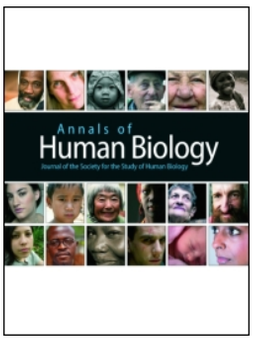

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SHORT REPORT

Analysis of medieval mtDNA from Napole cemetery provides new insights into the early history of Polish state

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ABSTRACT

Contemporary historical anthropology and classical archaeology are concerned not only with such fundamental issues as the origins of ancient human populations and migration routes, but also with the formation and development of inter-population relations and the mixing of gene pools as a result of interbreeding between individuals representing different cultural units. The contribution of immigrants to the analysed autochthonous population and their effect on the gene pool of that population has proven difficult to evaluate with classical morphological methods. The burial of one individual in the studied Napole cemetery located in central Poland had the form of a chamber grave, which is typical of Scandinavian culture from that period. However, this fact cannot be interpreted as absolute proof that the individual (in the biological sense) was allochtonous. This gives rise to the question as to who was actually buried in that cemetery. The ancient DNA results indicate that one of the individuals had an mtDNA haplotype typical of Iron Age northern Europe, which suggests that he could have arrived from that area at a later period. This seems to indirectly confirm the claims of many anthropologists that the development of the early medieval Polish state was significantly and directly influenced by the Scandinavians.

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Introduction

The beginnings of the Polish state in the early Middle Ages gave rise to many anthropological hypotheses concerning the influence of foreign cultures (populations) on the development of the Polish state structures and elites. One of those hypotheses suggests the presence of migrants from the Scandinavian culture area in the Polish territories in that period (Cattaneo, 2009; Skrok, 2013). One of the arguments supporting the above theory is the fact that chamber graves, typical of Scandinavia, have been found in several medieval burial grounds in Poland (Graslund, 1981). In Europe, outside of Scandinavia, such graves have also been identified in Germany, Ukraine and Russia (Janowski, 2011). In addition to the site discussed in this paper, chamber graves have been discovered at the following Polish sites: Cedynia (Malinowska-Łazarczyk, 1982), Sowinki (Krzyszowski, 1995), Kałdus (Chudziak, 2002) and Bodzia (Buko & Sobkowiak-Tabaka, 2011). Chamber graves are a form of burial in which a wooden structure made of horizontal or vertical logs and resembling a small house or chamber is erected over the deceased person's body (Eisenschmidt, 1994). Another special feature of these graves is their large size (as compared to typical burials) and depth, sometimes of up to 1.5 m. Additionally, such graves often exhibit multi-level complex stratigraphy with horizontal and vertical structural elements.

Classical anthropology is unable to conclusively answer the question of whether the individuals inhumed in the studied cemetery are allochtonous, which is suggested by the rich and unusual burial furnishings found in grave 7 in Napole, Chełmno Land (Bojarski, 2014). The archaeologically determined burial style and grave goods (cultural elements) may not be treated as absolute proof that the individuals (in the biological sense) were allochthonous. To address the above questions, fragments of mitochondrial genome were analysed with a focus on hypervariable regions (HVR), which is frequently used in similar studies into human origins.

Materials and methods

Ancient samples

Selected tooth samples were obtained from skeletal remains of two adult male individuals (graves 7 and 8), unearthed at an early medieval cemetery in Napole (53°09′07″N 18°57′06″E) with no signs of enamel damage. The archaeological site studied comprises a long hill (a few hundred metres), well exposed in the surrounding area (Bojarski, 2014). It consists of two great settlements outside a stronghold from

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Supplemental data for this article can be accessed here.

