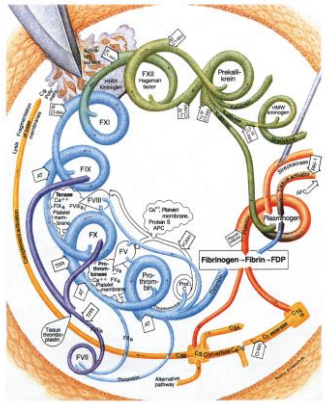
MTLA Workshop

Hämophilie und ihre Diagnostik



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Hämophilie, ein Überblick

Ursachen
Molekulare Mechanismen
Genetik

Lausanne, 22.02.2021

Hämophilie

- Aus dem Altgriechischen: αἷμα *haima:* Blut
- Aus dem Griechischen: φιλία *philia*: Neigung
- Bluterkrankheit: Erbkrankheit bei der die Blutgerinnung gestört ist
- Blut aus Wunden gerinnt nicht oder nur langsam
- Spontane Blutungen treten ohne sichtbare Wunden auf

Quelle: World Federation of Hemophilia

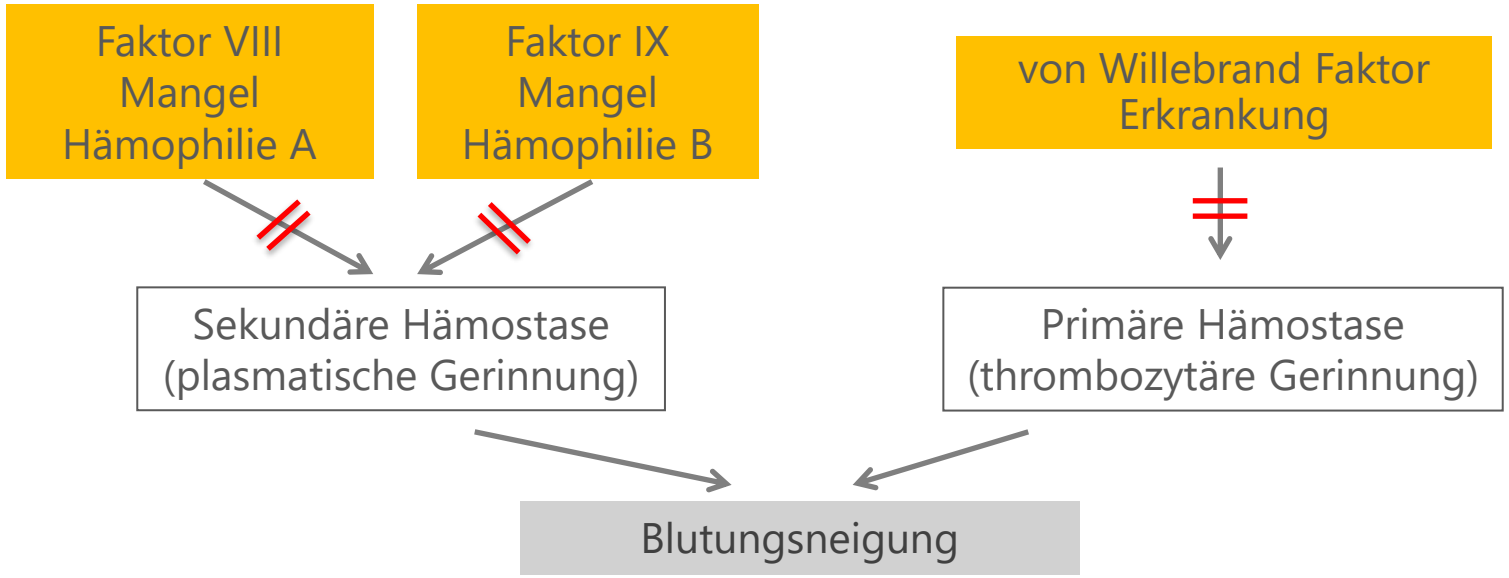
Geschichte der Hämophilie

2. Jhd. n. Chr.:	erste Erwähnung im Talmud
17. Jhd.:	Therapieversuche mit Schafsblut, Dionys
1803:	Beschreibung einer Familie Shepard mit hämorrhagischer Disposition, nur Männer betroffen, JC Otto
1813:	Beschreibung einer Familie mit hämorrhagischer Disposition, nur Männer betroffen, J Hay
1828:	Begriff Hämorrhaphilie wird geprägt, später Hämophilie, F Hopff
19. Jhd.:	Therapieversuche mit Vollbluttransfusionen, SD Lane
Um 1900:	Lebenserwartung Hämophiler 14-16 Jahre
1926:	Erste Beschreibung der Hereditären Pseudohämophilie: “Frauen und Männer gleichermaßen betroffen“, EA von Willebrand

