Int J Legal Med (2001) 114 : 194–196

CASE REPORT

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Identification of the skeletal remains of Martin Bormann by mtDNA analysis

Received: 13 March 2000 / Accepted: 20 June 2000

Abstract Contrary to statements of an eye-witness who reported that Martin Bormann, the second most powerful man in the Third Reich, died on 2 May 1945 in Berlin, rumours persisted over the years that he had escaped from Germany after World War II. In 1972, skeletal remains were found during construction work, and by investigating the teeth and the bones experts concluded that they were from Bormann. Nevertheless, new rumours arose and in order to end this speculation we were commissioned to identify the skeletal remains by mitochondrial DNA analysis. The comparison of the sequence of HV1 and HV2 from the skeletal remains and a living maternal relative of Martin Bormann revealed no differences and this sequence was not found in 1500 Caucasoid reference sequences. Based on this investigation, we support the hypothesis that the skeletal remains are those of Martin Bormann.

Key words Martin Bormann · MtDNA · Identification

Introduction

Martin Bormann was the second most powerful man in the Third Reich. He was the private secretary of Adolf Hitler, leader of the NSDAP, leader of the party chancellery, government minister and political chief of the so-called Volkssturm (Wistrich 1995). In the last days of the Second World War in May 1945, as the Soviet army invaded Berlin, he decided to attempt to escape from Germany. For this reason he left Hitler's bunker in Berlin on the night of 1–2 May, together with companions, and was never seen again. In the course of investigations by the allied forces Artur Axmann and Erich Kempka, two of these companions, reported that Bormann died near the Lehrter railway station, possibly by committing suicide with poison. However, rumours persisted over the years that he had escaped to South America or elsewhere. Since the beginning of the 1970s the police force has followed up 6400 leads on the whereabouts of Bormann, and 16 men suspected of being Martin Bormann have been temporarily arrested. As one of the top Nazi criminals, Bormann was charged with war crimes and found guilty and sentenced to death in his absence by an international military tribunal in Nuremberg in 1949.

In 1972, during construction work near the Lehrter railway station, the skeletal remains of two males were found. By investigating the teeth and the bones and comparing the results with notes from the doctor and dentist of Bormann, experts from the police dental clinic of Berlin and the Institute of Legal and Social Medicine in Berlin concluded at the time that one of these was Bormann. Nevertheless, new rumours arose in 1996 when the book "Operation James Bond" by the British author C. Creighton, a former British agent, was published in which it was claimed that Bormann was smuggled out of Berlin in 1945. For this reason the German general public prosecutor and the Bormann family decided to commission the identification of the remains by DNA analysis. Attempts to determine nuclear DNA markers from the bone failed. An 83-year-old female cousin of Bormann provided a sample for comparison.

In recent years, DNA analysis, especially mtDNA analysis, has been shown to be a powerful tool in forensic identification (Piercy et al. 1993; Wilson et al. 1995; Weichhold et al. 1998; Pfeiffer et al. 1999). Because of the maternal segregation, the high copy number and the insensitivity to degradation in comparison to chromosomal DNA, mtDNA analysis is very powerful for the identification of human remains (Sullivan et al. 1992; Holland et al. 1993; Gill et al. 1994).

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Materials and methods

General principles

To exclude the possibility of contamination, the examination of bone and blood samples was done in succession. After we obtained the mtDNA sequences of the two hypervariable regions from the bone samples, we tested the reference blood sample. All mtDNA analyses were performed by two different persons in two different laboratories. All results were compared with mtDNA databases containing all the sequences analysed in our laboratories, including the sequences of the laboratory staff. Extraction and amplification were carried out in separate rooms. All reagents were sterile, aliquoted and only used once. Negative controls were tested in parallel in all experiments.

