

The Boy in the Temple: Genetic Analysis of a Royal Mystery

by

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Part I – Background

Wars and extravagances on the part of French royalty in the 1700s led to increasing resentment by the citizens of France, which reached a peak in 1789 with the storming of the Bastille in Paris. The monarchy was deposed and a new government based on liberty, equality and fraternity began. During that time the French royal family (the Bourbon family; Louis XVI, his wife Marie-Antoinette, and their children Marie-Therese-Charlotte and Louis-Charles) was forcibly moved from the Palace of Versailles to Paris. In June 1791, under the cover of darkness and in disguise, the family attempted to escape the revolutionaries. During their first day's journey they were recognized and by the time they reached Varennes the revolutionaries had caught up with them. The family was arrested and returned to Paris where they were eventually imprisoned in the Temple (a medieval fortress). King Louis XVI was convicted of treason and beheaded in January of 1793. Following his father's death Louis-Charles was separated from his family in the Temple and placed in the care of the Temple commissioner. His mother, Marie-Antoinette was convicted of treason and beheaded in October 1793. Marie-Therese-Charlotte remained alone in the Temple until 1795 when she was released into the care of her aunt. According to the official records Louis-Charles died of tuberculosis on June 8, 1795. Since then, the official version of his death has been repeatedly questioned.

Did Louis-Charles die as reported or was it a substitute who died in 1795, while Louis-Charles escaped out of France? At the beginning of the 19th century several individuals claimed to be the son of Louis XVI. One of these, Karl Wilhelm Naundorff, could apparently provide sufficient circumstantial evidence to convince ex-members of the court of Versailles, including Agathe de Rambaud, Louis' childhood nurse, of his descent. Marie-Therese-Charlotte on the other hand never believed that he was her brother. In 1836, Naundorff was exiled to England when he attempted to sue Marie-Therese-Charlotte for property. He lived for several years in London and died in 1845 in Delft (the Netherlands) where he was buried under the name Louis Charles, Duc de Normandie, "Louis XVII." In 1863, the Dutch authorities permitted his descendants to use the name "de Bourbon," the name of the French royal family.

Since then, there has been much speculation about the real identity of Naundorff. In many publications about "the mystery of Delft," he is considered a swindler and a charlatan. Naundorff was his own witness, but formal proof of his identity was lacking. He apparently bought his identity from a German whose antecedents could not be traced. This finally led to the opening of Naundorff's coffin in 1950 in Delft for a study of the skeletal remains. A lock of hair from the bottom of his coffin, and the right humerus were removed. The humerus was used to investigate whether Naundorff's death was due to poisoning with arsenic. Since 1950, it has been kept in the archives of the Dutch Forensic Laboratory in Rijswijk. The hair samples from Naundorff were stored in two sealed envelopes in the Delft town archives. These remains were officially made available in 1993 for DNA analysis and were used in an attempt to determine the identity of Naundorff (Jehaes et al., 1998).



What analysis could be done to determine if Naundorff truly was Prince Louis-Charles (Louis XVII), son of King Louis XVI and Queen Marie-Antoinette of France?

Part II – Biological Sampling for DNA Analysis

The European royal families have a rich intertwined history. Marie-Antoinette's parents were Maria Theresa and Francis I Holy Roman Emperor (Habsburg family). They had 15 children, who themselves were married to and produced children with other royal families. Some of those individuals are provided below. Initials in parentheses refer to individuals included in the DNA study that we will analyze.

