# When Life Played Dice with Royal Blood

*Anindita Bhadra*

**Haemophilia is a blood clotting disorder that is genetically transmitted. In the 19th century, this disease made an appearance in the British royal family, and eventually spread across Europe through marriage within the royalty. This review traces the path of the 'tainted blood' through the European royalty and comments on how the disease played a role in the Russian revolution. Being a scientist, I also felt it my responsibility to explain the basic biology of the disease itself for the enthusiastic reader, finally concluding the story where history met science to divulge an episode of modern history which is like a page out of a thriller.**

I was planning my next lesson for the evolution course at IISER-Kolkata, and the topic at hand was inbreeding. I wanted to use a short video for this topic, and was looking for one on the internet quite late in the night. My plan was to find a short video of 5–10 minutes and download it for the next morning's class. I thought that haemophilia, the 'royal disease', would be a good example to talk about as a case study of the appearance and spread of a recessive mutation in a family line, and its spread in a family tree enhanced by inbreeding. This was an example that I remembered from my own undergraduate days – the case of haemophilia in Queen Victoria's bloodline was a favourite example for 'problems' of pedigree analysis. My search led me to a particularly nice documentary from *Discovery Knowledge* on *YouTube*, and I was hooked for the next hour or so. The documentary triggered a process of inquiry, and I began reading up on one of my favourite subjects – history. I realized that scientific understanding of the disease had been aided by its occurrence in the royal family, as research in this field flourished with royal patronage. History of science can be as interesting as science itself, something that we forget as students of science – a thought that led to the writing of this article.

**Anindita Bhadra is a faculty member at the Department of Biological Sciences, IISER Kolkata. She is a behavioural biologist, working with free-ranging dogs in India. She is interested in understanding dog behaviour in a natural habitat, and the evolution of dog-human relationships, which can help to understand the evolution of dogs, as well as develop better management strategies for stray dog populations. Anindita is the founding chair of INYAS (Indian National Young Academy of Science), and is interested in science popularization and science education.**

**Keywords**
Haemophilia, genetic disorder, Royal disease, blood, history.

Haemophilia was first diagnosed in Queen Victoria's eighth child, Prince Leopold.

## The Royal Disease

In the 19th century, haemophilia came to be known as the 'royal disease', due to its appearance and spread in the European royalty [1]. It is speculated that haemophilia first appeared in the royal bloodline of Europe in Queen Victoria (1837–1901) of Britain [2]. Since her father was not a haemophiliac and there had been no reported case of haemophilia in her mother's family, it is probable that the mutation occurred during spermatogenesis (the process of sperm production in the male) in her father, Edward, Duke of Kent. This is made more likely by the fact that Edward was in his fifties when Victoria was conceived [3]. It has also been speculated that the origin of haemophilia in the royal bloodline was a spontaneous mutation in the X-chromosome of Victoria [4], which was then passed on to two of her five daughters – Princess Alice and Princess Beatrice – and her eighth child, Prince Leopold, Duke of Albany. Thus began a story of inheritance that 'tainted' much of the royal blood of Europe, and played a crucial role in turning the face of history.

The birth of Prince Leopold (1853–1884) was a milestone in medical history, as Queen Victoria chose to use chloroform during her labour, and thus, the use of anaesthesia during childbirth came to be sanctioned [5]. Chloroform was quite a recent discovery, having been first used as an anaesthetic in 1947 by James Young Simpson, on himself as a test subject. Leopold was a weak child, prone to bruises, and was diagnosed with haemophilia in 1858–59 [6]. Haemophilia had been described as early as the 10th century as a hereditary bleeding disease which affects males in a family, and was scientifically described in the early 19th century. However, no treatment for haemophilia had existed during Prince Leopold's time, and indeed the exact cause of the disease was not known to science. Though the royal family kept Leopold's disease a closely guarded secret, his ailment also triggered research on haemophilia, and helped to improve the understanding of the disease. In fact, two of the eminent haemophilia researchers of the time were Sir William Jenner and John Wickham Legg, the first being the Queen's personal physi-

cian and a family friend who attended on Leopold all his life, and the second was Leopold's personal physician in 1866–67 [7–9]. Prince Leopold's death from a minor fall, at an early age of 31-years, triggered further discussion on haemophilia among the medical community, though the Prince was not mentioned directly in these discussions. Both, *The Lancet* and *The British Medical Journal*, published obituaries on the demise of the Duke of Albany, without mentioning haemophilia, but carried a full article on haemophilia in the same issue [10].

The haemophilia gene spread along the royal pedigree, and through marriage, entered the bloodlines of the German and Spanish royal families.

