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BIOL 294

Romanov Hemophilia

History

1. Nicholas II was the last Romanov to hold power in Russia. What was his title?

   Nicholas II was the Tsar of Russia

2. How long had the Romanov family been in power in Russia?

   The Romanov family had been in power for over 300 years

3. Nicholas II abdicated the throne. Who took power then?

   His brother, Grand Duke Michael

4. What happened to Nicholas II and his family after he abdicated the throne?

   They were held in exile in Yekaterinburg, Russia.

5. One of the reasons that the family of Nicholas II was executed (vs. just imprisoned) was because there was a fear that the White Russian Army would save them. Who was the White Russian Army?

   The White Army was composed of volunteers opposed to the Bolshevik government and would have therefore been variably loyal to the Romanovs'.[1]

Hemophilia

One of the pedigree charts found at the end of this assignment comes from the Module PowerPoint lecture notes.

6. How was Alix, the wife of Nicholas II, related to Queen Victoria of England? (Look at the pedigree chart carefully.)

   Tsarina Alix was a direct descendent of Queen Victoria, her granddaughter to be exact. Tsarina's parents were Louis IV and Alice, who was one of the daughters of Queen Victoria and Prince Albert.

7. On what chromosome is the gene that, when mutated, causes hemophilia and how does this contribute to its inheritance pattern?

   The hemophilia gene is on the X-chromosome; therefore, the gene would be expressed in all males born to a carrier female, but not all females would be carriers.

Both Queen Victoria and Alix are designated as being carriers for hemophilia.

8. What does it mean to be a carrier for a disease?

A carrier for a disease has the genetic potential for the genetic mutation but does not express the gene itself. Being a carrier for a gene is associated with recessive genes, because if the disease was dominant everyone who had the gene would present the symptoms completely. Being a carrier can also affect children, and most carriers have the potential to pass on the disease to their children.

9. Why aren't males considered carriers for hemophilia?

Males cannot be carriers for hemophilia because the disorder is associated with the X-chromosome. This means that males will always present the disease if their mother is a carrier, because they got their one X-chromosome from her. Males also do not pass on X-linked diseases to their sons, because the disease is not attached to the Y-chromosome from the father.[2,3]

10. In a couple of sentences, describe the physiology of the disease hemophilia. (Yes, I know it is severe bleeding because the blood cannot clot. But WHY can't the blood clot? Be *very* specific.)

Hemophilia is a blood disorder caused by a deficit of specific clotting proteins, called clotting factors. Most disorders that fall in this category happen due to shortages of factor VIII or factor XI, and this shortage is caused by a mutation in the gene that slows production of the protein down. This lack of proteins can impact the body and make it difficult to form blood clots, which is where the severe bleeding comes from.[2,3]

An interesting thing to point out is that there are individuals with an opposite problem to hemophilia, and instead the clot too much! These individuals (and me!) suffer from a Factor V Leiden mutation, which produces too much factor V protein and can lead to excess clotting.[4]

11. What type of hemophilia (A or B) is (probably) represented in the pedigree chart?

Hemophilia B is probably represented because they found evidence that the disease-causing mutation was in the F9 gene after examining Tsarevich Alexi's genetic material. Since the location of the mutation is known, as is the type of hemophilia it causes, it can be inferred that this mutation came from his mother, and therefore from Queen Victoria, because there is little possibility that the mutation swapped from the F8 gene to the F9 gene in one generation.

12. Describe the mutation (at the molecular level) that apparently caused hemophilia in Alix, (and probably all of the European families that had hemophilia). Be *very* specific.

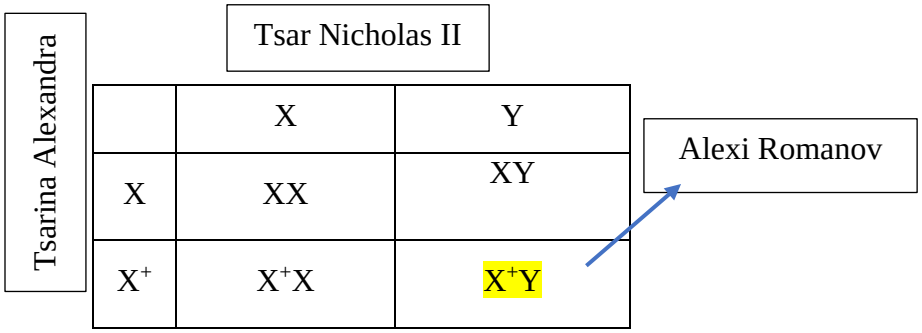
The F9 mutation was created by a casual substitution, a change in a single nucleic acid for another, in the splice acceptor site of exon 4 of the F9 gene found on the X-chromosome.