Maria Theresa and Holy Roman Emperor Francis I produced 15 children; only the last few daughters are listed here:

1. Johanna-Gabriella (JG)
2. Maria-Josepha (MJ)
3. Maria-Carolina (CA)
4. Marie-Antoinette (MA)

Marie-Antoinette (MA) married Louis XVI (Bourbon family) of France and they produced four children:

1. Marie-Therese-Charlotte who married Louis Antoine (her cousin)
2. Louis Joseph Xavier Francois who died from tuberculosis before the revolution
3. Louis-Charles (Louis XVII)
4. Sophie who died as infant

Maria-Carolina (CA) married Ferdinand IV and they produced 17 children, of which seven survived to adulthood; two are of interest here:

1. Maria-Teresa (female)
2. Maria-Amelie (female)

1. Maria-Teresa married Franz II and produced Maria-Leopoldinia (female)
 - a. Maria-Leopoldinia married Dom Pedro I and produced Francisca (female)
 - b. Francisca married Francois (male, son of Maria-Amelie) and produced Francoise (female)
 - c. Francoise married Robert, duke of Chartres (male) and produced Marie d'Orleans (female)
 - d. Marie d'Orleans married Valdemar (male) and produced Margaret (female)
 - e. Margaret married Rene of Bourbon-Parma (male) and produced two children:
 - f. Anna Antoinette Françoise Charlotte (female) (A)
 - g. Andre de Bourbon Parme (male) (AB)
2. Maria-Amelie married Louis Phillipe and produced three children:
 - a. Francois (male) who married Francisca (female, from above)
 - b. Ferdinand (male) who married Helena and produced Robert, Duke of Chartes (above)
 - c. Louise-Marie (female) (LM) who married Leopold I and produced Charlotte (CH)



