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Genetic analysis of the presumptive blood from Louis XVI, king of France

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ABSTRACT

A text on a pyrographically decorated gourd dated to 1793 explains that it contains a handkerchief dipped with the blood of Louis XVI, king of France, after his execution. Biochemical analyses confirmed that the material contained within the gourd was blood. The mitochondrial DNA (mtDNA) hypervariable region 1 (HVR1) and 2 (HVR2), the Y-chromosome STR profile, some autosomal STR markers and a SNP in HERC2 gene associated to blue eyes, were retrieved, and some results independently replicated in two different laboratories. The uncommon mtDNA sequence retrieved can be attributed to a N1b haplotype, while the novel Y-chromosome haplotype belongs to haplogroup G2a. The HERC2 gene showed that the subject analyzed was a heterozygote, which is compatible with a blue-eyed person, as king Louis XVI was. To confirm the identity of the subject, an analysis of the dried heart of his son, Louis XVII, could be undertaken.

1. Introduction

Different studies have focused on the ancient DNA analysis of historical individual remains, to gain information regarding their possible identification and also shed new light on historical mysteries. These include the analysis of the remains of the Romanov family [1,2], the putative evangelist Luke [3], the American outlaw Jesse James [4], the heart of Louis XVII, son of Louis XVI, king of France [5], the Italian poet Francesco Petrarca [6], or the astronomer Nicolaus Copernicus [7]. In the aforementioned studies, mummified or skeletal remains have been associated to particular individuals by different means, usually funerary information. In some cases, body remains are not even available and yet, the potential of the paleogenetic techniques can allow us to obtain the genetic profile of some notorious historical characters.

After the execution of Louis XVI in January 21st, 1793, eyewitnesses stated that many people from the crowd dipped their handkerchiefs in the king's blood and kept these objects as mementos [8]. An Italian family has owned for more than a hundred years – as demonstrated by a letter addressed to the director of the Musée Carnavalet in Paris, January 31st, 1900 – a dessicated gourd that presumably contained one of these handkerchiefs. The gourd, belonging to the species *Cucurbita moschata*, measures 23.7 cm in height, 15.2 cm at the base diameter and 7.2 cm at the top diameter, and was originally used as a bottle-gourd for gunpowder. It had been richly decorated with a pyrographic technique (Figs. 1 and 2). The portraits of prominent lead actors during the French revolution, including Georges Danton, Jean Paul Marat, Camille Desmoulins, Louis-Sébastien Mercier, Joseph Ignace Guillotin, Maximilien Robespierre, Bernard-René de Launay, Jacques de Flesselles and Joseph Foullon, are depicted, as well as some royalists, including, among others, the king Louis XVI, the queen Marie Antoinette, the Dauphin Louis XVII and the finance minister, Jacques Necker. Perhaps the most interesting information on the origin of this object is depicted in some text boxes between the portraits. In one of these it is stated: "Maximilien Bourdaloue on January 21st, dipped his handkerchief in the blood of the king after his beheading" (Fig. 3). In another section it is explained that the author of the gourd's decoration was Jean Roux, from Paris, and that the work was finished on September 18th, 1793. The final purpose of this object seems to have been economic. In another text box it can be read that the gourd had been crafted as a gift to "the Eagle" (maybe referring to Napoleon) and that a 500 francs profit is expected for it.

What looks superficially like a dark and dried substance can be seen within the gourd. A general genetic test on the putative blood

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Fig. 1. Picture of the gourd containing the presumptive blood of Louis XVI, depicting the portraits of French revolution leaders J. Danton, P. Marat, and C. Desmoulins.

remains has been conducted, screening the mitochondrial DNA (mtDNA) hypervariable region 1 (HVR1) and 2 (HVR2), the Y-chromosome STR profile and some autosomal STR markers. Our goal was to explore the genetic homogeneity of the sample and the reproducibility of the results, but also to characterize the putative genetic profile of Louis XVI for possible future comparisons, for instance with his son, Louis XVII, through the analysis of his presumed dried heart.

Fig. 2. Picture of the gourd with the portraits of M. de Launay, Flesselles, and Foullon.

Fig. 3. Text-box on the gourd explaining – in French – the origin of the blood sample. English translation: "Maximilien Bourdaloue on January 21st, dipped his handkerchief in the blood of the king after his beheading".

2. Materials and methods

2.1. DNA extraction

Five samples were scraped from the inside of the gourd and send to two different ancient DNA laboratories in Bologna and Barcelona for genetic analysis. In Barcelona, DNA was extracted from one of the samples following a protocol described elsewhere [9]. In short, 50 mg of sample were incubated overnight at 50 °C in a lysis solution (0.5% SDS, 50 mM TRIS, and 1 mg/mL of proteinase K in H_2O). Subsequently the DNA was extracted with phenol–chloroform and concentrated using a Centricon-30 filter column (Millipore) up to a 30 μl volume. In Bologna, the remaining gourd samples were extracted using the QIAmp DNA Micro Kit (QIAGEN, Hilden, Germany), following the manufacturer' instructions. Standard precautions to avoid contamination in ancient DNA were adopted during the experimental procedures [10,11]

2.2. Confirmatory blood test

A chromatographic method based on silica paper was applied in Bologna on three different powder samples of few milligrams: one sample diluted in 10 μl of sterile water, another sample diluted in 20 μl of 5% ammonia and incubated at room temperature for 30 min [12] and a third sample left overnight in 200 μl of lysis buffer and proteinase K provided in QIAmp DNA Micro Kit (QIAGEN) [12]. After a incubation at 120 °C for 10 min, an alcoholic benzidine spray reagent in conjunction with a hydrogen peroxide spray was used to detect blue spots, that correspond to blood residues [12].

2.3. Mitochondrial DNA

In Barcelona, the mitochondrial DNA (mtDNA) hypervariable region 1 (HVR1) was amplified by polymerase chain reaction (PCR) in two overlapping fragments (with the L16,055–H16,218 and L16,209–H16,378 primers, numbered according to the Cambridge Reference Sequence), along with extraction and negative controls to monitor against possible contamination. The amplification was based in a two-steps PCR, as described in Krause et al. [13]. The reaction was performed in a total volume of 20 μl, containing: 5 μ of DNA extract, 1X PCR buffer, 2.5 mM $MgCl_2$, 500 mM of each dNTP, 2 U of AmpliTaq Gold DNA Polymerase (Applied Biosystems), 150 nM of each primer in the first multiplex step and 1.5 μM of each primer in the second step. The annealing