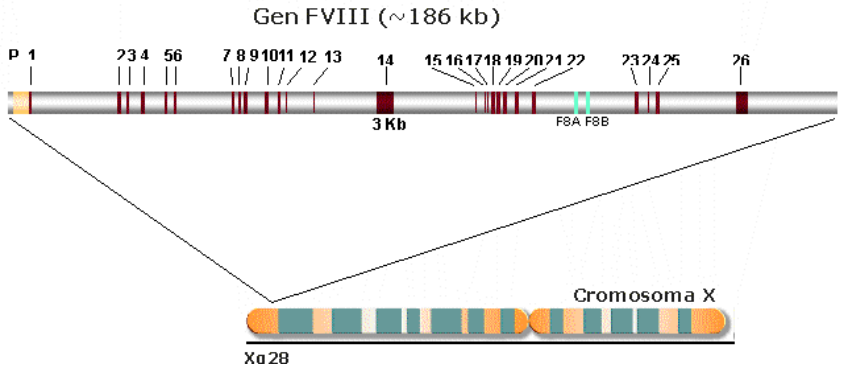
L’ hemofília de les famílies reials europees

IX Jornada d'actualització sobre l'hemofília

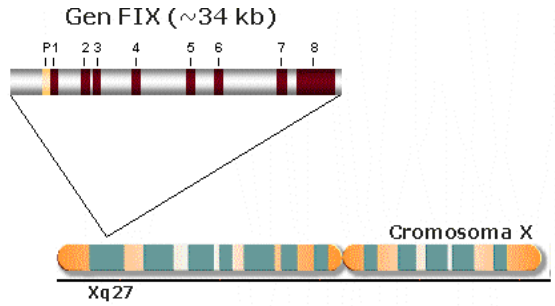
Carme Altisent
Unitat d’Hemofília. Barcelona

Vall d'Hebron

Tipus i graus d'hemofília



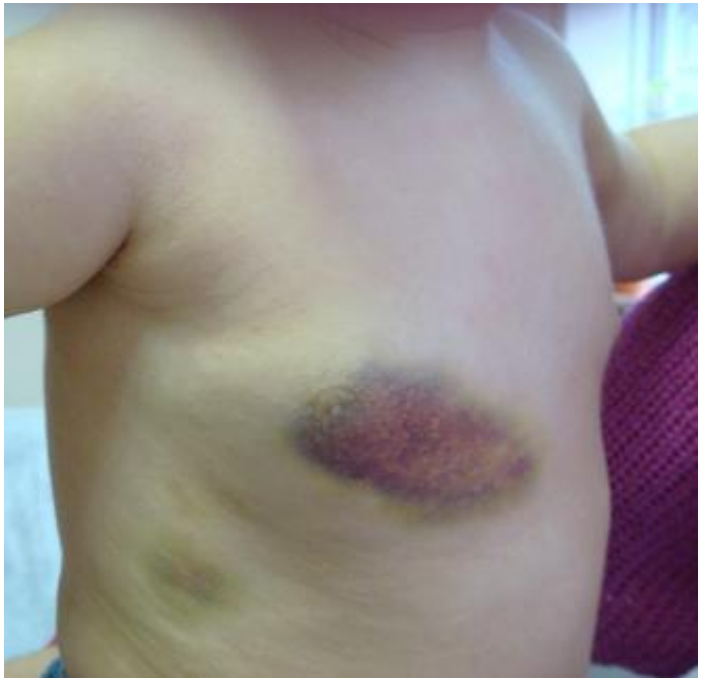
- A: Factor VIII
- B: Factor IX



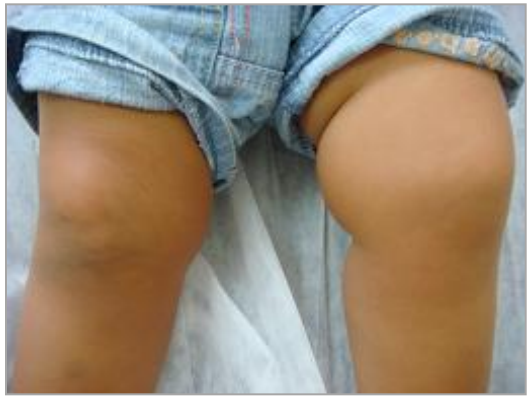
- Greu: <1%
- Moderada: 1-5%
- Lleu: 6-40%

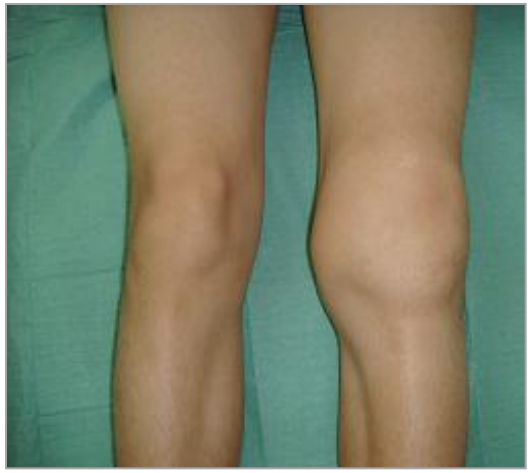
Hemorràgies

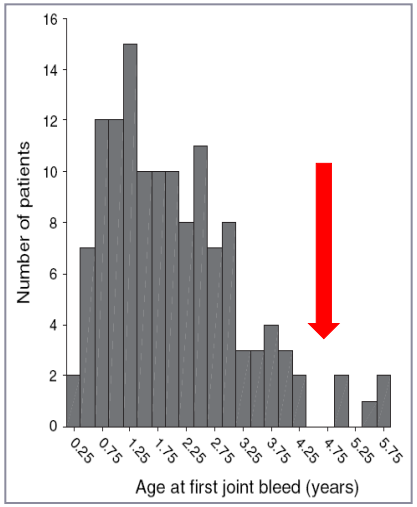
- Greu
  - Espontànies
  - Traumatismes mínims
- Moderada
  - Traumatismes lleus o moderats
- Lleu
  - Assimptomàtica
  - Estudi familiar
  - Estudi d'hemostàsia
  - Sagnat postoperatori

