



# INTERDISCIPLINARIA ARCHAEOLOGICA

## NATURAL SCIENCES IN ARCHAEOLOGY

homepage: <http://www.iansa.eu>



# Genetic Kinship and Sex Determination of Early Modern Period Human Remains from a Defunct Graveyard in the Former Village of Obora (Located on Šporkova Street in Prague's Lesser Town District)

Jana Nováčková<sup>a\*</sup>, Otakara Řebounová<sup>b</sup>, Dana Kvítková<sup>c</sup>, Martin Omelka<sup>b</sup>, Vlastimil Stenzl<sup>c</sup>

<sup>a</sup>*Institute of Archaeology CAS, Letenská 4, Prague, Czech Republic*

<sup>b</sup>*Prague City Archives, Archivní 6, Prague, Czech Republic*

<sup>c</sup>*Institute of Criminalistics, Bartolomějská 310/12, Prague, Czech Republic*

## ARTICLE INFO

### Article history:

Received: 1<sup>st</sup> May 2019

Accepted: 15<sup>th</sup> October 2019

DOI: <http://dx.doi.org/10.24916/iansa.2019.2.4>

### Key words:

Early Modern Period  
ancient DNA (aDNA)  
genetic analyses  
short tandem repeats  
Y-chromosome  
autosome

## ABSTRACT

The main aim of this study was to determine genetic kinship and genetic sex of individuals buried either in the same grave, multi-level grave, or neighbourhood grave. Success of genetic analyses is based on the quantity and quality of extracted aDNA, which can be compromised by degradation of DNA and possible contamination by modern DNA. We analysed archaeological skeletal remains from an Early Modern period graveyard belonging to the Church of St. John the Baptist in the former village of Obora, one of the most honourable Early Modern period archaeological sites in the Czech Republic. Most of the 906 excavated anatomically-laid burials are dated to the years 1730s–1770s. The results of 23 analysed individuals (divided into 4 groups) revealed that individuals are not blood relatives. Studies of historical written sources provide information that the parish affiliation at the time of death had a crucial role in choosing the place for burial. Genetic analyses increased success rate of sex determination to 91% compared to 61% determined by morphological methods. We were thus able to determine the genetic sex of children, an evaluation that cannot be made by morphological methods.

## 1. Introduction

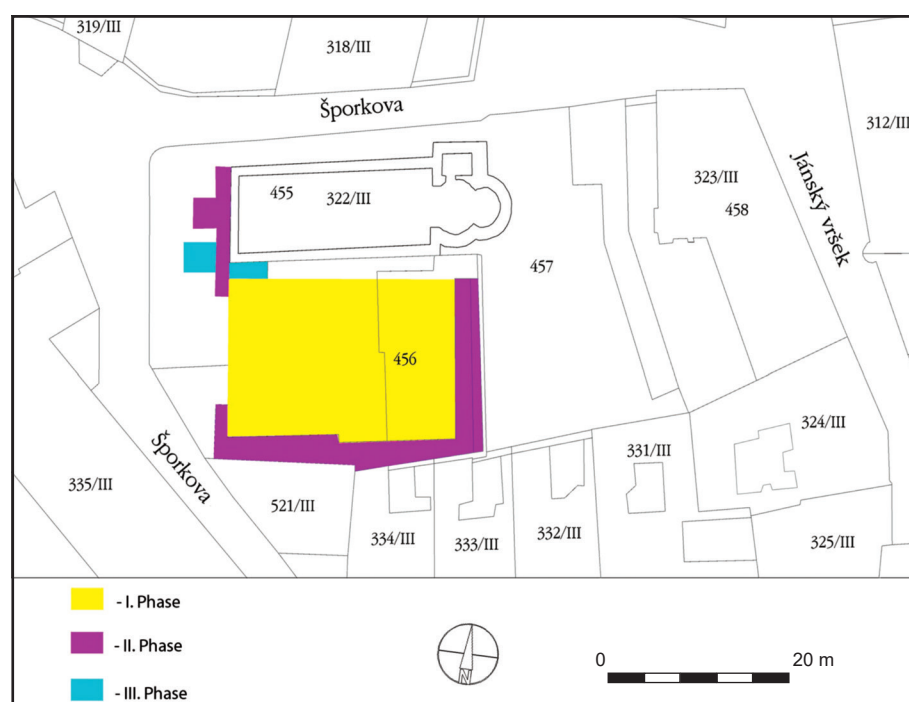
The implementation of genetic analyses into studies of archaeological skeletal remains can provide information about genetic kinship (Ciu *et al.*, 2015; Deguilloux *et al.*, 2014) and the genetic sex of children, when incomplete and poorly-preserved skeletons (Álvarez-Sandoval *et al.*, 2014; Lassen *et al.*, 2000; Tierney, Bird, 2014) cannot be reliably determined with different methods. Analyses of ancient DNA (aDNA) have been previously used in demographic studies of skeletal archaeological remains from several archaeological sites in the Czech Republic, for example, by Boberová *et al.*, 2012, Bravermanová *et al.*, 2018, or Frolík *et al.*, 2017. The determination of genetic kinship among the buried individuals would give an important insight into understanding funerary practices, and the social and demographic structures of historical cultures.

Additional useful information can also be obtained from written historical sources, such as civil and parish registers, testaments and chronicles.

The quality of genetic analyses of aDNA are negatively influenced by two major problems: its degradation into small fragments; and the contamination of aDNA with modern DNA. Firstly, over time, the DNA will become damaged and broken into small fragments due to its inhospitable environmental conditions (Hofreiter *et al.*, 2001, pp. 353–354; Pääbo *et al.*, 2004, pp. 654–660). Secondly, contaminant DNA can come from individuals who were in contact with the skeletal remains (archaeologists, anthropologists, or geneticists in the laboratory), as well as from chemical reagents, laboratory, or cross-sample contaminations. While working with our samples for genetic analyses, we followed the instructions published by Yang and Watt (2005).

Archaeogenetic research of genetic kinship is based on analyses of uniparental markers (Y-chromosome and mitochondrial DNA) and autosomal STR markers

\*Corresponding author. E-mail: [novackova.janka@gmail.com](mailto:novackova.janka@gmail.com)



**Figure 1.** The plan of three phases of excavations at the defunct graveyard of the Church of St. John the Baptist and ground plan of the church. The archaeological rescue excavation was carried out only on the part of graveyard in which construction work took place on (Omelka, 2006b, unpublished). Drawn by Martin Omelka.

(Deguilloux *et al.*, 2014; Juras *et al.*, 2017; Melchior *et al.*, 2010; Simón *et al.*, 2011). Each marker has its own unique mechanism of heritability from parent to offspring, and can reveal or exclude genetic relationships at different levels. We analysed the skeletal remains from a defunct graveyard of the Church of St. John the Baptist in the former village of Obora, situated at Šporkova Street no. 322/III in Prague, the capital of the Czech Republic. Genetic kinship and genetic sex was determined from the results of autosomal and Y-chromosomal STR markers.