13. How could the mutation you described in #12 result in a faulty gene product? Be *very* specific in your description.

The mutation would have come about by a single base substitution on the DNA held within the X-chromosome. This substitution has been located in an experiment in 1990 and seems to have been caused by a switch of cytosine (c) for adenosine (a) at position 10,401.[5] This mutation

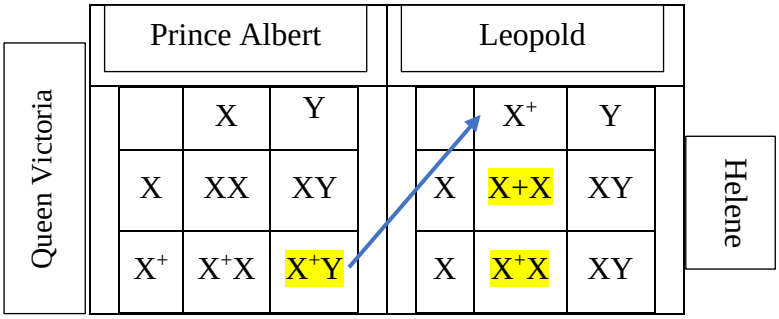
changed a proline for glutamine at position 50, which changed the gene's expression. This singular protein change led to the effects of the hemophilia disease, and because the mutation resides on the X-chromosome, it is passed on from parents to their children.

14. The Romanov's son, Alexis, had hemophilia. Describe how Alexis genetically acquired hemophilia. (Use a Punnett square. You can either draw a table or line up the genotypes.)



If we draw out the cross, we can see that the hemophilia gene (denoted here as X^+) is only carried by Tsarina Alexandra, Alexi's mother. Tsar Nicholas II does not carry the gene and is therefore X/Y. Since we know he acquired the gene from his mother, who is a carrier of the gene, she has to be heterozygous for the disorder, X/X^+. So, by doing a cross we can see that there is a 50% chance Alexi will inherit the X^+ gene, because we know he also has to inherit his father's Y-chromosome to be genetically male. Therefore, because we know Alexi was a hemophiliac, we know that he had to inherit the diseases gene from his mother and consequently has to be X^+/Y so that the diseases presents itself.

15. Using a Punnett square (again, draw a table or line up the genotypes), explain why only males in the pedigree chart have hemophilia. (Choose at least one of the males represented in the pedigree chart and show his parents in the Punnett square.)



The above cross shows the potential outcome for the children of Queen Victoria and Prince Albert. From the pedigree chart, we know that their son Leopold expressed hemophilia, and half of their daughters (Alice and Beatrice) were carriers. The cross demonstrates this, because we can see that only the males have one X-chromosome from an affected mother, which would automatically make the disease present itself. The females, however, can make up for the disease by having that extra X chromosome, whether it is maternal or paternal. However, the potential for them to pass on the disease to their children is still there because they carry that mutated gene. If they acquire the paternal X-chromosome that comes from a diseased father, they will be a carrier for the disease. This is also why males born to diseased fathers do not show the

disease, none of Leopold's sons are affected. Their mother is a normal female, and she is not a carrier, therefore they do not receive an affected X-chromosome from their mother. We can also use this cross to assume that if Leopold's sons had passed on their genes, all of their children would be unaffected, as they weren't carrying an affected maternal X-chromosome.

16. Is it possible for a female to inherit hemophilia, and, if so, how?

While it is not common, it is possible. Because the hemophilia gene is overruled by the normal X-chromosome, any heterozygous female is not likely to present with the disease. These females, however, may express certain symptoms related to excess bleeding. The possibility of a woman being affected, rather than a carrier, happens if the woman is a homozygote for the disease. If both inherited X-chromosomes have the gene on them, then the female will inherit the disease as her body has no way to compensate for the deficiency. Also, if the woman has any form of aneuploidy, she is more likely to present the disease, especially if she only has one X-chromosome.[2,3]

There is also the possibility that the body could deactivate the X-chromosome that does not carry the disease, turning it into a barr body. But this is probably rarer than an individual being homozygous for the disease.