**The Royal Pedigree**

Leopold had a hard time finding a match for himself, and finally after the intervention of his mother, the Queen, he married Helen of Waldeck at 29 years of age [11]. They had a daughter, Alice of Athlone, and a posthumously-born son, Charles Edward, the future Duke of Saxe–Coburg and Gotha. Alice inevitably inherited the haemophilia gene from her father, and was thus, a carrier. She married Prince Alexander of Teck, and they had three children, of which the youngest, Prince Maurice of Teck died when he was only six months old. Thus, it is not known if the child had inherited haemophilia from his mother. The eldest child, Princess May, was probably not a carrier as neither her two daughters nor her son showed any signs of the disease. Prince Rupert, Alice's second child, was haemophiliac, who died in a car accident at the age of 21. Thus, haemophilia touched German royalty briefly through Prince Leopold and died in his descendants with his grandson, Prince Rupert ([12], *Figure* 1).

Princess Beatrice (1857–1944) was the youngest child of Queen Victoria, and by the time of her birth, the queen was already worried about the tainted blood of the royal family. Beatrice married Prince Henry of Battenberg, a descendant of the House of Hesse into which Princess Alice was married. Beatrice and Henry had four children – Alexander Mountbatten, Victoria Eugénie, Leopold Mountbatten and Maurice of Battenberg [13]. The two younger sons were haemophiliac, and both died unmarried. Princess Eugénie of Battenberg married King Alfonso XIII and be-

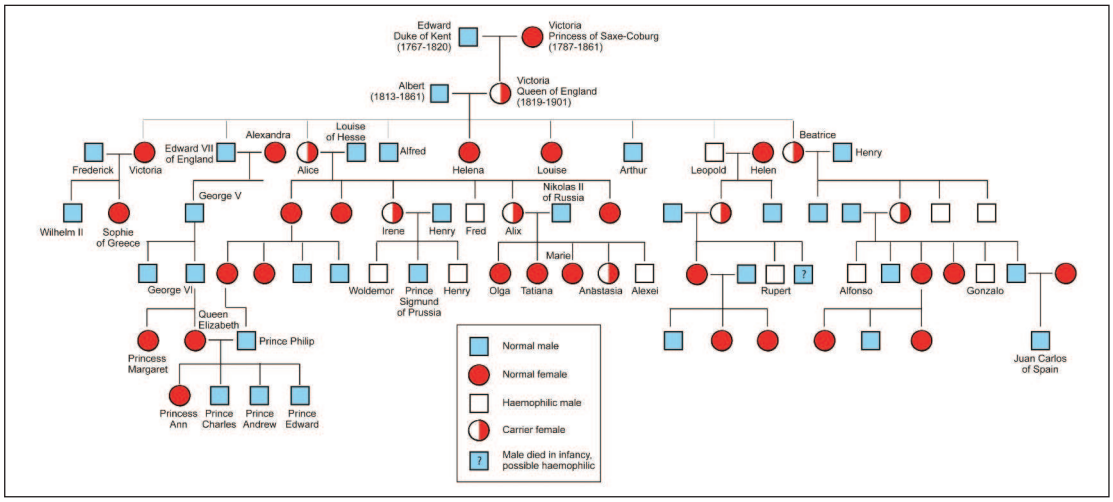


**Figure 1.** Tracking haemophilia in Queen Victoria and her descendants. (Adapted from [12]).

came the Queen of Spain. Eugénie was a carrier of haemophilia, and she carried the dreaded disease into the royal family of Spain, passing it on to two of her sons, the eldest, Don Alfonso, and the youngest, Don Gonzalo (*Figure* 1). Alfonso married twice but had no children, and died after minor injuries in a car accident at the age of 31. Once again, haemophilia claimed a royal life in Europe. Gonzalo and his sister Beatrice had a minor car accident when he was 19, in which neither of them were hurt. But it soon became evident that Gonzalo was bleeding internally, and died two days later. Since neither prince had left any heirs, and the two daughters of Eugénie were not haemophilia carriers, the tainted blood of Victoria did not proceed further down the generations of the Spanish royalty that anyway lost power and glory with the victory of the Republicans in 1931 [14].

Princess Alice (1843–1878) was Queen Victoria's third child and a carrier of haemophilia, which became evident only much later. She married the Grand Duke Louis IV of 'Hesse and by Rhine'. Prince Friedrich, their fifth child, died at three years of age due to heavy bleeding after a fall, the first sign that the dreaded disease had touched the German royalty. Alice had four daughters and three sons, of which only one was haemophiliac. Her daughter Irene of Hesse and by Rhine married Prince Heinrich of Prussia and they had three sons, Woldemar, Sigmund and Heinrich,