Otto JC: An account of an hemorrhagic disposition existing in certain families. Med. Repository 6:1-4, 1803
Hay J: Account of a remarkable haemorrhagic disposition, existing in many individuals of the same family. N Engl J Med Surg 2:221-225, Jul 1813
Lane SD: Haemorrhagic diathesis: successful transfusion of blood. Lancet 1:185, Oct 31, 1840
Von Willebrand EA: Hereditär pseudohemofili. Finska Lak Sallsk Handl 67(2):87-112, Feb 1926

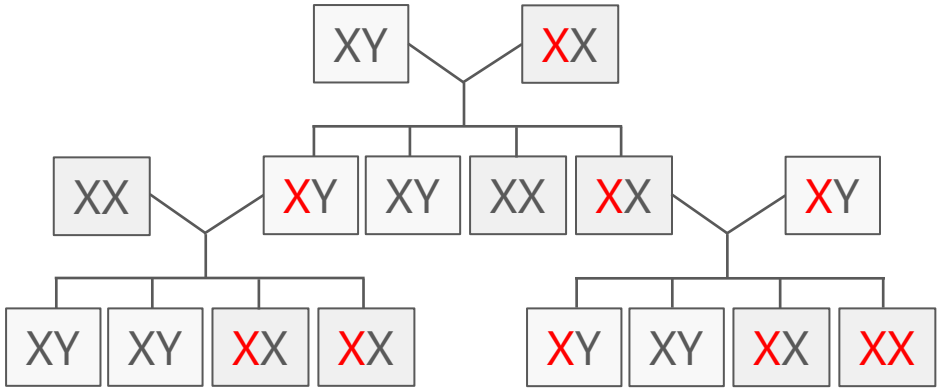
Arten der Hämophilie

- Hämophilie A, Mangel an F VIII-Aktivität, X-chromosomal rezessiv
- Hämophilie B, Mangel an F IX-Aktivität, X-chromosomal rezessiv
- Von Willebrand Erkrankung, Mangel oder Defekt an VWF, autosomal dominant (Typ 1, Typ 2) oder autosomal rezessiv (Typ 3)

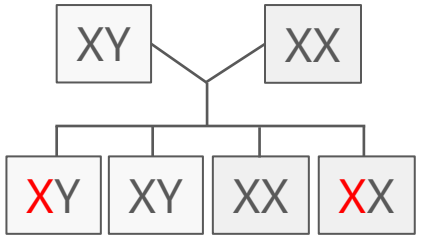


Vererbung der Hämophilie A und B

- X-chromosomal rezessiv
- Das merkmal-tragende Gen liegt auf dem X-Chromosom
- Rezessiv bedeutet “zurücktretend”



X-chromosomale Vererbung



Spontanmutation

In bis zu 50 % der Fälle Spontan-mutation in der Generation der Großeltern bzw. Eltern