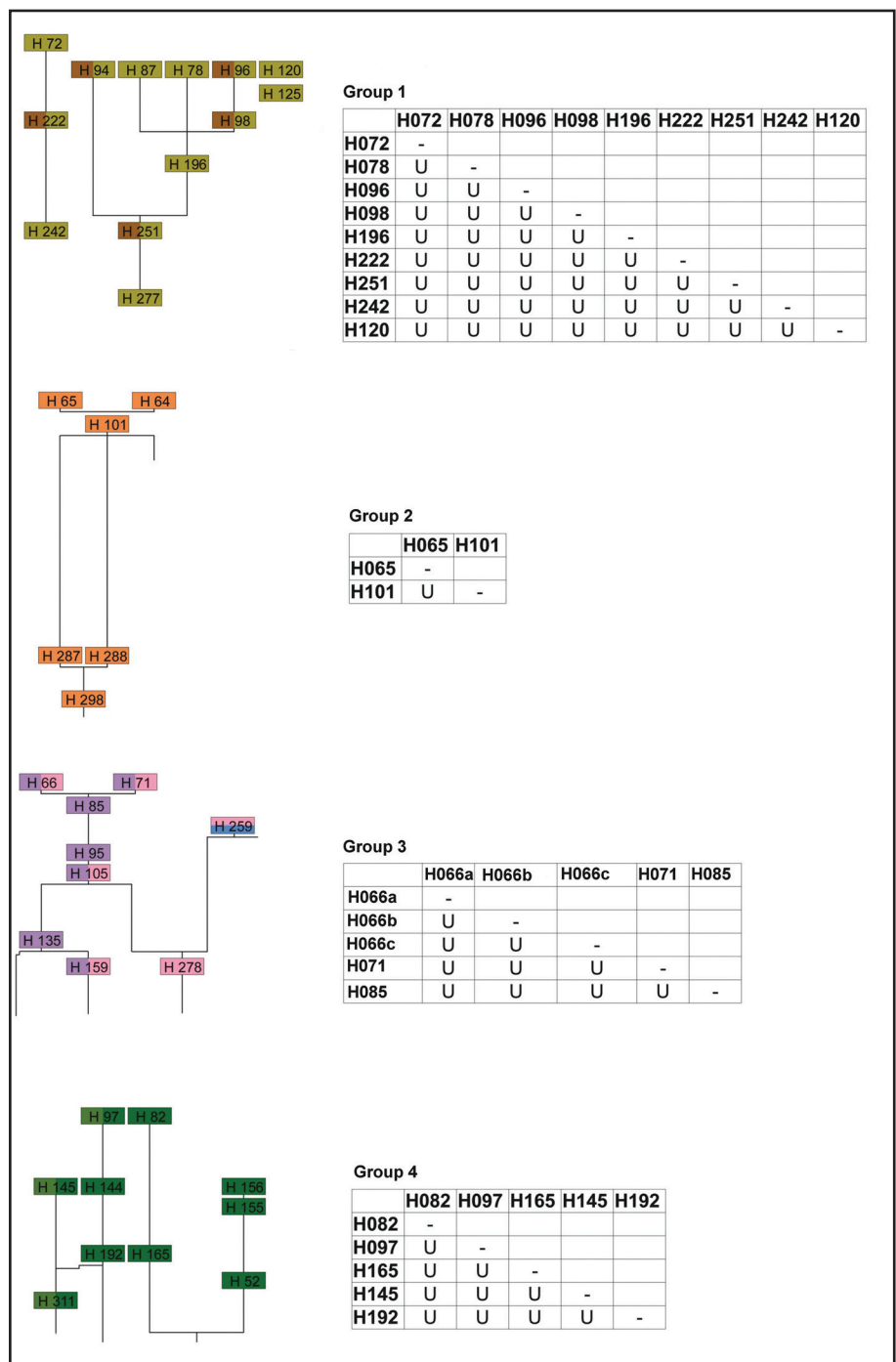
The site of Obora used to be a village located near Prague castle in the quarter known as Prague's Lesser Town. The first written record referencing Obora is dated to the years 1278–1282, but previous excavation has uncovered fragments dated to between the 9<sup>th</sup>–10<sup>th</sup> century (Dragoun, 1988a; 1988b; 1991). Obora was assigned to Prague in the 1650s, and its Church of St. John the Baptist was incorporated into the parish district of the Church of St. Wenceslas. The church with its graveyard was closed in 1784, and rebuilt into a residential building (Omelka, 2009). Skeletal remains of 906 anatomically-laid burials or parts thereof, that were dated to the years 1730s–1770s according to their grave goods, were excavated and documented during the archaeological rescue excavation conducted by the Department of Archaeology of the National Heritage Institute in Prague in the year 2002 (study no. 30/02) and 2004 (study no. 30/04) – Figure 1. The archaeological location in Šporkova Street is one of the most valuable Early Modern period archaeological sites in the Czech Republic due to the assemblage collection of grave goods and preserved written historical sources, providing great possibilities to study: funerary customs among the burgher citizens of the time (Omelka, Řebounová, 2017); other manifestations of Baroque religiousness (Omelka,

Řebounová, 2011; 2014); as well as social and demographic structures among this population (Omelka, Řebounová, 2012b). Several articles were published (mainly in Czech peer-reviewed journals) regarding artefacts found in the grave, including goods such as rings (Omelka, Šlancarová, 2007), beads (Omelka, Řebounová, 2008), crosses (Omelka *et al.*, 2009; 2010), pins (Omelka *et al.*, 2011), a medallion (Omelka, 2006a; Omelka, Řebounová, 2012a; 2016) and buttons (Omelka *et al.*, 2018). Pilot results of genetic analyses of 11 individuals were presented at the International conference “Internationale Tagung der Österreichischen Gesellschaft für Mittelalterarchäologie 2018” in Sankt Pölten (Austria) (Nováčková *et al.*, in press). In the present study, we increased the number of analysed individuals to confirm or reject the hypothesis that the pattern of funerary practices of Early Modern society, as suggested by the pilot study, would hold up under further examination.

## 2. Material and methods

We analysed a total of 46 samples (bones and teeth) from 23 individuals (Table 1), of which 12 individuals (group 3 and group 4) are newly published, and 11 individuals (group 1 and group 2) were previously published (Nováčková *et al.*, in press). Individuals were divided into four groups (Table 1) according to their stratigraphic relationships in the graveyard (Figure 2). The groups contain the genetic material of men, women and children, except for group 2, where two children (newborn and 18 months old) were buried just above an adult woman. Multi-level graves contained skeletal remains of adult women, men and children and so there is a high probability that they are members of one family (for example,

**Figure 2.** Flowcharts by Jiří Vachuda. The flowcharts are parts of unpublished documentation of research in Šporkova Street, schematically representing the position of graves on the burial site in the geometrically defined sectors. Each sector is designated by a different colour. Some skeletons intersected more than one sector, and so are labelled using more than one colour. The results of the computer analysis of genetic kinship are the nearby flowcharts.