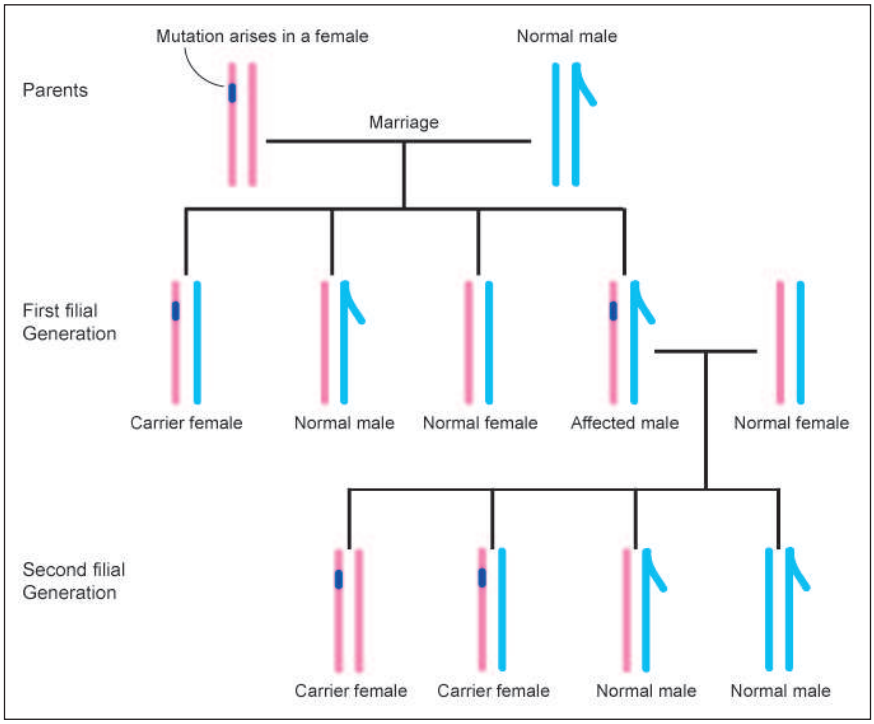


**Figure 2.** Inheritance pattern of recessive X-linked mutations in humans, which is applicable for the case of haemophilia.

of which two were haemophiliacs, thus proving Irene to be a carrier (see *Figure* 2 for the inheritance pattern of the haemophilia gene mutation). While Prince Heinrich died at four years of age, Prince Woldemar survived till he was 56, quite a remarkable age for a haemophiliac at that time. Though Woldemar had married, he had no issues, and thus, haemophilia did not get carried further down in Irene's descendants. Alice's sixth child, Princess Alexandra (Alix) of Hesse and by Rhine, married Tsarevich Nicholas in 1894 to become Alexandra Feodorovna [15], the Tsarina of Russia. This single event was later to become the turning point of Russian history.

In the first seven years of their marriage, Alexandra gave birth to four daughters, and became more and more desperate for a son and male heir to the throne. Tsarevich Alexei was born in 1904, bringing much happiness to the royal family, until it was noted that little Alexei was bleeding abnormally from the navel. The dreaded disease had touched the Russian royalty, and Alix knew no peace. The news of the Tsarevich's haemophilia was kept a

Grigory Rasputin was thought to have the capacity to heal through prayer, and seemed to sooth the ailing Tsarevich Alexei. The Tsarina began to increasingly depend on him for her son's well-being.

closely guarded secret, with only the immediate family and the doctors being privy to the news. Alix not only suffered to see her son's ailment, but also went into depression, knowing that she was responsible for her son's pain. In her search for a possible cure for her son and peace, she met the *strannik* (pilgrim) Grigory Rasputin in 1907. Rasputin was thought to have the capacity to heal through prayer, and was called upon by the Tsar to attend to his suffering son. The next day, the boy felt much better, in spite of the fact that the doctors had given up on his survival [16]. The desperate Tsarina grew increasingly dependent on Rasputin for her son's survival and well-being. In October 1912, the Tsarevich went through a serious crisis in Spala, and was given the last sacrament. In her desperation, Alix contacted Rasputin, and he responded by telegram, saying, "The little one will not die. Do not allow the doctors to bother him too much." Alexei actually recovered from the crisis, and this served to increase the Tsarina's dependence on Rasputin. While some believed that Rasputin cured through hypnosis, others believed that he was drugging the Tsarevich, but nothing could explain his ability to cure the boy from a distance of 2600 km [17].

Rasputin propagated the message of peace and earned the disfavour of several members of the aristocracy and the Duma because he continuously advised the Tsar against war on the eve of World War I. The Russian aristocracy hated Rasputin because of his proximity to the Tsar's family, and especially because of the control he had over the Tsarina. When Nicholas himself led the Russian army to the war on 23rd August 1915, the country was left under the rule of the Tsarina, and through her, Rasputin. The hatred that the Russians had for Rasputin only increased after this, and a conspiracy that had been hatched in the summer of 1914 culminated in Rasputin's much-discussed murder in December 1916. However, the discord between the Tsar and his country did not end with this death, but the existing rift only increased. In early 1917, the first pulse of the Russian revolution was felt, and soon led to the Tsar abdicating his crown and supreme power in March 1917. In the Act of Abdication, he

wrote: "*Not desiring to be separated from Our beloved son, We bequeath Our heritage to Our brother, the Grand Duke Michael Alexandrovich, and give him Our blessing. We abjure him to govern in perfect accord with the representatives of the nation sitting in the legislative institutions, and to take a sacred oath in the name of the beloved Fatherland*." The country was like 'a rudderless ship at the mercy of the waves' with the masses feeling lost. Germany had been aiding the Russian revolution in order to destroy Russia from within, and they provided support to Lenin and the Bolsheviks. The Tsar and his family were assassinated at their residence, Ipatiev House in Yekaterinburg, on the night of July 16–17, 1918 by the Bolshevik party, leading to the end of monarchy in Russia [18].