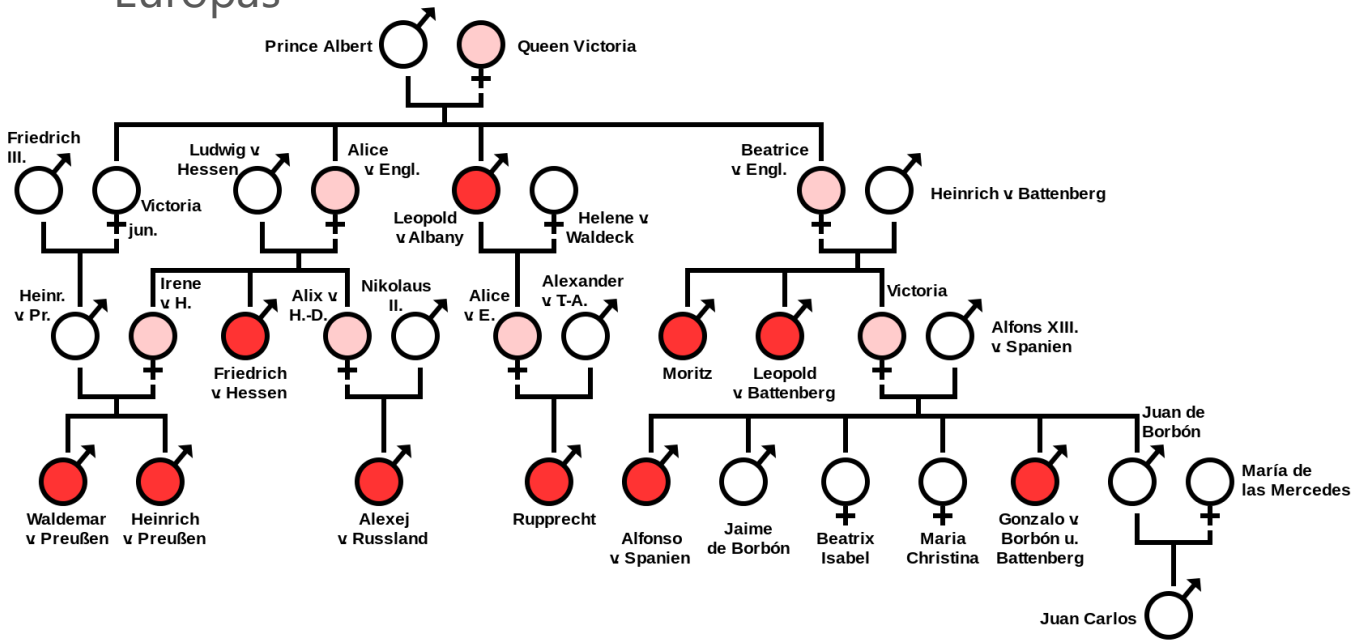
XX = Konduktorin

XY = Hämophiler Mann

XX = Hämophile Frau

Royal Disease

- Ausgehend von Queen Victoria von England (1819-1901)
- Verbreitung der Hämophilie in verschiedene Königshäuser Europas



Queen Victoria mit Prince Albert und ihren Kindern.
https://www.zdf.de/assets/queen-victoria-eine-koenigliche-familiensaga-102~768x432?cb=1464736865097, Jan 2017

Royal Disease war Hämophilie B

- Analyse von Knochenfragmenten der 1918 ermordeten Familie Romanov zeigt Splicestellen-Mutation auf F9 Gen, die zu schwerer Hämophilie B führt.

Genotype Analysis Identifies the Cause of the "Royal Disease"

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Ellen L. W. Kittler,[1] Yuri K. Moliaka[1]

Genotype Analysis Identifies the Cause of the "Royal Disease"

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DNA variations underlying a specific phenotype or pathology from the past may vanish into history along with carrier individuals or populations. Occasionally, however, these "mutation relics" can be recovered from biological remains and used to reconstruct historical events or solve medical mysteries.

The "royal disease," a blood disorder transmitted from Queen Victoria (1819–1901) to European royal families, contributed to pivotal events in European history and is one of the most striking examples of X-linked recessive inheritance. Although the disease is widely recognized to be a form of hemophilia (a blood clotting disorder), its molecular basis has never been identified (fig. S1). Genealogical analysis suggests that the royal disease mutation was transmitted from Russian Empress Alexandra (granddaughter of Queen Victoria) to her son, Crown Prince Alexei, who suffered from severe bleeding beginning at infancy (*1*, *2*). Our recent DNA analysis revealed that the remains of all members of the Nicholas II Romanov family murdered in 1918 have been found, including the remains of Alexei (*3*).

