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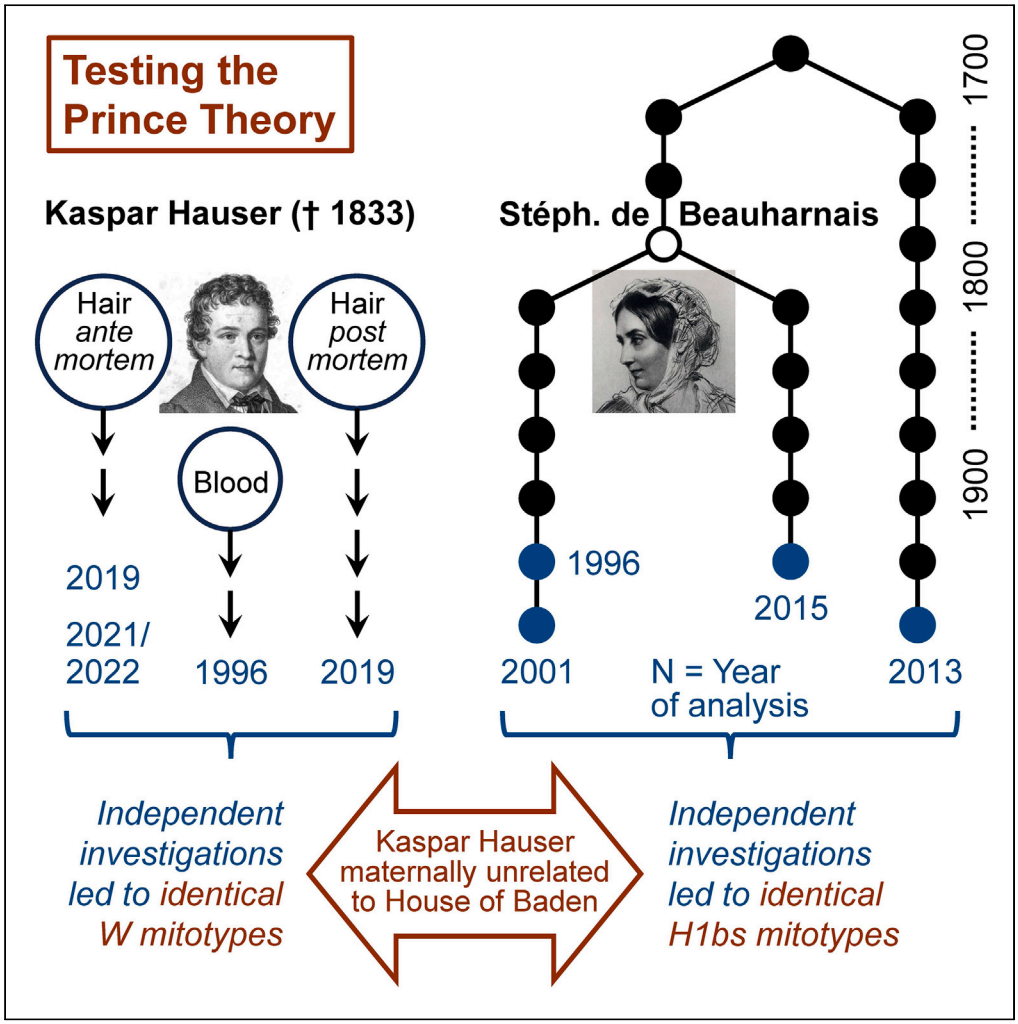

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Highlights

Independent genetic mtDNA analyses in three laboratories gave identical results

Hair and blood samples attributed to Kaspar Hauser yielded the same mtDNA

Kaspar Hauser's mtDNA is unambiguously different from the "Baden lineage"

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Kaspar Hauser's alleged noble origin – New molecular genetic analyses resolve the controversy

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SUMMARY

Kaspar Hauser's parentage has been the subject of research and debate for nearly 200 years. As for his possible aristocratic descent through the House of Baden, there is suspicion that he was swapped as a baby, kidnapped, and kept in isolation to bring a collateral lineage to the throne. In the last 28 years, various genetic analyses have been carried out to investigate this possible aristocratic origin. Previous results using less sensitive Sanger and electrophoresis-based methods were contradictory, and moreover, the authenticity of some samples was disputed, thus leaving the question open. Our analyses using modern capture- and whole genome-based massively parallel sequencing techniques reveal that the mitochondrial DNA haplotypes in different samples attributed to Kaspar Hauser were identical, demonstrating authenticity for the first time, and clearly different from the mitochondrial lineage of the House of Baden, which rules out a maternal relationship and thus the widely believed "Prince theory".

INTRODUCTION

Brief sketch on Kaspar Hauser's public life

If not mentioned otherwise, we follow in the historical part mainly Feuerbach (1832),[1] Daumer (1873),[2] and Pies (1966).[3] Anselm Ritter von Feuerbach and Georg Friedrich Daumer knew Kaspar Hauser personally; Hermann Pies has analyzed and published (in several volumes) most of the available official documents on the subject.

Kaspar Hauser (Figure 1) was a youth, who appeared seemingly out of nowhere in the Bavarian city of Nuremberg on Whit Monday, May 26, 1828. Estimated to be 16 years of age, his appearance was lubberly, he seemed hardly able to walk, and he could barely speak. He carried a letter from an anonymous writer who said that the boy was given into his custody in 1812 and that he had kept him in total isolation. When interrogated by the police, he could not answer any of their questions nor could he provide account of himself. To everyone's surprise, however, he was able to write "Kaspar Hauser" in clearly readable letters, though, as he explained later, without knowing their meaning. Although it was quite clear that it was not his real name, he continued to use it.

In the following months, several distinguished individuals took care of him. Among them were Jakob Friedrich Binder, Mayor of Nuremberg; Baron Gottlieb von Tucher, jurist at the Town Court of Nuremberg; Anselm Ritter von Feuerbach, President of the Court of Appeal in Ansbach; and particularly Professor Georg Friedrich Daumer, a high-school teacher, poet, and philosopher. All these men (and several others) wrote detailed reports on Kaspar's initial condition and his development. At his arrival, he was in good physical health (attested by the physicians Carl Preu and Johann Carl Osterhausen), and his poor motor skills soon improved, although he remained relatively weak. Likewise, his verbal skills developed quickly, but he continued to speak in a childish way for some time. He was therefore not considered physically or mentally disabled, but the suspicion arose that he had been entirely neglected for a very long time.

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Figure 1. Portrait of Kaspar Hauser by Johann Kreul (published by Anselm Ritter von Feuerbach, Dollfuß 1832)

According to Kaspar's own account, for as long as he could remember, he sat in a small, dark dungeon without ever getting to see anybody else. His only companions were two horses and a dog, all made of wood. His nourishment consisted of bread and water, but he never saw the person who supplied it during his sleep. Apparently, hygiene support also took place while he was asleep, supported by opium doses (commercially available as laudanum) in his drinking water. A few days before his release, a man appeared and taught him how to walk, parrot a few sentences (e.g., "I would like to become such a rider as my father was"), and write "Kaspar Hauser". The unknown man took him on a march leading to Nuremberg, where he left him.

Taking into account Kaspar Hauser's initial limited abilities and his subsequent development, he was considered to have been incarcerated at the age of 3–4 years. His detractors argue that no child would survive such treatment without physical harm if at all. The dubiety of his dungeon story is a key argument for Kaspar Hauser's many critics, who take this as a proof of his fraudulence.[4–12] Despite such reservations, Kaspar Hauser became a celebrity, a subject of curiosity of the Biedermeier society, a feature in Germany's newspapers, and an object of interest to Nuremberg's visitors. Moreover, his unknown origin and doubtful past fueled speculation that he might be of noble origin.

A year and a half after Kaspar Hauser's appearance, a dramatic event occurred that is viewed differently by his supporters and critics: on the afternoon of October 17, 1829, he was found lying in a pool of his blood in the cellar of Prof. Daumer's house. His forehead showed a wound, two inches in length, that was apparently inflicted by a sharp instrument—a murder attempt, say his supporters,[1–3,13–15] and a self-inflicted injury to boost his declining public attention, say his critics. He recovered within a few weeks, but his life changed considerably as the King of Bavaria, Ludwig I (1786–1868), ordered that, henceforth, he had to be guarded day and night by two policemen. Some authors interpreted this as evidence that Kaspar Hauser was not just a neglected youth, but a person of political relevance.

For more details about his short public life, we refer the interested reader to the extensive historical literature about Kaspar Hauser (see the earlier text). In this article, we limit the description to two further aspects: (1) Kaspar Hauser moved to the nearby city of Ansbach in December 1831, where he lived in the house of a strict teacher, Johann Georg Meyer; (2) on December 14, 1833, five and a half years after his appearance, he returned home with a stab wound in his chest, from which he died three days later—murder, say his supporters, and accidental death after another self-inflicted injury, say his critics. The arguments for the two views fill discussions in the pertinent literature.

