ARTICLE

Royal Hemophilia

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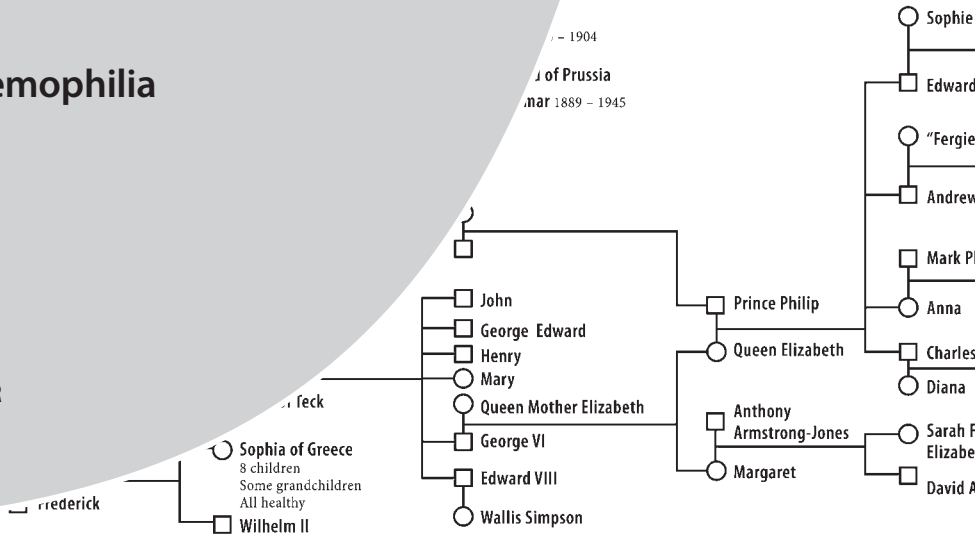

Abstract

The mutation that caused hemophilia in European royal families during the 19th century has been characterized and presents excellent teaching opportunities.

Key Words: *Hemophilia; introns; Queen Victoria; point mutations; frameshift mutations.*

Queen Victoria of England was a carrier for hemophilia. She had nine children; one of her sons had hemophilia, and at least two of her daughters are known to have been carriers of this mutation. One of her granddaughters, Alexandra, married Tsar Nicholas II of Russia and passed the mutation for hemophilia on to the heir to the Russian throne, with devastating consequences for political stability. Many historians believe that the success of the Boshevik Revolution was possible because the Russian royal family was distracted by caring for their son Alexis. Alexis, the heir to the throne, had hemophilia, and his many bleeding episodes were a major concern to his parents. Recent work has identified this mutation. The results are surprising and very useful in teaching molecular biology.

Queen Victoria of England was a carrier for hemophilia.

Two Genes on the X Chromosome Code for Proteins Required for Blood Clotting

Blood clotting is a multistep process, requiring a cascade of chemical reactions. Each of these reactions is catalyzed by an enzyme, and each of these enzymes is coded for by a gene. Two of the enzymes required for the blood-clotting cascade are coded for by genes on the X chromosome, Factor VIII and Factor IX. Details of the blood-clotting cascade can be found in a biochemistry textbook such as Berg et al. (2012).

Factor VIII is a large protein, coded for by a gene on the X chromosome. Factor IX is a smaller protein, also coded for by a gene on the X chromosome (Figure 1). Mutations in either of these genes cause hemophilia inherited in a sex-linked manner. The gene for Factor VIII contains 26 exons, or coding regions, whereas the gene for Factor IX contains 8 exons.

The Russian Royal Family

Tsar Nicholas of Russia married Alexandra, a granddaughter of Queen Victoria. They had five children, four phenotypically normal girls and a boy, Alexei, who had hemophilia (Figure 2). The entire family was killed in 1918 during the Bolshevik Revolution. Recent discovery of their graves made their tissue available for sequencing. This work, done by Rogaev et al. (2009), was technically difficult because only tiny amounts of tissue were available. This made it possible, but difficult, to obtain enough DNA for accurate sequencing. Sophisticated methods, such as using multiple primers for polymerase chain reaction and massively parallel sequencing methods, were required. These are discussed in the Rogaev paper.

