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CASE REPORT

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Wonder or fake—investigations in the case of the stigmatisation of Therese Neumann von Konnersreuth

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Abstract We investigated two compresses used by Therese Neumann (T.N.), a woman who lived from 1898 until 1962 in Konnersreuth, Germany. The compresses were soaked with blood during the appearance of stigmata on T.N.'s body on a Friday. T.N. became very popular among the faithful in Germany at this time. The question was whether this blood was from T.N. herself or from a family relative or an animal. The comparison of the HV1 and HV2 mtDNA sequence obtained from the compresses with the sequences from a reference sample from a maternally related niece of T.N. revealed an identity. Furthermore, we obtained a short tandem repeat (STR) profile from the bloodstains that were identical with the STR profile from a gummed envelope. The envelope contained a letter written by T.N. in the 1930s. Therefore, our investigations gave no indication for any manipulation.

Keywords Stigmata · Stigmatisation

Introduction

Therese Neumann (T.N.) lived from 8 April 1898 until 18 September 1962 in Konnersreuth, district Oberpfalz, Germany. It is reported that she was cured from severe illnesses several times (such as blindness and paralysis). From a medical point of view, recovering from some of these illnesses seemed to be impossible at the time. Since she could see again on the day of the beatification of Therese of Lisieux, whom T.N. revered much, it was assumed that she was cured upon the intercession of St. Therese of Lisieux. Besides this, further phenomena in her life were visions of historic–religious events, the ability to speak Aramaic, the language in Palestine at the time of Jesus, and the report that she lived for decades of years without any food except for receiving the holy communion [4]. T.N.'s stigmatisation started with a wound near her heart on 4 March 1926. On the following Fridays, with each so-called suffering, more bleeding stigmata appeared. During these sufferings, she was not only bleeding but she also had visions of the life of Jesus Christ (Fig. 1). Until Easter in 1926, further wounds had appeared on both hands and feet (Fig. 2), and these stigmata never healed. The wounds were visible for the rest of her life in the palm and on the back of her hands as well as on the soles and the upper sides of her feet. Until her death in 1962, the bleeding of the stigmata connected with the Friday sufferings occurred 780 times (http://www.thereseneumann.de).

During her life, she became very popular, especially in the district of Oberpfalz and also in the rest of Germany, and her admirers called her "Therese Neumann von Konnersreuth." Since these phenomena cannot be explained scientifically, some doubts still exist that she might have faked the phenomena that made her popular [7]. For this reason, we were commissioned by the Abteilung für Selig- und Heiligsprechungsverfahren des Bistums Regensburg (Department of Beatification and Canonisation of the Bishopric from Regensburg) to investigate whether the blood on the compresses originated from T.N. herself, relations or an animal.

Materials and methods

Stain material

We received the following stain material: two compresses (gauze bandages), approximately 10×10 cm in size, each with a reddish-brown stain in the middle of approximately 8 cm diameter. The compresses consisted of approximately 15 layers of gauze, of which all layers were stained. The compresses had been stored in the vicarage of Konnersreuth. No information as to the age of the compresses could be provided.

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Fig. 1 Therese Neumann during a so-called "Fridays suffering" (photo taken from http://www.thereseneumann.de with permission of Marie Theres Neumann)

Furthermore, we received two envelopes that were sent by T.N. The first letter was stamped on 16 March 1932 and the second letter on 24 November 1937.

A saliva sample of a maternally related niece of T.N. was also used.

DNA extraction

General principles. To exclude cross-contamination, examinations of the stain samples and the reference saliva sample were done in succession. All results were compared with mtDNA databases containing all sequences ever analysed in our laboratory, including the sequences of our laboratory staff. Extraction and amplification were carried out in separate rooms. All reagents were sterile, aliquoted and used only once. Negative controls were carried out in parallel in all experiments.

For the DNA extraction, only the inner layers of the compresses were used to avoid contamination due to handling of the compresses. Approximately 0.5×1 cm was cut out, and 2 ml of lysis buffer (10 mM Tris, 100 mM NaCl, 2% SDS, 10 mM EDTA) containing 400 μg/ml proteinase K was added. Similarly, 0.5×1 cm from the gummed flap of the envelopes and from the stamps was cut out and processed in parallel. The samples were incubated at 56°C for 2 days. After lysis, the samples were extracted with phenol/chloroform, DNA was precipitated with 2.5 vol ethanol and the pellet was resuspended in 50 μl aqua bidest. Negative controls without sample were processed in parallel.