As for Kaspar Hauser's background and origin, there are mainly two opposing theories, him being (1) an abducted prince of the Grand Duchy of Baden, Germany,[2,3,13–16] or (2) a liar and fraud, fooling the honorable but naive folks in Nuremberg and Ansbach.[4–12] The main argument for the latter theory is the peculiar dungeon story, as detailed earlier.

The key elements for the prince theory are based on the following facts. On September 29, 1812, Grand Duchess Stéphanie de Beauharnais (1789–1860), adopted daughter of Napoleon Bonaparte (1769–1821) and wife of the reigning Grand Duke Carl (1786–1818), gave birth to an apparently healthy boy in Karlsruhe (Figure 2). Being the first son of the noble couple, he would have become the next Grand Duke of Baden. However, he (officially) died at the age of 18 days before even receiving a name. The most popular version of the prince theory states that the second wife of the antecedent grand duke Carl Friedrich (1728–1811), Countess Louise Caroline von Hochberg (1768–1820), had replaced the two-week-old prince with a fatally ill son of one of her employees. The substituted baby died and was buried as the prince, while the real prince lived on in secrecy and later became Kaspar Hauser. Stéphanie and Carl further had daughters, but no surviving male descendant, which is why one of Countess Hochberg's sons came to the throne of Baden in 1830 (Figure 2). Again, we limit the review of arguments in favor of or against this theory to the minimum here, but note that it would have been difficult to abduct and replace the prince in his fathers' castle.

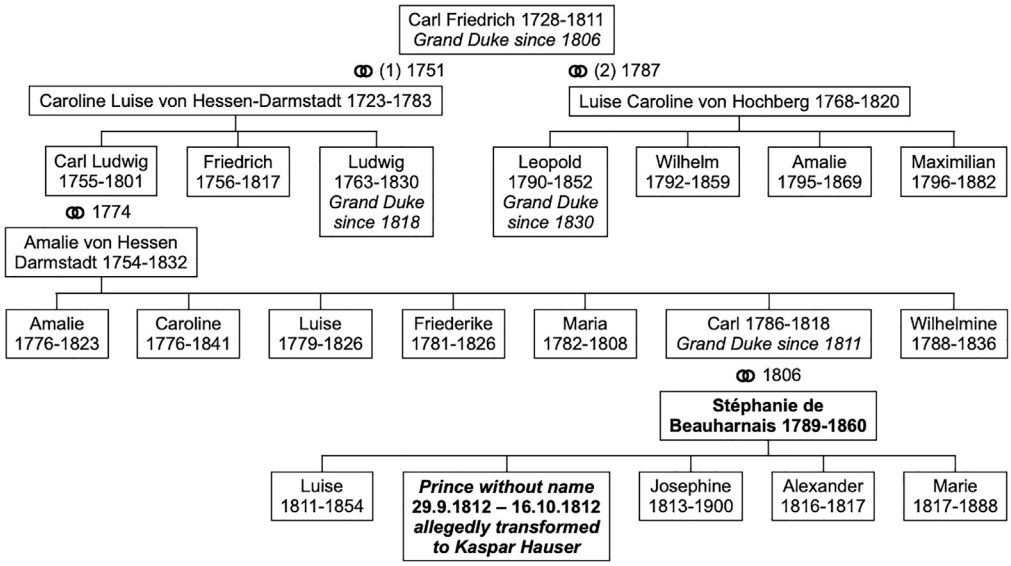

Figure 2. Extract of the genealogical tree of the House of Baden showing the position of the prince who was allegedly transformed to Kaspar Hauser

Kaspar Hauser died from his injuries on December 17, 1833. With his death, speculations about his history and ancestry did not end. On the contrary, Kaspar Hauser's life became one of the most mysterious riddles in German history, with the assumption that he was the kidnapped and replaced Prince of Baden being the most popular solution. In the mid-1990s, this led to molecular genetic investigations of samples assigned to Kaspar Hauser as well as samples donated from members of the Baden family.

The Munich Institute of Legal Medicine (Germany) and the Forensic Science Service (FSS, United Kingdom) excluded Kaspar Hauser from being maternally related to the House of Baden by comparing mitochondrial DNA (mtDNA) results from a bloodstain from the underwear that Kaspar Hauser was supposedly wearing during the incident in 1833 and the mtDNA from reference samples from two descendants of Stéphanie de Beauharnais.[17] However, subsequent mtDNA analyses carried out at the Münster Institute of Legal Medicine (Germany) in 2001/2002 on hair samples and other investigated items attributed to Kaspar Hauser yielded weak sequences representing mitotypes different from those reported in the study by Weichhold et al.,[17] thus questioning the earlier evidence.[18] Moreover, the investigators concluded that the mtDNA found in the hair samples did not exclude Kaspar Hauser from being maternally related to Stéphanie de Beauharnais.[18]

In order to solve this stalemate and motivated by the successful introduction of massively parallel sequencing (MPS) technologies in the 2010s that demonstrated significantly improved success rates in old and challenging forensic specimens compared to electrophoretic methods,[19–21] this study reports on the reanalysis of the case in two independent laboratories by typing new batches of hair samples attributed to Kaspar Hauser with primer extension capture (PEC) and shotgun MPS.

RESULTS

Early Sanger-based sequencing resulted in ambiguous findings

The mtDNA analyses performed in the early 2000s were typically restricted to the two hypervariable segments (HVS-I and HVS-II) of the mtDNA control region (CR) and involved the amplification and direct Sanger-based sequencing of HVS-I and II.[22] In 2001 and 2002, such mtDNA analyses were performed in Münster on specimens attributed to Kaspar Hauser including his hair (ante and post-mortem) and swabs from a bloodstain on his trousers and from a hat, the latter two being kept in the Kaspar Hauser Museum in Ansbach. These analyses resulted in weak HVS-I/II sequences that were identical to each other, except for an observation of 16316G in the mtDNA of the bloodstain, and a 16316R in one of the swabs from the hat (Table 1, MS1–MS5), as well as differences in the less discriminatory length heteroplasmic variants of the HVS-II polyC-stretch around position 310, which we further ignore here for comparison purposes according to the recommendations of the ISFG.[23] The mitotypes found in the hair samples and on the hat represent the most common West Eurasian mitotype 263G 315.1C[24] with an expected frequency of ~15% in the partial HVS-I/II sequence range 16212–16365 180–341 in West Eurasia (EMPOP V4R13; https://empop.online).[25] Relative to the mtDNA of the Baden lineage, this mitotype differed only at position 16220 in the analyzed range (Baden lineage: 16220C 263G 315.1C; ~0.02% in the West Eurasian subset of EMPOP), which was interpreted as inconclusive result in the early 2000's based on the ISFG[26] and EDNAP[27] recommendations. Advances in our understanding of the mtDNA phylogeny over the past two decades indicate that the transversion at 16220 relative to the rCRS on a haplogroup H background (263G 315.1C) points toward the presence of haplogroup H1bs.[28] This would be unambiguously different from the main haplogroup H motif 263G 315.1C that was found in some of the analyzed specimens and would therefore indicate an exclusion.

Table 1. Summary of samples, methods, and mitochondrial DNA results in the Kaspar Hauser case

Sample assignment[a]	Year(s) (sampling)	Year(s) (analysis)	Interpretation range (reduced to HVS-I/HVS-II)[d]	Mitotypes[b]										
				16220	16223	16292	16316	73	189	195	204	207	263	315.1
rCRS				A	C	C	A	A	A	T	T	G	A	–
Maternal relatives of St. de Beauharnais	1996–2015[c]	1996–2016	16024-16362 73–340[e,f]	C									G	C
KH bloodstain underwear (MC1)	1996	1996	16024-16400 35–400[e]		T	T		G	G	C	C	A	G	C
KH bloodstain underwear (BI1)	1996	1996	16024-16400 62–226[e]		T	T		G	G	C	–	A	ns	ns
KH hair pool am (Feuerbach) (MS1)	2001	2001/2002	16283-16365 192–329[e]	ns	ns			ns	ns				G	C
KH hair pool pm (Feuerbach) (MS2)	2001	2001/2002	16212-16365 180–341[e]					ns					G	C
KH hair pool (Ansbach) (MS3)	2001	2001/2002	16217-16365 183–340[e]					ns					G	C
KH bloodstain trousers (Ansbach) (MS4)	2001	2001/2002	16220-16365 181–340[e]				G	ns					G	C
KH hat brim 2 (Ansbach)[j] (MS5)	2001	2001/2002	16230-16365 181–340[e]				R	ns					G	C
KH hair shaft 1 am (Feuerbach) (IN1a)	2014[k]	2019	73-203 279–340[g]	ns	ns	ns	ns	G	G	C	ns	ns	ns	C
KH hair shaft 2 am (Feuerbach) (IN1b)	2014[k]	2019	73-169 214–340[g]	ns	ns	ns	ns	G	ns	ns	ns	ns	G	C
KH hair shaft 3 am (Feuerbach) (IN1c)	2014[k]	2019	73–325[g]	ns	ns	ns	ns	G	G	C	C	A	G	C
KH hair shaft 4 am (Feuerbach) (IN1d)	2014[k]	2019	16167-16303 73–340[g]		T	T	ns	G	G	C	C	A	G	C
KH hair shaft 1 pm (Feuerbach) (IN2a)	2014[k]	2019	–	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KH hair shaft 2 pm (Feuerbach) (IN2b)	2014[k]	2019	96–203[g]	ns	ns	ns	ns	ns	G	C	ns	ns	ns	ns
KH hair shaft 3 pm (Feuerbach) (IN2c)	2014[k]	2019	–	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns	ns
KH hair shaft 4 pm (Feuerbach) (IN2d)	2014[k]	2019	16284-16365 73–340[g]	ns	ns	T		G	G	C	C	A	G	C
KH hair shaft 1 pm (Diterich) (IN3a)	2014[k]	2019	16024-16365 73–340[g]		T	T		G	G	C	C	A	G	C
KH hair shaft 2 pm (Diterich) (IN3b)	2014[k]	2019	16024-16106 73–340[g]	ns	ns	ns	ns	G	ns	ns	ns	ns	G	C
KH hair shaft 3 pm (Diterich) (IN3c)	2014[k]	2019	16024-16365 73–340[g]		T	T		G	G	C	C	A	G	C
KH hair shaft 4 pm (Diterich) (IN3d)	2014[k]	2019	16197-16365 73–340[g]		T	T		G	G	C	C	A	G	C
KH hair shaft am (Feuerbach) 6681340 (PO1)[l]	2001[m]	2021/2022	16024-16365 73–277 300-329 331–340[h,i]		T	T		G	G	C	C	A	G	C

