NATIONAL CENTER FOR CASE STUDY TEACHING IN SCIENCE

The Fate of the Romanovs: A Genetic Mystery

by
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Part I – Introduction

After having four daughters, the birth of Alexei Nikolaevich on August 12th of 1904 was met with much rejoicing by Tsar Nicholas II. Finally, an heir to the House of Romanov and the throne of the Russian Empire! After Alexei's umbilical cord was cut, however, the baby continued to bleed for hours, and his blood did not clot. As he grew, trivial injuries such as bruises, a nosebleed, or a cut became potentially life-threatening (Britannica, 2026).

Alexei had inherited "the royal disease," so called because of its widespread prevalence amongst royals throughout Europe in the late 19th and early 20th centuries (Figure 1).

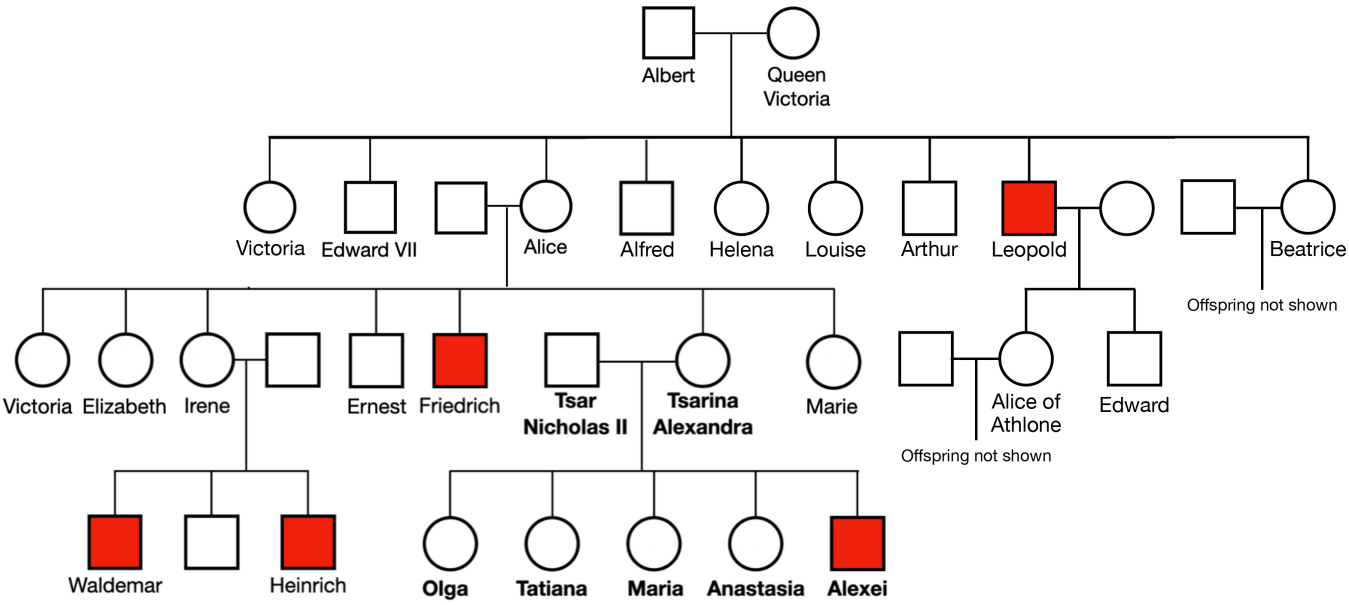

Figure 1. Pedigree showing transmission of the "royal disease" from Queen Victoria of England to her descendants in European royal families. Adapted from Rogaev et al. (2009).

Questions

1. Is the mode of inheritance of the "royal disease" most likely dominant or recessive? Explain your reasoning and include any underlying assumptions that you are making about the inheritance of this disease.

2. This disease appears to affect males more commonly than females. What might be an explanation for this phenomenon?

3. Given your answers to the previous questions, which family members are possible carriers?

4. If Tsar Nicholas II and Tsarina Alexandra had another child:
   a. What are the chances of having an unaffected son? Show your calculations.

   b. What are the chances of having a carrier daughter? Show your calculations.

5. No one in the royal lineage before Queen Victoria is reported to have had the royal disease. How might a new (*de novo*) mutation be passed down from Queen Victoria to her children?

Part II – Revolution and the Mystery of the Romanovs

We now know that the "royal disease" is hemophilia, a disease that impairs the blood clotting response needed to stop bleeding. In about one-third of cases there is no family history of the disorder, suggesting that this genetic disease arises due to a spontaneous mutation (CDC, 2025). In the case of the royal disease, this mutation may have arisen as a germ line mutation in Queen Victoria or Queen Victoria's father, Prince Edward (Rogaev et al., 2009).