Sequencing

Rogaev et al. (2009) compared the sequences of the Factor VIII and Factor IX genes with the normal human genes, known from the Human Genome Project. All the exons in both genes were completely normal. They then looked at the introns, the noncoding parts of the gene. All introns in the Factor VIII gene were normal. However, the gene for Factor IX contained a point mutation from A to G in the third base before the end of the intron between exons 3 and 4 (Figure 3).

Most mutations in introns do not affect either proteins or phenotype. But because this mutation occurred at 3 base pairs before the beginning of exon 4, it was part of the splice site for this intron. When messenger RNA from the gene with the mutated intron is spliced, two extra bases are left in the final messenger RNA molecule. This means that two bases that are normally the end of the intron are treated like the beginning of exon 4 instead of being spliced out. Because this

The American Biology Teacher, Vol. 75, No. 9, pages 652–656. ISSN 0002-7685, electronic ISSN 1938-4211.
DOI: 10.1525/abt.2013.75.9.5

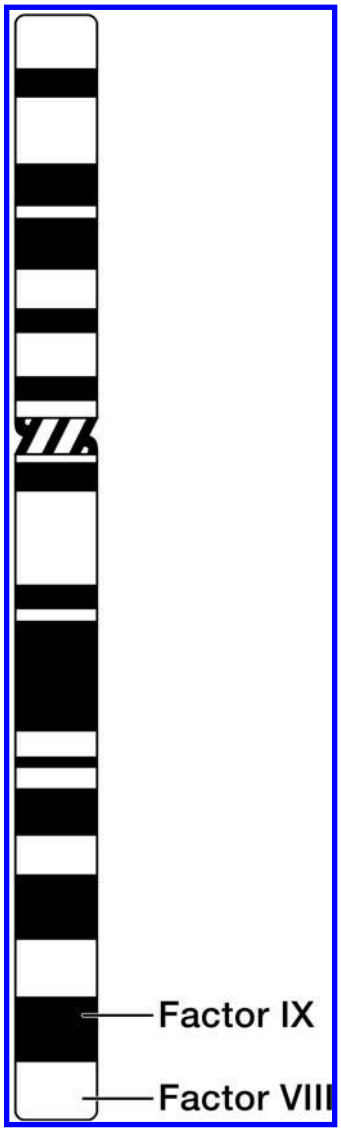

Figure 1. Partial map of the human X chromosome (idiogram copyright 1994 by David Adler, http://www.pathology.washington.edu/research/cytopages/idiograms/human; used with permission).

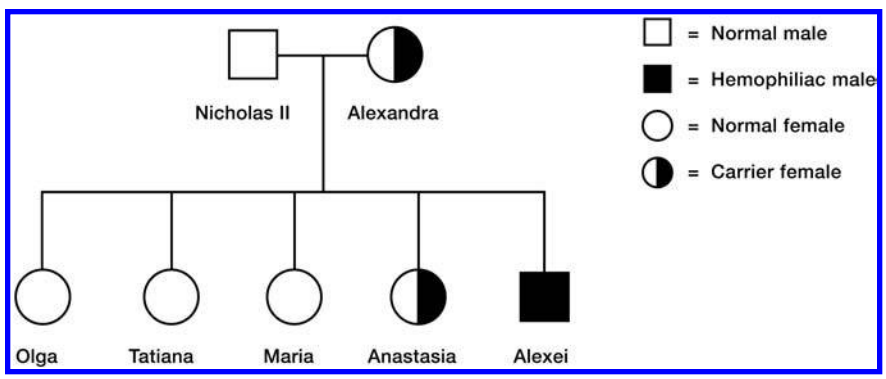

Figure 2. The Russian royal family. Alexandra was a granddaughter of Queen Victoria. Alexei had hemophilia, so it was assumed that his mother, Alexandra, was a carrier. The work of Rogaev et al. (2009) confirmed that Alexandra was a carrier and showed that Olga, Tatiana, and Maria were normal, while Anastasia was a carrier.

