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BIOL 294

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01/26/2023

# Romanov

The Romanov family were an imperial dynasty that ruled Russia. Numerous members of their family inherited the Russian throne over three centuries from 1613 to 1917. Queen Victoria and Prince Albert had 9 children. Those children were named Victoria, Alice, Edward VII, Alfred, Louise, Arthur, Leopold, Beatrice, and Helena. Alice married Louis IV and had 5 children. Leopold married Helene and had three children. One of the daughters of Alice and Louis IV, Alix married Nicholas II. Nicholas II was the last member of the Romanov family to inherit the Russian throne, his title was "Emperor of All the Russias" and the "Last Tsar of the Russian Empire". The Bolsheviks murdered Nicholas II and his family then Vladimir Lenin, the leader, took control in place of Nicholas II. Nicholas II's family including his children were also executed by the Bolsheviks.

Nicholas II wife, Alix, is Queen Victoria's granddaughter, and both are carriers for hemophilia. Hemophilia is a condition where a clotting factor of the blood is missing so the time for blood to clot is significantly increased. The affected person will typically have severe bleeding from a slight injury. There are two types of hemophilia. Hemophilia A is most common and occurs when the individual does not have enough clotting factor VIII. Hemophilia B is less common and occurs when the individual lacks clotting factor IX. The Romanov family most likely had hemophilia B.

Alix: XXi X=unaffected allele
Nicholas II: XY Xi= allele for hemophilia

| | X | Xi |
|---|---|---|
| X | XX | XXi |
| Y | XY | XiY |

Looking at the Punnett Square above, a child will have a 25% chance of being a son with hemophilia. Since Alix is a carrier for hemophilia, meaning she only has one affected X allele, she could pass that allele down to her child and the father determines whether to pass down an unaffected X, creating a daughter who is a carrier for the disease, or a son who has the disease. In this case, the father passed down the "Y" chromosome creating an affected son.

Mother: XXi
Father: XY

| | X | Xi |
|---|---|---|
| X | XX | Xxi |
| Y | XY | XiY |

Mother: XX
Father: XiY

| | X | X |
|---|---|---|
| Xi | XiX | XiX |
| Y | XY | XY |

Referring to the two Punnett Squares above, only males have hemophilia because hemophilia is X-linked. Meaning females (XX) have an extra X allele to counteract the one allele for hemophilia. Therefore, they are carriers. They can pass down the affected allele but not show the phenotype of hemophilia. Males (XY) only have one "X" chromosome, so they only need one affected allele to present with hemophilia.

It is possible for a female to inherit hemophilia if her father has hemophilia and her mother is a carrier for hemophilia. The daughter would have to inherit the allele for hemophilia, that is carried on the X chromosome, from her mother and father. Another way a female can inherit hemophilia is if she inherits an allele for hemophilia from only one parent, but the other X chromosome is missing such as in Turners Syndrome or her clotting factors on the other X chromosome are ineffective. At that time in history, the topic of genetics was rising in popularity but there was still minimal knowledge about genetic testing. Someone could be identified as a carrier for a gene whether their children display the phenotype for a specific trait, whether it is desirable or not. Throughout the 20th century, research and testing about genetics continued to grow exponentially.

Mother: XXi
Father: XiY

| | X | Xi |
|---|---|---|
| Xi | XXi | XiXi |
| Y | XY | XiY |

According to the Punnett Square above, there is a 25% chance that a mother who is a carrier and a father with hemophilia will have a daughter that is a carrier. As shown, there is a 25% chance of a carrier mother and a father with hemophilia having a daughter who is a carrier. If we were to calculate the probability of having four daughters in a row who are carriers, we would multiply this probability. (25%) x (25%) x (25%) x (25%) = 0.39%. It is very unlikely to have four daughters in a row that are all carriers.

Alix: XXi
Nicholas II: XY

X=unaffected allele
Xi= allele for hemophilia

| | X | Xi |
|---|---|---|
| X | XX | XXi |
| Y | XY | XiY |

None of Alexis' sisters had hemophilia because the father did not have hemophilia, so he did not possess an affected X chromosome to pass onto any of his daughters. The daughters may have obtained an affected X chromosome from the mother, but this would result in the daughter being a carrier but not present with hemophilia.