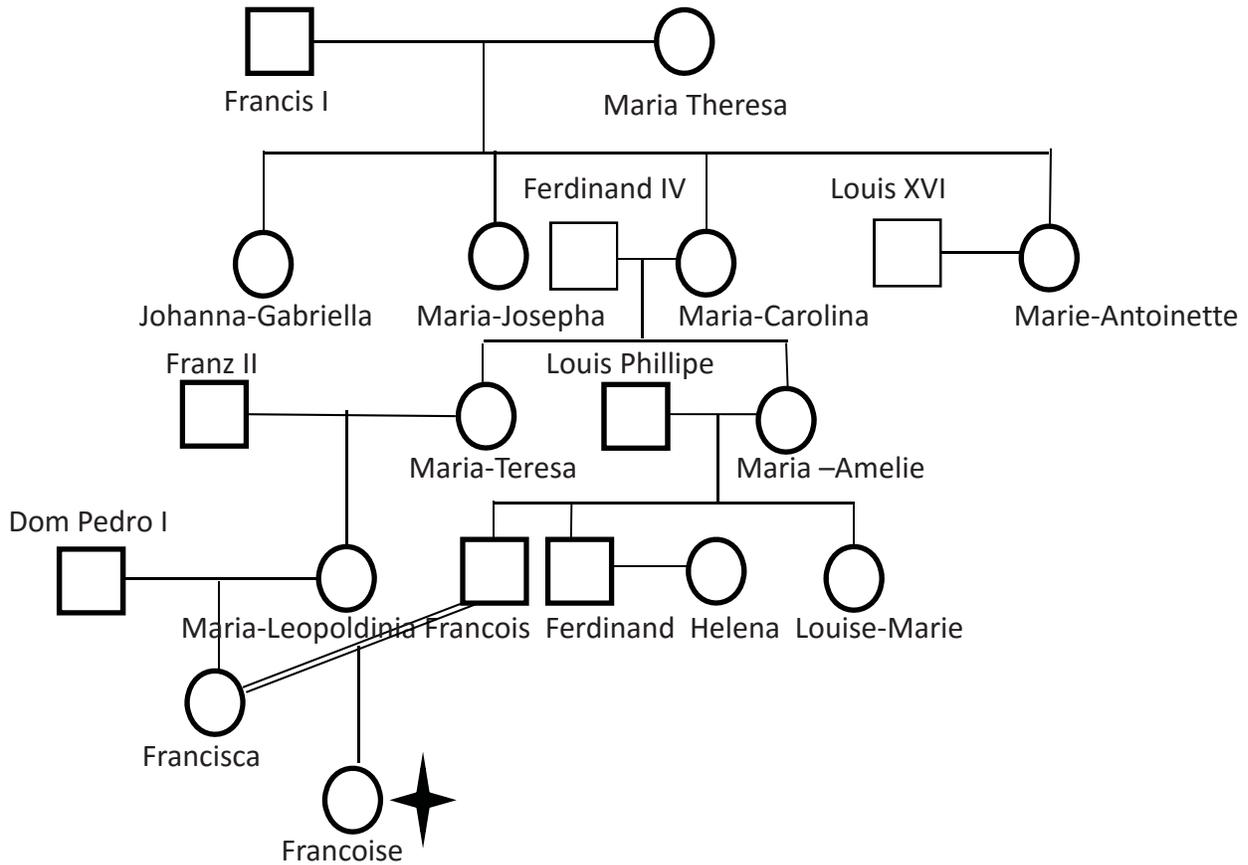
Activity 1 – Pedigree Preparation

Produce a pedigree for this large, extended (and somewhat inbred) family. Place an asterisk (*) next to the individuals who were included in the DNA study (indicated above by the use of initials in parentheses, JG, M, CA, MA, etc.).

Activity 2 – Alleles Identical By Descent

Which members of this family can trace their lineage back to a common ancestor through both parents? These individuals may have inherited two identical alleles from that ancestor, and so those alleles would be considered identical by descent (IBD).

