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## DNA analysis in the case of Kaspar Hauser

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**Abstract** In 1828 a mysterious young man appeared in Nürnberg, Germany, who was barely able to speak or walk but could write down his name, Kaspar Hauser. He quickly became the centre of social interest but also the victim of intrigue. His appearance, his origin and assassination in 1833 were, and still are, the source of much debate. The most widely accepted theory postulates that Kaspar Hauser was the son of Grand Duke Carl von Baden and his wife Stephanie de Beauharnais, an adopted daughter of Napoleon Bonaparte. To check this theory, DNA analysis was performed on the clothes most likely worn by Kaspar Hauser when he was stabbed on December 14th, 1833. A suitable bloodstain from the underpants was divided and analysed independently by the Institute of Legal Medicine, University of Munich (ILM) and the Forensic Science Service Laboratory, Birmingham (FSS). Mitochondrial DNA (mtDNA) was sequenced from the bloodstain and from blood samples obtained from two living maternal relatives of Stephanie de Beauharnais. The sequence from the bloodstained clothing differed from the sequence found in both reference blood samples at seven confirmed positions. This proves that the bloodstain does not originate from a son of Stephanie de Beauharnais. Thus, it is becoming clear that Kaspar Hauser was not the Prince of Baden.

**Key words** Kaspar Hauser · Ancient DNA · Mitochondrial DNA · Sex determination

### Introduction

In 1828, Kaspar Hauser appeared in Nürnberg, Germany. According to his later descriptions, he was kept alone in a dungeon as long as he could remember. Speculation quickly surrounded the origin of this man and the possibility that he was the son of Grand Duke Carl von Baden and his wife, Stephanie de Beauharnais. Their first-born son had died soon after birth. The suggestion arose that the newborn Prince had been exchanged with a sick child in order to change the succession to favour another branch of the family. If this theory was right and the Prince was secretly raised, he would have been of the same age as Kaspar Hauser and therefore Kaspar Hauser could be the prince of Baden. This theory gives a motive for the fact that Kaspar Hauser was stabbed only five years after his public appearance. DNA analysis has been performed to check if he really was a Prince of Baden.

In the last few years, identification of human remains by DNA analysis has proven to be a powerful tool in forensic investigations. Analysis of mtDNA is especially useful, because it is present at a high copy number in cells [1] and is more likely to survive for prolonged periods compared to chromosomal DNA. It has been successfully applied to human remains such as a 7000-year-old human brain [2] or an approximately 5000-year-old man who was found mummified in an alpine glacier [3]. MtDNA is inherited only from the mother to the child and thus all members of a family who are maternally related would have the same mtDNA type. Because of this unique pattern of inheritance, mtDNA is very useful in identification cases [4–6]. MtDNA analysis and analysis of Y-chromosomes are methods in determining family relationships when a gap of several generations exists between an ancestor and a living descendant but mtDNA analysis is the only option in determining maternal family relationships such as in the identification of the remains of the Romanov family [7]. Therefore, we applied mtDNA analysis to a suspected bloodstain from Kaspar Hauser and maternally related descendants of Stephanie de Beauharnais to check if a relationship can be demonstrated.

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In order to perform DNA analysis, authentic material from Kaspar Hauser was required. Suitable material for analysis would be the remains of Kaspar Hauser from the grave situated in Ansbach in a public cemetery. An exhumation would have been possible but was not performed because it was doubtful if the gravestone was still above the correct grave due to disturbances during the war. Therefore, it was not clear if bones found in the grave would originate from Kaspar Hauser or from other persons buried nearby.

The museum of Ansbach owns clothes which were worn by Kaspar Hauser when he was stabbed on December 14th, 1833. These clothes were seized by the prosecutor after his murder and were kept at the court until 1888. The clothes were then given to the Historical Society of Ansbach. They were photographed and exhibited for the first time shortly before 1930. In Spring 1996 the clothes were inspected by members of the Institute of Legal Medicine, University of Munich (ILM) and the Forensic Science Service Laboratory (FSS), Birmingham. The clothes seemed fairly clean and in a good state of preservation. The upper clothing was seen to bear a single stab cut, penetrating the several layers of cloth in the region of the left chest. The bloodstains on the clothing had the same outline as those in the pictures from the 1920s, but appeared faded. Bloodstaining was associated with the cut marks and also appeared to have run down and collected along the top edge of the underpants, where there was a substantial stain. The fabric of the underpants had two layers in the upper region where the bloodstain was situated. The bloodstain was cut out and divided for independent analysis in each laboratory. The inner part of the fabric was chosen because it was least likely that contamination from outside or environmental exposure such as sunlight would have occurred.