**Figure 3.** Skeletal remains of individuals H66 and H71 (in blue). Grave H66 contained skeletal remains of an adult female with additional bones (in green) of a child and adult male. Photographed by Jiří Vachuda.



**Table 1.** The list of individuals analysed.

No.	ID	Sex determined anthropologically	Age determined anthropologically	Analysed part of skeleton	Concentration of aDNA (pg/ul)	Inventory number	Group
1.	H72/I	female	30–40	tooth	33.4	P7A 19 001	1
2.	H72/II			bone	4.64		
3.	H78/I	undetermined	2–3	tooth	1.55	P7A 19 007	1
4.	H78/II			bone	36		
5.	H94/I	undetermined	juvenis	tooth	4.22	P7A 19 021	1
6.	H94/II			bone	5.25		
7.	H96/I	female	50–60	metatarsal	0.24	P7A 19 023	1
8.	H96/II			rib	8.03		
9.	H98/I	female	30–40	tooth	2.46	P7A 19 025	1
10.	H196/I	male	maturus	tooth	15.3	P7A 19 122	1
11.	H196/II			rib	11.6		
12.	H222/I	male	40–50	ulna	394	P7A 19 162	1
13.	H222/II			tooth	366		
14.	H251/I	female	30–40	tooth	2.11	P7A 19 173	1
15.	H251/II			tooth	0.9		
16.	H120/I	undetermined	newborn	ulna	9.06	P7A 19 047	1
17.	H120/II			rib	66		
18.	H242/I	undetermined	juvenis	bone	4.32	P7A 19 162	1
19.	H242/II			rib	2.12		
20.	H64/I	undetermined	18 months	tooth	1.26	P7A 18 993	2
21.	H64/II			bone	2.54		
22.	H65/I	undetermined	newborn	humerus	35.7	P7A 18 994	2
23.	H65/II			rib	68.1		
24.	H101/I	female	maturus	tooth	47.4	P7A 19 028	2
25.	H101/II			rib	1.25		
26.	H66a/I	female	maturus	tooth	6	P7A 18 995	3
27.	H66a/II			tooth	17.8		
28.	H66b/I	undetermined	juvenis	rib	11.4		
29.	H66b/II			bone	2.04		
30.	H66c/I	male	maturus	tooth	2.79		
31.	H66c/II			tooth	31.3		
32.	H71/I	undetermined	newborn	femur	2.62	P7A 19 000	3
33.	H71/II			os petrosum	69.2		
34.	H85/I	female	20–30	tooth	0.5	P7A 19 014	3
35.	H85/II			tooth	5.95		
36.	H85/III			tooth	2.16		
37.	H82/I	undetermined	newborn	radius	3.8	P7A 19 011	4
38.	H82/II			phalang	2.93		
39.	H97/I	female	50–60	calf bone	2.49	P7A 19 024	4
40.	H97/II			phalang	1.29		
41.	H165/I	female	40–50	tooth	35.2	P7A 19 092	4
42.	H165/II			os petrosum	396		
43.	H145/I	male	adultus	os petrosum	317	P7A 17 072	4
44.	H145/II			tooth	17.5		
45.	H192/I	male	adultus	tooth	24.9	P7A 19 118	4
46.	H192/II			metacarpus	1.97		

see Figure 3). Samples of teeth and bones were taken from different parts of the skeletons, depending on their state of skeletal preservation. For detailed information about the samplings, see Table 1. Sampling took place in the National Museum in Prague, where the remains are deposited.

Samples were analysed in several independent steps in four separate rooms (mechanical cleaning; extraction of aDNA; quantification and PCR amplification; post-PCR sequencing). Blank controls were added to each step/reaction to monitor for possible contamination resulting from the lab procedures, but revealed no evidence thereof.

### 2.1 Mechanical cleaning and extraction of aDNA

Samples were rinsed using 96% ethanol and ultra clean water. Bone and teeth surfaces were sanded using either a Dremel Multi (Dremel) electric mini sander, or manually using sandpaper due to a sample's preservation. Bones were cut into small pieces and ground into powder using a 6870 Freezer Mill (Spex Sample Prep). Subsequently, 70mg of the bone powder was incubated in a lysis buffer (0.5M EDTA [pH 8.0], Proteinase K and 0.5% SDS) at 56°C in a UVP HB-1000 Hybridizer Hybridization Oven (Analytik Jena US LLC) for 24 hours. Finally, aDNA was extracted using a MinElute PCR Purification Kit (Qiagen) according to a modified protocol published by Yang *et al.* (1998) and Anderung *et al.* (2008).

### 2.2 Quantification of aDNA

The success of the extraction of preserved aDNA and the amount of extracted aDNA was determined using a real-time PCR quantification Plexor HY System (Promega) kit on a LightCycler 480 RealTime PCR Instrument (Roche). Samples were prepared in duplex reactions. The Plexor HY kit contains primer for a target of a 133bp sequence from a testis-specific protein, Y-encoded (TSPY) locus on chromosome Y, providing means of determining genetic sex.

### 2.3 Amplification and sequencing of aDNA

Samples were analysed for 23 autosomal STR markers, amelogenin X and Y, and 23 Y-chromosomal STR markers using four commercially-available kits: the PowerPlex ESX 17 System, the PowerPlex ESI 17 Pro System, the PowerPlex 16 System, and the PowerPlex Y23 System (all from Promega Corporation). Amelogenin X and Y loci were used to determine genetic sex. Each sample was analysed in several independent amplifications and sequencing reactions using peqSTAR 96X Universal Gradient cycler (VWR Pqlab) and the Applied Biosystems 3130xl Genetic Analyzers instrument (15kV injection for 15s at POP4 polymer) (Applied Biosystems). Samples were prepared according to the manufacturer's recommendation with 32 amplification cycles instead of the 30 that were recommended by the manufacture protocol.

### 2.4 Data analyses

Raw data from capillary electrophoresis were analysed with GeneMapper IDX software (Applied Biosystems). Results

of autosomal and Y-chromosomal STR markers were used for genetic kinship and genetic sex determination among buried individuals. Results of STR markers were evaluated and computed by several software programs: Mlrelate (Kalinowski *et al.*, 2006), Familias 3 (Kling *et al.*, 2014), Network 5 (Bandelt *et al.*, 1999), and Network Publisher 2.1.2.5 (Fluxus Technology Ltd.) A phylogenetic network of Y-chromosomal STR markers was constructed only for markers that were successfully genotyped in all male samples. The network was constructed using Median joining (Bandelt *et al.*, 1999), and the final tree was redrawn by Network Publisher 2.1.2.5 (Fluxus Technology Ltd). Genetic kinship between samples in all groups was computed by one to one for all samples using software Familias 3, that differentiated between five categories of genetic relationship (parent-offspring, full siblings, half siblings, cousins and second cousins) as well as unrelated individuals using Blind search by calculating a likelihood ratio (Kling *et al.*, 2014) and ML-Relate (Kalinowski *et al.*, 2006) that determined three close relationships (parent-offspring, full siblings and half siblings) and unrelated individuals (Kalinowski *et al.*, 2006).