Samples, sample preparation and DNA extraction

Bone slices, 2 cm thick, were removed from the middle of the right femur and the left tibia, cleaned with emery paper and pulverised in a bone mill (Retsch, Haan, Germany). To 0.5 g of each sample, 2 ml lysis buffer was added (1 M Tris, 1 M KCl, 10% SDS, 0.5 M EDTA) containing 0.4 M DTT and 2 mg proteinase K and incubated at 56 °C for 2 days. After lysis the samples were extracted with phenol/chloroform, DNA was precipitated with 2.5 vols ethanol and the pellet was resuspended in 50 µl aqua bidest. The isolated DNA was further cleaned using the glassmilk-based Geneclean kit (Bio 101, La Jolla, Calif.). Negative controls without bone samples were processed in parallel.

DNA from the blood sample of the maternal relative of Martin Bormann was extracted using the QIAamp Blood kit (Qiagen, Hilden, Germany).

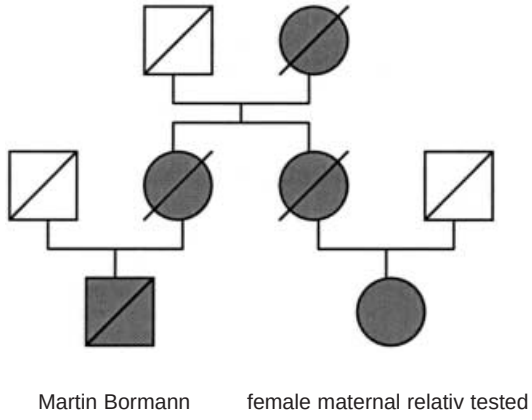

Fig. 1 Pedigree of the Bormann family

Amplification and sequencing of mtDNA

Amplification of PCR products for sequencing was carried out according to Holland (1998), except that AmpliTaq Gold (PE Corporation) was used. For each hypervariable region two overlapping segments were amplified, resulting in a total of four amplification reactions per sample. For the PCR reaction 10% of the DNA extracted from 0.5 g bone and 1 µl from the blood DNA sample were used. Cycle conditions were carried out in a PE 9600 GeneAmp PCR System using an initial pre-incubation at 95 °C for 12 min, followed by 35 (for bone) or 32 (for blood samples) cycles of 95 °C for 30 s, 57 °C (primer sets II, III, IV) or 60 °C (primer set I) for 40 s, 72 °C for 20 s and a final extension step at 72 °C for 10 min. The yield of the amplification was checked by agarose gel electrophoresis and ethidium bromide staining. Subsequently, the PCR products were cleaned with the QIAquick PCR purification kit from Qiagen (Hilden, Germany). Sequence analysis was performed with the ABI PRISM BigDye Terminator Cycle Sequencing Ready reaction kit (with AmpliTaq DNA Polymerase FS, PE Corporation) in both directions. The amplification and sequencing primers used were according to the Armed Forces DNA Identification Laboratory protocol (Holland 1998):

1. Primer set I: F15971 5′-TTA ACT CCA CCA TTA GCA CC-3′ – R16258 5′-TGG CTT TGG AGT TGC AGT TG-3′
2. Primer set II: F16144 5′-TGA CCA CCT GTA GTA CAT AA-3′ – R16410 5′-GAG GAT GGT GGT CAA GGG AC-3′
3. Primer set III: F00015 5′-CAC CCT ATT AAC CAC TCA CG-3′ – R00285 5′-GTT ATG ATG TCT GTG TGG AA-3′
4. Primer set IV: F00155 5′-TAT TTA TCG CAC CTA CGT TC-3′ – R00389 5′-CTG GTT AGG CTG GTG TTA GG-3′

After sequencing, excess terminators were removed with Centri-Sep columns (Amicon, Beverly, Mass.). The sequencing products were analysed on a 310 CE sequencer (ABI, PE Corporation).