We have now analyzed extracts of degraded DNA from the same remains (skeletal bone specimens) by applying multiplex target amplification and massively parallel sequencing (MPS) methods, which allowed us to retrieve and characterize nuclear gene sequences. We used the Alexandra specimens for initial mutation screening of genes for blood coagulation factor VIII, *F8* (26 exons), and factor IX, *F9* (8 exons), both located on the X chromosome. Mutations in these two genes are the most common cause of hemophilia. Because the DNA samples were limited in quantity and quality, we designed multiple pairs of primers for short amplicons combined into multiplexing polymerase chain reaction (PCR) sets for the *F8* and *F9* genes (*4*). We analyzed the amplicons from primary or secondary PCR by using MPS in conjunction with conventional sequencing. In parallel, we included MPS of the complete mitochondrial DNA genome as a control for potential contamination and unambiguous identification of the sample (*4*).

We found no evidence for nonsynonymous missense or small insertion-deletion mutations in either *F8* or *F9* genes in the specimens. However, we detected an A-to-G intronic mutation located three base pairs upstream of exon 4 (intron-exon boundary IVS3-3A>G) in the *F9* gene that could be pathogenic. Typical for a heterozygous carrier, both wild-type and mutant alleles were detected in specimens from Alexandra. The specimens from Alexei contained only the single mutant allele, indicating that he was hemizygous for the mutation, whereas one of his sisters (presumed to be Anastasia) was a heterozygous carrier (Fig. 1 and figs. S2 and S3). Bioinformatics analysis predicts that the IVS3-3A>G mutation at this evolutionarily conserved nucleotide creates a cryptic splice acceptor site (*4*), which shifts the open reading frame of the *F9* mRNA, leading to a premature stop codon (Fig. 1B).

To evaluate the effect of the mutation on RNA splicing, we used an in vitro exon trap assay in combination with MPS (fig. S4). We found that 99.98% of transcripts from the mutant *F9* allele were generated by splicing at the mutant site. Hemophilia manifests in a severe form if less than 1% of factor VIII or IX is functional (*5*).

We conclude that the royal disease is a severe form of hemophilia B, known also as "Christmas disease," caused by a mutation creating an abnormal splicing site in the *F9* gene (*4*).

References and Notes

1. R. F. Stevens, *Br. J. Haematol.* **105**, 25 (1999).
2. C. Ojeda-Thies, E. C. Rodriguez-Merchan, *Haemophilia* **9**, 153 (2003).
3. E. I. Rogaev *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **106**, 5258 (2009).
4. Materials and methods are available as supporting material on *Science* Online.
5. G. C. White *et al.*, *Thromb. Haemost.* **85**, 560 (2001).
6. Single-letter abbreviations for the amino acid residues are as follows: C, Cys; D, Asp; E, Glu; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; S, Ser; V, Val; and Y, Tyr.
7. We thank T. Andreeva, A. Goltsov, I. Morozova, A. Tazeldinov for technical support; V. Gromov and N. Nevolin for assistance with the bone samples; S. Denisov and A. Mitronov for bioinformatics; and A. Chikunov for general support.

Supporting Online Material

www.sciencemag.org/cgi/content/full/1180660/DC1
Materials and Methods
SOM Text
Figs. S1 to S4
Tables S1 to S3
References

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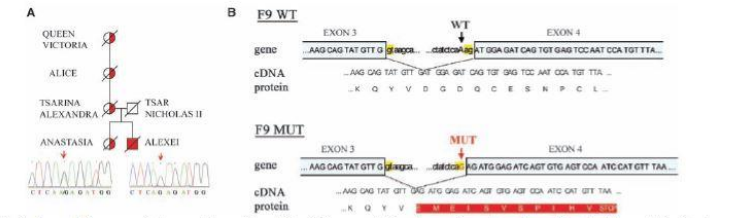