[a]MC … ILM Munich, BI … FSS Birmingham, MS … IFM Münster, IN … ILM Innsbruck, PO … IBB Potsdam; am, ante mortem; pm, postmortem; KH, Kaspar Hauser.

[b]MtDNA data are displayed relative to the rCRS. Empty space, identical to rCRS; –, no nucleotide reported; ns, no sequence information; Length variants in C-runs around 310 were not considered.

[c]ILM München, 1996; IFM Münster, 2001; Genomics Research Center, Houston, 2013; Forensic Genetics and Molecular Archaeology, Leuven, 2015. Tested maternally related individuals depicted in Figure S2.

[d]The interpretation ranges varied between laboratories, but all samples comprised the positions which are specified in the table. The reported ranges were reduced to the regions of the two hypervariable segemnbts HVS-I and HVS-II.

[e]Sanger-based sequencing technology.

[f]PCR-based massively parallel sequencing.

[g]PEC (primer extension capture)-based massively parallel sequencing.

[h]Whole-genome massively parallel sequencing.

[i]Mitotype reported here only relative to the indicated positions in the top line.

[j]The samplings from hat brim 1 and 3 yielded only partial mitotypes.

[k]IN1, IN2, and IN3 were transferred in total from Münster to Innsbruck in 2019.

[l]In cooperation with GGB Leicester; investigation and analysis in Potsdam and Leicester were done in a "blinded" manner, i.e., the investigators did not know from which historical person the hairs originated.

[m]PO1 was separated from the remainder of MS1 and transferred from Münster to Potsdam in 2020.

We note that these Sanger-based sequencing results from 2001/2002 have not been published scientifically and are therefore included here for completeness.

MPS-based sequencing results

The mtDNA-specific real-time PCR quantitation assay targeting a fragment size of 143 bp did not yield detectable amounts of DNA in the extracts of the 12 hair shaft samples analyzed in Innsbruck in 2019 (data not shown). Nevertheless, the PEC MPS analyses targeting the mtDNA CR yielded interpretable results in ten (IN1a–d, IN2b, IN2d, and IN3a–d) of the 12 investigated hair shafts attributed to Kaspar Hauser (Tables 1 and S1) with mean read lengths from 96 to 128 bp. The extraction blank and the negative control samples yielded no interpretable sequence reads that would indicate cross contamination (average read depth: negative control 0.0033; extraction blank 0.3461). One sample (hair shaft 2 postmortem [pm] Feuerbach, IN2b) resulted in a short interpretable sequence only (108 bp); the other nine hair shaft samples yielded mitotypes between 276 and 1,122 bp in the CR (1,122 bp corresponds to the size of the entire CR [16024–576]; Table S1). The mitotypes observed in the ten at least partially successfully analyzed hair shafts were all concordant to each other in their comparable ranges (Tables 1 and S1). The highest sequence coverage was obtained for hair shafts 1 and 3 pm (Diterich, IN3a, IN3c) that yielded the identical full CR mitotype 16223T 16292T 16519C 73G 189G 195C 204C 207A 263G 315.1C (length variation in the HVS-II C-stretch around position 310 disregarded). This particular mitotype is also identical in the comparable regions to the mtDNA sequences reported earlier for a bloodstain from the underwear attributed to Kaspar Hauser (Table 1, MC1 and BI1).[17] The other eight PEC-based mitotypes gave concordant results.

A reference sample provided by Stéphanie Bougaran, who is maternally related to Stéphanie de Beauharnais (Figure S2), yielded the same haplogroup H1bs CR mitotype 16220C 263G 315.1C as reported for the reference samples in the study by Weichhold et al.[17]

One hair shaft sample taken from Kaspar Hauser ante mortem (labeled with "6681340"; Tables 1 and S1 PO1) and one other hair shaft sample, unrelated to this case from an institute's internal museum specimen for teaching purposes (dummy control; labeled with "7463316"; Table S1 PO2), were further investigated in Potsdam in 2021/2022 using single-stranded library generation followed by MPS.[29] The DNA extract from sample 6681340 (PO1) resulted in a low read-depth (13.29 reads on average), partial (97.99%) mitogenome sequence that fully matched the PEC MPS-based results obtained for the hair shaft samples attributed to Kaspar Hauser (Tables 1 and S1) in the comparable regions. The DNA extract from the dummy control 7463316 (PO2), which accompanied the shipment of hair sample 6681340 (PO1) as a blind control unrelated to the case, yielded a mitogenome sequence (23.37 reads on average) with 99.27% of the mitogenome covered that belonged to haplogroup I2d and was identical in the comparable sequenced regions to the results of a previous analysis performed on this sample in Innsbruck (Table S1).

Statistical analysis

The mitotypes found in hair samples in 2019 (Table 1; IN1a–IN1d, IN2b, IN2d, and IN3a–IN3d) and in 2021/2022 (PO1) corroborate the mtDNA results obtained from a bloodstain from the underwear in 1996 (Table 1; MC1, BI1). All mitotypes are identical to each other in the comparable sequenced regions. Since different sequencing ranges were obtained for the various specimens, we focus the statistical evaluation on the mitotypes of MC1, IN3a, and PO1, which are independent results from each other in terms of sampling, analyzing laboratory, time of analysis, and method used. The mitotypes of MC1, IN3a, and PO1 share a common control region sequence range of 16024–16400 35–277 300–329 331–400 and the common mitotype 16223T 16292T 73G 189G 195C 204C 207A 263G 315.1C (Table S1). This mitotype belongs to haplogroup W (of West Eurasian provenience) and was observed 20 times among 17,320 West Eurasian EMPOP sequences in the query range (EMPOP V4, R13). To provide a conservative estimate, we add our observations to the database (x+3/n+3), summing up to 23/17,323, which equals 0.1327%. The addition of a conservative 99.9% confidence interval (BetaInv) results in an upper limit of 0.2424% or 1:410 for the probability that a randomly selected person of the West Eurasian population matches this mitotype. Consequently, the probability that the investigation of sample IN3a yielded the same sequence as sample MC1 is 1:410, if this sequence is *not* Kaspar Hauser's. The probability that the analysis of both samples IN3a and PO1 yielded the same mitotype as sample MC1 is 1:410 × 1:410 = 1:168,100 or 0.00059% if this sequence is *not* Kaspar Hauser's. We note that the results obtained in 2001/2002 are not considered here (see discussion section).

DISCUSSION

The fate of Kaspar Hauser has become a focus of public interest and scientific research throughout the past two centuries. The sudden appearance of this young man and his account about his early life gave rise to speculations about his origin and past.