The power that Rasputin could wield over the Tsarina and the Tsar because of the Tsarina's belief that he alone could remedy her son's sufferings, was the final nail in the coffin that sealed the fate of the Romanovs.

One can always argue that the Russian revolution was inevitable, as was the rise of democracy and the eventual disintegration of the USSR [19]. The stage for the revolution was set with the World War I in the backdrop and the growing dissent of the people of Russia against Tsar Nicholas II. Nicholas firmly believed in the ideal of the ruler being a saintly and fatherly figure to his people. The Age of Enlightenment had brought in ideas of democracy and dignity among the intellectuals of the Russian society, and the increasing demands of the people for democratic participation in the government led to the formation of the Duma in 1906 [20]. However, the Tsar still reigned supreme, and the Duma was under his control, creating further dissent. The power that Rasputin could wield over the Tsarina and the Tsar because of the Tsarina's belief that he alone could remedy her son's sufferings, was the final nail in the coffin that sealed the fate of the Romanovs. Ironically, Queen Victoria's haemophilia was instrumental, though subtly, in paving the path of history. With the death of Alexandra and her offspring, the Russian line of inheritance of the mutation also perished.

## Haemostasis

The clotting of blood following vascular injury is essential for survival, and thus, a disease that affects this important

Blood clotting involves a cascade of proteins and platelets, the smallest cells in the blood. During the process of clotting, liquid blood is converted into a gel form through the mediation of coagulation factors.

phenomenon can prove to be lethal. The process of stopping blood flow from the injury is known as haemostasis (haemo = blood; stasis = standing). When an injury cuts open blood vessels and damages endothelial cells (cells lining the inner wall of the blood vessels), the smooth muscles in the walls of the blood vessels undergo spasms, constricting the vessels. This process – known as vasoconstriction – reduces the flow of blood through the injured vessel, and can stop bleeding if the affected blood vessel is thin. For thicker vessels though, vasoconstriction can only slow the drainage of blood from the site of injury.

Injured endothelial cells release a glycoprotein known as VWF, the von Willebrand factor [21], which is also present in the alpha-granules of platelets. Platelets are the smallest cells in blood, biconvex in shape and without a nucleus; they look like tiny discs under normal conditions [22]. They exist in the inactivated form in blood flowing through undamaged vessels. When the endothelial lining is damaged, fibrils of collagen protein are exposed. Platelets bind to the VWF released by the injured endothelial cells, and the VWF, in turn, binds to the collagen. This process stops the platelets from flowing away from the region of injury, and is known as adhesion [23]. The adhered platelets undergo the process of activation mediated by thrombin, which binds to and activates protease-activated G protein receptors on the surface of platelets. During the activation process, the platelets lose most of their cytoskeletal structure, release the contents of their alpha and dense granules, swell like balloons, and develop spiky extensions [22] which help them to bind to each other, forming the platelet plug (*Figure* 3). This is the second stage of haemostasis.

The third stage of clotting sets in if the platelet plug is not able to stop the bleeding. Several proteins, known as coagulation factors, are involved in this process. The clotting or coagulation factors are present in blood in their inactivated states, and are activated in a sequential manner during the clotting process through a clotting cascade. During the process of clotting, liquid blood is converted into a gel form through the mediation of the coagulation factors. The activated platelets express binding sites for fibrinogen; each