In the early 1900s, there was no cure for hemophilia, leaving the Tsar Nicholas and Tsarina Alexandra fearful for their child Alexei. At the age of five, the young prince was assigned guards to watch over him constantly to ensure he did not injure himself. In desperation, Tsarina Alexandra turned to a self-proclaimed holy man, Grigori Rasputin (Figure 2). Rasputin claimed to be able to heal the royal disease, and quickly became a powerful and manipulative force within the Russian royal court (Britannica, 2025).

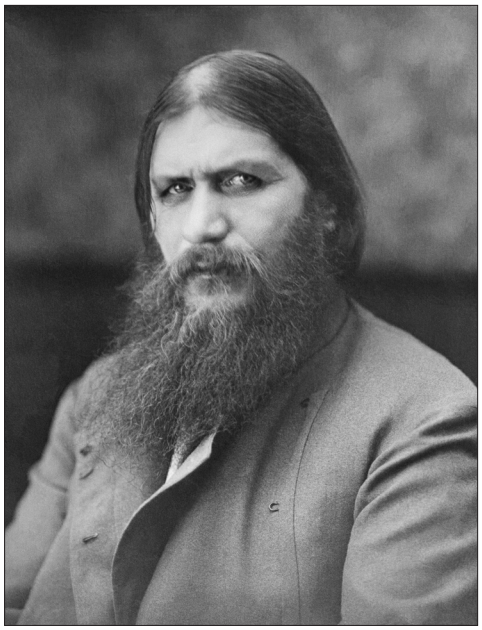

Figure 2. Grigori Rasputin, c. 1910, public domain.

With Russia already in turmoil due to an economic collapse, many laid the blame on the royal family. The favoritism shown to Rasputin only deepened public mistrust of the unpopular Romanovs. As a result, Rasputin was assassinated in the winter of 1916, and the February Revolution in 1917 forced Tsar Nicholas II from the throne. In 1918, the entire imperial family was executed (Britannica, 2025).

After the death of the Romanovs, rumors swirled that some of the children may have escaped execution. Many women famously came forward claiming to be the Grand Duchess Anastasia. The idea that Anastasia had somehow escaped became the basis for movies, plays, and even a Broadway musical. However, archaeologists eventually discovered two mass graves thought to contain the remains of the Romanov royal family. Mitochondrial DNA (mtDNA) testing was used to confirm a maternal relationship between some of the remains and a blood sample from Prince Philip (1921– 2021, husband of Queen Elizabeth II and a descendant of Queen Victoria) (Gill et al., 1994) (see Figure 3, next page).

This, along with nuclear DNA testing (see Figure 4, next page), confirmed that one grave contained the remains of Tsarina Alexandra, Tsar Nicholas II, and three of their children (Gill et al., 1994). The second grave was found to contain the remaining two children.

DNA source	Selected sequence of hypervariable region 1 (HRV1) of mitochondrial DNA
Prince Phillip, blood sample	T T C C C C A C C T T C
unidentified adult, femur skeleton 1	T T C C C C A C C C T C
unidentified adult, femur skeleton 2	C T C C T C A C C T T T
unidentified child, femur skeleton 3	T T C C C C A C C T T C
unidentified adult, femur skeleton 4	C C C C C C A T T T T T
unidentified child, femur skeleton 5	T T C C C C A C C T T C
unidentified child, femur skeleton 6	T T C C C C A C C T T C
unidentified adult, femur skeleton 7	T T C C C C A C C T T C
unidentified adult, femur skeleton 8	C T C C C C A C C C T T
unidentified adult, femur skeleton 9	C T C T C T G C C T C T
Anna Anderson, hair sample	C C C C C C A T C C T T

Figure 3. Summary of mtDNA differences compared to a reference sequence. DNA sequences of hypervariable mtDNA region 1 determined from amplified bone DNA extracts from the mass graves or fresh blood samples. Adapted from Gill et al. (1994) and (1995).