With the emergence of fluorescence-based mtDNA Sanger sequencing in the 1990s, it became popular to attempt genetic identification of unknown or putatively known remains with these methods.[30] The first molecular genetic analyses in the case of Kaspar Hauser were published in 1998 by researchers of the Munich Institute of Legal Medicine (Germany) and the FSS (United Kingdom).[17] Both laboratories used tissue cuttings with blood from underwear that Kaspar Hauser was supposedly wearing during the incident on December 14, 1833. The underwear consisted of two layers of fabric that were stuck together around the bloodstain. These layers were separated under controlled humidity of 80% and cuttings (approx. 10 cm^2) were taken from the inner layer, which was better protected from extraneous environmental conditions and occasional handling procedures. For the genetic analyses, both laboratories targeted the two hypervariable segments (HVS-I and HVS-II) of the mtDNA CR, as was common practice at the time. The protocols differed slightly between the two groups, but they both were able to yield mtDNA sequences for an overlapping range of 16024–16365 and 73–226 relative to the reference sequence. They reported two concordant mtDNA sequences for the bloodstain with the consensus mitotype 16223T 16292T 73G 189G 195C (204C) 207A 263G 315.1C

(Table 1; MC1, BI1). The mtDNA analyses performed on maternally related reference samples of the Baden lineage (Figure S2) yielded the HVS-I/II mitotype 16220C 263G 315.1C (Table 1). The authors concluded that seven differences existed between the two mitotypes, which represented an unambiguous result excluding the donor of the blood sample from the Baden lineage.[17]

While the authenticity of the reference samples was beyond doubt, uncertainties arose over the authenticity of the underwear and the bloodstains on the underwear that was on museal display for many decades. There were rumors that the bloodstains had been manipulated repeatedly for museal purposes, which, if true, could have impacted the mtDNA results.

This prompted an independent set of mtDNA analyses, again using Sanger sequencing, in 2001/2002 on various samples attributed to Kaspar Hauser, including hair samples and swabs from his trousers (blood) and from his hat. We note here that this time, the owners of the samples at the Kaspar-Hauser Museum in Ansbach were not agreeing with cutting material from the trousers or the hat, but allowed only for abrasions, which are known to result in less DNA yield as cuttings would. Since Sanger-based mtDNA sequencing of old hair samples was not always successful, the hairs were pooled to increase input mtDNA amount and thus the chances of yielding useful results, despite the fact that it was already known at the time that mtDNA sequences could differ between hairs from the same person due to mosaicism and for other reasons.[22] While the Sanger-based mtDNA sequencing analyses on the maternal reference samples (blood, buccal swab, and hair) in 2001/2002 confirmed the results from the earlier testing in 1996,[17] the pooled hair shafts attributed to Kaspar Hauser yielded weak, different, and partially inconsistent results (Table 1; MS1–MS5). The majority of the experiments on the pooled hair shafts resulted in the most common West Eurasian HVS-I/II mitotype 263G 315.1C that is known to represent a wide bouquet of lineages within haplogroup R0.[24] The swab from the bloodstain, however, showed an additional substitution at 16316 on top of the common R0 motif that has been observed in some haplogroup H lineages, e.g., H27 (Phylotree b.17[28]). A mixture of the R0 mitotype and the variant including 16316G was apparently observed in a swab of the hat. In summary, the 2001/2002 Sanger-based sequencing results of the hair shafts and the swab of the trousers' bloodstain and from the hat were weak and discordant to the mitotype generated from the blood on the underwear in 1996.[17] Importantly, they were also not conclusive among the different investigated items either.

The most common West Eurasian mitotype found in some of the samples in 2001/2002 differed to the maternal House of Baden lineage by a single position (16220). In accordance with the forensic guidelines for mtDNA interpretation at the time, a single difference would not support a full exclusion of two otherwise identical mtDNA sequences from belonging to the same lineage. Depending on the tissue at hand and particularly for hair shaft samples, a single difference was regarded as inconclusive (in some legislation even as inclusive) with respect to the direct relatedness of two mtDNA sequences.[26,27]

The results obtained in 2001/2002 were interpreted based on nominal differences between mtDNA sequences only and did not take phylogenetic considerations into account. This is different today, as it is known and generally accepted that mitotypes carry a phylogenetic signature. Haplogrouping, the process of allocating mtDNA sequences to haplogroups,[31] has become a routine practice in forensic genetics. The bespoke difference at 16220 constitutes a transversion relative to the rCRS (A16220C) and is rarely observed in the human mitochondrial phylogeny. It is indicative of haplogroup H1bs. Outside of H1bs, 16220C has only been observed in a few other lineages, e.g., HV12a, V, J2b1, and F3b, confirming the rare occurrence of this variant. Therefore, the apparent similarity between the Sanger-based sequencing results of the hair shaft samples and the House of Baden lineage in 2001/2002 is restricted to the two hypervariable segments only. If larger regions of the mitogenome had been analyzed, more discrepant positions between the two lineages would have been observed based on phylogenetic expectations, and the two mitotypes would not have been considered similar.

The emergence of MPS-based sequencing revolutionized mtDNA analyses in the field of forensics as well as other fields of genetic research. The advantages of MPS over conventional Sanger-based sequencing have aptly been discussed.[21] One particular strength that has been taken advantage of in ancient DNA research for more than a decade now is the amenability of this method to sequence smaller (mt)DNA fragments than can be targeted with PCR-based approaches. One of these methods, PEC, was originally developed to sequence mitogenomes from ancient Neanderthal remains[32] and was later adapted to suit the forensic genetic workflow.[20] PEC allows for much shorter mtDNA fragments to be captured, enriched, and sequenced compared to PCR-based library methods, which opened the door for the successful application of this method to forensic cases involving highly degraded mtDNA.[33] Comparable success was reported using other hybridization capture methods that also target smaller mtDNA fragments than those typically used for PCR.[33]

These successful applications motivated the revision of this case in 2019, and PEC MPS-based analyses were carried out on new batches of hair shaft samples attributed to Kaspar Hauser (Table 1; IN1, IN2, and IN3). In contrast to earlier Sanger-based sequencing efforts, individual rather than pooled hair shafts were now analyzed. Of 12 selected single hair shafts derived from three different sources, ten yielded interpretable results. The quality of these sequence data was high and reproducible in various ways. First, the genetic data were sound and confirmed with generally high sequence coverage and read depth. Second, hair samples stemming from the same hair strand resulted in identical mtDNA sequences in the comparable ranges. Third, hair samples attributed to one person derived from three different collections again yielded the same results in the overlapping regions, and finally, fourth, the mtDNA consensus sequence of all the investigated hair samples from this study matched the results of the blood sample that was also attributed to the same individual, i.e., Kaspar Hauser.[17]

Furthermore, two hair shafts, one assigned to Kaspar Hauser (PO1) and one unrelated to this case (dummy control, 7463316; PO2) were subjected to another round of independent testing in Potsdam by a laboratory specialized in ancient DNA analyses. The hair shafts were anonymized and no information on their origins was revealed. The only information given to the laboratory lead and staff was that one of the two samples originated from a historical person. The identity of this historical person—Kaspar Hauser—was revealed to the personnel involved in the lab work and the analysis only after they had presented their results. So, these results have been obtained

without any bias or influence by expectations about what sequence might emerge from the samples under investigation, or which theory the results might prove or disprove (principle of "blinding", which is a common practice in clinical research). The dummy control hair shaft (7463316, PO2) unrelated to this case resulted in a haplogroup I2d mitotype confirming earlier results obtained in Innsbruck in the comparable sequenced regions (Table S1); the hair shaft collected from Kaspar Hauser ante mortem (6681340, PO1) yielded a haplogroup W mitotype that was identical to the results obtained by PEC MPS in the comparable sequence ranges as well as to earlier results obtained from the bloodstain of the underwear.[17]

The apparent discrepancy between the recent identical MPS-based data obtained in two different laboratories using different library preparation techniques on the one hand and the 2001/2002 Sanger-based sequencing results on the other hand can be explained by the different mtDNA fragment sizes that were targeted with the respective methods. Mean read lengths observed with PEC MPS were between 96 and 128 bp, that is, below the size targeted with PCR-based Sanger sequencing. Therefore, Sanger-based sequencing by no means could have picked up the genuine mtDNA within the hair shafts but more likely extraneous DNA, which is why the results obtained in 2001/2002 for samples MS1–MS5 were not considered for the statistical evaluation. In contrast, even old blood samples, like the ones investigated in 1996, generally contain more mtDNA that is usually less degraded than in hair shafts and thus can also yield reliable results with Sanger-based HVS-I/II sequencing.[22] It is by now widely accepted that MPS-based methods, particularly capture-based technologies, are superior to PCR-based sequencing approaches in old and challenging samples, a fact that also explains the results obtained in this study.

To statistically evaluate the results obtained in this case, the analyses of three samples were considered that can be regarded as entirely independent from each other. These are bloodstain from underwear MC1 (Munich 1996), hair shaft IN3a (Innsbruck 2019), and hair shaft PO1 (Potsdam 2021/2022). These three samples are from different sources, they were delivered independently to the respective laboratories, and they were analyzed in different years and using different technology by different scientists. The blood sample MC1 was cut from an underwear assigned to Kaspar Hauser and analyzed in 1996; the strand from which hair shaft IN3a was taken was cut from Kaspar Hauser's head after his death and analyzed in Innsbruck in 2019; and the strand from which hair shaft PO1 was taken was collected from Kaspar Hauser while alive and analyzed in Potsdam in 2021/2022. Equally important, the transmission routes of the two strands until today are traceable and distinctly different. Finally, the individual hairs investigated in Potsdam have never been in Innsbruck and vice versa. There is no reasonable possibility that any contamination or laboratory error that might have affected the result from one of the samples might have as well affected those from the other two.