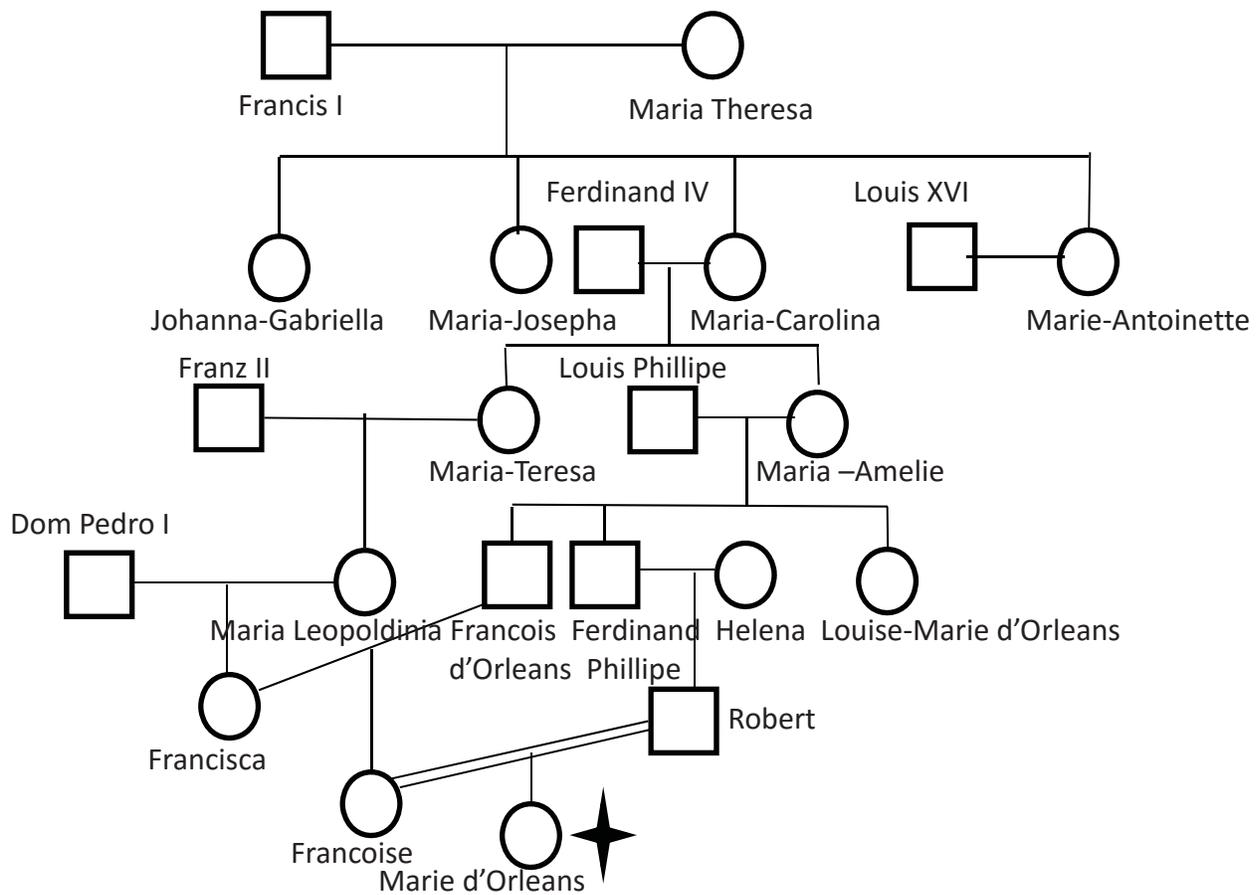
Abbreviated Pedigree for Françoise



Inbreeding Coefficient for Françoise (★)

- Who is Françoise's common ancestor through both lines of her descent?
- Draw the loop on the abbreviated pedigree. How many relatives (excluding Françoise) are in the loop?
- What is the inbreeding coefficient (F) for Françoise?
- Describe in your own words what this value means.

Abbreviated Pedigree for Marie d'Orleans



Determining the inbreeding coefficient for Marie d'Orleans (★):

- Who are Marie d'Orleans common ancestors through both lines of her descent?
- How is calculating her inbreeding coefficient (F) going to be different than calculating F for Françoise?
- Draw the loops on the abbreviated pedigree. How many relatives (excluding Marie d'Orleans) are in each loop?
- What is the inbreeding coefficient (F) for Marie d'Orleans?

Part III – DNA Analysis

Naundorff's claim was that he was Louis-Charles (Louis XVII). DNA analysis allowed researchers to revisit this claim. Hair samples and the right humerus from Naundorff were removed from his coffin during the restoration of his burial place in 1950 in Delft. Maria Theresa, a dotting mother, kept locks of hair from her children and grandchildren in her rosary. Samples were available for Johanna-Gabriela (JG), Maria-Josepha (MJ), Maria-Carolina (CA) and Marie-Antoinette (MA). Two separate samples of hair from Marie-Antoinette, obtained from different verifiable sources, were included in the study. A hair sample was also available from Louise-Marie (LM), granddaughter of Maria-Carolina (CA) and Louise-Marie's daughter Charlotte (CH). Anna Antoinette Françoise Charlotte (A) (queen of Romania) provided a blood sample and her brother Andre de Bourbon Parme (AB) provided a hair sample.

Questions

1. What DNA sequences could provide useful information concerning the claim being made?
2. Genetically, what do the individuals with stars on the pedigree have in common?



The researchers analyzed three different types of DNA sequences, one from the X and Y chromosomes and two from the mitochondrial genome (mtDNA).

- X-Y homologous amelogenin gene: the sequence of this gene differs between males and females.
- mtDNA sequences
 - The control region of the mitochondrial DNA, also referred to as the d-loop, contains two regions called the hypervariable regions (HVR1 and HVR2), which vary considerably in sequence among individuals.
 - HaeIII Restriction Site Polymorphism: a common polymorphism (T-to-C transition) occurs at position 16,519 between HVR1 and HVR2, which creates an *Hae*III cut site.

The mtDNA d-loop sequences were compared to the Anderson consensus sequence of the human mitochondrial genome.

Questions

3. What information would the XY amelogenin sequence provide for each individual sample?
4. Why was mtDNA the appropriate DNA to use for this analysis?
5. Why are mtDNA sequences used rather than autosomal DNA sequences?
6. In general, could Y chromosome sequences provide any further information? If so, what type of information? Is that information critical to this case?