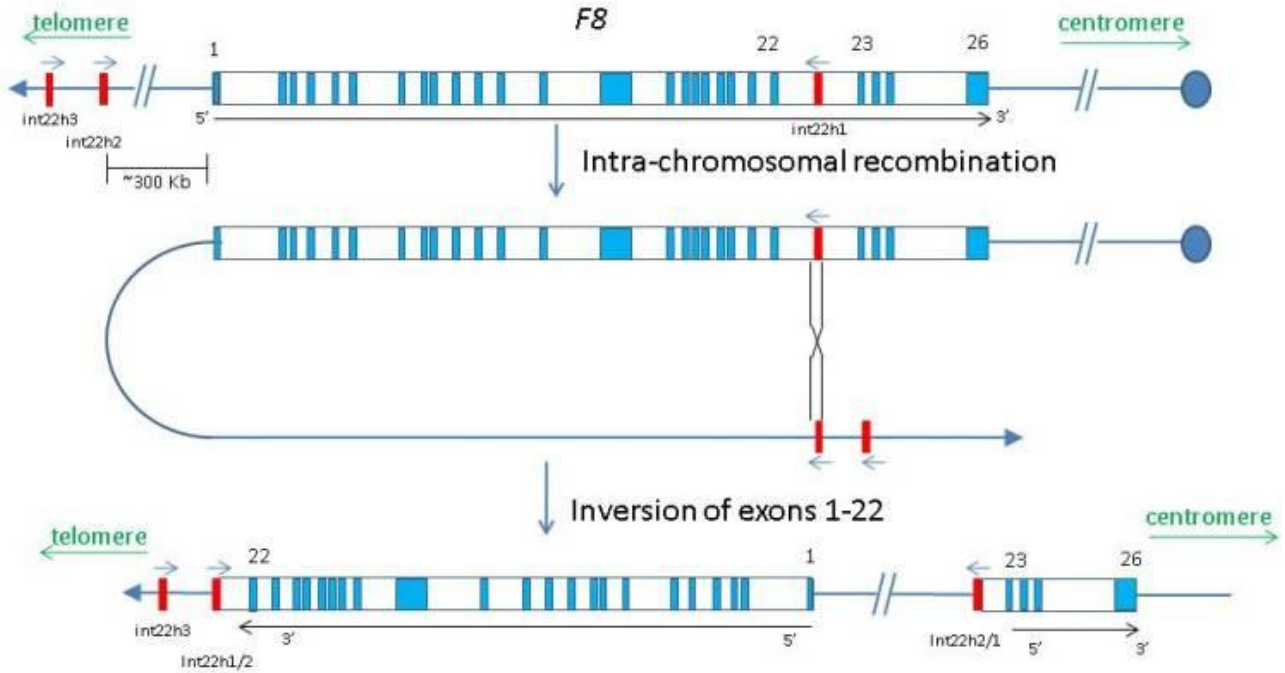
Fig. 1. The royal disease was likely caused by a point mutation in *F9*, a gene on the X chromosome that encodes blood coagulation factor IX. (**A**) Partial pedigree of the royal family, showing transmission of the mutation from Queen Victoria to Empress Alexandra and from Alexandra to Prince Alexei, her hemophilic son. Alexei was hemizygous for the mutation, whereas Alexandra and one of her daughters, putative Grand Duchess Anastasia, were heterozygous carriers. (**B**) The A-to-G mutation occurs just upstream of exon 4 in the *F9* gene and is predicted to create a new splice acceptor site that could lead to production of a truncated factor IX protein (*6*). WT indicates wild type.

Rogaev EI, Grigorenko AP, Faskhutdinova G, Kittler ELW, Moliaka YK: Genotype Analysis Identifies the Cause of the „Royal Disease". Science 326: 817, Nov 2009

Mutationen bei Hämophilie

Inversionen: Umkehrung der Transkriptionsrichtung

- Intron-22-Inversion: intrachromosomale Rekombination, Auseinanderbrechen des F8-Gens und umgekehrte Anlagerung der Introns 1-22.



Quelle: http://www.nibsc.org/image.ashx?assetid=2248a950-d4f1-4798-bf52-df3a104fb3e9, Jan 2017

Verlaufsformen der Hämophilie

Verlaufsformen	FVIII/FIX Akt.	Klinischer Phänotyp
Schwere Hämophilie	< 1 %	Ausgeprägte Blutungsneigung, Neigung zu Spontanblutungen
Mittelschwere Hämophilie	1 - 5 %	Weniger ausgeprägte Blutungsneigung, bei Restaktivität > 3 % Gelenkblutungen nur noch selten
Leichte Hämophilie	5 - 15 %	Blutungen meist nur in Risikosituationen (z.B.: OP, Verletzungen)
Subhämophilie	15 - 50 %	Keine spontanen Blutungen

Oldenburg J, Pötzsch B, Madlener K. Hämostaseologie, 2. Auflage, S. 335-345