mRNA will go to the cytoplasm and be translated into the Factor IX protein, this point mutation has the same effect as a frameshift mutation (an insertion or deletion of 1 or 2 base pairs). Every amino acid is changed in the Factor IX protein after the mutation. People with this mutation make normal Factor VIII protein. Their Factor IX protein contains a normal version of the part of the protein coded for by the first three exons. Eleven amino acids of the fourth exon are produced, but they are completely different from the normal sequence. After these 11 amino acids, there is a stop codon, so most of the fourth exon as well as the fifth, sixth, seventh, and eighth exons are not translated into protein at all (Figure 3). This truncated Factor IX protein is not functional, resulting in severe hemophilia. There are 6 billion base pairs in a diploid human genome of 46 chromosomes. It is remarkable that a single-base-pair change, in a genome of 6 billion base pairs can result in such a devastating disease. Even in this royal family, no hemophiliac male lived past his thirties. A dry lab illustrating this change is presented in the Appendix.

This work also tells us which of the Tsar's daughters were carriers for hemophilia. Alexei, as predicted, had one mutated Factor IX gene on his single X chromosome. Alexandra, his mother and Victoria's granddaughter, had one normal Factor IX gene and one mutated Factor IX gene. The three oldest daughters – Olga, Tatiana, and Maria – had two normal Factor IX genes and were not carriers, whereas Anastasia had one normal Factor IX gene and one mutated Factor IX gene and was a carrier (Figure 2).

This mutation can be very useful when teaching about sex-linked inheritance, as well as about introns and their role in the genome. Most eukaryotic genes are made of exons and introns. The exons code for the order of amino acids in the protein. Introns are DNA sequences that are translated into messenger RNA and then spliced out before the final mRNA is sent to the cytoplasm. It is estimated that ≤40% of the DNA in the human genome consists of introns, whereas only ~1.5% of the DNA in the human genome is found in exons and actually codes for proteins. In general, intron sequence is not highly conserved, because most mutations in introns do not affect the order of amino acids in a protein and, therefore, do not affect the folding or properties of proteins. As a result, mutations in introns are usually not selected against by natural selection. However, the nucleotide sequences at the ends of introns are recognized by the enzymes that splice out the introns. These sequences are highly conserved because mutations in them change the proteins coded for by the genes. As seen in this real case, a mutation at the end of an intron can cause a faulty splice, resulting in an abnormal protein and a very serious disease.

In studying a mutation that causes hemophilia, you would expect the mutation to occur in an exon, a coding part of the gene, and you would expect the mutation to change the order of amino acids in the protein. This mutation, however, occurs at the end of an intron and changes the splice site for that intron. This results in a two-base insertion in the final mRNA, leading to the truncated protein discussed here. This mutation provides a novel way of discussing the nature of introns, how they are spliced out, and why the DNA sequences at the ends of introns are critical and highly conserved.