Figure 1. (a) Plan view of Napole Cemetery, analysed graves are marked with grey and arrows (modified from Bojarski 2014). (b) Location of the Napole burial site in the territory of present-day Poland.

the 10th–13th century (Supplementary Figure 1) and one small cemetery with 24 skeletal graves identified (Figure 1). There was the centre of the settlement micro-region in the basin of the lower Drwęca situated next to a long-distance communication route from Rus to Pomerania (Supplementary Figure 2) (Bojarski, 2014). The chronological frames of this burial were set between the end of the 11th and the turn of the 12th and 13th century according to the tombs' equipment (Bojarski, 2014). Notably, one grave in the Napole cemetery certainly contained evidence of a wooden structure, which allows this particular one to be qualified as a supposed chamber grave (Bojarski, 2014). Unfortunately, the skeleton excavated from grave #13 is not available, therefore could not be analysed in parallel with others at the molecular level. All other skeletal remains from this cemetery are also considered as lost. The regularity of grave cavities and homogenous equipment unequivocally suggests short-term use of the necropolis by the local, small community (Bojarski, 2014).

Extraction of aDNA

The teeth of individuals, collected and transferred to sterile containers, were delivered to the aDNA laboratory of the Department of Molecular Biology, Medical University of Łódź and kept frozen until the beginning of the DNA isolation procedure. Each tooth was at first cleaned mechanically, in order to remove surface contamination and washed in 5% NaClO, then in 96% ethanol. Each side of the tooth was exposed to UV light for 30 minutes. Afterwards, teeth were ground to powder in a freezer mill SPEX Sample Prep 6770 (0.4–0.6 g) and incubated in 0.5 M EDTA, pH = 8.0, for 48 hours. Proteinase K and PTB were added and suspensions were incubated at 56 °C for the next 2 hours. Subsequently, the solution was subjected to DNA isolation in MagNA Pure® Compact Nucleic Acid Purification System (Roche, Switzerland).

Mitochondrial DNA analysis

The hyper-variable regions I and II (HVR) of the mtDNA control region were amplified as five overlapping fragments using primer pairs which yielded products of 186, 171, 136, 130 and 156 bp. PCR was performed in 25 µl with 3–4 µl of extract, using standard reagents including KAPA HiFi PCR (Kapa Biosystems, Wilmington, MA), with a profile of 45 cycles and annealing at 53.5–57 °C. Approximately 3 µl of the amplicons were visualised on silver-stained 10% polyacrylamide gel. After cleaning on spin columns (Clean-up, A&A Biotechnology, Gdynia, Poland), PCR products were extended using the BigDye® 3.1 termination-ready reaction mix (Applied Biosystems, Waltham, MA). Each sequencing reaction (20 µl) contained 4 µl of BigDye® mix, 30 ng of primer and 50–70 ng of the amplicon. The cycling conditions were as follows: initial denaturation at 95 °C for 5 minutes was followed by 36 cycles at 95 °C for 30 seconds, 56 °C for 8 seconds and 60 °C for 4 minutes. Extension products were cleaned on spin columns (ExTerminator, A&A Biotechnology), dried in a Speed-Vac system, re-suspended in 20 µl of deionised formamide and analysed using ABI Prism 3130™ Genetic Analyser. Sequences were edited and analysed using BioEdit and MEGA 4: Molecular Evolutionary Genetics Analysis.

Table 1. Results of mtDNA from Napole samples.

Subject	Sex	Age	Coding sequence*	HVR I and II haplotype* (67–256; 16125–16400)	HG
Napole samples					
Grave 8	♂	*Maturus*	7028T, 4529T	73G 16129A 16223T 16304C 16391A	I
Grave 7	♂	*Adultus/Maturus*	7028C	16304C	H
People involved in aDNA analysis					
T.P.	—	—	7028T	73G 16223T 16297C 16298C 16327T	—
K.J.	—	—	7028T	73G 152C 16189C 16270T 16291T	—
A.M.	—	—	7028T	73G 16224C 16284G 16311C 16319A	—
T.K.	—	—	7028T	73G 16126C	—

*Polymorphic sites are numbered according to the revised Cambridge Reference Sequence (rCRS) (Andrews et al. 1999).

Moreover, in order to confirm macrohaplogroup H and I the sequencing reactions were performed on two amplified mtDNA coding regions for SNP 7028 and 4529. Sequence of all primers pairs used in mtDNA analysis are presented in Supplementary Table I.