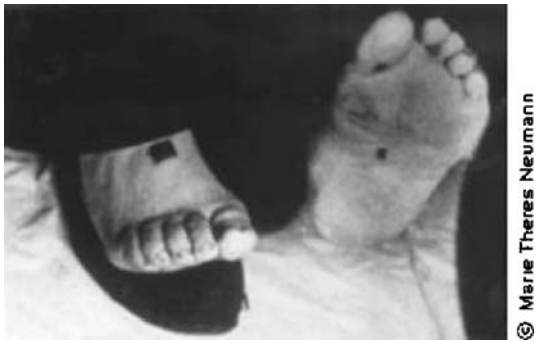

Fig. 2 Stigmatisation on the feet of Therese Neumann (photo taken from http://www.thereseneumann.de with permission of Marie Theres Neumann)

DNA from the saliva sample of the maternal relative of T.N. was extracted using the chelex method [12].

Amplification and sequencing of mtDNA

Amplification and sequencing was carried out according to Vigilant et al. [11] and Holland et al. [5]. For each hypervariable region, two overlapping segments were amplified, resulting in a total of four amplification reactions per sample. For all amplifications, AmpliTaq Gold (Applied Biosystems, Weiterstadt, Germany) was used. Two or 10 μl of the DNA extracted from the bloodstains or the envelope and 1 μl from the reference DNA sample were added to the PCR. Cycle conditions were as follows (PE 9600 GeneAmp PCR System, Applied Biosystems): preincubation at 95°C for 12 min, followed by 35 (for the stain samples) or 32 (for the reference samples) cycles of 95°C for 30 s, 57°C (primer set 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 ABI Prism BigDye Terminator Cycle Sequencing Ready reaction kit (Applied Biosystems) in both directions. The amplification and sequencing primers used were according to the Armed Forces DNA Identification Laboratory protocol [5]—primer set I: F15971 5′-TTA ACT CCA CCA TTA GCA CC, R16258 5′-TGG CTT TGG AGT TGC AGT TG; primer set II: F16144 5′-TGA CCA CCT GTA GTA CAT AA, R16410 5′-GAG GAT GGT GGT CAA GGG AC; primer set III: F00015 5′-CAC CCT ATT AAC CAC TCA CG, R00285 5′-GTT ATG ATG TCT GTG TGG AA; primer set IV: F00155 5′-TAT TTA TCG CAC CTA CGT TC, R00389 5′-CTG GTT AGG CTG GTG TTA GG.

After sequencing, excess BigDye terminators were removed with Centri-Sep columns (Amicon, Beverly, MA, USA). The sequencing products were separated on a 310 capillary electrophoresis system and analysed with the SeqScape software (Applied Biosystems).

STR analysis

STR analysis was carried out using the genRES MPX-2 multiplex Kit (Serac, Bad Homburg, Germany) for the simultaneous amplification of D3S1358, VWA, FGA, TH01, ACTBP2, D21S11, D8S1179, D18S51 and amelogenin [6]. PCR was carried out according to the kit protocol except that 32 cycles instead of 30 were used. PCR products were analysed using the 310 capillary system from Applied Biosystems according to the manufacturer's instructions. Raw

data were analysed using the GeneScan and GenoTyper Software (Applied Biosystems).

Presumptive test

The Sangur test was performed with the Combur-3-test E from Roche Diagnostics, Mannheim, Germany, which is used for the detection of blood in urine and is based on a modified benzidine method. For the detection of dried blood, the following protocol was used: the test stick is first moistened with water and then rubbed on the stain. In the presence of blood, the colour of the stick changes from yellow to green after a few seconds.

Results and discussion

To confirm the presence of blood on the compresses, we performed the Sangur test, which was positive for both compresses. For the determination of the species, an Ouchterlony test was performed [8]. A positive reaction was observed only with anti-human sera, whereas the antisera against pig, cow and chicken gave no results. Therefore, no evidence for the presence of blood of these animals could be obtained.

In the next step, we determined the HV1 and HV2 sequence of the mitochondrial DNA from both compresses. To reduce the size of the amplicons, two sets of primers were used for each hypervariable region. All four primer sets allow amplification of about 250 bp long PCR products.