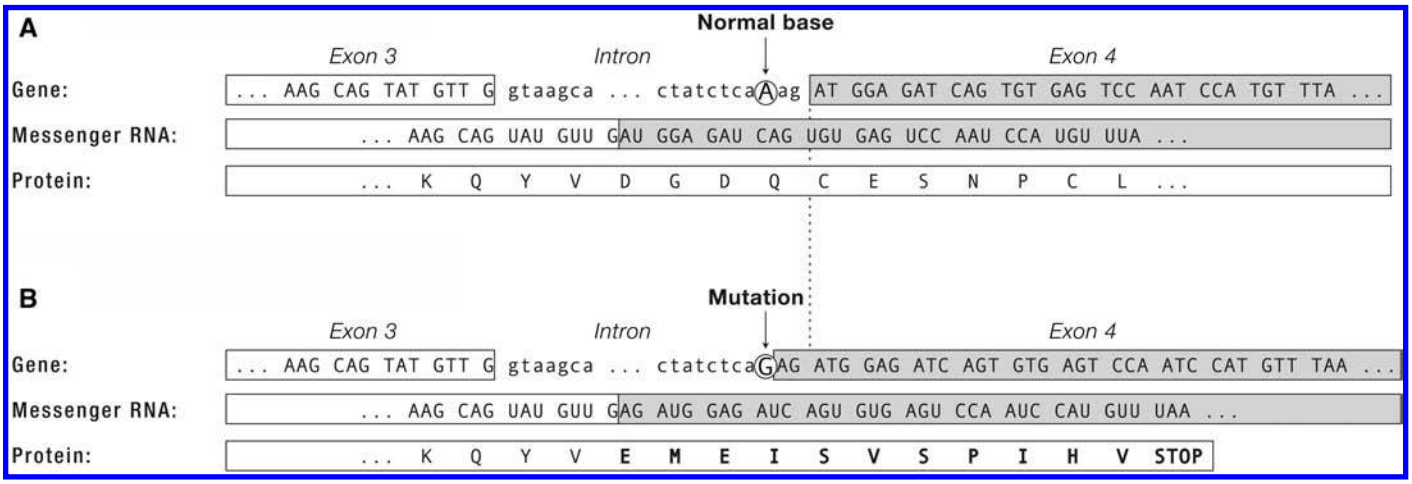

Figure 3. The mutation that caused hemophilia in Queen Victoria and her descendants. Figure 3A shows the sequence of DNA in the normal Factor IX gene at the end of exon 3 and the beginning of exon 4, with the ends of the intron between them. The next lines show the messenger RNA coded for by the normal gene, followed by the sequence of amino acids coded for by this part of the normal gene. Figure 3B shows the sequence of DNA in the mutated Factor IX gene, with the point mutation (A to G) highlighted. This mutation alters the splicing, so that the last two bases of the intron (AG) are now included in the mRNA, as shown. The next lines show the messenger RNA and the order of amino acids in the mutated protein. Notice that every amino acid after the faulty splice is different, and that after 11 altered amino acids there is a stop codon, so the last part of the protein is not produced. The changed amino acids are shown in bold. The point mutation, A to G, three base pairs before the beginning of exon 4, results in two bases being added to the messenger RNA, as shown. The single-letter abbreviations for the amino acids are as follows: A = alanine, C = cysteine, D = aspartic acid, E = glutamic acid, F = phenylalanine, G = glycine, H = histidine, I = isoleucine, K = lysine, L = leucine, M = methionine, N = asparagine, P = proline, Q = gluamine, R = arginine, S = serine, T = threonine, V = valine, W = tryptophan, and Y = tyrosine. (Figure modified from Rogaev et al., 2009; reprinted with permission.)

Figure 4 shows a partial pedigree of Queen Victoria and her descendants. This family tree can lead to many interesting questions, some of which are presented below. Answers are in italics.

1. Why is there no hemophilia in the present British royal family?

   Because Queen Victoria's son, King Edward VII, got her normal X chromosome and did not have hemophilia. Therefore, he had no mutant gene to pass to his descendants.

2. Three of Queen Victoria's daughters had no descendants with hemophilia. Could any of them have been a carrier?

   Her oldest daughter, Victoria, had a healthy son and a daughter who was probably not a carrier given that she had eight healthy children as well as healthy grandchildren. However, there is a significant chance that Victoria was a carrier. The first two children of her mother, Queen Victoria, were normal. If the Queen had not had any more children, nobody would ever have known that she was a carrier. The chance that a woman who is a carrier would have two healthy non-carrier children is 1 in 4. Therefore, there is 1 chance in 4 that Queen Victoria's daughter Victoria was a carrier. Queen Victoria's second daughter, who was not known to be a carrier, was Helena. Helena had two daughters and no grandchildren. Therefore, we have no information about whether or not she was a carrier, so her chance of being a carrier is 1 in 2. Similarly, Louise had no children, so her chance of being a carrier is also 1 in 2.

