trøndelag, Telemark, Troms, Oppland, Hedmark	NOR_Vik	56	NOR_Vik	0.00029	0.02615	0.01680	0.03300	0.00555	0.00591	0.00487	0.01482	0.01495	0.00279	0.00000	0.01498	0	0.23472	0.24351	0.23419	0.32756	0.13356	0.12030	0.02509	0.02027	0.02638	0.08351	Krzewińska et al. 2015 (56)
Europe, north	Denmark	medieval Danes and Vikings: Galgedil, Kongemarken, Risby	DAN_Vik.med	29	DAN_Vik.med	0.03475	0.02474	0.01497	0.03971	0.02704	0.00767	0.02013	0.01722	0.04188	0.01754	0.01498	0.00000	0.00968	0.19704	0.22519	0.20332	0.27978	0.12517	0.11832	0.03735	0.02968	0.01906	0.07507	Melchior et al. 2010 (29)
Europe, north	Iceland	early medieval Icelander	ICE_med	73	ICE_med	0.02017	0.01799	0.01261	0.03843	0.00072	0	0	0.01083	0.01394	0.00460	0	0.00968	0.00000	0.23389	0.24348	0.23162	0.34068	0.15191	0.13537	0.01287	0.02298	0.02338	0.08465	Helgason et al. 2009 (73)
Asia, north	Russia - Eastern Siberia	Yakuts	RUS_Yakuts	57	RUS_Yakuts	0.19287	0.11529	0.15648	0.23164	0.11712	0.17975	0.24147	0.24673	0.22667	0.14676	0.23472	0.19704	0.23389	0.00000	0.04431	0.04954	0.02327	0.07019	0.06209	0.09727	0.10751	0.12421	0.10225	Crubézy et al. 2010 (57)
Asia, central	Mongolia	Xiongnu period: Hsiung-nu	MON_Xion	46	MON_Xiongnu	0.19654	0.10441	0.16693	0.26904	0.12445	0.21258	0.26476	0.28769	0.23341	0.16954	0.24351	0.22519	0.24348	0.04431	0.00000	0.01662	0.02082	0.11251	0.10561	0.09799	0.09621	0.13468	0.06604	„Keyser-Tracqui (2003: 4); Kim et al. 2010 (3)"
Asia, central	China-Inner Mongolia	Wanggus, Upper Capital - Zhenzishan	CHIN_Wang_Zhen	33	CHIN_Wang-Zhen	0.19924	0.09246	0.17642	0.28168	0.15272	0.19771	0.27838	0.31633	0.26514	0.17219	0.23419	0.20332	0.23162	0.04954	0.01662	0.00000	0.06109	0.08824	0.09307	0.10395	0.09867	0.12247	0.07706	„Fu et al. 2007 (12); Fu et al. 2009 (21)"
Asia, central	China-Inner Mongolia	Tuoba Xianbei	CHIN_Xian	16	CHIN_Xianbei	0.27629	0.16189	0.22321	0.32318	0.20387	0.28572	0.37441	0.46755	0.41711	0.23434	0.32756	0.27978	0.34068	0.02327	0.02082	0.06109	0.00000	0.09538	0.08524	0.13602	0.13248	0.20258	0.08281	Changchun et al. 2006 (16)
Asia, north	Russia	Early-Late Krotovo	SIB_KROT	26	SIB_KROT	0.12372	0.06757	0.10031	0.15619	0.09372	0.13048	0.16145	0.19418	0.20739	0.11145	0.13356	0.12517	0.15191	0.07019	0.11251	0.08824	0.09538	0.00000	0	0.05851	0.05133	0.08813	0.07251	Molodin 2012 (26)
Asia, north	Russia	Andronovo	SIB_AND	24	SIB_AND	0.10514	0.06025	0.08323	0.13032	0.06140	0.10847	0.13655	0.16515	0.17718	0.09312	0.12030	0.11832	0.13537	0.06209	0.10561	0.09307	0.08524	0	0.00000	0.03892	0.04050	0.08030	0.06741	Molodin 2012 (20), Allentoft 2015 (4)
Asia, north	Russia	Baraba, Late-Bronze Age	SIB_BAR	19	SIB_BAR	0.03750	0	0.00231	0.04717	0	0.01110	0.02840	0.05000	0.02959	0.00250	0.02509	0.03735	0.01287	0.09727	0.09799	0.10395	0.13602	0.05851	0.03892	0.00000	0	0.00582	0.02008	Molodin 2012 (19)
Asia, north	Russia	„Solenoozernaia; Tatarka; Oust-Abakansty; Bogratsky; Afontova Gora"	MIN_BRAge	20	MIN_BRAge_KAR	0.02540	0	0	0.05086	0	0.02554	0.02795	0.03946	0.03533	0.01707	0.02027	0.02968	0.02298	0.10751	0.09621	0.09867	0.13248	0.05133	0.04050	0	0.00000	0.00763	0.00465	„Keyser et al. 2009 (11); Allentoft et al. 2015 (9)"
Asia, central	Kazakhstan, Russia	„Ak-Mustafa; Izmaylovka; Oi-Zhaylau-III; Vodokhranilische; Zevakinskiy; Birlik; Molaly, Shubarat; Kok-Mardan; Solyanka; Bulanovo; Stepnoe VII; Bofshekaragankii; Kapova cave; Tanabergen II"	KAZ_BRAge_IAge	34	KAZ_BRAge_IAge	0.02846	0	0.00014	0.02404	0.01621	0.01689	0.03213	0.04266	0.01745	0.00126	0.02638	0.01906	0.02338	0.12421	0.13468	0.12247	0.20258	0.08813	0.08030	0.00582	0.00763	0.00000	0.05572	„Lalueza-Fox et al. 2004 (26); Allentoft et al. 2015 (8)"
Asia, central	Altai Republic, Mongolia	Scytho-Siberian	ALT_Scyt	22	ALT_Scyt	0.07087	0.03838	0.04632	0.12490	0.03623	0.08633	0.10615	0.12344	0.12339	0.07108	0.08351	0.07507	0.08465	0.10225	0.06604	0.07706	0.08281	0.07251	0.06741	0.02008	0.00465	0.05572	0.00000	„Ricaut et al. 2004 (2); Gonzalez-Ruiz et al. 2012 (16); Allentoft et al. 2015 (1); Pilipenko et al. 2010 (3)"