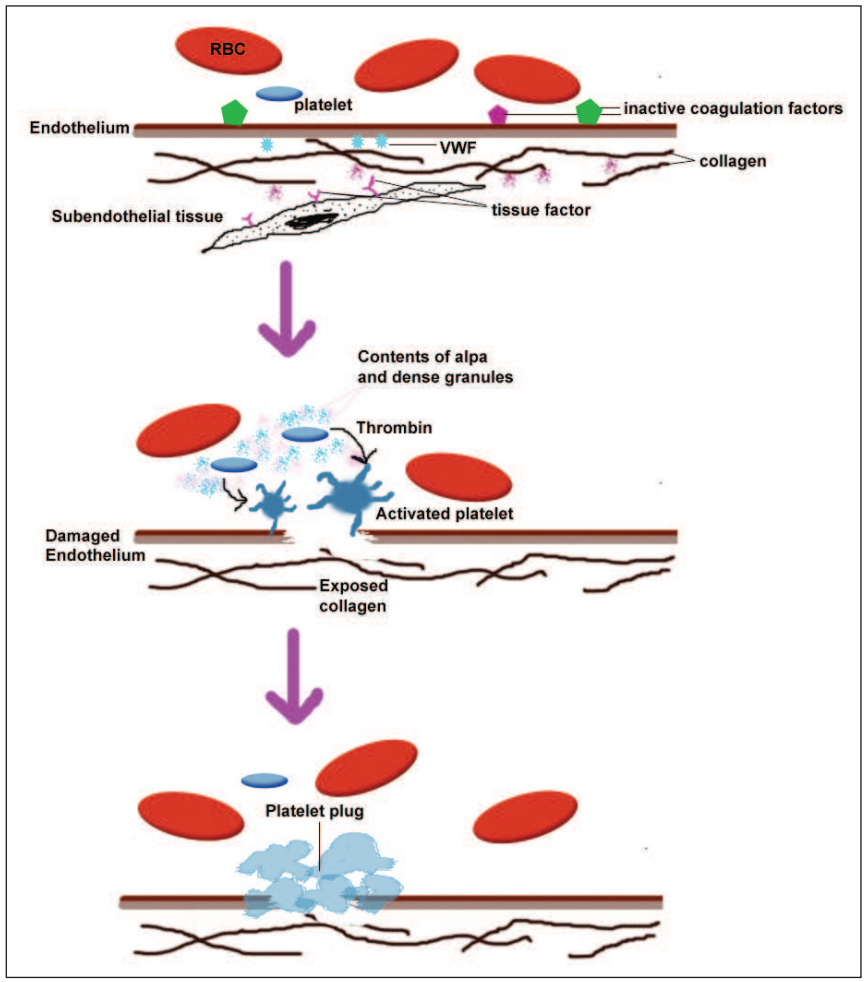


**Figure 3.** A schematic representation of the formation of a platelet plug during haemostasis.

fibrinogen molecule connects two activated platelets. Thrombin mediates the conversion of the soluble fibrinogen to insoluble fibrin monomers, which are connected to each other by calcium. The fibrin polymers form a loose mesh, stabilized by coagulation factor XIII. This mesh of fibrin traps red blood cells, leading to the formation of a clot that stops blood flow. Interestingly, though thrombin mediates the formation of fibrin from fibrinogen, leading to clot formation, fibrin fibres inhibit thrombin activity through a negative feedback loop. As the clot grows, this negative feedback intensifies, leading to reduction in thrombin activity. This prevents excessive clotting that can lead to a clogging of the blood vessel.

Two different pathways can initiate the clotting process – a very rapid, extrinsic pathway, or a slower, and more complex intrinsic pathway.

## The Clotting Cascade

The clotting cascade is a fascinating phenomenon in which more than a dozen molecules interact in a coordinated, orchestrated fashion, to enable the process of clotting. Two different pathways can initiate the clotting process – a very rapid, extrinsic pathway, or a slower, and more complex intrinsic pathway (*Figure* 4). In the extrinsic pathway, trauma activates Factor VII and triggers the release of a lipoprotein known as tissue factor (TF) from blood vessels. TF binds to the activated Factor VII which is a protease with two substrates, Factor IX and Factor X; it acts on Factor X to activate it. In the intrinsic pathway, if blood enters tissue spaces due to an injury, Factor XII (Hageman factor) circulating in the blood is activated by contact with the collagen. The activated Factor XII acts on Factor XI, which in turn acts on