Authentication of aDNA and contamination prevention

All steps of the molecular biology processing were carried out in the laboratory that is dedicated to the work with ancient DNA and free of the other molecular analysis. All procedures were followed as previously described (Witas et al., 2013). Instead of laborious and expensive cloning of representative samples we decided for sequencing of multiple isolates from the same specimen, as suggested by Winters et al. (2011). At least two teeth from every individual were subjected to isolation of DNA (two independent extractions were carried out from each tooth). If any of the obtained sequenograms differed from the others, such results were not taken into consideration and remnants were not further processed. People who were involved in the sample processing or worked in the lab were mtDNA tested (Table 1). Blank and mock PCR controls were included in each amplification step to monitor against contamination (if positive the whole procedure was repeated). The detailed information on the number of isolations, PCR and sequencing reactions is given in Supplementary Table II.

Results and discussion

The mtDNA sequences of two analysed specimens (each performed in four independent replicates) were identical, which confirms both the reproducibility of acquired data and the sufficient quality of isolated aDNA. Results for the individual from grave 7, which was characterised by relatively rich grave goods (consisting of an iron knife and a spearhead (Bojarski, 2014)), are typical of this part of the world. Haplotype (7028C, 16304C), classifying the individual to haplogroup H, was highly abundant across Europe (also in Poland) in ancient populations ranging from the Neolithic to the Middle Ages (Brandt et al., 2013; Juras et al., 2014; Lorkiewicz et al., 2015; Melchior et al., 2010; Ploszaj et al., 2015; Witas et al., 2015) and it still remains widespread today (Mielnik-Sikorska et al., 2013). Thus, while this haplotype does not allow for specific conclusions, it certainly does not preclude a Scandinavian origin as, according to the literature, it was present also in Iron Age Denmark (Melchior et al., 2008).

A much more unique mtDNA haplotype was found in the individual from grave 8, who was rather tall for a medieval man (169.8 cm tall) (Florkowski, 1992). The obtained mtDNA haplotype (4529T, 7028T, 16129A 16223T 16304C 16391A, belonging to haplogroup I) is now extremely rare in Europe (0.1%) (Parson & Dur, 2007). In the contemporary Polish population, none of the 1253 HVR I haplotypes determined to date match the medieval one identified in Napole (Malyarchuk et al., 2002; Grzybowski et al., 2007; Mielnik-Sikorska et al., 2013). Moreover, such a haplotype has not been found among the data collected by our team from a variety of archaeological sites and periods between the Neolithic and the Middle Ages (Lorkiewicz et al., 2015; Witas et al., 2015) or among the recently published data on ancient mtDNA from the present-day area of Poland (Juras et al., 2014). This indicates that the individual in question is probably allochtonous. Moreover, the chamber grave (no. 13) unearthed at that site, typical of the Scandinavian culture area, suggests that some members of the small population using the cemetery may have come from northern Europe. Analysis of mtDNA data from this part of Europe clearly shows that haplogroup I is characteristic of ancient Scandinavian populations, where its frequency was 12.5% (Melchior et al., 2010), more than 6-times higher than today (1.9%) (Helgason et al., 2000). Among the several haplotypes belonging to haplogroup I identified in northern Europe for different historical periods, only one is identical to the haplotype found in Napole. The haplotype in question was identified in an individual (designated as B5) from the Iron Age Bøgebjerggård settlement in present-day Denmark (Melchior et al., 2008). The identified haplotype might represent descendants of the individual, who arrived in Poland during migrations after Iron Age as suggested by archaeologists (Harck & Lübke, 2001). The absence of that haplotype from the contemporary Polish population may be due to the fact that mtDNA is inherited maternally. It should be remembered that allochthonous individuals were more often male than female, which led to the disappearance of the atypical haplogroups from the Polish mtDNA pool over the subsequent generations. Another likely scenario is that we found the individual belonging to the population of Polish residents carrying such a haplogroup in those days, whose mtDNA haplotype disappeared as a consequence of, for e.g. genetic drift.

The results reported in this paper seem to confirm the claims of many anthropologists and archaeologists that not only was the process of Polish state formation significantly influenced by Scandinavian culture, but people from that part of the continent actually migrated to the area of present-day Poland. It should be stressed that this is the only ancient mtDNA analysis to date concerning this type of cemetery.

Further research into burial grounds containing chamber graves is needed to verify the occurrence of a steady flow of immigrants to the territories inhabited by ancient Polish populations.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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