## 3. Results

The success of the genetic analyses depends on the quantity and quality of the extracted aDNA (Table 1). Genetic sex and genetic kinship was evaluated for individuals that were successfully genotyped in several independent reactions of two or three different samples from one individual. Samples H64 and H94 were excluded from the statistical analyses, since sample H64 failed to be successfully genotyped, and sample H94 did not provide reliable results (results from two different parts of the skeleton gave different results). The skeletal remains of H94 were excavated during two different archaeological excavations and it is possible that the two parts of the skeleton were completed incorrectly.

### 3.1 Genetic kinship

Genetic kinship was determined from the result of autosomal (Table 2) and Y chromosomal STR markers (Table 3). STR profiles listed in Table 2 and Table 3 are summaries of all performed analyses from all kits, as well as all samples analysed for the same individual. The success rate of STR marker detection was increased using three different available autosomal kits. Ancient DNA is degraded into small fragments over time, and it is necessary to analyse small fragments (Allentoft *et al.*, 2012; Pääbo, 1989). The advantage of using the PowerPlex ESX 17 System and PowerPlex ESI 17 Pro System kits as complements is that while they contain the same markers, the primers are designed to complement one another, with a different final marker length. Markers that are long in the first kit are short in the second. The stratigraphic relation between individuals within the burial grounds is shown on flowcharts below (Figure 2). Genetic analyses revealed only unrelated

**Table 2.** Results of autosomal STR markers (NA – results not available; XX – female; XY – male).

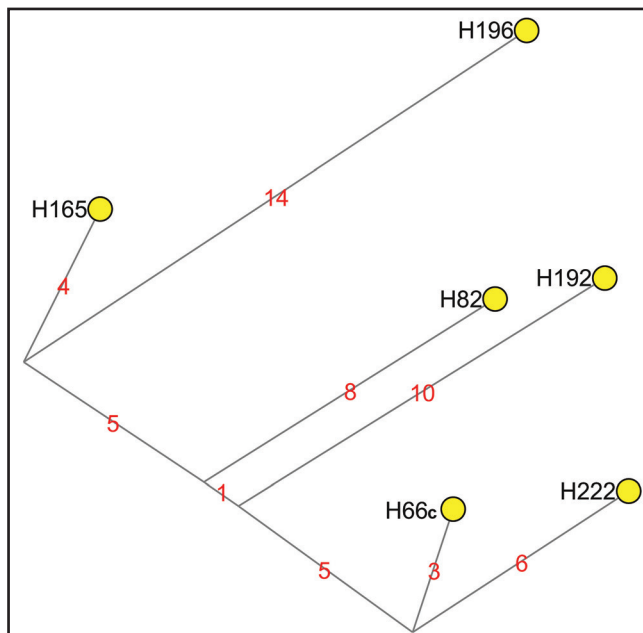
	Amel	D3S1358	D19S433	D2S1338	D22S1045	D16S539	D18S51	D1S1656	D10S1248	D2S441	TH01	Vwa	D21S11	D12S391	D8S1179	FGA	SE33	Penta E	D5S818	D13S317	D7S820	CSF1PO	Penta D	TPOX
H72	XX	16; 17	15; 13	16; 17	15	12; 13	15; 17	12; 17.3	14	14	8; 9	16; 19	30	17; 19	12; 14	23; 25	14; 21	12	9; 11	8; 12	11	11	9	11
H78	XX	14; 16	13; 15	NA	15	11	13; 14	12; 15.3	13; 16	10	6; 8	17; 18	28	15	14; 15	24	NA	NA	11; 12	10; 12	8; 11	NA	NA	9; 11
H94													NA											
H96	XX	15; 18	13	NA	11; 16	NA	NA	11; 16	14	10	7; 8	16; 17	28	19; 24	11	NA	NA	NA	13	11	NA	NA	NA	8
H98	XX	14; 17	13	20; 25	15; 16	9; 11	13; 17	12; 16	15	10; 11	7; 9	15; 19	NA	15; 19	12; 13	20; 24	17	13	11	14	9	NA	NA	8
H196	XY	15; 17	15	24	16	12; 12	12; 14	13; 17.3	14	11; 11	7; 8	16; 18	29; 30	22; 24	13; 16	19; 26	20; 27.2	NA	11; 12	11; 12	11; 12	12	NA	8; 9
H222	XY	14; 16	13; 14	17; 19	15; 18	11	12; 14	16	13; 15	10; 12	7; 7	17; 18	28; 29	18; 24	12; 13	21; 22	19.2; 21.2	NA	11	8	10; 12	12	NA	9
H251	XX	15; 16	12	16	15	12	18	12; 14	14	10; 11.3	8; 9.3	17; 18	28; 30	NA	13; 14	22; 25	25.2; 17	NA	11	8; 11	NA	NA	NA	NA
H242	XX	14; 17	12	17; 18	14; 16	11; 12	12; 14	11; 15	14	10; 11	9; 9.3	20	30; 30.2	20; 22	NA	NA	18	NA	NA	NA	10	11	NA	NA
H64													NA											
H65	XX	16; 17	14	23	16	9; 13	12	12; 15	13	11	6; 7	18	29; 31	18; 20	11; 12	23; 25	20	7	11	11; 12	10	10	NA	10; 11
H120	XX	15; 18	14; 15	20	14; 15	11; 12	11; 12	11	15; 17	11; 14	8; 9.3	15; 18	31.2	18; 19	12; 13	20	23.2; 25.2	NA	10; 13	12	11	NA	NA	8; 12
H101	XX	18	14; 15.2	17	12; 16	11	15; 18	15.3; 18.3	13	14	6; 9.3	16	29; 32.2	22	14	19; 20	24.2; 30.2	10; 18	11; 12	11	10	13	8; 12	11
H66a	XX	15; 17	11; 13	23; 24	16	9; 11	12; 14	11; 12	13; 16	11; 11.3	6; 9	14; 17	28; 29	17; 19	10	22; 23	18; 27.2	11; 16	NA	11; 12	11	10	10	8; 11
H66b	XX	15; 18	13	NA	11; 17	9; 12	15	13; 17.3	16	10	6; 9.3	14; 19	28; 31	18	11	NA	NA	13	11	12	11	NA	NA	8; 14
H66c	XY	13; 16	15; 17	17	12; 17	11; 12	15; 17	18.3	13; 14	10; 11	6; 8	16; 18	27; 32.2	18	13; 15	20.2; 23	21.2; 30.2	NA	NA	NA	NA	NA	NA	NA
H71	XX	16; 17	15	17; 20	11; 15	11; 13	12; 14	12; 16	13; 14	11	6; 9.3	16; 18	28; 30.2	15; 18	14; 15	25	NA	5; 15	11; 12	8	8; 10	12	9; 12	8; 12
H85	XX	14; 17	13; 14.2	23; 24	11; 17	8; 13	12; 14	12; 17	12; 13	11	7; 9.3	14; 17	29; 30	20; 21	10; 14	19; 25	15; 19	5	9; 13	8; 13	8; 12	13	9; 13	8
H82	XY	16; 18	14; 16.2	17; 23	15; 16	11; 13	13; 14	11; 15	14	14	NA	17; 18	28; 30	15	10; 14	22; 23	16; 25.2	NA	10; 12	8; 11	8; 9	10	NA	8
H97	XX	15	12; 13	17	16; 17	12	NA	NA	16	14	7	16	NA	NA	15; 17	23; 24	NA	NA	11	11; 14	10; 12	12	NA	NA
H165	XY	14; 15	14; 15.2	23; 25	11	11; 14	15; 17	16	13; 17	11; 14	9; 9.3	15; 16	29; 30	17; 20	13	21; 23	25.2; 28.2	5; 12	12	10; 12	10	10	11	8
H145	XX	14; 15	11; 14	23	15	10; 12	11; 13	15; 16	12; 13	10; 14	6; 9.3	16; 17	30; 32.2	18	12; 15	23; 23.2	26.2; 27.2	7	9; 12	11	10	NA	NA	11
H192	XY	16; 20	14; 15.2	18; 19	16; 17	12	13; 15	15; 15.3	13; 15	10; 11	9; 9.3	15; 18	29; 30	16; 17	13; 15	21; 23	16; 20	NA	10; 13	11; 12	11; 12	11	9; 13	8; 9

