

Part II: Hemophilia

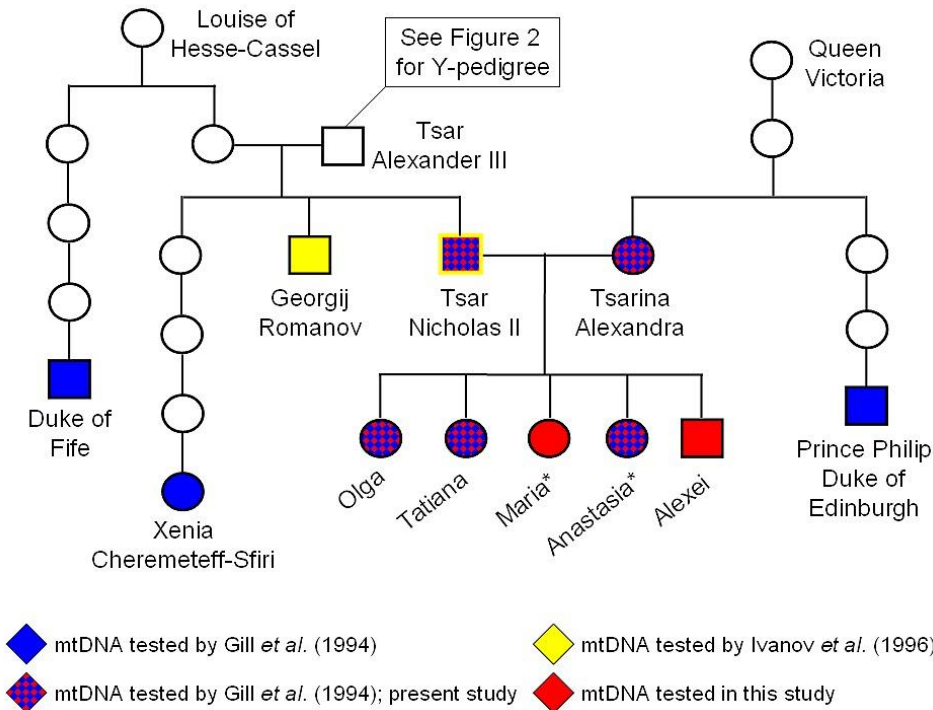
1. The chromosome that causes hemophilia A and hemophilia B is the X chromosome as the mutant allele diseases are X-linked recessive diseases with red-green color blindness.
2. The mutation responsible for plaguing royal European families at the beginning of the 20th century and Tsarevich Alexei/Alexis is a substitution mutation in the splice acceptor site of exon 4 in the Factor IX gene with mutant alleles causing hemophilia B. Genetic tools and biotechnology were used to identify this causal mutation from a grave discovered in 2007 with Nicholas II's and Alix's son Alexei and one daughter who were buried in it.
3. A splice-site mutation occurs at a splice site or the boundary between an exon and intron. The substitution mutation in the factor IX gene can disrupt RNA splicing, resulting in the loss or lack of exon 4 that causes coagulation factor IX deficiency characterized by hemophilia B. Splice-site mutations result in the loss of exons or addition introns not spliced out that changes the protein-coding sequence and results in a faulty gene product for factor IX. The splice-site mutation prevents the RNA processing of mature mRNA from precise splicing and results in a faulty protein product with an aberrant protein-coding sequence.
4. A woman who is a carrier for hemophilia is heterozygous for the X-linked recessive mutant allele 'h' on the X chromosome. The genotype for a carrier of hemophilia is $X^H X^h$.

Additional source used:

National Cancer Institute. (n.d.). NCI Dictionary of Genetics Terms. National Cancer Institute, <https://www.cancer.gov/publications/dictionaries/genetics-dictionary/def/splice-site-mutation>

Part III: Molecular Analysis of People in a Mass Grave

5. Traits controlled by genes on mtDNA display maternal inheritance. This is why Alix as the mother instead of the father Nicholas II had mtDNA testing to identify three females as her daughters.
6. The mtDNA of living relative HRH Prince Philip was used to identify Alix and three of her daughters from the mass grave because he is maternally related to Alix by her sister. As a direct descent of Alix as her grandnephew, HRH Prince Philip shared maternal inheritance with Alix and her children with his mtDNA. HRH Prince Philip is known in today's world as husband to Queen Elizabeth II, grandfather to eight, and great-grandfather to twelve as of now. HRH Prince Philip's more well-known grandchildren are Prince William, Prince Harry, Princess Beatrice, and Princess Eugenie as they are the children of Prince Charles or Prince Andrew and are closer in line to the British throne.



Coble, M.D. et al. Mystery Solved: The Identification of the Two Missing Romanov Children Using DNA Analysis. *Plos One*; <https://doi.org/10.1371/journal.pone.0004838> (2009).

7. Alix's and Nicholas II's children missing from the mass grave were only son Alexei and one of their daughters. According to Russian experts, the missing daughter is Maria, while according to U.S. experts, the missing daughter is Anastasia without a DNA reference from each sister. The two missing children from the mass grave of the Romanov family were found in a grave 70 meters away from the mass grave in 2007.

8. The Duke of Fife and Princess Xenia Cheremeteff Sfiri are maternal relatives of Nicholas II, so their mtDNA can be used to identify him in the mass grave. Regardless of whether the maternal relatives are biological females or males, the uniparental (maternal) inheritance of mtDNA is what is significant in this mtDNA analysis. As shown in the Romanov pedigree above, the Duke of Fife is the great grandnephew of the daughter of Louise of Hesse-Cassel who mated with Tsar Alexander III, the father of Nicholas II. Nicholas II and the Duke of Fife share mtDNA with Nicholas II's mother and the Duke of Fife's great-grandmother and great-great-grandmother. Princess Xenia Cheremeteff-Sfiri is the great-grandniece of Nicholas II, and they share mtDNA with the princess's great-grandmother and Nicholas II's mother.

9. A single point heteroplasmy at position 16169 was found in the mtDNA of Nicholas II but not in the Duke of Fife or Princess Xenia Cheremeteff-Sfiri. The Duke of Fife and Princess Xenia Cheremeteff-Sfiri were fixed for 16169 T. Nicholas II's brother, Grand Duke Georgij Romanov, also shares the same point heteroplasmy at 16169, but Nicholas II had a mostly C/t ratio while his brother had a mostly T/c ratio.

10. The term given to the existence of two or more genetically distinct mitochondria in the cell is heteroplasmic as an example of heteroplasmy. A cell with more than one genotype (wild-type or mutant) of an organellar genome is said to be heteroplasmic. On the other hand, a cell with one type of mtDNA (wild-type or mutant) is said to be homoplasmic regarding mitochondria.

Additional sources used:

Batchelor, T. (2021). How Prince Philip's DNA helped solve a Russian Romanov murder mystery. The Independent, <https://www.independent.co.uk/news/world/world-history/prince-philip-family-russia-murder-b1829742.html>

Gibson, K. (2023). All About Queen Elizabeth and Prince Philip's 8 Grandchildren. People, <https://people.com/royals/queen-elizabeth-prince-philip-grandchildren/>

Goldberg, M.L., Fischer, J., Hood, L., and Hartwell, L. (2020). Genetics: From Genes to Genomes 7th Edition. McGraw-Hill Higher Education.