17. Some historians speculate that Alexis' hemophilia condition could have led to the Russian Revolution. Explain. ***You should look up the faith healer Rasputin and read about his relationship to the Romanov family.***

Alexi's condition made his childhood difficult. As he suffered from the disease inherited from his relative, Queen Victoria, his day-to-day life was painful and there was little that could be done. This is when Rasputin came into the picture, claiming that he could heal the suffering prince, and he did help. However, Rasputin also claimed to have become Tsarina Alexandra's advisor, and as such, he was used as a way for other political parties to undermine Tsar Nicholas II's power. The political influence Rasputin gained was not contradicted by the Tsarina, who had taken control of the domestic policy during World War I per her husband's wishes, though she wasn't very good at it. The Tsarina was also under speculation of having relations with Rasputin, though this was never confirmed, and as such she fell under even more scrutiny. Unfortunately, the influence Rasputin had over the Tsarina would lead to her family's downfall, and this caused the Tsar and his family to lose political clout, giving the Russian Revolution the time to grow in power and influence. Another important piece was that before his death, Rasputin gave a prophecy that should he die, the imperial family would fall as well.[6-8]

Molecular Analysis of People in a Mass Grave

18. Two "graves" were discovered near Yekaterinburg, Russia. Describe the number of bodies in each grave.

The first grave has nine skeletons in it, five members of the royal family and four servants.

The second grave, after careful recovery and examination, could have had no less than two people in it. One was female, about 15-19 years old, and the other was probably a male around 12-15 years old.

19. When were these graves discovered?

One grave was discovered in the 1970s by Dr. Alexander Avdonin but was kept secret until the fall of the Soviet Union in 1991.

The second grave was found in 2007, about 70 meters from the first grave.

20. What type of testing was done to confirm sex and familial relationships among the remains found in the mass grave?

Nuclear DNA testing confirmed the sex and familial relationship of the remains using five different STR markers from genetic material taken from the remains. These tests included examining mtDNA, STR, and Y-STR markers.

21. Genetically, what does STR "stand" for? Be very specific in your answer.

STR stands for short tandem repeats, small repeats of DNA sequences that involve a repetition of a specific set of nucleotides. They can be 1-6 base pairs in sequence, and many together can lead to mutation or polymorphic changes. They are also known as microsatellites and can be up to 100 nucleotides long.[9] They are also easily tested for familial relation because the mutation rate and mutation type can be found in both parent and child.

22. HRH Prince Philip, the Duke of Edinburgh, provided mitochondrial DNA used to identify Alix and her three daughters. HRH Prince Philip, the Duke of Edinburgh, is married to Queen Elizabeth II of England. Wait, isn't Queen Elizabeth II related to Queen Victoria? So why was ***Prince Philip's*** mitochondrial DNA used? (To help you answer this question, look at the second pedigree chart.)

His Royal Highness Prince Philip is more closely related to Tsarina Alexandra (Alix) than Queen Elizabeth II. Both Tsarina Alexandra and Prince Philip came from a maternal bloodline carried under the pairing of Alice and Louis IV. Alix was a direct daughter of Alice and Louis, as was HRH Prince Philip's grandmother. The passage of the maternal mtDNA would have been the same, as the HRH Prince Philip's mother was the daughter of one of Tsarina Alexandra's unnamed sisters.

Queen Elizabeth II, however, is related to Queen Victoria through the paternal line. Her father was the one related to Queen Victoria, therefore she would have inherited different maternal mtDNA, and therefore did not inherit the same mtDNA as Tsarina Alexandra.

23. Who was missing from the mass grave (the one with the most skeletons)?

Missing from the mass grave was Tsarevich Alexi and one of his sisters. The identity of the sister was a source of contention between the Russians, who believed it to be Maria, and the Americans, who believed the missing individual was Anastasia.

Molecular Analysis of People in a Mass Grave, cont.

24. The Duke of Fife and Princess Xenia provided mitochondrial DNA used to identify Nicholas. One of these is a female and another is a male. Does that matter? What general statement can you make about their genetic relationship to Nicholas and Alexandra? Are these people still living?

The fact that the mtDNA was taken from one male and one female had no impact on the test results, as both The Duke of Fife and Princess Xenia shared a maternal link to Nicholas II. This would have linked the two individuals back to a single woman, who's mtDNA would have been the same across all of her maternally descended family members. This means that Nicholas would have shared the mtDNA with these two individuals allowing him to be correctly identified, while Tsarina Alexandra did not. Princess Xenia is no longer living, she died in 1960, and The Duke of Fife, James Carnegie, died in 2015.[10,11]

25. What was discovered in the mitochondrial DNA of Nicholas that was not identified in either the Duke of Fife or Princess Xenia?

The mtDNA of Tsar Nicholas II had a mutation that caused a slight change in ratios of C/T, which his maternal relatives did not have. His relatives had a fixed ratio at position 16169 while Nicholas II was mostly C/t.

26. What is the term given to the existence of two (or more) genetically different mitochondria in the cell?

The term is heteroplasmy. For Tsar Nicholas II, the single point heteroplasmy happened at position 16169.