3. The Russian royal family – Nicholas and Alexandra and their five children, Olga, Tatiana, Marie, Anastasia, and Alexis – were killed by the Bolsheviks. Their graves were unknown until one of the people involved told where they were in a deathbed confession. At this time, their bodies were found. They were identified because they had the same mitochondrial DNA as Prince Philip, the husband of the present Queen Elizabeth. Why was this an appropriate method of identification, and why didn't they use DNA from Queen Elizabeth?

   Mitochondrial DNA is inherited from the cytoplasm of the egg. Therefore, it is passed only from mother to child and can be traced in the straight female line of descent. Alexandra's mother was Alice, one of Queen Victoria's daughters. Therefore, Alexandra and her five children represent a straight female line of descent from Queen Victoria and would have Queen Victoria's mitochondrial DNA. Prince Philip can also trace his lineage to a straight female line of descent from Queen Victoria. His mother was the daughter of Mary Victoria, who was the daughter of Alice, a daughter of Queen Victoria. So Prince Philip would be expected to have Queen Victoria's mitochondrial DNA, the same as Alexandra and her five children. The fact that the mitochondrial DNA of both Prince Philip and Alexandra and her five children were found to be the same confirmed their identity. By contrast, Queen Elizabeth is descended from Queen Victoria, but not in a straight female line. Her great-grandfather was Edward VII, a son of Queen Victoria, so she would not have the same mitochondrial DNA as Queen Victoria.

This mutation is of great historical interest. Because it is unusual, it can be used to teach advanced concepts in a high-interest setting.