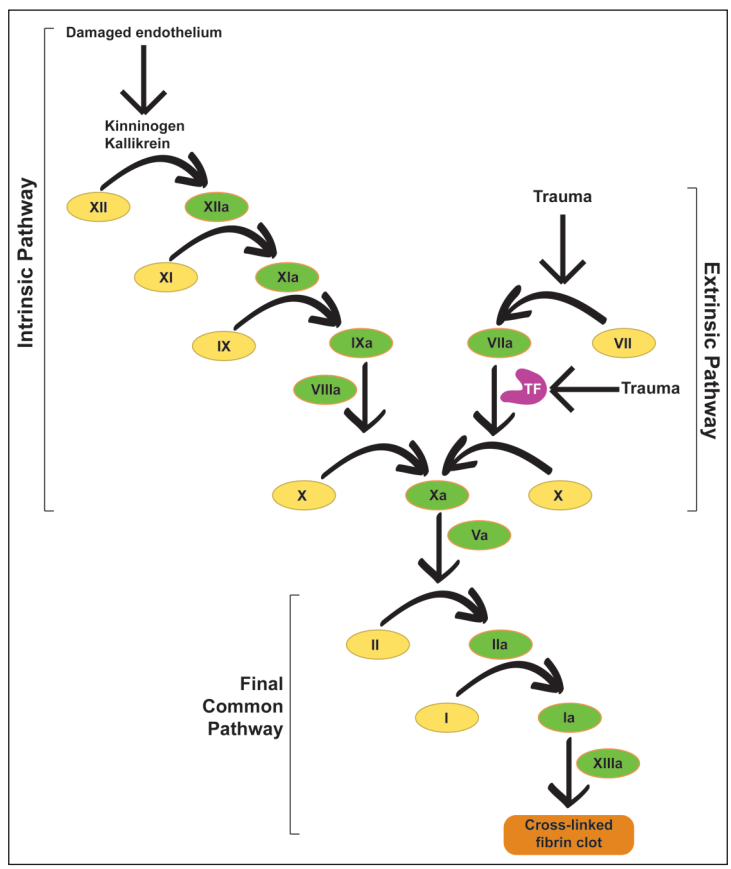


**Figure 4.** The clotting cascade: Inactive coagulating factors are yellow, and the active forms are green. Of these, Factor II is prothrombin, Factor IIa is thrombin, Factor I is fibrinogen and Factor Ia is fibrin.

Factor IX, which acts on Factor X, activating it. The activated Factor X, thus created by one or both the pathways, activates Factor V, which is also known as prothrombinase. This enzyme converts prothrombin (Factor II) to thrombin, which is a key player in the clotting process, as discussed above. The clotting process has several positive feedback loops, which help to amplify an initial small reaction manifold in a short span of time. Factor VIII (antihaemophilic factor) is present in the blood bound to VWF, which stabilizes factor VIII and helps to localize it to the site of injury. Thrombin and Factor Xa (activated Factor X) cleave Factor VIII from VWF through proteolysis, thus activating Factor VIII [24]. The active Factor VIIIa binds with Factor IX to stimulate the formation of more Factor Xa. As thrombin production increases, it enhances the production of Factors V, VIII and XI. Factor XI catalyzes the production of Factor IXa [25, 26].

Haemophilia is caused by mutations in genes that encode for coagulation factors. Haemophilia A is caused by a mutation in gene *F8* and Haemophilia B by a mutation in gene *F9*. These are sex-linked mutations in the X-chromosome.

## Haemophilia

Haemophilia is an X-linked recessive disorder that results in impaired blood clotting, caused by mutations in either of two genes encoding for coagulation factors. Haemophilia can be of two types: haemophilia A is caused by a deficiency of Factor VIII, due to a mutation of the gene *F8*. The gene *F9* is responsible for the production of Factor IX, and a mutation in this gene causes haemophilia B [27]. Mutations in the genes *F8* and *F9* either produce faulty coagulation factors or produce these in low numbers, leading to excessive bleeding due to faulty clotting of blood. Thus, haemophilia is known as a bleeding disease. Patients not only suffer from excessive bleeding from even minor injuries, but also can bleed internally, especially at the joints, leading to a lot of pain and distress. Since this is a sex-linked mutation on the X-chromosome, females are affected by the disease only when both copies of the X-chromosome bear the mutation. Since this is a rare event, females bearing a single copy of the mutation are carriers of the disease, while males are affected as they are hemizygous for the X-chromosome, having only a single X-chromosome paired with a Y-chromosome. Hence, males are affected more by haemophilia than females, and healthy females

Geli Ryabov, a filmmaker, discovered the grave of the Romanovs, where they had been buried by their assassins.

(carriers) can pass this disease on to their sons, following the pattern of X-linked inheritance (*Figure* 2).

**History Meets Science**

The remains of the Romanovs had not been found for years after their assassination, though a Russian investigator, Nicholas Sokolov had found some valuables a few months after the assassination at a site which he thought was the grave. It was assumed that the bodies had been destroyed. Eleven people had been shot – Nicholas II and his wife Alexandra, their children – Alexis, Olga, Tatiana, Maria and Anastasia, the chambermaid Anna Demidova, the cook Ivan Kharitonov, the butler Alexis Troup, and the royal physician, Dr Botkin. The assassinators had thrown the naked bodies of the dead in a hastily dug hole in the road and left burst cans of sulphuric acid on them so that they would be destroyed. However, the sulphuric acid had leached into the soil, dissolving only the soft tissue. Hence, on 30th May, 1979, when the filmmaker, Geli Ryabov and his team eventually discovered the grave, the skeletons were black-green in colour. Ryabov had spent nearly three years investigating the assassination of the Romanovs. His investigations took him to Leningrad, where he met Yakov Yurovsky's son Alexander and daughter Rima. Yurovsky had been at the helm of the assassination as the executioner, and he had made a detailed report of the event for the Soviet Government. He is later known to have recounted the event in graphic detail to the Soviet historian Michael Petrovsky in 1920, and also had made notes in 1922, which later became known as the 'Yurovsky note' [28]. His son provided these notes to Ryabov, and with the help of these, some old photographs and Sokolov's book, *The Murder of the Tsar's Family* [29], Ryabov pieced together the answer to the puzzle of the Romanovs' grave [30]. Ryabov intended to make a documentary of the assassination and of the eventual finding of the grave. Though he did not ultimately make this film himself, he did feature in a documentary made by *National Geographic* [31] on the Romanovs.