The assumption that the common mitotype observed in MC1, IN3a, and PO1 would not belong to Kaspar Hauser requires that each of the three investigations of these samples was affected by at least one of three possible errors:

(1) The investigated sample (blood or hairs) does not originate from Kaspar Hauser, but from a different person (erroneous historical tradition).
(2) The observed mitotype does not originate from the sample (blood or hairs), but represents contamination.
(3) There was a laboratory error (e.g., mix-up of samples).

Due to the independence of the three investigations, it can be excluded that any individual error would affect more than one investigation. Hence, it would be an entirely coincidental finding that there were errors in all three investigations leading to identical (but erroneous) results. An estimate for the probability that the three investigations yielded just by chance the same mitotype, which does not belong to Kaspar Hauser, can be derived from the probability of this sequence in a suitable database (EMPOP). A conservative estimate of the probability that the common mitotype of samples MC1, IN3a, and PO1 resulted from any kind of errors and does not represent Kaspar Hauser's mtDNA can be expressed as 1:168,100 or 0.00059%. It follows that the probability of this mitotype being indeed Kaspar Hauser's calculates as 1-1/168,100 = 0,999994 or 99.9994%. With that same probability, we can exclude the possibility that Kaspar Hauser is a son of Stéphanie de Beauharnais.

There is now redundant evidence provided from various samples and three independent technologies that Kaspar Hauser's mtDNA belongs to the West Eurasian haplogroup W[34] and is thus unambiguously different from the confirmed haplogroup H1bs lineage observed in the House of Baden. Based on the assignment of the investigated samples to Kaspar Hauser, and there is strong historical and extremely strong statistical evidence supporting this, it can be excluded that he was the exchanged and kidnapped Prince of Baden.

We note that it is not possible to derive specific information about Kaspar Hauser's geographical origin from his mtDNA sequence other than that it is of West Eurasian provenance. Hesse's assertion of having proven an origin from Tyrol by comparing Kaspar Hauser's mtDNA with that of one or two randomly selected Tyrolian women (with 8 or 9 mismatches)[35] is not based on agreed scientific facts and concepts. The mitotype found in Kaspar Hauser's samples between 1996 and 2022, although relatively rare, is shared by many people across Europe. Therefore, his possible origin cannot be narrowed down to a specific region like Tyrol just on the basis of his mtDNA sequence.

Regardless of the genetic investigations conducted in this and previous research and their conclusions, Kaspar Hauser remains a phenomenon that will continue to enrich our social and scientific life. Various scientific concepts have been developed under his acronym, such as the Kaspar Hauser experiment (behavioral biology), the Kaspar Hauser syndrome (medicine/psychology), the Kaspar Hauser complex (psychoanalysis), or the Kaspar Hauser effect (educational science), which are all based on the history and fate of an individual of still unknown identity and descent. The inscription on his grave—*Here lies Caspar Hauser, riddle of his time, unknown his birth, mysterious his death, 1833*—continues to hold true until future genetic and genealogic analyses may uncover this secret.

Conclusions

This study resolves previous conflicting results on the mtDNA of Kaspar Hauser, the mysterious foundling of Nuremberg. Using modern capture- and whole genome-based MPS techniques on different hair samples attributed to him, the same mitotype was found, demonstrating authenticity of his remains for the first time. This mitotype is clearly different from the mtDNA found in several individuals maternally related to Kaspar Hauser's presumed mother Stéphanie de Beauharnais, Grand Duchess of Baden, which rules out the "Prince theory."

Limitations of the study

The samples available to this study were analyzed in an exhaustive way using the most modern technologies available at the time. There were limitations though, particularly with respect to sample availability. The first round of experiments in Munich and Birmingham in 1996 was performed on cuttings of bloodstains from the underwear assigned to Kaspar Hauser.[17] Cuttings are known as one of the richest sources for DNA in forensic settings as they provide access to the entire biological material. For the subsequent analyses performed in Münster, Munich, and Freiburg in 2001/2002, the latter two were involved in the analytical process, no cuttings were provided from the bloodstain of the trousers or the hat. The laboratories were supplied with abrasions and swabs, which represent alternative sampling techniques that are however known to result in reduced yields of biological material and hence DNA. This fact is a strong indicator why the mtDNA sequencing results in 2001/2002, particularly from the blood of the trousers, resulted only in weak mtDNA sequences. Had the laboratory been given access to an even small cutting of this item, they would have been in a much better position to generate more reliable mtDNA data.

This sampling limitation also has further consequences. Bloodstains typically include much more nuclear DNA than hair shafts do. With the provision of cuttings from the trousers, the laboratories would have had realistic chances to generate nuclear DNA profiles, e.g., SNP profiles that would render themselves useful to further investigate the origin and fate of the young man named Kaspar Hauser, e.g., through investigative genetic genealogy.

This limitation also applies to the new analyses performed in 2019. No further samples could be taken from the items assigned to Kaspar Hauser in the Ansbach Museum, not even mild abrasions. Also, this study did not have access to human remains from Kaspar Hauser, who had been buried in Ansbach. Additional analyses could have possibly been performed in this regard using nuclear DNA markers that might have increased the evidential value of the conducted research.

Regarding the historical implications of our results, it can be excluded with an extremely high probability that Kaspar Hauser was a son of Stéphanie de Beauharnais, as the popular prince theory had claimed. However, we still have no clue who he really was, and we want to caution the reader not to mistake our results as evidence for the competing theory that Kaspar Hauser was a liar and a fraud. He may have been a crime victim even if he was not a prince of the Grand Duchy of Baden.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

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AUTHOR CONTRIBUTIONS

Conceptualization, W.P., M.H., D.P., C.H., and B. Brinkmann; methodology, C.A., T.K., M.P., C.B., A.K., G.H., B. Bayer, G.W., and T.S.; software, C.A., T.K., and M.P.; validation, W.P.; formal analysis, W.P., C.A., T.K., G.H., T.S., and S.L.-B.; investigation, W.P., D.P., and C.H.; resources, W.P., T.K., S.L.-B., H.P., M.H., D.P., C.H., and B. Brinkmann; data curation, W.P., C.A., T.K., and G.H.; writing – original draft preparation, W.P., T.K., and D.P.; writing – review and editing, all authors; visualization, W.P. and D.P.; supervision, W.P., D.P., and C.H. All authors have read and agreed to the published version of the manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