**Table 3.** Results of Y-chromosomal STR markers (NA – results not available).

	DYS76	DYS3891	DYS448	DYS38911	DYS19	DYS391	DYS481	DYS49	DYS533	DYS438	DYS437	DYS70	DYS635	DYS390	DYS439	DYS392	DYS643	DYS393	DYS458	DYS385a	DYS385b	DYS456	YGA1A-H4
H196	18	13	20	32	NA	11	30	11	12	10	NA	18	24	24	12	11	10	13	16	14	16	15	NA
H222	17	13	18	28	14	12	22	12	12	12	15	18	23	22	11	13	NA	13	18	11	14	16	12
H66c	17	13	19	30	NA	10	22	12	12	NA	NA	17	24	NA	NA	NA	NA	13	16	11	14	NA	NA
H82	18	13	19	29	14	9	23	12	NA	NA	NA	17	20	NA	12	11	9	12	19	13	15	NA	NA
H165	16	13	20	30	14	10	26	12	10	10	16	21	21	23	11	11	12	13	17	14	15	14	11
H192	18	13	20	30	14	10	22	12	12	10	14	19	21	24	13	11	NA	13	16	16	18	17	11

relationships between analysed samples within all groups. The matrix generated by M1-Relate software (Figure 2), provides information that individuals in all groups are not blood-related.

Six individuals were determined by a signal for amelogenin Y as a male and were analysed for Y-chromosomal STR markers (Table 3). All identified male individuals differed considerably from each other in terms of observed alleles (Figure 4), providing no evidence of any father-son relationships, nor of a common close male ancestor.



**Figure 4.** The phylogenetic network constructed for Y-chromosomal STR markers (yellow rings-individuals; red numbers-number of mutations).

### 3.2 Sex determination

The presence/absence of a signal for amelogenin Y locus was used to determine the genetic sex of skeletal remains (XX – female and XY – male). Six individuals determined

as a male by the presence of a signal for amelogenin Y were successfully genotyped for Y-chromosomal STR markers. Due to the fact that the amelogenin Y locus can be affected by allelic drop-out, we also took the result of the amplification TSPY gene in the Plexor HY kit into consideration. All samples that did not have a signal for amelogenin Y were also not amplified for the Y-chromosomal TSPY gene, and genetic sex was classified as a female (Table 4). Our results were compared with the morphological findings of studies performed by Milan Stloukal from the Department of Archaeology of the National Museum in Prague. His unpublished morphological examinations of skeletal remains are archived in the Department of Archaeology in the National Heritage Institute in Prague. The anthropological sex and age of the skeletal remains were determined using methods in accordance to the protocol by Ferembach *et al.* (1979). We observed two cases of discordance (for individuals H145 and H165) between morphological and genetic findings (see Table 4), and thus were able to increase the rate of sex determination from 61% for morphological findings (14 individuals) to 91% (21 individuals) for genetic findings. We were also able to determine the sex of children that could not be evaluated by morphological methods. Both individuals H145 and H165 were poorly preserved, having seriously damaged skeletons and fragmented skulls.

### 4. Discussion

The results of the genetic analyses confirmed the hypothesis about the funerary practices of Early Modern period burghers, which was based on the study of historical written sources such as death registers, parish registers and testaments. Historical written sources did not provide any clear information about the existence of family graves on the bourgeois graveyard of St. John the Baptist church in the in Early Modern period village of Obora. Genetic analyses revealed that the individuals, who were buried in the same

**Table 4.** Results of morphological and genetic sex determination; discordances between morphological and genetic findings are labelled in red.

No.	ID	Sex determined antropologically	Sex determined genetically	No.	ID	Sex determined antropologically	Sex determined genetically
1.	H72	female	female	13.	H101	female	female
2.	H78	undetermined	female	14.	H66a	female	female
3.	H94	undetermined	undetermined	15.	H66b	undetermined	female
4.	H96	female	female	16.	H66c	male	male
5.	H98	female	female	17.	H71	undetermined	female
6.	H196	male	male	18.	H85	female	female
7.	H222	male	male	19.	H82	undetermined	male
8.	H251	female	female	20.	H97	female	female
9.	H120	undetermined	female	21.	H165	female	male
10.	H242	undetermined	female	22.	H145	male	female
11.	H64	undetermined	undetermined	23.	H192	male	male
12.	H65	undetermined	female				

multi-level graves or in neighbouring graves, are not blood-related members of a single family.

The evidence from parish and civil registers suggests that bourgeois (middle class) members of society were buried in accordance to the parish affiliation of the given house which was the site of their death. Division of a family at the time of death was not unusual in that period. As a case in point, we can mention the burials of the young boy Johann Roßlaw and his mother. Although the boy died on 3<sup>rd</sup> May 1764 and his mother on 22<sup>nd</sup> May 1764, they were not buried in the same grave, nor even at the same graveyard as a result of the different parish affiliation of the houses in which their death took place. The boy died at the “House of Three Swallows” and was buried at the graveyard of the Church of St. John the Baptist, but his mother died at “Schumann House”, and was buried at the graveyard of the Church of St. Lawrence. Her daughter Rosina died in October 1764 at the house “U Jedličků” and was buried at the graveyard of the Church of the St. Lawrence (Prague City Archives, Collection of Matrices, sign. MIK Z4, fold 19–20 and 22; Omelka, Řebounová, 2012a, p.239). A very similar example was in the case of the Roßenfeld family. Three of the five children were buried at the graveyard of the Church of St. Lawrence and two at the graveyard of the Church of St. John the Baptist. It was also probably due to their different parish affiliations (Prague City Archives, Collection of Matrices, sign MIK Z3, fold 310 and 313; sign MIK Z4, fold 20 and 31).