The self-proclaimed faith healer Rasputin was summoned by the Romanovs' to heal Alexis with his hemophilia. The Romanov's were grateful to Rasputin for healing Alexis so the family started to think highly of him. Rasputin started to have more influence on the family than

just on Alexis, so much so that the government were concerned Rasputin was straying the royal family away from their original beliefs and values.

Romanov Part B

Part II: Hemophilia

1. The chromosome that contains the gene that causes hemophilia is the X chromosome.
2. The mutation that caused hemophilia in the European families is a substitution mutation in the splice acceptor which is found in exon 4 within the F9 gene.
3. If there is a mutation in the splice acceptor, then the correct intron will not be removed from the mRNA sequence to create mature mRNA. There will be extra unnecessary genes if the intron is not removed. The individual with this mutation will not possess the clotting factor because the gene sequence is not correct. The protein formed will be abnormal, making it unable to perform the normal function of clotting blood, resulting in hemophilia.
4. The genotype for a carrier of hemophilia is $X^H X^h$.

Part III: Molecular Analysis of People in a Mass Grave

5. Mothers pass down mitochondrial DNA to their offspring while males do not; so Alix passed down her mitochondrial DNA to her children. This explains why mitochondrial DNA testing shows Alix's mitochondrial DNA helping to identify the three females rather than Nicholas II's.
6. HRH Prince Philip's mitochondrial DNA was used because he was the only living person related to Tsarina. HRH Prince Philip was married to Queen Elizabeth II of England and Queen Elizabeth is related to Queen Victoria. Comparing the mitochondrial DNA between HRH Prince Philip and Tsarina would confirm a connection, with the mitochondrial DNA, to Alix and the three daughters.
7. Alexi and Anastasia were missing from the mass grave.
8. When the mitochondrial DNA is being analyzed, the sex of the person the DNA is coming from does not matter, however if the it matters when determining comparing the mitochondrial DNA to possible relatives or other samples. There was a link so the mitochondrial DNA of Duke of Fife and Princess Xenia has been passed down to Nicholas II, meaning there is a maternal connection.
9. Heteroplasmy was identified at a single location at the 16169 position.
10. Heteroplasmy is the term given to the existence of two or more genetically different mitochondria within a cell.

Romanov Part C

Part III: Molecular Analysis of People in a Mass Grave

1. There were nine bodies in the grave discovered near Yekaterinburg and two child bodies were found in a separate grave.
2. These graves were discovered in 1991.
3. The White Russian Army was a group of people that fought against communist beliefs during the Russian Civil War
4. DNA testing was performed on the bones in the mass grave and blood testing was performed on the living family members to determine the relationship to the remains.
5. "STR" stand for short tandem repeats which is used in the analysis of DNA with repetitive base pairs.
6. The three types of DNA used to test the remains in the second grave were mitochondrial DNA, Y-STR testing, and autosomal STR.
7. The type of testing used to identify Alexis would be Y-SRT testing.
8. DNA was taken from the femur to identify Alexis.
9. No, Anastasia was not in the same grave that Alexis was found in. She was found in the grave that contained the nine bodies, of which were Anastasia's sisters.

Part IV: Who Wants to Be Anastasia?

10. Anna Anderson claimed to be Anastasia, along with many women claiming to be Anastasia. After DNA testing of Anna Anderson, it was revealed that she was not related to the Royal Family.
11. Anna Anderson settled in Charlottesville because of health issues that were treated at Charlottesville's Martha Jefferson Hospital.
12. Anna Anderson married J.E. Manahan who was an American professor.
13. Nuclear DNA was taken from Anna's hair and bowels.
14. Nuclear DNA was taken from Nicholas' and Alix' bones.
15. STR analysis and mtDNA analysis was performed on Anna, Nicholas, and Alixs' DNA.
16. Anna Anderson's mtDNA was compared to the mtDNA of Carl Maucher and the Duke of Edinburgh.
17. A hypervariable region of mtDNA is a location where base pairs repeat or have substitutions.
18. The mtDNA revealed that Anna was not related to the Royal Family or Anastasia.
19. Nicholas' and Alixs' children were all accounted for by 2007.

Part V: Current Hemophilia Treatment

20. A current treatment for hemophilia is to replace the clotting factors that the body can not synthesize itself. These clotting factors can come from human blood or be made synthetically in a laboratory.