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REFERENCES

1. Von Feuerbach, A. (1832). Kaspar Hauser. Beispiel eines Verbrechens am Seelenleben des Menschen (J. M. Dollfuß, Ansbach).
2. Daumer, G.F. (1873). Kaspar Hauser. Sein Wesen, seine Unschuld, seine Erduldungen und sein Ursprung in neuer, gründlicher Erörterung und Nachweisung (Verlag von Alfred Coppenrath).
3. Pies, H. (1966). Kaspar Hauser. Eine Dokumentation (Verlag C. Brügel & Sohn).
4. Merker, F.,C. (1831). Nachrichten über Caspar Hauser, aus authentischen Quellen und Betrachtungen über deren Beweiskraft für die Einkerkerungs-Geschichte des Jünglings (L. W. Krause'sche Buchhandlung).
5. Stanhope, P. H. (1835). Materialien zur Geschichte Kaspar Hausers (Akademische Buchhandlung von J. E. B. Mohr, Heidelberg).
6. Meyer, J. (1872). Authentische Mittheilungen über Caspar Hauser (Ansbach: Verlag von Fr. Seybold).
7. Mittelstädt, O. (1876). Kaspar Hauser und sein badisches Prinzenthum (Verlagsbuchhandlung von Fr. Bassermann).
8. Von der Linde, A. (1887). Kaspar Hauser. In Eine neugeschichtliche Legende (Verlag von Chr (Limbarth, Wiesbaden)).
9. Striedinger, I. (1933). Neues Schrifttum über Kaspar Hauser. Z. Bayer. Landesgesch. *6*, 415–484.
10. Mistler, J. (1971). Gaspard Hauser, un drame de la personnalité (Librairie Arthème Fayard).
11. Trautz, F. (1974). Zum Problem der Persönlichkeitsdeutung: Anlässlich des Kaspar-Hauser-Buches von Jean Mistler. Francia *2*, 715–731.
12. Schreibmüller, W. (1991). Bilanz einer 150jährigen Kaspar Hauser-Forschung. Genealogisches Jahrbuch *31*, 43–84.
13. Klee, F. (1929). Neue Beiträge zur Kaspar Hauser-Forschung (J. L. Schrag Verlag).
14. Mayer, J., and Tradowsky, P. (1984). Kaspar Hauser, das Kind von Europa (Urachhaus, Stuttgart).
15. Leonhardt, U. (1987). Prinz von Baden genannt Kaspar Hauser (Eine Biographie (Wunderlich, Reinbek)).
16. Von Feuerbach A. Memoire über Kaspar Hauser. Wer möchte wohl Kaspar Hauser sein? In: Feuerbach L., editor. Anselm Ritter von Feuerbach's Leben und Wirken. 2nd vol. Leipzig: Verlag von Otto Wigand; 1852. p. 319–333.
17. Weichhold, G.,M., Bark, J.,E., Korte, W., Eisenmenger, W., and Sullivan, K.,M. (1998). DNA analysis in the case of Kaspar Hauser. Int. J. Legal Med. *111*, 287–291. https://doi.org/10.1007/s004140050173.
18. Brinkmann, B. (2006). Neuester Stand der Forschung der Gerichtsmedizin und Pathologie der Universität Münster (Leipzig: Anselm von Feuerbach, Kaspar Hauser, Reprint-Verlag).
19. Templeton, J.E.L., Brotherton, P.,M., Llamas, B., Soubrier, J., Haak, W., Cooper, A., and Austin, J.,J. (2013). DNA capture and next-generation sequencing can recover whole mitochondrial genomes from highly degraded samples for human identification. Investig. Genet. *4*, 26. https://doi.org/10.1186/2041-2223-4-26.
20. Eduardoff, M., Xavier, C., Strobl, C., Casas-Vargas, A., and Parson, W. (2017). Optimized mtDNA Control Region Primer Extension Capture Analysis for Forensically Relevant Samples and Highly Compromised mtDNA of Different Age and Origin. Genes *8*, 237. https://doi.org/10.3390/genes8100237.
21. Marshall, C., Sturk-Andreaggi, K., Daniels-Higginbotham, J., Oliver, R.,S., Barritt-Ross, S., and McMahon, T.,P. (2017). Performance evaluation of a mitogenome capture and Illumina sequencing protocol using non-probative, case-type skeletal samples: Implications for the use of a positive control in a next-generation sequencing procedure. Forensic Sci. Int. Genet. *31*, 198–206. https://doi.org/10.1016/j.fsigen.2017.09.001.
22. Wilson, M.,R., Allard, M.,W., Monson, K., Miller, K.W.P., and Budowle, B. (2002). Recommendations for consistent treatment of length variants in the human mitochondrial DNA control region. Forensic Sci. Int. *129*, 35–42. https://doi.org/10.1016/s0379-0738(02)00206-2.
23. Parson, W., Gusmão, L., Hares, D.,R., Irwin, J.,A., Mayr, W.,R., Morling, N., Pokorak, E., Prinz, M., Salas, A., Schneider, P.,M., et al. (2014). DNA Commission of the International Society for Forensic Genetics: revised and extended guidelines for mitochondrial DNA typing. Forensic Sci. Int. Genet. *13*, 134–142. https://doi.org/10.1016/j.fsigen.2014.07.010.
24. Bodner, M., Amory, C., Olivieri, A., Gandini, F., Cardinali, I., Lancioni, H., Huber, G., Xavier, C., Pala, M., Fichera, A., et al. (2022). Helena's Many Daughters: More Mitogenome Diversity behind the Most Common West Eurasian mtDNA Control Region Haplotype in an Extended Italian Population Sample. Int. J. Mol. Sci. *23*, 6725. https://doi.org/10.3390/ijms23126725.
25. Parson, W., and Dür, A. (2007). EMPOP–a forensic mtDNA database. Forensic Sci. Int. Genet. *1*, 88–92. https://doi.org/10.1016/j.fsigen.2007.01.018.
26. Carracedo, A., Bär, W., Lincoln, P., Mayr, W., Morling, N., Olaisen, B., Schneider, P., Budowle, B., Brinkmann, B., Gill, P., et al. (2000). DNA commission of the International Society of Forensic Genetics: guidelines for mitochondrial DNA typing. Forensic Sci. Int. *110*, 79–85. https://doi.org/10.1016/s0379-0738(00)00161-4.
27. Tully, G., Bär, W., Brinkmann, B., Carracedo, A., Gill, P., Morling, N., Parson, W., and Schneider, P. (2001). Considerations by the European DNA profiling (EDNAP) group on the working practices, nomenclature and interpretation of mitochondrial DNA profiles. Forensic Sci. Int. *124*, 83–91. https://doi.org/10.1016/s0379-0738(01)00573-4.
28. Van Oven, M., and Kayser, M. (2009). Updated comprehensive phylogenetic tree of global human mitochondrial DNA variation. Hum. Mutat. *30*, E386–E394. https://doi.org/10.1002/humu.20921.
29. Paijmans, J.L.A., Fickel, J., Courtiol, A., Hofreiter, M., and Förster, D.,W. (2016). Impact of enrichment conditions on cross-species capture of fresh and degraded DNA. Mol. Ecol. Resour. *16*, 42–55. https://doi.org/10.1111/1755-0998.12420.
30. Gill, P., Ivanov, P.,L., Kimpton, C., Piercy, R., Benson, N., Tully, G., Evett, I., Hagelberg, E., and Sullivan, K. (1994). Identification of the remains of the Romanov family by DNA analysis. Nat. Genet. *6*, 130–135. https://doi.org/10.1038/ng0294-130.
31. Bandelt, H.,J., van Oven, M., and Salas, A. (2012). Haplogrouping mitochondrial DNA sequences in Legal Medicine/Forensic Genetics. Int. J. Legal Med. *126*, 901–916. https://doi.org/10.1007/s00414-012-0762-y.
32. Briggs, A.,W., Good, J.,M., Green, R.,E., Krause, J., Maricic, T., Stenzel, U., Lalueza-Fox, C., Rudan, P., Brajkovic, D., Kucan, Z., et al. (2009). Targeted retrieval and analysis of five Neandertal mtDNA genomes. Science *325*, 318–321. https://doi.org/10.1126/science.