The mtDNA sequence of compress 1 showed five heteroplasmatic positions [3], indicating a possible contamination of the sample (Table 1). However, the sequence of compress 2 matched the sequences of the reference sample from a maternally related niece of T.N. This sequence was not present among the sequences of 1,674 Caucasian individuals stored in the FBI mtDNA database (http://www.fbi.gov/hq/lab/fsc/backissu/april2002/mt.mdb). Therefore, no evidence was obtained that the blood on compress 2 was from an unknown unrelated person. Our mtDNA data support the hypothesis that the blood on the compress is from a person maternally related to the niece of T.N.

For the gummed flap of envelope 1, we obtained a sequence with many heteroplasmatic positions (Table 1), which indicates the presence of at least two sequences in the sample, e.g. contamination. Nevertheless, the sequence found for compress 2 and for the niece of T.N. could be present in the mixture as well since all positions that deviated from the Anderson reference [1] appeared heteroplasmatic in the sequence from the envelope. For the stamp of envelope 1, no mtDNA could be amplified. The stamp and the gummed flap of envelope 2 gave two different mtDNA sequences that differed from the sequence of the niece; therefore, no further investigation of autosomal DNA was carried out on this material.

After sequencing the mtDNA, we tried to obtain STR profiles from the two bloodstains and the gummed flap of envelope 1. Only the blood on compress 2 gave a STR profile, whereas no results were obtained for compress 1. Furthermore, the gummed flap from envelope 1 gave a profile as well. The profile from the envelope and from the compress almost revealed an identity (Table 2 and Fig. 3). In two systems, the profile from the envelope exhibited a minor extra allele; however, the major alleles were identical with the profile of the compress. At the locus D18S51, the profile from the compress was 14/16 and the envelope showed only allele 16. Since the peak height was below the threshold for homozygous profiles, an allelic drop-out could not be excluded. For ACTBP2, more than four minor peaks were observed, and this locus was excluded from the evaluation. In the amelogenin locus, only the X allele was observed indicating a female stain donor.

The frequency of the profile (without SE33 and D18S51) was estimated at approximately 1 in 900 million. It is therefore highly likely that the blood on the compress and the major component of the biological material on the envelope were from the same female person.

Table 1 MtDNA sequences of the blood stain samples, the envelop and the maternal relative of T.N. compared to the Anderson reference sequence (Anderson et al. 1981)

Region	Position	Anderson	Niece of T.N. 16020–16370	Compress 1 16020–16370	Compress 2 16020–16370	Envelope flap 1 16020–16370
HV1	16188	C	C	C	C	A/C[a]
	16218	C	T	T/C[a]	T	T/C[a]
	16261	C	C	C	C	C/T[a]
	16278	C	C	C	C	C/T[a]
	16291	C	C	C	C	C/T[a]
	16298	T	C	C/T[a]	C	C/T[a]
Region	Position		060–380	060–380	060–380	060–380
HV2	72	T	C	C	C	T/C[a]
	194	C	T	T/C[a]	T	C/T[a]
	263	A	G	G	G	G
	309.1	–	C	C[a]	C	C[a]
	315.1	–	C	C[a]	C	C

[a]Heteroplasty
–, No base in reference sequence

Table 2 Autosomal typing results for the envelope 1 and the bloodstain on compress 2

DNA system	Gummed flap envelope 1	Bloodstain compress 2
D3S1358	16/16	16/16
VWA	16/18	16/18
FIBRA	23/24	23/24
TH01	(6)/8/9	8/9
SE33	ma	27.2/29.2
D8S1179	13/14	13/14
D21S11	(30)/30.2/31	30.2/31
D18S51	16[a]	14/16
Amel	X/X	X/X

Data enclosed in parentheses indicate weak signal
ma=multiple alleles
[a]Allelic drop-out?

Therefore, our investigation of the mtDNA and the autosomal STR loci gave no indications of any manipulation on the blood compresses. The fact that the extent of contamination seen during mtDNA sequencing was not observed during autosomal STR typing could be due to different sensitivities of both methods. A contamination with a single "modern" cell on the stain material results in 100–1,000 contaminating mtDNA molecules.