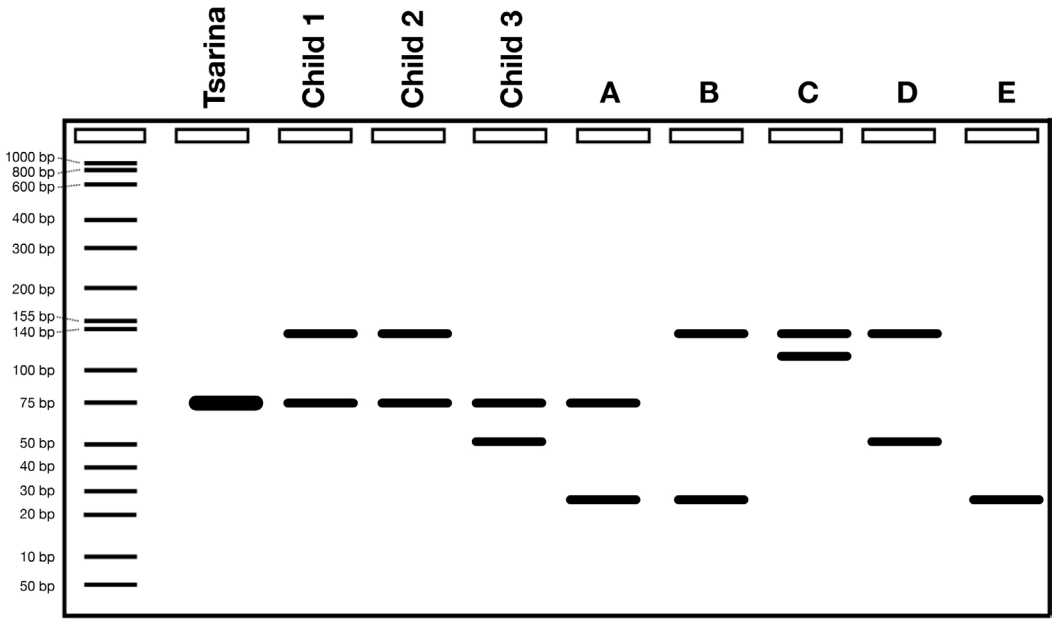

Figure 4. Analysis of skeletal remains at a short tandem repeat (STR) nuclear genome locus HUMTHO1. Lane 1 shows a molecular weight marker. Adapted from Gill et al. (1994).

Questions

1. How does the mitochondrial genome differ from the nuclear genome?

2. How is mitochondrial DNA inherited?

3. Notice the relationship between Prince Phillip and the Tsarina shown in Figure 5. Why were the researchers able to accurately identify the Tsarina's remains using Prince Philip's blood sample?

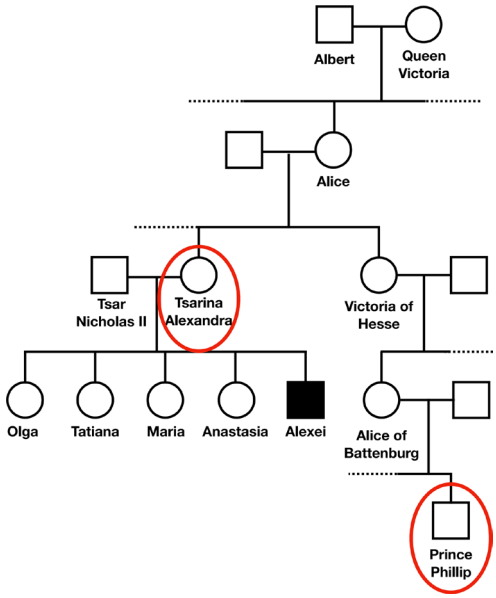

Figure 5. Pedigree. Adapted from Rogaev et al. (2009).

4. Refer to Figure 3 to answer the following:
   a. Based on the sequence of hypervariable mtDNA region 1, as well as the source of the DNA, which femur sample likely came from the Tsarina?

   b. Are the unidentified children (represented by femur samples 3, 5, and 6) related to the Tsarina? How can you tell?

   c. Of the half-dozen women who claimed to be the lost Grand Duchess Anastasia, German-born Anna Anderson became the most infamous. Although Anna died before genetic testing was possible, a tissue sample was obtained posthumously and compared to the Romanov DNA samples. Anna's hypervariable mtDNA region 1 sequence is shown at the bottom of Figure 3. Could Anna be the lost Grand Duchess Anastasia? Why or why not?

5. Refer to Figure 4 to answer the following:
   a. Which gel lane (A–E) likely represents DNA from Tsar Nicholas II? Why do you think so?

   b. Why does the Tsarina's sample show a single band on this electrophoresis gel, rather than two?

Part III – Uncovering the Molecular Mechanism Behind the Royal Disease

The last known descendant of Queen Victoria with the royal disease died in 1940. Because of this, it was unclear exactly what kind of hemophilia plagued the royal families. Using the Romanov remains, researchers amplified and sequenced the two genes most often mutated in hemophilia: blood coagulation factor VIII (F8) and blood coagulation factor IX (F9), both located on the X chromosome. Using this method, Rogaev et al. (2009) found a single nucleotide variant in F9 associated with hemophilia (Figure 6).