Other useful sources of funerary practices can be gleaned from testaments. Testaments of aristocratic and the richest, social-bourgeoisie class individuals, who were usually buried in church interiors, were written very precisely, containing detailed information about where exactly they want to be buried and even with whom they wish to be buried after death (Král, 2005; Nováčková *et al.*, 2019, in press). The graveyard of the Church of St. John the Baptist was mainly used to bury citizens belonging to the middle or lower-middle social classes, and individual testaments (if written) usually only specified the name of the church. We can mention, for example, the testament of František Dispach. He died in 1766 in his house in Lesser Town, which belongs to the parish district of the Church of St. John the Baptist. His testament was written only several months before his death, and he wanted to be buried at the graveyard of the Church of St. John the Baptist or the graveyard of the Church of St. Wenceslas. Finally, he was buried at the graveyard of the Church of St. Wenceslas (Manuscript Collection, sign 4764, fol. A21–A22) and not at the graveyard of the Church of St. John the Baptist, where his five children had been buried before him (Anna died in 1731, Vaclav in 1737, Theresie in 1743, Ludmila in 1743, and Antonie in 1750). All his children died in their father's house (Prague City Archives, Collection of Registries, sign MIK 3, fol. 185, 211, 253, 256 and 281). There is also mention of Anna Dispachova, who died in 1742 in the house “At the White Angel”, and was buried at the graveyard of the Church of St John the Baptist, but the relationship with František Dispacha is not clear from the register (Archive of the City of Prague,

Collection of Registries, sign MIK 3, fol. 236). It is evident that family relationships were not taken into consideration when members of one family were buried.

According to the data from historical written sources and from the results of genetic analyses, there is no indication that people from the Early Modern period's lower and middle social classes (*i.e.* most of the people buried at graveyard of the Church of the St. John the Baptist) of purposefully-buried members of one family were buried in one multi-level grave, or in neighbourhood grave sites, or even in the same graveyard, as was the very common practice of the aristocracy and the richest among the population (Král, 2005).

## 5. Conclusion

In this study we have applied an interdisciplinary approach to investigate genetic kinship, genetic sex and the funerary practices of an Early Modern period bourgeois society. Genetic analyses are a powerful method for sex determination in skeletons of children, as well as in badly-preserved and incomplete skeletons of adults (Álvarez-Sandova *et al.*, 2014; Lassen *et al.*, 2000; Tierney, Bird, 2014), where morphological methods provide unreliable or no results (Álvarez-Sandova *et al.*, 2014; Lassen *et al.*, 2000; Tierney, Bird, 2014). We observed a contradiction between morphological and genetic methods in the sex determination of two separate skeletons of buried individuals: individuals H145 and H165 were poorly preserved, resulting in an unreliable morphological determination of sex. In such cases, genetic analyses are a more exact method to determine the sex of skeletal remains than are morphological treatments. By implementing genetic analyses, the number of successfully-determined individuals increased from 14 (61%) to 21 (91%); in addition, we were able to determine the genetic sex of children that could not be determined through morphological methods.

Genetic analyses are a crucial tool in determining the genetic kinship of archaeological skeletal remains. The skeletal samples used for genetic analyses were chosen according to their relative stratigraphic positions within the burial grounds, and divided into four groups. Skeletons of adults and children buried in the same multi-level grave, or in very close proximity, have a high probability of being members of the same family. Genetic analyses of autosomal and Y-chromosomal STR markers revealed, however, that the individuals analysed were not blood-relatives. These results of genetic analyses are in accordance with and confirm the hypothesis based on the evidence provided by written historical sources (civil and parish registers and testaments). There is evidence that some members of families of middle and lower social classes were buried in different graveyards: because they had died in different houses belonging to a different parish affiliation. The tradition of founding family graves at that time is well documented among aristocratic families and the more wealthy inhabitants, who were usually buried together; however, this would be in the interior of the

church. On the other hand, the majority of baroque inhabitants of the past village of Obora buried at the graveyard of the Church of St. John the Baptist were probably buried there wherever a free place was available and according to their parish affiliation, without taking blood relationships into consideration.

## Acknowledgements

The research of family relationships of skeletal remains was supported by a grant from the Ministry of the Interior of the Czech Republic (VI20162020015). We are grateful to the National Museum in Prague for allowing us to sample the human remains in their possession.

