Forensic Science International: Genetics 15 (2015) 90–97

Contents lists available at ScienceDirect

Forensic Science International: Genetics

journal homepage: www.elsevier.com/locate/fsig

Genetic research at a fivefold children's burial from medieval Berlin

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ARTICLE INFO

Keywords:
Medieval multiple burial
St. Peters square (Petriplatz)
Ancient DNA
Medieval population of Berlin

ABSTRACT

Berlin originated from the two twin cities Berlin and Cölln, which both were founded at the beginning of the 13th century. However the real date of their foundation as well as the origin of the first settlers is still unknown. On the Berlin site the historic city center is still visible in the Nikolaiviertel, but the medieval origin of Cölln disappeared almost completely. In 2007 a large scale excavation, which comprised an area of about 1700 m^2 of the historical center of the St. Peters church, recovers the remains of Cölln's first citizens and span a period of 500 years of medieval population. Here we present the first genetic analysis of a fivefold children's burial from excavations in Berlin. The genetic data unveiled next to ancestry and eye color data also the kinship and the gender of the five individuals. Together with the archeological context the new gained information help to shed more light on the possible reasons for this burial.

1. Introduction

During the medieval eastward expansion (Östlicher Landesausbau) many areas were occupied which had been the territories of the Slavs from the 7th until the 11th century. The medieval eastward expansion started in the 12th century and continued till the 14th century. Many new settlements and towns were established by the colonizers in this time. It is known from written sources that in some places Slavs participated in this foundation but their real influence and their approximate numbers are unknown. One of the newly founded settlements was Germany's capital, Berlin, which has its origins in the medieval towns Berlin and Cölln on either side of river Spree. Cölln was first mentioned in the records in the year 1237, but recent archeological excavations indicate settlement began around the year 1200 [1]. Besides the uncertainties regarding the date of the initial settlement foundation, the origin of the first settlers is unknown. No written sources remain from the starting years of Berlin and Cölln are lost. All knowledge about the act of foundation of Berlin was already lost in the 15th century [2]. Only archeological research can provide new information regarding the beginnings of the city.

From 2007 to 2009 a large scale excavation was carried out at St. Peters Square (Petriplatz), in the former city center of Cölln, with St. Peter's church in the middle, surrounded by its graveyard. Excavation of the graveyard revealed 3122 graves containing the remains of 3717 skeletal individuals dating from about 1200 to 1717, when the cemetery was closed to protect public health [3]. In addition to the high number of skeletons recovered, which represent a significant fraction of the medieval to post-medieval population of Cölln, the excavated part of the cemetery also revealed a series of phenomena. For example, it contained a high number of multiple burials with graves containing up to 12 individuals, all carefully orientated in West–East direction.

In order to test the DNA preservation of the excavated skeletons, we investigated a fivefold burial that contained children who died at the ages of 2–10 years. The obtained genetic data, consisting of mtDNA sequences and genomic STR markers, help to clarify the kinship and sex of all five individuals. When combined with archeological and osteological data, genetic analysis enables us to shed more light on the reasons behind multiple burials and on medieval funeral traditions. The results of this study provide the first insights into the genetic composition of the burials excavated from St. Peters graveyard and demonstrate the potential for further investigation of the founding population of Berlin.

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http://dx.doi.org/10.1016/j.fsigen.2014.10.022
1872-4973/

2. Methods

2.1. Bone samples and non-osteological analysis

Bone and teeth samples from the multiple burial of five children (context number 3191) were provided by the Landesdenkmalamt Berlin. Burial 3191 had been excavated and recorded following the principles of single context recording. The burial was recorded with photographs and conventional survey techniques as well as with 3D-Laserscan (Fig. 1). There was no textile or wood preserved but a pair of bronze tweezers was found in the top fill of the grave above skeletons A–C (Supplement Material S1) [4]. Following excavation, the skeletons were stored in boxes under dry conditions at room temperatures of between 5 and 18 °C. The date of the burial was determined by radiocarbon dating of bone from individual C who was shown to have died between 1411 and 1445. The radiocarbon dating was carried out in the Klaus-Tschira-Lab of the Curt-Engelhorn-Zentrum für Archäometrie in Mannheim (cal 1 Sigma 1421–1439; cal 2 Sigma 1411–1445, [5]). X-radiography was performed in order to check the long bones for any possible indication of malnutrition or structural anomalies within the children's skeleton.