33. Zavala, E.,I., Thomas, J.,T., Sturk-Andreaggi, K., Daniels-Higginbotham, J., Meyers, K.,K., Barrit-Ross, S., Aximu-Petri, A., Richter, J., Nickel, B., Berg, G.,E., et al. (2022). Ancient DNA Methods Improve Forensic DNA Profiling of Korean War and World War II Unknowns. Genes *13*, 129. https://doi.org/10.3390/genes13010129.
34. Palanichamy, M.,G., Sun, C., Agrawal, S., Bandelt, H.,J., Kong, Q.,P., Khan, F., Wang, C.,Y., Chaudhuri, T.,K., Palla, V., and Zhang, Y.,P. (2004). Phylogeny of mitochondrial DNA macrohaplogroup N in India, based on complete sequencing: implications for the peopling of South Asia. Am. J. Hum. Genet. *75*, 966–978. https://doi.org/10.1086/425871.
35. Hesse, G. (2017). Kaspar Hauser, das Kind von Europa, der Sohn des Pfarrers und Botanikers Wolfgang Hechenberger aus Tirol, spricht Latein. Und kommt aus (Berlin: Deutsche Literaturgesellschaft).
36. Hellmann, A., Rohleder, U., Schmitter, H., and Wittig, M. (2001). STR typing of human telogen hairs–a new approach. Int. J. Legal Med. *114*, 269–273. https://doi.org/10.1007/s004140000175.
37. Niederstätter, H., Köchl, S., Grubwieser, P., Pavlic, M., Steinlechner, M., and Parson, W. (2007). A modular real-time PCR concept for determining the quantity and quality of human nuclear and mitochondrial DNA. Forensic Sci. Int. Genet. *1*, 29–34. https://doi.org/10.1016/j.fsigen.2006.10.007.
38. Parson, W., Eduardoff, M., Xavier, C., Bertoglio, B., and Teschler-Nicola, M. (2018). Resolving the matrilineal relationship of seven Late Bronze Age individuals from Stillfried, Austria. Forensic Sci. Int. Genet. *36*, 148–151. https://doi.org/10.1016/j.fsigen.2018.07.005.
39. Strobl, C., Churchill Cihlar, J., Lagacé, R., Wootton, S., Roth, C., Huber, N., Schnaller, L., Zimmermann, B., Huber, G., Lay Hong, S., et al. (2019). Evaluation of mitogenome sequence concordance, heteroplasmy detection, and haplogrouping in a worldwide lineage study using the Precision ID mtDNA Whole Genome Panel. Forensic Sci. Int. Genet. *42*, 244–251. https://doi.org/10.1016/j.fsigen.2019.07.013.
40. Roth, C., Parson, W., Strobl, C., Lagacé, R., and Short, M. (2019). MVC: An integrated mitochondrial variant caller for forensics. Aust. J. Forensic Sci. *51*, S52–S55. https://doi.org/10.1080/00450618.2019.1569150.
41. Andrews, R.,M., Kubacka, I., Chinnery, P.,F., Lightowlers, R.,N., Turnbull, D.,M., and Howell, N. (1999). Reanalysis and revision of the Cambridge reference sequence for human mitochondrial DNA. Nat. Genet. *23*, 147. https://doi.org/10.1038/13779.
42. Bandelt, H.,J., and Parson, W. (2008). Consistent treatment of length variants in the human mtDNA control region: a reappraisal. Int. J. Legal Med. *122*, 11–21. https://doi.org/10.1007/s00414-006-0151-5.
43. Dür, A., Huber, N., and Parson, W. (2021). Fine-Tuning Phylogenetic Alignment and Haplogrouping of mtDNA Sequences. Int. J. Mol. Sci. *22*, 5747. https://doi.org/10.3390/ijms22115747.
44. Gansauge, M.,T., Gerber, T., Glocke, I., Korlevic, P., Lippik, L., Nagel, S., Riehl, L.,M., Schmidt, A., and Meyer, M. (2017). Single-stranded DNA library preparation from highly degraded DNA using T4 DNA ligase. Nucleic Acids Res. *45*, e79. https://doi.org/10.1093/nar/gkx033.
45. Meyer, M., Kircher, M., Gansauge, M.,T., Li, H., Racimo, F., Mallick, S., Schraiber, J.,G., Jay, F., Prüfer, K., de Filippo, C., et al. (2012). A high-coverage genome sequence from an archaic Denisovan individual. Science *338*, 222–226. https://doi.org/10.1126/science.
46. Basler, N., Xenikoudakis, G., Westbury, M.,V., Song, L., Sheng, G., and Barlow, A. (2017). Reduction of the contaminant fraction of DNA obtained from an ancient giant panda bone. BMC Res. Notes *10*, 754. https://doi.org/10.1186/s13104-017-3061-3.
47. Paijmans, J.,L.,A., Baleka, S., Henneberger, K., Taron, U.,H., Trinks, A., Westbury, M.,V., and Barlow, A. (2017). Sequencing single-stranded libraries on the Illumina NextSeq 500 platform. Preprint at arXiv. https://doi.org/10.48550/arXiv.1711.11004.
48. Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows- Wheeler transform. Bioinformatics *25*, 1754–1760. https://doi.org/10.1093/bioinformatics/btp324.
49. Milne, I., Stephen, G., Bayer, M., Cock, P.J.A., Pritchard, L., Cardle, L., Shaw, P.,D., and Marshall, D. (2013). Using Tablet for visual exploration of second-generation sequencing data. Brief. Bioinform. *14*, 193–202. https://doi.org/10.1093/bib/bbs012.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Chemicals		
AmpliTaq Gold DNA Polymerase with Gold Buffer and MgCl2	Thermo Fisher Scientific	Cat#4311806
GeneAmp™ dNTP Blend (2.5 mM ea)	Thermo Fisher Scientific	Cat#N8080260
Bovine Serum Albumin solution	Merck	Cat#B8667
Water, LiChrosolv (2.5 L)	vWR	Cat#1.15333.2500
AMPure XP	Beckman Coulter	Cat#A63882
Ethanol absolute, EMPROVE	vWR	Cat#1.00986.2500
Dynabeads MyOne Streptavidin C1	Thermo Fisher Scientific	Cat#65001
UltraPure 5M NaCl	Thermo Fisher Scientific	Cat#A57006
UltraPure 1 M Tris-HCl Buffer, pH 7.5	Thermo Fisher Scientific	Cat#15567027
UltraPure 0.5M EDTA, pH 8.0	Thermo Fisher Scientific	Cat#15575020
UltraPure SSC, 20X	Thermo Fisher Scientific	Cat#15557044
UltraPure SDS Solution, 10%	Thermo Fisher Scientific	Cat#15553027
TWEEN 20	Merck	Cat#P7949-100ML
18MΩ H2O	PURLAB FLEX system	https://www.bvs-wassertechnik.at
Tris-HCL 1M, pH 8	Thermo Fisher Scientific	Cat#15568-025
NaCl 5M	Thermo Fisher Scientific	Cat#AM9759
CaCl2 1M	Thermo Fisher Scientific	Cat#J63122.AE
SDS 10%, ultrapure Invitrogen	Thermo Fisher Scientific	Cat# 24730020
HPLC-water	A. Hartenstein	Cat#CW20
Proteinase K 20 mg/mL	Qiagen	Cat#19131
DL Dithiothreitol (DTT)	Sigma Aldrich	Cat#10197777001
Isopropanol	A. Hartensetin	Cat#CP50
Qubit dsDNA HS Assay Kit	Thermo Fisher Scientific	Cat#Q32854
Tape Station reagents D1000	Agilent	Cat#5067-5583
Tape Station Tape D1000	Agilent	Cat#5067-5582
Nuclease free water	New England Biolabs	Cat#B1500S
EDTA solution 0.5M	VWR	Cat#E177-500ML
Tween 20 100%	A. Hartenstein	Cat#CT21
SDS 20%	Thermo Fisher Scientific	Cat#AM9820
SSC Buffer. 20X	Roth	Cat#1054.1
Dynabeads MyOne Streptavidin C1	Thermo Fisher Scientific	Cat#65001
T4 PNK 500U	New England Biolabs	Cat#EK0031
Klenow Fragment 300U	Thermo Fisher Scientific	Cat#EP0051
T4 RNA Ligase Buffer	New England Biolabs	Cat#B0216L
Fast AP 1000U	Thermo Fisher Scientific	Cat#EF0651
T4 DNA Ligase 5U	Thermo Fisher Scientific	Cat#EL0011
T4 DNA Ligase 30U	Thermo Fisher Scientific	Cat#EL0013
Accuprime Pfx Polymerase	Thermo Fisher Scientific	Cat#12344024
dNTP 25 mM	Biozym	Cat#350600502
SYBR green PCR MasterMix	Thermo Fisher Scientific	Cat#4309155
ATP 100 mM	Thermo Fisher Scientific	Cat#R0441

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REAGENT or RESOURCE	SOURCE	IDENTIFIER
Critical commercial assays		
Ion Xpress Plus Fragment Library Kit	Thermo Fisher Scientific	Cat#4471269
Ion Xpress Barcode 1-96	Thermo Fisher Scientific	Cat#4474517
Bioanalyzer High Sensitivity DNA Analysis Kit	Agilent Technologies	Cat#5067-4626
Ion Library TaqMan Quantitation Kit	Thermo Fisher Scientific	Cat#4468802
Ion S5 Precision ID Chef & Sequencing Kit	Thermo Fisher Scientific	Cat#A33208
PrepFiler Forensic DNA Extraction Kit	Thermo Fisher Scientific	Cat#4463351
MinElute PCR Purification Kit	Qiagen	Cat#28004
Oligonucleotides		
Biotinylated primer	Microsynth AG	
F15910 5′ CACCAGTCTTGTAAACCGGAGAT 3′		
F16068 5′ CACCCATCAACAACCGCTATGTA 3′		
F16341 5′ TACAGTCAAATCCCTTCTCGTCC 3′		
F16229 5′ ATCACACATCAACTGCAACTCC 3′		
F16405 5′ TCCTCCGTGAAATCAATATCCCG 3′		
F16535 5′ CCCACACGTTCCCCTTAAATAAG 3′		
F47 5′ GCATTTGGTATTTTCGTCTGGGG 3′		
F130 5′ GTCTTTGATTCCTGCCTCATCCT 3′		
F261 5′ CCGCTTTCCACACAGACATCATA 3′		
F364 5′ AAGAACCCTAACACCAGCCTAAC 3′		
F446 5′ ACATTATTTTCCCCTCCCACTCC 3′		
F544 5′ CGAACCAACCAAACCCCAAAG 3′		
F626 5′ GGCTCACATCACCCCATAAACA 3′		
F737 5′ CACGATCAAAAGGGACAAGCATC 3′		
Enrichment Control Primer	Microsynth AG	
F8342 5′ GAACCAACACCTCTTTACAGTGA 3′		
IS7 5′ ACACTCTTTCCCTACACGAC 3′		
IS8 5′GTGACTGGAGTTCAGACGTGT 3′		
CL53 5′CGACGCTCTTC-ddC 3′		
CL73 5′ACACTCTTTCCCTACACGACGCTCTTCC 3′		
TL136 5′SpacerC12-AA[SpacerC12]CTTCCGATCT NNNNNNN-AmC6 3′		
CL78 5′ [Phos]AGATCGGAAG[SpcC3][SpcC3] [SpcC3][SpcC3][SpcC3][SpcC3][SpcC3][SpcC3] [SpcC3][SpcC3][BtnTg] 3′		
CL72 5′ACACTCTTTCCCTACACGACGCTCTTCC 3′		
Gesaffelstein 5′ GGAAGAGCGTCGTGTA GGGAAAGAGTGT 3′		
Software and algorithms		
Torrent Suite Software	Thermo Fisher Scientific	
Converge Software	Thermo Fisher Scientific	
Integrative Genomics Viewer	Broad Institute	https://igv.org/
EMPOP mtDNA database	EMPOP	https://empop.online/

RESOURCE AVAILABILITY

Lead contact

Further information and request for resources should be directed to and will be fulfilled by the lead contact, Walther Parson (walther.parson@i-med.ac.at).

Materials availability

This study did not generate new unique reagents.

Data and code availability

- Mitogenome data are available in the supplementary material.
- This paper does not report original code.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon reasonable request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

Hair samples

Except for MS3 analyzed in Münster (2001/2002), the history of the hairs investigated between 2001 and 2022 is well known. For an overview see also Figure S1.