## References

- ALLENTOFT, M.E., COLLINS, M., HARKER, D., HAILE, J., OSKAM, C.L., HALE, M.L., CAMPOS, P.F., SAMANIEGO, J.A., GILBERT, M.T., WILLERSLEV, E., ZHANG, G., SCOFIELD, R.P., HOLDAWAY, R.N., BUNCE, M., 2012. The half-life of DNA in bone: measuring decay kinetics in 158 dated fossils. *Proceedings Biological Sciences*, 279(1748), 4724–4733.
- ÁLVAREZ-SANDOVAL, B.A., MANZANILLA, L.R., MONTIEL, R., 2014. Sex Determination in Highly Fragmented Human DNA by High-Resolution Melting (HRM) Analysis. *PLoS ONE*, 9(8), e104629.
- ANDERUNG, C., PERSSON, P., BOUWMAN, A., ELBURG, R., GÖTHERSTRÖM, A., 2008. Fishing for ancient DNA. *Forensic Science International: Genetics*, 2(2), 104–107.
- BANDELT, H.J., FORSTER, P., RÖHL, A., 1999. Median-joining networks for inferring intraspecific phylogenies. *Molecular Biology and Evolution*, 16(1), 37–48.
- BOBEROVÁ, K., DROZDOVÁ, E., PÍŽOVÁ, K., 2012. Application of Molecular Genetic Methods in Anthropological and Paleodemographic Studies of Fragmentary and Damaged Skeletal Material from Rescue Excavations. *Journal of Life Sciences*, 6(9), 961–969.
- BRAVERMANOVÁ, M., DOBISÍKOVÁ, M., FROLÍK, J., KAPOVÁ, S., STRÁNSKÁ, P., SVĚTLÍK, I., VANĚK, D., VELEMÍNSKÝ, P., VOTRUBOVÁ, J., 2018. Nové poznatky o ostatcích z hrobů K1 a K2 rotundy sv. Víta na Pražském hradě – New findings on the remains from graves K1 and K2 from the St. Vitus Rotunda at Prague Castle. *Archeologické rozhledy*, 70, 260–293.
- CUI, Y., SONG, L., WEI, D., PANG, Y., WANG, N., LI, C., FENG, B., TANG, W., LI, H., REN, Y., ZHANG, C., HUANG, Y., HU, Y., ZHOU, H., 2015. Identification of kinship and occupant status in Mongolian noble burials of the Yuan Dynasty through a multidisciplinary approach. *Philosophical Transactions of the Royal Society B: Biological Sciences*, 370(1660), 20130378.
- DAMGAARD, P.B., MARGARYAN, A., SCHROEDER, H., ORLANDO, L., WILLERSLEV, E., ALLENTOFT, M.E., 2015. Improving access to endogenous DNA in ancient bones and teeth. *Scientific Reports*, 5, 11184.
- DEGUILLOUX, M., PEMONGE, M., MENDISCO, F., THIBON, D., CARTRON, I., CASTEX, D., 2014. Ancient DNA and kinship analysis of human remains deposited in Merovingian necropolis sarcophagi (Jau Dignac et Loirac, France, 7<sup>th</sup>–8<sup>th</sup> century AD). *Journal of Archaeological Science*, 41, 399–405.
- DRAGOUN, Z., 1988a. Archeologický výzkum rotundy sv. Jana Křtitele pod Pražským hradem v r. 1986 a 1987 – Archaeological Excavation of the Rotunda of St. John the Baptist below Prague Castle in 1986–1987 (in Czech). *Archaeologia historica*, 13, 403–416.
- DRAGOUN, Z., 1988b. Praha 1 – Malá Strana, Jánký vršek, Šporkova ulice – Prague 1 – Lesser Town, Jánký vršek, Šporkova street (in Czech). *Pražský sborník historický*, 21, 184–185.
- DRAGOUN, Z., 1991. Praha 1 – Malá Strana, Jánký vršek – Prague 1 – Lesser Town, Jánký vršek, Šporkova street (in Czech). *Pražský sborník historický*, 24, 195–196.
- FEREMBACH, D., SCHWIDETZKY, I., STLOUKAL, M., 1979. Empfehlungen für die Alters- und Geschlechtsdiagnose am Skelett. *Homo*, 30, 1–32.
- FROLÍK, J., STRÁNSKÁ, P., VOTRUBOVÁ, J., EMMEROVÁ, B., VANĚK, D., 2017. People “on the Margin”: A Medieval Cemetery in Český Brod – Malechov (Central Bohemia). *Interdisciplinaria Archaeologica: Natural Sciences in Archaeology*, 8(1), 59–75.
- GAMBA, C., JONES, E.P., TEASDALE, M.D., McLAUGHLIN, R.L., GONZALES-FORTES, G., MATTIANGELI, V., DOMBRÓCZKI, L., KÓVÁRI, I., PAP, I., ANDERS, A., WHITTLE, A., DANI, J., RACZKY, P., HIGHAM, T.F.G., HOFREITER, M., BRADLEY, D.G., PINHASI, R., 2014. Genome flux and stasis in a five millennium transect of European prehistory. *Nature Communications*, 5(5257), 5257.
- HANSEN, H.B., DAMGAARD, P.B., MARGARYAN, A., STENDERUP, J., LYNNERUP, N., WILLERSLEV, E., ALLENTOFT, M.E., 2017. Comparing Ancient DNA Preservation in Petrous Bone and Tooth Cementum. *PLoS ONE*, 12(1), e0170940.
- HOFREITER, M., SERRE, D., POINAR, H.N., KUCH, M., PÄÄBO, S., 2001. Ancient DNA. *Nature Reviews Genetics*, 2(5), 353–359.
- JURAS, A., CHYLENSKI, M., KRENZ-NIEDEBALA, M., MALMSTROM, H., EHLER, E., POSPIESZNY, L., LUKASIK, S., BEDNARCZYK, J., PIONTEK, J., JAKOBSSON, M., DABERT, M., 2017. Investigating kinship on Neolithic post-LBK human remains from Krusza Zamkowa, Poland using ancient DNA. *Forensic Science International: Genetics*, 26, 30–39.
- KALINOWSKI, S.T., WAGNER, A.P., TAPER, M.L., 2006. ML-Relate: a computer program for maximum likelihood estimation of relatedness and relationship. *Molecular Ecology Notes*, 6(2), 576–579.
- KEYSER-TRACQUI, C., LUDES, B., 2005. Methods for the study of ancient DNA. *Methods in molecular biology*, 297, 253–264.
- KLING, D., TILLMAR, A.O., EGELAND T., 2014. Familias 3-Extensions and new functionality. *Forensic Science International: Genetics*, 13, 121–127.
- KRÁL, P., 2005. Tod, Begräbnisse und Gräber. Funeral ritus des böhmischen Adels als Mittel der Representation und des Andenkens. In: M. Hengere, ed. *Macht und Memoria. Begräbniskultur europäischer Oberschichten in der Frühen Neuzeit*. Köln, Weimar, Wien: Böhlau, pp. 421–448.
- LASSEN, C., HUMMEL, S., HERRMANN, B., 2000. Molecular sex identification of stillborn and neonate individuals (“Traufkinder”) from the burial site Aegerten. *Anthropologischer Anzeiger*, 58(1), 1–8.
- MELCHIOR, L., LYNNERUP, N., SIEGISMUND, H.R., KIVISILD, T., DISSING, J., 2010. Genetic Diversity among Ancient Nordic Populations. *PLoS One*, 5(7), e11898.
- NOVÁČKOVÁ, J., OMELKA, M., ŘEBOUNOVÁ, O., STENZL, V., in press. Begräbnispraxis des Pragerbarocken Bürgertums im Licht der DNA-Analyse. In: *Internationale Tagung der Österreichischen Gesellschaft für Mittelalterarchäologie 2018*. Sankt Pölten. Wien: Beiträge zur Mittelalterarchäologie in Österreich.
- OMELKA, M., 2006a. Nález neobvykle členěného medailonu s vyobrazením kříže svatého Benedikta a Zachariášova požehnání ze Šporkovy ulice čp. 332/III v Praze – The Finds of Unusually Divided Medallion with a Motif of St. Benedict's cross and Zachary's Blessing from Šporkova Street, House No. 322/III in Prague (in Czech). *Archeologica Pragensia*, 18, 144–152.
- OMELKA, M., 2006b. *Investorská zpráva o archeologickém výzkumu Praha 1 – Malá Strana, Šporkova ulice čp. 332/III – Investor's report about archaeological research in Prague 1–The Lesser Town, Šporkova Street no. 322/III*. Unpublished manuscript deposited in the Archive of the National Heritage Institute, ú. o. p. in Prague, Archive of the Department of Archaeology.
- OMELKA, M., 2009. Hřbitov u kostela sv. Jana v Oboře ve Šporkově ulici čp. 332/III na Malé Straně v Praze – The Cemetery of the Church of St. John in Šporkova Street (no.322/III), the Lesser Town, Prague (in Czech). *Stoletá Praha*, 25, 93–101.
- OMELKA, M., PETŘÍK, J., PROKEŠ, L., ŘEBOUNOVÁ, O., ŠLANCAROVÁ, V., 2018. Soubor knoflíků ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana) – An Assemblage of Buttons from the Defunct Cemetery at the church of Saint John the Baptist in Obora (Prague Lesser Town) (in Czech). *Archeologie ve středních Čechách*, 22, 709–744.