Fig. 1. Context no. 3191, fivefold burial of children (individuals A–E). The arrow indicates North direction. Each red and white unit of the measuring stick presents 10 cm in length. The board shows the name of the excavation, the context number and the date. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

2.2. Contamination prevention

To prevent any possible risk of contamination during the handling and analysis of the ancient remains the following precautions were taken: all processing was performed in a pre-PCR room and facilities were dedicated only to investigations of skeletal remains. Prior to preparation of the sample the area and all surfaces were decontaminated with a UV-aircleaner (MWG Biotech, Ebersberg, Germany), 1 M HCl (Roth, Karlsruhe, Germany) and Sterillium (Bode Chemie, Hamburg, Germany). All consumables and instruments, including the extraction robots used for grinding and the DNA extraction of the samples were decontaminated by UV-light before and after usage. All stages of the DNA extraction were carried out with disposable gloves, masks, and hair-nets as well as with a long and clean lab coat with wristbands. All laboratory staff as well as all excavators, archeologist and anthropologist, who were handling the material, provided their DNA samples for comparison of the STR and mtDNA data. Furthermore, open tube, positive and negative controls were included into the extraction and PCR reactions. DNA extractions as well as PCR reactions were carried out in at least duplicates and from different bones or teeth (Table 1).

2.3. Sample preparation, DNA extraction and quantification

Specimens were taken from the femora, the temporals and the teeth, which showed the best preservation and the strongest bone structure (Table 1). All specimens were washed with ethanol (J.T. Baker, Deventer, The Netherlands) and distilled water (J.T. Baker, Deventer, The Netherlands) as a measure to remove possible contaminations. Bone samples were cut into small cubes and decontaminated once again with ethanol. Dried specimens were mechanically ground to powder using a swing mill MM2000 (Retsch, Haan, Germany).

DNA extraction was carried out after a protocol, which was developed by the research group of historical anthropology and human ecology at the Georg-August-University in Goettingen (https://www.uni-goettingen.de/de/136161.html) and partly published in Grumbkow et al. [6]. In detail, each extraction was performed with 250 mg of bone powder, which were split into 2 ml Eppendorf tubes, each with 125 mg. Next, 1800 μl 0.5 M EDTA (Sigma-Aldrich, Steinheim, Germany) was added to each sample tube and incubated for 18 h at 37 °C. For lysis 25 μl of 600 mAU/ml Proteinase K (Qiagen, Hilden, Germany) was added to each sample and incubated at 56 °C for 2 h. After lysis, 25 μl of 1% SDS (Sigma Aldrich, Steinheim, Germany) was added and incubated for 5 min at 65 °C. All incubation steps were carried out on the thermomixer comfort heating block with 1100 rpm of shaking (Eppendorf, Hamburg, Germany). Lysate was recovered by centrifugation at 3300 rcf and then transferred to a 50 ml falcon tube containing 16 ml of PB buffer with 100 μl sodium acetate buffer pH 5.2 (Sigma-Aldrich, Steinheim, Germany). Next, the lysate–PB buffer mix was transferred to the spin columns from the MinElute® PCR Purification Kit (Qiagen, Hilden, Germany). Due to the exceeded lysate volume, the volume of the spin columns was enlarged by attaching 20 ml extenders (Qiagen, Hilden, Germany). The columns with the extenders and the lysate were placed on the QIAvac 24 Plus device and lysate was drawn through the column membrane by a vacuum, which was built up with a vacuum pump in combination with the QIAvac Connecting System (Qiagen, Hilden, Germany). Vacuum was applied till all lysate has passed