One strand was cut from Kaspar Hauser's head while he was still alive (date unknown). From this strand, samples MS1 and IN1a-d were taken in 2001 and 2014. In 2020, PO1 was separated from the remainder of MS1. The strand is in possession of the descendants of Anselm Ritter von Feuerbach, one of Kaspar Hauser's most important friends and author of one of the first books on the subject. Another strand in possession of the Feuerbach family was cut from Kaspar Hauser's head after his death in December 1833 (in accordance with common practices at the time). From this strand samples MS2 and IN2a-d were taken in 2001 and 2014.

Another strand cut from Kaspar Hauser's head after his death took a different route. It was first kept by the Meyer family in Ansbach, where Kaspar Hauser had lived at the time. In 1911, Dr. Julius Meyer (1835–1913), son of the teacher Johann Georg Meyer and author of several books about Kaspar Hauser, gave the strand to the writer Sophie Höchstetter (1873–1943), whose father had lived in the house of the Meyers as a student and who later wrote a novel about Kaspar Hauser (published in 1925). She passed the strand on to her younger companion Carola Freifrau von Crailsheim-Rügland (1895–1982), also a writer. In 1977, the latter left it to Helmut Diterich, editor of a newspaper in Ansbach. The transfers of 1911 and 1977 are documented by letters of which the authors of this manuscript have copies. In 2002, Helmut Diterich passed the items on to his son, the diplomat Werner Diterich, who provided about 15 hairs of the strand for genetic analyses to Dietmar Pfründer on September 18, 2014. Unfortunately, not much later, Werner Diterich passed away. Until the time of writing, his widow could not locate either the strand (with about 100 remaining hairs) nor the originals of the accompanying letters from 1911 and 1977, which is why we have to consider these items of evidence as lost. However, there are some hairs left from the sample that Werner Diterich handed over to Dietmar Pfründer, since only four of these hairs were used for the analysis in Innsbruck (IN3a-d).

Loose hair samples from the strands reported to be collected from Kaspar Hauser at different times and occasions (see above) were provided to the Innsbruck laboratory in May 2019 in three envelopes for mtDNA analysis. Envelope 1 contained hair shafts (approx. 6–7 cm in length) collected from Kaspar Hauser ante mortem (date unknown), assets Family Feuerbach; envelope 2 contained hair shafts (approx. 6–7 cm in length) collected from Kaspar Hauser postmortem (1833), assets Family Feuerbach; envelope 3 contained hair shafts (approx. 4–5 cm in length) collected from Kaspar Hauser postmortem (1833), assets Werner Diterich.

Another strand attributed to Kaspar Hauser, but without a known history (personal communication of the museum director to Dietmar Pfründer on Sept. 25, 2020), is stored and displayed in the Kaspar Hauser Museum in Ansbach. Some hairs of this strand were investigated in 2001/2002 (MS3). Unfortunately, a permission to take another sample from this strand was denied, which is why it could not be re-investigated in 2019 and 2021/2022.

The institute in Potsdam received two hair samples: (1) one hair sample was taken from the remainders of the samples used in 2001/2002 in Münster, specifically from the strand "Feuerbach *ante mortem*", sampling 1 (MS1); (2) another hair sample *unrelated* to this case (dummy control) was taken from the internal museal specimen for teaching and research purposes at the institute in Innsbruck. To allow for unbiased analyses, the two samples were "blinded" with respect to their origins, i.e., they were transferred in identically looking Petri dishes without any accompanying information regarding their origins. The hair strand assigned to Kaspar Hauser was coded "6681340", the sample unrelated to this case (dummy control) was coded "7463316". Also, the nature of the case and the circumstances were not revealed to the collaborators (no mention of Kaspar Hauser) until the completion and reporting of the mitotypes of both hair samples in Potsdam.

Reference sample

Buccal swab samples were provided by Stéphanie Bougaran, who is maternally related to Grand Duchesse Stéphanie de Beauharnais (Figure S2). The analysis was ordered via https://www.igenea.de and performed by a contract laboratory (Genomics Research Center, Houston) in 2013.

Informed consent statement

There is a written contract between the "Arbeitskreis Kaspar-Hauser-Freunde" and the IFG Münster from 2014. This contract regulates the examination and publication of the data on the two hair strands "Feuerbach". The current owner of these two strands is a descendant of the Feuerbach family, who gave permission (on July 27, 2014 in Ansbach), in the presence of three members of the "Arbeitskreis Kaspar-Hauser-Freunde", to sample 15–20 hairs from each strand for DNA analysis (photo documentation available).

Werner Diterich gave permission to sample individual hairs from a strand of hairs from Kaspar Hauser that were in his possession on September 18, 2014 (Email communication and photo documentation are available).

Stéphanie Bougaran, maternally related to Grand Duchesse Stéphanie de Beauharnais (Figure S2), has volunteered to have her mtDNA sequenced under informed consent. She has provided explicit permission to use her mtDNA and name in publications.

METHOD DETAILS

Analyses performed in Innsbruck

Preparation of hair segments and DNA extraction

Hair samples were inspected microscopically and none of them showed roots. Four hair shafts were selected from each of the three envelopes for further molecular genetic analyses (IN1a-d, IN2a-d, IN3a-d; Tables 1 and S1; Figure S1). The surfaces of these hair shafts were mechanically cleaned with double-distilled water. Hair shafts were cut into small pieces (approx. 1 cm) and subjected to mild lysis by incubation in 900 μL lysis buffer (10 mM Tris pH 8.0, 100 mM NaCl, 1 mM $CaCl_2$, 2% SDS, 800 μg/mL proteinase K) at 56°C for 2 h (under agitation). The hair segments were further washed three times (by pipetting up and down) in 800 μL lysis buffer. The purified hair segments were completely lysed using the protocol outlined in Hellmann et al[36] and DNA was extracted on a Qiagen EZ1 Advanced XL Nucleic Acid Automated Purification System (Qiagen, Hilden, Germany) following the manufacturer's recommendations. MtDNA was quantified using a real-time quantitative PCR approach and a 143 bp mtDNA target sequence according to Hesse et al.[35]

Primer Extension Capture of DNA from hair samples

The extracted DNA was subjected to Primer Extension Capture Massively Parallel Sequencing (PEC MPS) following the protocol detailed in Eduardoff et al[20] and adapted in Niederstätter et al.[37] In brief, positive control DNA was sheared for 40 min with the Ion Shear Kit (Thermo Fisher Scientific (TFS), Waltham, MA, USA) and the sequencing library was prepared using the IonPlus Fragment Library Kit (TFS) according to the manufacturer's protocol. DNA extracted from the hair segments was directly subjected to library preparation as described above. The libraries were amplified using the IonPlus Fragment Library Kit (TFS) and 10 PCR cycles according to manufacturer's protocol. The PEC reactions were performed according to Parson et al[38] and the libraries were sequenced on an Ion S5 (TFS) with automated template preparation using the IonChef pipeline[39] (TFS) according to manufacturer's protocol.

Data analysis

MtDNA sequence variant interpretation was performed manually from the raw data using MVC.[40] Sequence variants were reported relative to the rCRS[41] based on phylogenetic alignment considerations according to Bandelt et al[42] and Parson et al.[23] Length variation in polynucleotide stretches around positions 16193 and 309 were excluded from analysis and reporting. Haplogroups were estimated based on Phylotree, build 17 (www.phylotree.org)[28] using the haplogrouping function in EMPOP (https://empop.online)[25] and the fine-tuned algorithm SAM2.[43] Read depth (RD) analysis of aligned sequences with a minimum mapping quality of 30 was performed with an in-house developed python script.

The probability of the mitotype was estimated using the Westeurasian subset of the EMPOP database (V4R13; https://empop.online) and applying a one-sided confidence interval upper bound using the BetaInv function.

Analyses performed in Potsdam

DNA extraction

Hair samples were purified and digested as described in Hellmann et al[36] except that the hair was digested by incubation at 37°C for 2 h. DNA was then extracted using the PrepFiler Forensic DNA Extraction Kit (TFS) following the manufacturer's instructions.

Library preparation and MPS of two hair samples

Single-stranded libraries were prepared following the protocol from Gansauge et al[44] with an additional uracil removal step by incubation of the libraries with 0.5 Units/mL USER enzyme for 15 min as described in Meyer et al.[45] The optimal number of library amplification PCR cycles was determined using qPCR as described in Basler et al.[46] Libraries were then sequenced (62 Mio reads for 6681340, 18 Mio reads for 7463316) on an Illumina NextSeq 500 sequencing platform, using a 75 bp SE 500/550 High output Kit V2.5 (Illumina) following the procedures described in Paijmans et al.[47]

Data analysis

The trimmed reads were mapped to the rCRS (NC_012920) using bwa 0.7.17, and PCR duplicates were removed using SAMtools 1.12.[48] The mapped reads were filtered based on a mapping quality of >29 and their alignment to unique positions along the reference sequence. The assembly to the reference was visualized in Tablet[49] and mtDNA sequence variant interpretation was performed manually from the raw data in Leicester. Sequence variants were reported relative to the rCRS as above.