Results and discussion

The mtDNA sequence obtained from the skeletal remains found near the Lehrter railway station was compared with the sequence of a maternal relative of Martin Bormann (Fig. 1, Table 1). The female cousin and the bone sample had identical sequences in the HV1 (16024–16365) and HV2 (0073–0340) regions which were not seen in the 88 mtDNA sequences tested in our laboratory so far. To estimate the frequency of the sequence, we made a database search request at the Institute of Legal Medicine, Otto-von-Guericke University of Magdeburg (unpublished results) and the Armed Forces DNA Identification Laboratory (Budowle et al. 1999). The sequence was not found among the 1500 Caucasoid reference sequences present in the two databases (as of June 1998). Therefore, taken to-

Table 1 MtDNA sequences of the bone sample and the maternal relative of Martin Bormann compared to the Anderson reference sequence (Anderson et al. 1981, sequences determined between 16024–16365 and 73–340)

Sample	Positions within the hypervariable regions (HVR) of mtDNA											
	HVR1								HVR2			
	16126	16182	16183	16189	16291	16294	19296	19298	73	195	263	315.1
Reference sequence	T	A	A	T	C	C	C	T	A	T	A	
Right femur	C	C	C	C	T	T	T	C	G	C	G	C
Left tibia	C	C	C	C	T	T	T	C	G	C	G	C
Blood sample of relative	C	C	C	C	T	T	T	C	G	C	G	C

gether, the results from this investigation and the identification by teeth and bone support the hypothesis that the skeletal remains are those of Martin Bormann.

Acknowledgements We would like to thank N. Jimerson, M. Holland, D. Krause and H. Wittig for database research.

References

Anderson S, Bankier AT, Barrell BG, de Bruijn MH, Coulson AR, Drouin L, Eperon IC, Nierlich DP, Roe BA, Sanger F, Schreier PH, Smith AJ, Staden R, Young IG (1981) Sequence and organisation of the human mitochondrial genome. Nature 290: 457–465

Budowle B, Wilson MR, DiZinno JA, Stauffer C, Fasano MA, Holland MM, Monson KL (1999) Mitochondrial DNA regions HVI and HVII population data. Forensic Sci Int 103: 23–35

Gill P, Ivanov PL, Kimpton C, Piercy R, Benson N, Tully G, Evett I, Hagelberg E, Sullivan K (1994) Identification of the remains of the Romanov family by DNA analysis. Nat Genet 6: 130–135

Holland MM (1998) Amplification of mitochondrial DNA / The Armed Forces DNA Identification Lab. Protocol. Mitochondrial DNA sequence analysis in forensic casework – methods and current issues. Promega workshop proceedings from 9.6.98, Innsbruck, pp 1–4, 11

Holland MM, Fisher DL, Mitchell LG, Rodriquez WC, Canik JJ, Merril CR, Weedn VW (1993) Mitochondrial sequencing analysis of human skeletal remains: identification of remains from the Vietnam War. J Forensic Sci 38: 542–553

Piercy R, Sullivan KM, Benson N, Gill P (1993) The application of mitochondrial DNA typing to the study of white Caucasian identification. Int J Legal Med 106: 85–90

Pfeiffer H, Brinkmann B, Huhne J, Rolf B, Morris AA, Steighner R, Holland MM, Forster P (1999) Expanding the forensic German mitochondrial DNA control region database: genetic diversity as a function of sample size and microgeography. Int J Legal Med 112: 291–298

Sullivan KM, Hopgood R, Gill P (1992) Identification of human remains by amplification and automated sequencing of mitochondrial DNA. Int J Legal Med 105: 83–86

Weichhold GM, Bark JE, Korte W, Eisenmenger W, Sullivan KM (1998) DNA analysis in the case of Kaspar Hauser. Int J Legal Med 111: 287–291

Wilson MR, DiZinno JA, Polanskey D, Replogle J, Budowle B (1995) Validation of mitochondrial DNA sequencing for forensic casework analysis. Int J Legal Med 108 (2): 68–74

Wistrich RS (1995) Who's Who in Nazi Germany. Routledge Press, New York

ANNOUNCEMENT

19th International Congress on Forensic Genetics
28 August–1 September 2001, Münster, Germany

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