- OMELKA, M., ŠLANCAROVÁ, V., 2007. Soubor prstenů ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana) – A Collection of Rings from the Defunct Cemetery at the church of Saint John the Baptist in Obora, Prague – the Lesser Town (in Czech). *Archeologie ve středních Čechách*, 11, 671–709.
- OMELKA, M., ŘEBOUNOVÁ, O., 2008. Soubor korálek ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana) – A Collection of Beads from the Defunct Cemetery at the Church of St. John the Baptist in Obora, Prague–the Lesser Town (in Czech). *Archeologie ve středních Čechách*, 12, 606–679.
- OMELKA, M., ŘEBOUNOVÁ, O., 2011. Poznámky ke zbožnosti a pohřebnímu ritu malostranského barokního měšťanstva ve světle archeologických nálezů – Remarks on the Religiousness and Burial Rite of the Lesser Town Baroque Burghers in the Light of Archaeological Findings (in Czech). *Pražský sborník historický*, 39, 268–298.
- OMELKA, M., ŘEBOUNOVÁ, O., 2012a. Soubor medailonů a feniků se symbolikou sv. Benedikta ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana) – A Collection of Medallions and Pfenings with the Symbols of St. Benedict from the Defunct Cemetery at the Church of St. John in Obora (Prague–Lesser Town) (in Czech). *Archeologie ve středních Čechách*, 16/2, 983–1019.
- OMELKA, M., ŘEBOUNOVÁ, O., 2012b. A view of the archaeological context of the Lesser Town cemetery in Šporkova Street in Prague using Modern period iconography and written sources. *Studies in Post-Medieval Archaeology*, 4, 233–250.
- OMELKA, M., ŘEBOUNOVÁ, O., 2014. Barokní mariánské kultury na Malé Straně v zrcadle pražských archeologických nálezů náboženských medailů (s přihlédnutím k situaci v Čechách a na Moravě) – Baroque Maria's Cults in the Lesser Town in the Mirror of Prague Archaeological Finds of Religious Medals (with Consideration to the Situation in Bohemia and Moravia). *Pražský sborník historický*, 42, 243–268.
- OMELKA, M., ŘEBOUNOVÁ, O., 2016. Zboží pro chudé a bohaté – „originály“ a dobové „padělky“ náboženských medailů na příkladu nálezů z hrobových kontextů – Products for the Poor and for the Rich: “Originals” and “Fakes” of Religious Medals on the Example of Finds from Grave Contexts (in Czech). *Archaeologia historica*, 41, 309–325.
- OMELKA, M., ŘEBOUNOVÁ, O., 2017. Stav a perspektivy bádání novověkého pohřebního ritu v Čechách – The State and Perspectives of Research into Modern-Age Funeral Rites in Bohemia (in Czech). *Archaeologia historica*, 42, 117–133.
- OMELKA, M., ŘEBOUNOVÁ, O., ŠLANCAROVÁ, V., 2009. Soubor křížků ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana). I. Obecné formy kříže – A Collection of Crosses from the Defunct Cemetery at the Church of St. John in Obora, Prague–Lesser Town. I. General Forms of Crosses (in Czech). *Archeologie ve středních Čechách*, 13(2), 1001–1083.
- OMELKA, M., ŘEBOUNOVÁ, O., ŠLANCAROVÁ, V., 2010. Soubor křížků ze zaniklého hřbitova při kostelu sv. Jana v Oboře (Praha – Malá Strana). II. Speciální kříže – A Collection of Crosses from the Defunct Cemetery at the Church of St. John in Obora, Prague–Lesser Town. II. Special Crosses (in Czech). *Archeologie ve středních Čechách*, 14(1), 423–476.
- OMELKA, M., ŘEBOUNOVÁ, O., ŠLANCAROVÁ, V., 2011. Špendlík – před hradbou a za hradbou – The Pin within and without the Town (in Czech). *Archaeologia historica*, 36, 23–540.
- PÄÄBO, S., 1989. Ancient DNA: Extraction, characterization, molecular cloning, and enzymatic amplification. *Proceedings of the National Academy of Sciences of the United States of America*, 86(6), 1939–1943.
- PÄÄBO, S., POINAR, H., JAENICKE-DESPRES, V., HEBLER, J., ROHLAND, N., KUCH, M., KRAUSE, J., VIGILANT, L., HOFREITER, M., 2004. Genetic analyses from ancient DNA. *Annual Reviews of Genetics*, 38, 645–679.
- PINHASI, R., FERNANDES, D., SIRAK, M., CONNELL, S., ALPASLAN-ROODENBERG, S., GERRITSEN, F., MOISEYEV, V., GROMOV, A., RACZYK, P., ANDERS, A., PIETRUSEWSKY, M., ROLLEFSON, G., JOVANOVIĆ, M., TRINH HOANG, H., BAR-OS, G., OXENHAM, M., MATSUMURA, H., HOFREITER, M., 2015. Optimal Ancient DNA Yields from the Inner Ear Part of the Human Petrous Bone. *PLOS One*, 10(6), e0129102.
- SIMÓN, M., JORDANA, X., ARMENTANO, N., SANTOS, C., DÍAZ, N., SOLÓRZANO, E., LÓPEZ, J.B. GONZÁLEZ-RUIZ, M., MALGOSA, A., 2011. The presence of nuclear families in prehistoric collective burials revisited: The bronze age burial of montanissell cave (Spain) in the light of aDNA. *American Journal of Physical Anthropology*, 146(3), 406–413.
- TIERNEY, S., BIRD, J., 2014. Sex identification of human remains from an Irish Medieval population using biomolecular methods. *European Scientific Journal*, 2, 521–530.
- YANG, D.Y., ENG, B., WAYE, J.S., DUDAR, J.C., SAUNDERS, S.R., 1998. Improved DNA extraction from ancient bones using silica-based spin columns. *American Journal of Physical Anthropology*, 105(4), 539–543.
- YANG, D.Y., WATT, K., 2005. Contamination controls when preparing archaeological remains for ancient DNA analysis. *Journal of Archaeological Science*, 32(3), 331–336.

#### Other Sources

- Prague City Archives, Collection of Registries, sign MIK Z3, MIK Z4, MIK Z5.
- Prague City Archives, Manuscript Collection, sign 4764.
- The National Heritage Institute in Prague, Archive of the Department of Archaeology, Documentation of excavations no. 30/02 and 30/04.