## Materials and methods

### Laboratory organisation

To exclude the possibility of contamination, mtDNA analysis was performed independently by the ILM and the FSS and each sample was analysed in duplicate. All results were compared with mtDNA databases containing all analysed sequences from each laboratory, including mtDNA sequences of persons working in the laboratories. To minimise the possibility of contamination in the laboratories, all extractions were performed using sterile aliquots of buffers once only, the remainder being rejected after use. Extraction and amplification were carried out in different rooms to ensure that amplified products could not contaminate the extraction laboratory. Negative controls without DNA added to the extraction mixture were used in all experiments. All experiments were rejected on the rare occasion that a signal was obtained in the negative control.

### Sampling of bloodstained clothing

The fabric of the underpants had two layers in the upper region, where the substantial bloodstain was situated. In the region of the bloodstain, the two layers were stuck together. To separate the two layers of fabric, the underpants were put in a chamber with a controlled humidity of 80%. Under these conditions, the two sticky layers of fabric could be separated. Two pieces of approximately 10 cm<sup>2</sup> were removed from the inner layer of the bloodstain.

### DNA extraction

Both laboratories performed independent extractions from two pieces of fabric each of which was 4 cm<sup>2</sup>. At the ILM the pieces of fabric were first incubated overnight at 56 °C in 1 ml of mild lysis buffer I (0.02 M Tris-HCl, 0.01 M EDTA, 0.05 M KCl, 0.5% Tween 20 and 0.5 mg proteinase K) and then with 1 ml of lysis buffer II (0.02 M Tris-HCl, 0.01 M EDTA, 0.05 M KCl, 1% Tween 20, 1% NP-40, 0.04 M DTT, 1 mg proteinase K). After lysis the samples were extracted with phenol, phenol/chloroform and chloroform. DNA was precipitated with 2.5 vols ethanol and washed 3 times with 0.5 ml distilled water in a Microcon 30 concentrator (Amicon) for 30 min. Pieces of fabric without bloodstaining were extracted in the same way.

At the FSS the pieces of fabric were incubated overnight at 37 °C in 500 µl extraction buffer (0.01 M Tris HCl pH8.0, 0.01 M EDTA, 0.1 M NaCl, 2% SDS), 50 µl proteinase K (10 mg/ml) and 20 µl 1 M DTT. The buffer was recovered and the cloth re-extracted with 500 µl extraction buffer, 50 µl proteinase K and 20 µl DTT at 56 °C for 3 h. The buffer was again recovered. DNA was purified from both first and second recovered buffers with two washes each of phenol and phenol/chloroform and one of chloroform, precipitated with 2.5 vols of ethanol with incubation at -20 °C overnight. The DNA was collected by centrifugation at 13 000 rpm for 30 min, the pellet was washed with 70% ethanol and resuspended in 50 µl sterile deionised water. Results were obtained from the first and second extractions of the blood staining.

At the ILM blood samples from the maternal relatives of Stephanie de Beauharnais were extracted by a standard phenol/chloroform protocol [8]. At the FSS reference samples were extracted with Chelex 100 resin (Bio-Rad) [9].

### Sex determination

The sex of the bloodstained clothing was tested by amplification of a segment of the X-Y homologous gene, amelogenin [10]. At the ILM the size of PCR products was then determined with a 373A DNA Sequencer (Applied Biosystems) as previously described [11]. At the FSS, the sex of the blood was determined after agarose electrophoresis.

### Amplification and sequencing of mtDNA

At the ILM amplification of PCR products for sequencing was carried out in a single step reaction with 200–500 pg DNA of the blood samples or 1/10 of the main extractions from the bloodstained clothing as described [12] with the exception that AmpliTaqGold (Perkin Elmer) was used. The amplification conditions were 12 min at 95 °C in a first step, 30 s at 95 °C, 40 s at 57 °C (primer set II, III, IV) or 60 °C (primer set I) and 20 s at 72 °C for 32, 35 or 38 cycles depending on the amount of DNA. After controlling on ethidium bromide gels and purification, sequencing analysis of PCR products was performed using the AmpliTaqFS polymerase cycle sequencing with dye-labelled dideoxyterminators (Applied Biosystems). All results were confirmed by sequencing in both directions. The PCR amplification and sequencing primers for the mtDNA control region were [12]:

Primer set I: F15990 5' TTA ACT CCA CCA TTA GCA CC 3',  
R16239 5' TGG CTT TGG AGT TGC AGT TG 3',  
Primer set II: F16163 5' TGA CCA CCT GTA GTA CAT AA 3',  
R16391 5' GAG GAT GGT GGT CAA GGG AC 3',  
Primer set III: F00034 5' CAC CCT ATT AAC CAC TCA CG 3',  
R00266 5' GTT ATG ATG TCT TCT GTG TGG AA 3',  
Primer set IV: F00174 5' TAT TTA TCG CAC CTA CGT TC 3',  
R00370 5' CTG GTT AGG CTG GTG TTA GG 3'.

To check for human specificity, 50 ng DNA from cow, pig, sheep and chicken were amplified under the same conditions with 38 cycles. No visible PCR products were obtained in an ethidium bromide gel.