The first historically verified stigmatisation is attributed to Francis of Assisi, who lived in the 13th century, and several cases have been reported since then. To our knowledge, this investigation is the first to analyse blood evidence from such religious phenomena using forensic genetics techniques. However, many other historical cases without a religious background have been analysed with

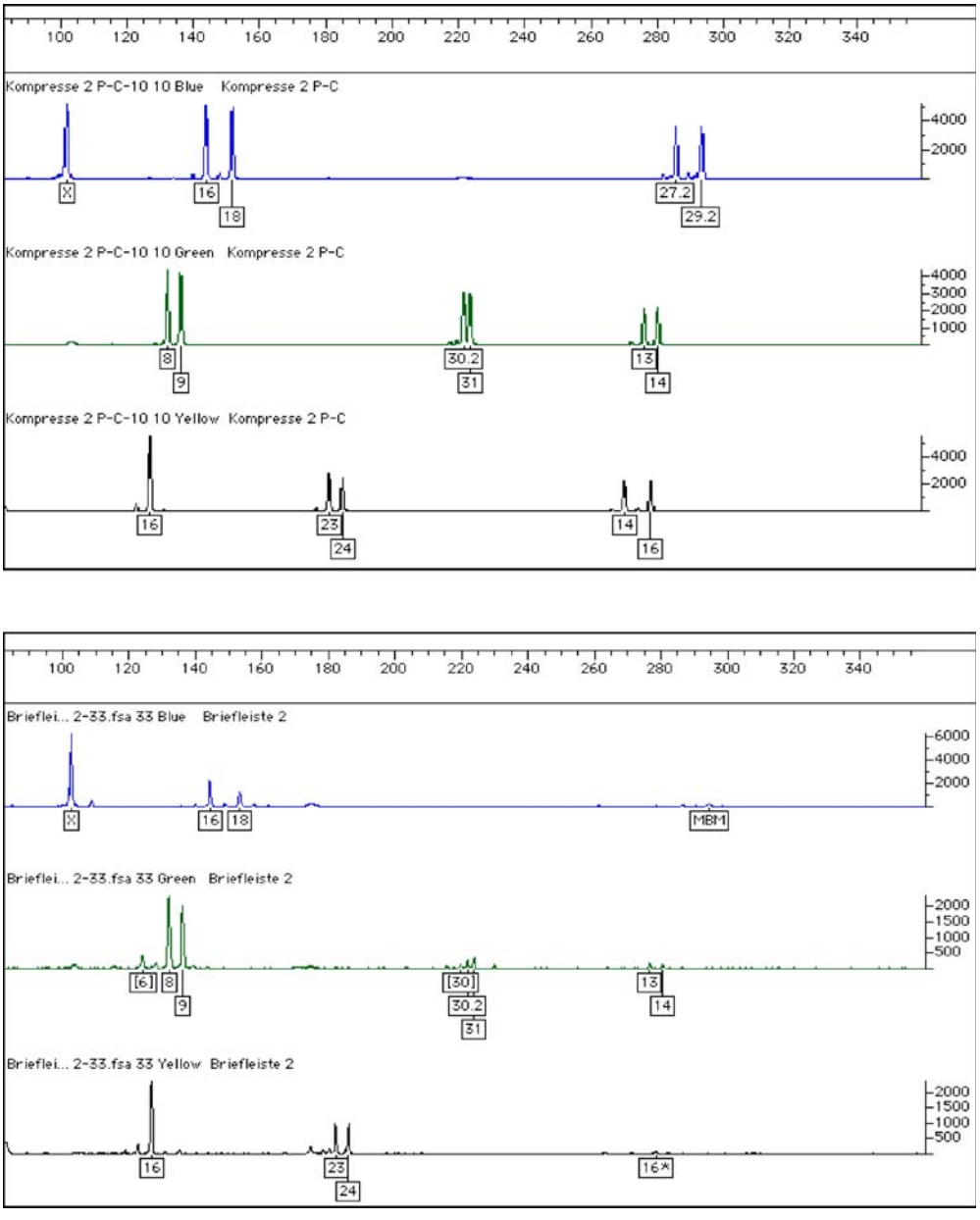

Fig. 3 STR profiles of the compress (*upper panel*) and of the gummed flap of envelope 1 (*lower profile*). Analysis was carried out using the genRES MPX-2 kit. Loci are amelogenin, vWA, ACTBP2 (*first row*, from *left* to *right*), TH01, D21S11, D8S1179 (*second row*) and D3S1358, FGA, D18S51 (*last row*)

mtDNA [2, 9, 10]. In our case of stigmatisation, no evidence for manipulation or faking could be found.

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