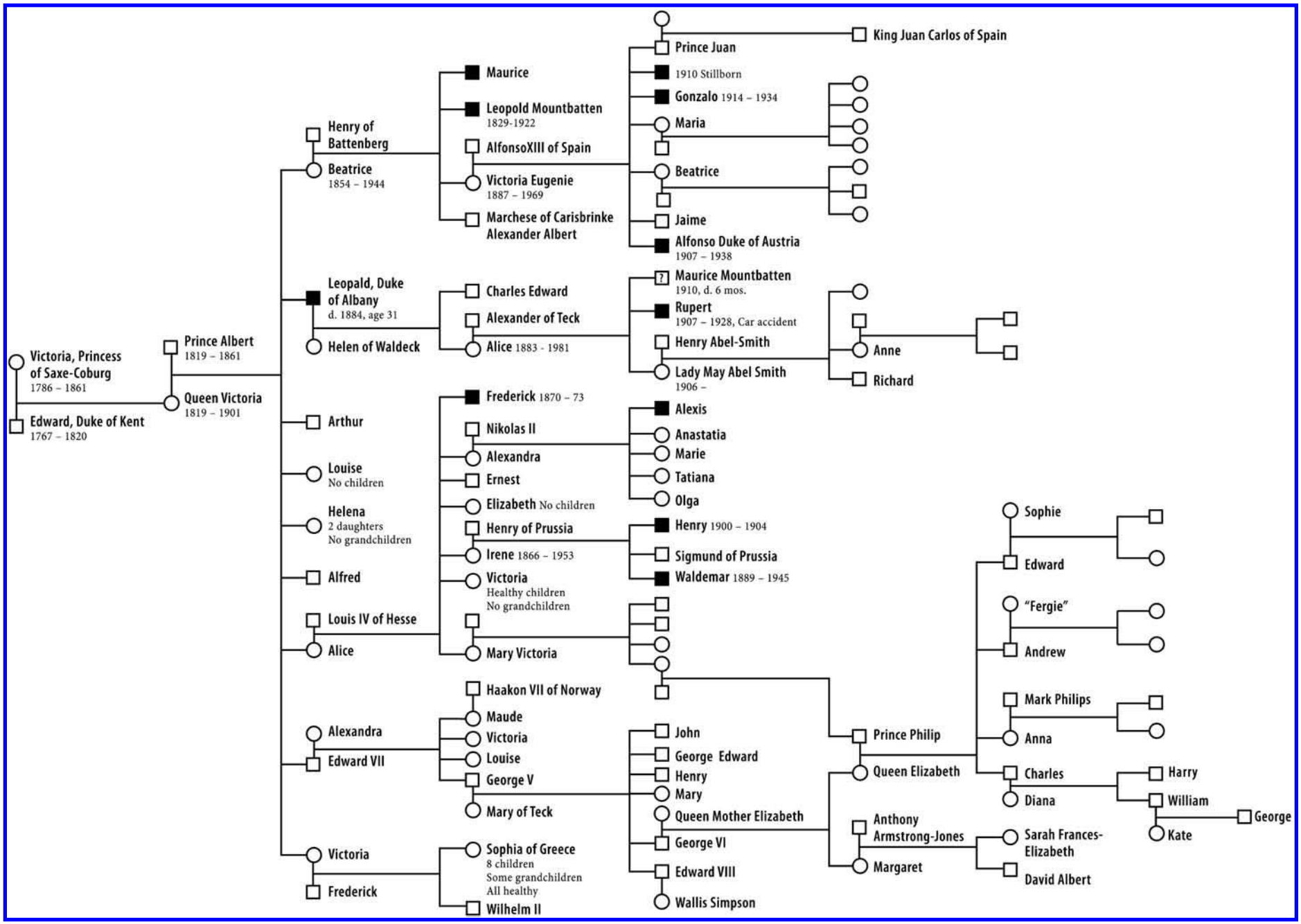

Figure 4. Partial pedigree of Queen Victoria and her descendants. Hemophiliac males are indicated by black squares.

○ Acknowledgments

Thanks to the Whitehead Institute's Partnership for Science Education and the Seminar Series for High School Teachers. Much of the material on the composition of the human genome came from lectures in this program. The Whitehead Institute for Biomedical Research is a leading nonprofit research and educational institution located in Cambridge, MA. Thanks also to Harvard University's Life Sciences–HHMI Outreach Program. Their lectures also provided much of the background material for this article. Thanks to Nadav Kupiek for expert preparation of the art work.

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Appendix. Answers are in italics.

The following is the order of nucleotides on one strand of a DNA molecule:

TTC GTC ATA CAA CTA CCT CTA GTC ACA CTC AGG TTA GGT ACA AAT T

1. What is the order of nucleotides in the mRNA molecule coded for by this DNA?

   AAG CAG UAU GUU GAU GGA GAU CAG UGU GAG UCC AAU CCA UGU UUA A

2. What protein does this mRNA code for?

   lysine glutamine tyrosine valine aspartic acid glycine aspartic acid glutamine cysteine glutamic acid serine asparagine proline cysteine leucine

3. If two bases, TC, are added to the original DNA strand after the 13th base (C), what mRNA would the new DNA code for?

   AAG CAG UAU GUU GAG AUG GAG AUC AGU GUG AGU CCA AUC CAU GUU UAA

4. What protein would this mRNA code for?

   lysine glutamine tyrosine valine ***glutamic acid methionine glutamic acid isoleucine serine valine serine proline isoleucine histidine valine STOP***

5. Compare the proteins in #2 and #4. How might these mutations be compared to those in the Factor IX gene leading to hemophilia?

   Every amino acid after the insertion has been changed. There is also a premature stop codon after 11 different amino acids. This means that the protein in #4 will be considerably shorter than the protein in #2. The original DNA is a small portion of the gene coding for Factor IX, a gene on the X chromosome that codes for a protein required for blood clotting. The two added bases in the final mRNA are the result of the actual mutation in the Factor IX gene that caused hemophilia in the descendants of Queen Victoria of England. The 2-base addition was caused by a point mutation at a splice site in an intron of the Factor IX gene, as discussed in this article. The Factor IX protein produced by the mutated gene is too short, and is nonfunctional, resulting in severe hemophilia. The altered amino acids are shown in bold.

6. Explain how a mutation in an intron can result in an apparent frameshift mutation as seen in this activity.

   A mutation in a splice site of an intron can cause the intron to be spliced out incorrectly. In this case, it results in the addition of two bases to the mRNA.