The unearthing of the remains of the Romanovs triggered off a

series of scientific investigations. The first and foremost objective was to prove beyond doubt that the skeletons did indeed belong to the Romanovs. The faces of the dead had been smashed before burial, and no soft tissue remained. Though eleven people had been assassinated, only nine skeletons were found in the grave. Reports by Russian forensic experts suggested that two of the Tsar's children were missing from the grave, eliciting speculations about whether they could have escaped the bloody fate met by the remaining family. In 1994, Gill [32] reported DNA analysis results of the nine skeletons. Sex identification and short-tandem repeats (STR[1]) analysis revealed that a family group was indeed present among the skeletons.

[1] STRs are microsatellites, which consist of hundreds of repeats of two to thirteen nucleotide units. These repeats, when scanned over a number of regions of DNA, can help to identify individuals, based on differences in their STR signatures, and is thus a very powerful tool in forensic science. Forensic laboratories typically use thirteen STR loci to generate genetic fingerprints of individuals [33].

Though most of our DNA is located in the nucleus of the cell, a cell organelle called mitochondria also carries a very small quantity of DNA. This is variously known as extra-chromosomal DNA, non-nuclear DNA or mitochondrial DNA (mt DNA). While 93% of the nuclear genome consists of non-coding DNA, only 3% of the mitochondrial genome is non-coding, and the mitochondrial genome has a mutation rate about hundred-fold higher than the nuclear genome. The most important distinction between the two kinds of DNA is in their inheritance patterns – mt DNA follows a maternal inheritance pattern (*Figure* 5), wherein the entire genome is inherited by the offspring from their mother through the cytoplasm of her ovum or egg cell, and paternal mt DNA is never inherited. Thus, the mt DNA is highly conserved in the maternal lineage of individuals. Because of its high rate of mutation and peculiar inheritance pattern, mt DNA is used in many genealogical studies, spanning a wide spectrum, from families to evolutionary trees [34–37]. Gill [32] used mt DNA analysis to report perfect sequence match between the putative Tsarina and her three daughters (from the skeletal remains) and a sample donated by the Tsarina Alexandra's grandnephew, Philip, Duke of Edinburgh, a living maternal relative. A similar analysis revealed a match between a certain sequence in the putative Tsar's mt DNA with that of two of his living maternal relatives. The report estimated the probability of the remains belonging to

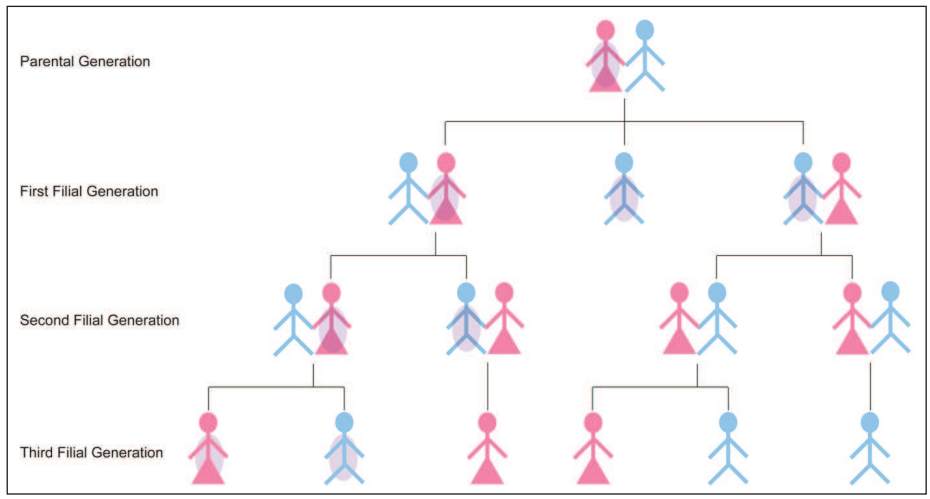


**Figure 5.** An illustration for maternal inheritance of mitochondrial DNA. The pink figures show females, blue figures show males. The figures marked with an oval bear the mitochondrial gene of concern. Paired pink and blue figures signify mating, and the black lines show offspring.

the Tsar to be 98.5%, but the Russian Orthodox Church demanded more evidence in support of the claim. This led to the exhumation of the Grand Duke Georgij Romanov, the Tsar's brother. His bone samples were analysed at the Armed Forces Institute of Pathology DNA Identification Laboratory in Maryland [38], and heteroplasmy of a single base in the mt DNA was found to be identical in both the putative Tsar and his brother's samples. The probability of the remains to be that of the Tsar was estimated to be over 100 million to 1, proving the identity of the Tsar beyond reasonable doubt to most of the world [38]. This led to the Russian Government giving the last Tsar of Russia and his family a state funeral; they were buried in the crypt of St Petersburg's St Peter and Paul Cathedral, exactly 80 years from the day of their assassination, on 17th July 1998. The Archbishop of St Petersburg did not attend the funeral, reflecting the vast rift between religion and science.