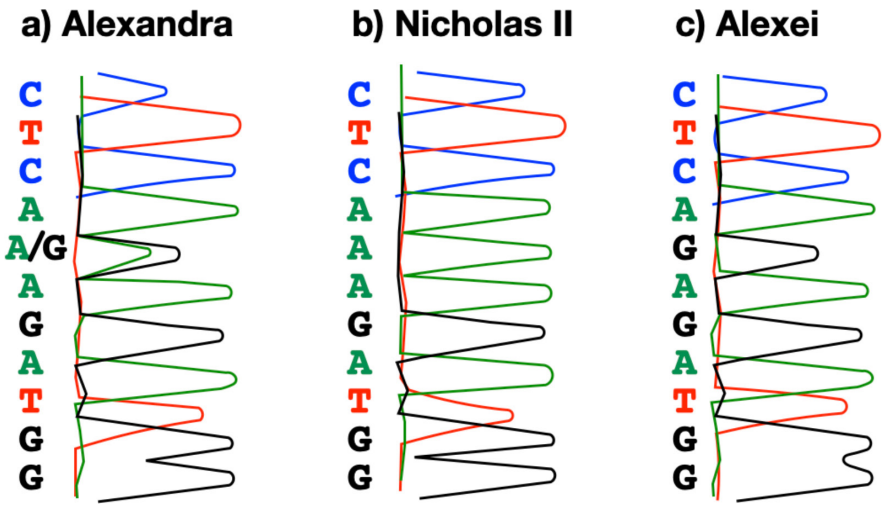

Figure 6. Chromatograms from conventional Sanger sequencing of a small portion of the F9 gene from Tsarina Alexandra (a), Tsar Nicholas (b), and Alexei (c). Adapted from Rogaev et al. (2009).

Questions

1. Based on these chromatograms, what difference in the DNA sequence do you see that may be correlated with hemophilia?

2. Why does Alexandra's sample show mixed peaks (A/G) in the chromatogram?

3. Hemophilia is a recessive disease. Why is Alexei affected by hemophilia despite having an unaffected, non-carrier father?

4. What are some ways in which a single nucleotide variant could lead to a non-functional protein?

Part IV – Conclusions

Rogaev et al. (2009) mapped the single nucleotide variant (SNV) to an intron just upstream of exon 4 of the F9 gene (Figure 7).

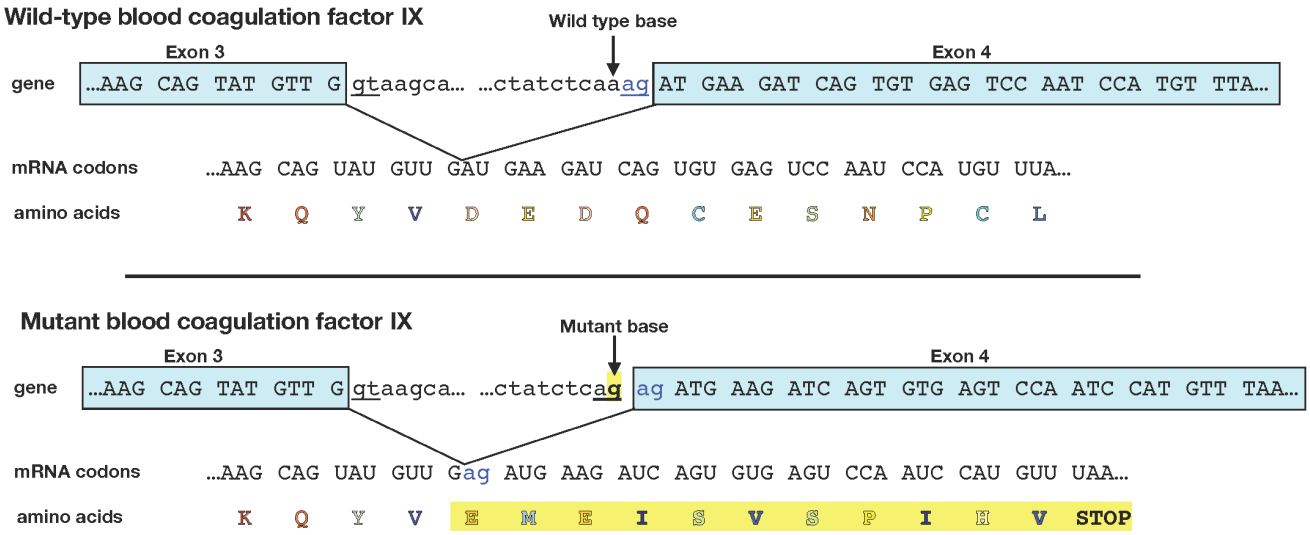

Figure 7. The A→G mutation occurs just upstream of exon 4 in the F9 gene. Adapted from Rogaev et al., 2009.

Questions

1. What is splicing? What happens if proper splicing does not occur?

2. The splicosome recognizes sets of "GU" and "AG" together in the right context to mark the beginning and end of an intron. Based on Figure 7, describe the effect the A→G SNP had on the factor IX protein sequence. Why did this lead to a truncated protein?

References

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