Mt F_{ST} values at loci and Hudson Fst matrix of 52 modern and 23 ancient populations

F_{ST} values were calculated between modern and ancient populations, 1000 Genomes non-genomic human SNPs downloaded from ftp.1000genomes.ebi.ac.uk/vol1/ftp/phase3/ and analysed using Arlequin v3.5.1.2. F_{ST} values are presented in the lower diagonal of the table, p-values are listed in the upper diagonal. Significant fst values are indicated with p values (<0.05).

genome-wide average values

| Region | Country | Population | Abbreviation | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | 11 | 12 | 13 | 14 | 15 | 16 | 17 | 18 | 19 | 20 | 21 | 22 | 23 | 24 | 25 | 26 | 27 | 28 | 29 | 30 | 31 | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | 47 | 48 | 49 | 50 | 51 | 52 | 53 | 54 | 55 | 56 | 57 | 58 | 59 | 60 | 61 | 62 | 63 | 64 | 65 | 66 | 67 | 68 | 69 | 70 | 71 | 72 | 73 | 74 | 75 | 76 | 77 | 78 | 79 | 80 | 81 | 82 | 83 | 84 | 85 | 86 | 87 | 88 | 89 | 90 | 91 | 92 | 93 | 94 | 95 | 96 | 97 | 98 | 99 | 100 | 101 | 102 | 103 | 104 | 105 | 106 | 107 | 108 | 109 | 110 | 111 | 112 | 113 | 114 | 115 | 116 | 117 | 118 | 119 | 120 | 121 | 122 | 123 | 124 | 125 | 126 | 127 | 128 | 129 | 130 | 131 | 132 | 133 | 134 | 135 | 136 | 137 | 138 | 139 | 140 | 141 | 142 | 143 | 144 | 145 | 146 | 147 | 148 | 149 | 150 | 151 | 152 | 153 | 154 | 155 | 156 | 157 | 158 | 159 | 160 | 161 | 162 | 163 | 164 | 165 | 166 | 167 | References |
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