27. What three types of DNA were used to test the remains found in a second grave?

The three types of DNA tested were mitochondrial DNA from Tsarina Alexandra, Autosomal STR genotypes from both the Tsar and Tsarina, and a 17-marker Y-STR haplotype from Tsar Nicholas II.

28. Of the three types of DNA you listed in #28, which one would have been used specifically to identify Alexis?

The identity of Tsarevich Alexi would have been found using the 17-marker Y-STR haplotype because it would have been attached to Alexi's Y-chromosome rather than the X-chromosome he inherited from his mother. This would give definite familial connection to Tsar Nicholas II if the DNA matched.

29. What was the source of the DNA used to identify Alexis?

The source of the tested DNA came from the femur bone of the male remains found in the second grave.

30. Was Anastasia in the grave in which Alexis was found?

There is no way to distinguish Anastasia from her sister Maria to tell which grave they were in. Alexi could have been buried with either girl, but while the DNA evidence does show their genetic relation, it cannot correctly identify the individual in the second grave with Alexi.

Who Wants to Be Anastasia?

Apparently, about 200 people have wanted to be Anastasia and have claimed to be her! One of the most famous imposters was a woman named Anna Anderson (Manahan).

31. Give a brief history (2-3 sentences) of Anna Anderson-both her claims and what is thought to be true.

Anna Anderson was one of many women who claimed to be the missing Princess Anastasia. She was able to garner support in her claim and had many people believing that she truly was the missing princess. However, there were still those that questioned her claim and believed her to be a Polish woman by the name of Franzisca Schanzkowska, who had disappeared in 1920.

32. Where in the US did Anna Anderson eventually settle and why?

Anna Anderson settled in Charlottesville, VA after marrying American history professor J.E. Manahan. She followed him to the US and the pair lived out their years together in Virginia.[12]

33. What were the sources of Anna Andersons's nuclear DNA?

Anna Anderson's nuclear DNA came from samples of her hair and intestines.

34. What were the sources of Nicholas' and Alix's nuclear DNA?

The sources of Nicholas' and Alix's nuclear DNA were their bones.

35. What type of analysis was done on DNA from Anna Anderson, Nicholas, and Alix?

There were a few different types of analysis done by different scientific institutes used on the DNA from Anderson, Nicholas II, and Alix. These included STR analysis using a quadruplex and mtDNA analysis using a modified solid phase sequencing protocol done by the Forensic Science Service. The Armed Forces Institute of Pathology investigated the DNA by mtDNA analysis using dye-terminator cycle sequencing, and Pennsylvania State University tested Anderson's hair via the Stoneking and Melton method, which used PCR.

36. Anna Anderson's mitochondrial DNA was compared to the mitochondrial DNA of what two "other" people?

The two other people Anderson's mtDNA was compared to were His Royal Highness the Duke of Edinburgh, a direct maternal descendent of Tsarina Alexandra, and Carl Maucher, the great nephew of Schanzkowska related through the maternal line.

37. A hypervariable region of the mitochondrial DNA was analyzed. Define a hypervariable region.

A hypervariable region is a portion of a genome that is predisposed to have higher levels of variation than other, similar areas. This variation can be from generation to generation, or even from different forms of mutation or recombination. The variability of these regions allows them to diversify quickly, especially because they are in direct contact with antigens.[13,14]

38. What were the conclusions from the mitochondrial DNA comparisons?

The conclusions of the mtDNA comparisons show that Anna Anderson was not related to a maternal relative of the Tsarina or of HRH Prince Philip, and therefore could not have been Princess Anastasia. Also, Anderson showed a maternal genetic connection to Carl Maucher, supporting the theory that Anna Anderson was Franzisca Schanzkowska.

39. The article which describes the analysis of Anna Anderson's DNA was published in 1995. When were all of Nicholas' and Alix's children finally accounted for?

All of the children were finally accounted for between 2007 and 2008, when the STR comparison of the remains found in the graves were tested. This was because up until 2007 they had not yet found the remains of all the bodies. When the second grave was uncovered, they believed they had found the son and the missing daughters, but the genetic relation was necessary for identity confirmation.

40. What did you learn from doing this assignment?

This assignment taught me both how interesting genetics can be and how it can answer questions we've had for years. Before 2007 I don't believe anyone would have thought we'd be able to test the remains in depth enough to find the missing princess, but we did, and the way genetic relationships worked into tracing DNA is just cool. This assignment also showed me that people like to find the truth enough to go back into previously taken data sets and rerun them, especially when an identity is contested. It was also really neat that the scientists were able to go and see who Anna Anderson really was.

Resources

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