Where were the remains of the two other children of the Tsar? Yurovsky's account reveals that while a pit was being dug for burying the corpses, a fire had been prepared, and two of the bodies of the Romanov children had been burnt in this fire. In July 2007, Sergei Plotnikov, a builder and member of an amateur history group that spent free summer weekends looking for the Romanov remains, stumbled over some bones [39], approxi-

mately 70 metres from the larger grave [35]. This led to the discovery of two more skeletons and further forensic investigations. Using mt DNA, autosomal STR and Y-chromosome STR, [35] proved beyond doubt that the newly discovered skeletons were that of the Tsarevich and one of his sisters.

Haemophilia, as discussed earlier, can be of two types, A and B, of which haemophilia A is more common, accounting for about 85% of all cases of blood clotting disorders and haemophilia B accounting for about 14%. Other clotting defects like the von Willebrand disease, Hageman factor deficiency, etc., account for the remaining 1% of bleeding disorders [40]. It had been assumed for long that the 'tainted blood' of Queen Victoria's progeny was due to haemophilia A, simply because of the higher abundance, and hence, higher probability of occurrence of the disease. Prince Waldemar of Prussia was the last known bearer of the royal disease, and he died in 1945, before the two forms of haemophilia were identified [41]. Hence, no living member of the royal pedigree could lead researchers to the origin of the disease in Queen Victoria's family. When the bones of Alexei were discovered and confirmed, the possibility of a probe into this question opened up, and newly-discovered techniques in molecular biology and biotechnology made the study possible. Rogaev *et al* [42] conducted an extensive study using multiplex target amplification and massively parallel sequencing (MPS) methods to retrieve and analyse nuclear DNA from the degraded genetic material of the skeletal remains. The Tsarina's specimens were first used to screen for *F8* and *F9* mutations in the X-chromosome, and following extensive genetic analysis, the authors identified an inversion mutation (IVS3-3A>G ) on the *F9* gene. While both, wild-type and mutant, forms of the gene were detected in Alexandra's specimens, the specimens from Alexei carried only the single mutant allele, showing him to be hemizygous for the mutation. The specimens from one of his sisters, presumed to be Anastasia, revealed heterozygosity for the allele, suggesting her to be a carrier for haemophilia, like her mother. The authors concluded that the royal disease "was a severe form of haemophilia

Modern science finally proved beyond doubt that the Royal disease was a severe form of haemophilia B – and not haemophilia A – as previously thought.

The occurrence of haemophilia in Queen Victoria's descendants was a matter of incredible chance, that made its mark in history.

B, known also as 'Christmas disease', caused by a mutation creating an abnormal splicing site in the *F9* gene" [42].

## A Hand Dealt by Fate

Let us revisit the origin of haemophilia in Queen Victoria. It has been speculated that the mutation either arose spontaneously in Victoria herself [4] or Victoria acquired the mutation through her father, which arose in him during spermatogenesis [40]. The mean mutation rate to haemophilia per chromosome per generation has been computed to be $4.1 \times 10^{-6}$, with the female-specific rate being $1.9 \times 10^{-6}$ and the male-specific rate being $2.1 \times 10^{-5}$ [43]. If we consider the first hypothesis, then the probability of the event of a spontaneous haemophilia mutation occurring in Victoria is one in half a million. Though the chances of the mutation occurring in males is higher, the probability of Victoria acquiring the mutation is probably much lower than in the previous case. The reason is simple: considering an average of 250 million sperms being released during ejaculation by a male, the probability of the haemophilia mutation occurring during spermatogenesis should be devalued by $2.5 \times 10^{-7}$ to incorporate the probability of the mutated sperm fertilizing the ovum during fertilization. The numbers we obtain are staggeringly low, and it is indeed intriguing to think that this rare chance event could have had such far-reaching consequences in the history of the European royalty. The results of the latest genetic studies that proved the royal disease to be haemophilia B, which results from a single point (inversion) mutation, rather than A, which is typically caused by a large inversion and is about five times more common than B, just increase the incredibility of the event [44]. The odds against haemophilia arising would put the greatest gambling games to shame, but the spread of haemophilia in the consecutive generations simply followed the normal trajectory of the disease. Within a span of 81 years, haemophilia, which had been identified, spread across the continent, touched the royalty of Britain, Germany, Spain, Denmark and Russia, and vanished for good in the fourth generation of Queen Victoria's descendants, leaving a trail of bloody deaths in its wake.

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*Address for Correspondence*
Anindita Bhadra
Behaviour and Ecology Lab
Department of Biological Sciences
Indian Institute of Science Education and Research
Kolkata, Mohanpur Campus
Mohanpur 741 246
West Bengal, India.
Email: abhadra@iiserkol.ac.in