Part IV – Was the Boy Louis-Charles?

Comparative mitochondrial DNA (mtDNA) analysis gave evidence that Naundorff's remains could not be identified as those of Louis XVII. But was the boy who died in 1795 actually Louis-Charles (proclaimed Louis XVII upon the death of Louis XVI) or a substitute?

In a follow-up study, mtDNA analysis was performed on the heart of the young boy who died in 1795. In order to obtain the strongest evidence possible, two laboratories independently analyzed the heart. The aim was to compare the mtDNA D-loop sequence of the heart with that of the maternal relatives already analyzed in the Naundorff case (Jehaes et al., 2001).

Biological Samples

In December 1999, a segment of the heart muscle and a piece of the aorta were removed from the heart presumed to be of the boy who died on June 8, 1795. After the autopsy, the physician stored the heart in distilled wine alcohol. After 8 to 10 years the alcohol was evaporated and the heart was further kept dry.

Analysis of the X-Y homologous amelogenin gene and an anatomical report indicated that the heart was from a male child between 5 to 12 years (Jehaes et al. 2001).

DNA Analysis of the mtDNA D-loop HVR1 and HVR2 regions and the HaeIII restriction site was conducted on the heart tissue.

Re-analysis of samples from Marie-Antoinette and Johanna-Gabriela were conducted for short PCR fragments (as in the 2001 tests) to re-examine the “missed” positions 152 and 194 in HV2 from the 1998 tests (? in Table 1). That analysis showed that Marie-Antoinette's DNA had a C at both positions T152 and C194, whereas Johanna-Gabriela had a C at position T152 and a T at position C194. The combined data from 1998 and 2001 is in the following Table 2.

Table 2. mtDNA sequence analysis from the Naundorff study and reanalysis of samples for Johanna-Gabriela and Marie-Antoinette.

Individual	Tissue Sample	HVR1	Haelll gain	HVR2			
		All positions	T16519	T152	C194	A263	N315.1
Louis XVII ?	heart	identical to Anderson	C	C	T	G	C
Johanna-Gabriela*	hair	identical to Anderson	C	C	T	G	C
Marie-Antoinette*	hair	identical to Anderson	C	C	C	G	C
Anna**	blood	identical to Anderson	C	C	T	G	C
Andre**	hair	identical to Anderson	ND	C	T	G	C

*New DNA extractions were analyzed after Naundorff study, ** from the Naundorff study, ND=not determined

Questions

1. If the boy was the son of Marie-Antoinette, what do we expect to see when comparing their mtDNA sequences?
2. Compare Marie-Antoinette's HVR2 sequence to the sequence from her sister Johanna-Gabriela. Why could Marie-Antoinette's sequence be different from her sister's but not from her son's?
3. Are the nucleotide substitutions found in members of the Habsburg family and the heart tissue of the boy consistent with the boy being related to this family through cytoplasmic inheritance?
4. Is the evidence conclusive proof that the heart is from Louis XVII?
5. Who else could the DNA sample have been from?
6. Would you want any further information to make a more conclusive decision? If so, what information? If not, what is the most concrete evidence in this case?

References

Jehaes E, Decorte R, Peneau A, Petrie J, Boiry P, Gilissen A, Moisan J, Van den Berghe H, Pascal O and Cassiman J. 1998. Mitochondrial DNA analysis on remains of a putative son of Louis XVI, King of France and Marie-Antoinette. *European Journal of Human Genetics* 6: 383–395.

Jehaes E, Pfeiffer H, Toprak K, Decorte R, Brinkmann B and Cassiman J. 2001. Mitochondrial DNA analysis of the putative heart of Louis XVII, son of Louis XVI and Marie-Antoinette. *European Journal of Human Genetics* 9: 185-190.

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