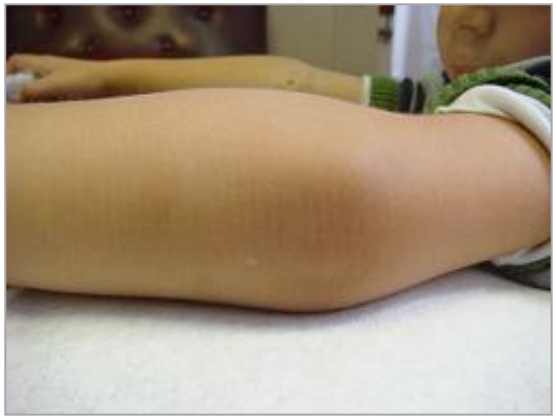


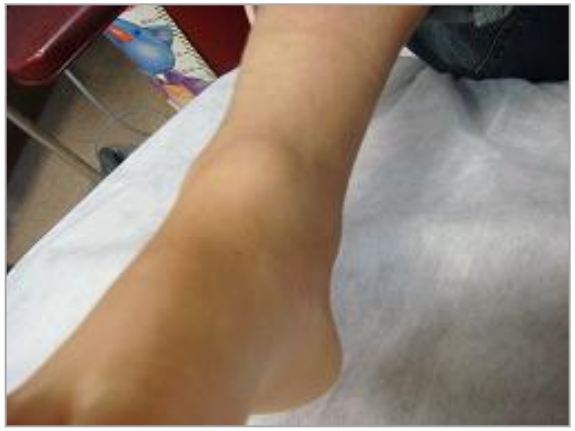
Hemartrosi






Number of patients
16
14
12
10
8
6
4
2
0
0.25
0.75
1.25
1.75
2.25
2.75
3.25
3.75
4.25
4.75
5.25
5.75
Age at first joint bleed (years)

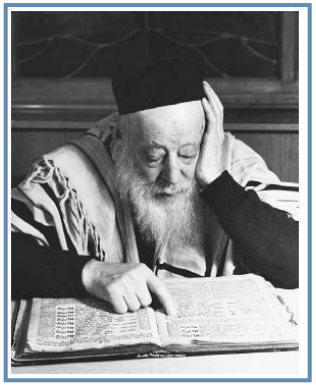




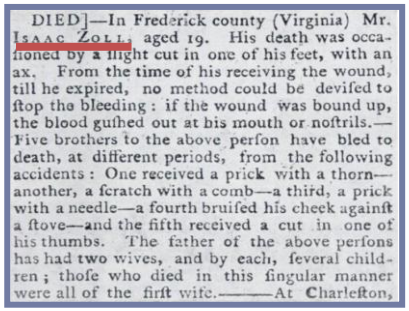
Coneixement ampli

- Herència
  - **Talmud, Yebanot 64 b**

    ...si el primer fill d'una dona és circumsisat i mor i el segon és circumsisat i mor, no s'hauria de practicar la circumcisió al tercer fill...



- Manifestacions clíniques
  - **Newspaper, on March 22, 1791**



DIED]—In Frederick county (Virginia) Mr. Isaac Zoll, aged 19. His death was occafioned by a flight cut in one of his feet, with an ax. From the time of his receiving the wound, till he expired, no method could be deviſed to ſtop the bleeding: if the wound was bound up, the blood guſhed out at his mouth or noſtrils.—Five brothers to the above perſon have bled to death, at different periods, from the following accidents: One received a prick with a thorn—another, a ſcratch with a comb—a third, a prick with a needle—a fourth bruiſed his cheek againſt a ſtove—and the fifth received a cut in one of his thumbs. The father of the above perſons has had two wives, and by each, ſeveral children; thoſe who died in this ſingular manner were all of the firſt wife.———At Charleſton,

- Tractament
  - **Samuel Lane.** Haemorrhagic diathesis. Successful transfusion of blood. **Lancet 1840; 35: 185-188**

Tractament

Subfraction I-O
Cryo-precipitates
Low purity pdFVIII concentrates
Intermediate-purity concentrates
High-purity concentrates
rFVIII available
rFIX available
Late 1950s
Mid 1960s
Early 1970s
Late 1970s
Early 1980s
Mid 1980s
Late 1980s
Early 1990s
Late 1990s
Early 2000s
Plasma fractionation
Donor/plasma screening for HBV
Heat treatment of pdFVIII
Heat-treated concentrates widely available
Immunoaffinity, treating with solvent or detergent, ion exchange
HIV/HCV screening
Qualification of donors, inventory hold, nucleic acid testing, nanofiltration

Esperança de vida

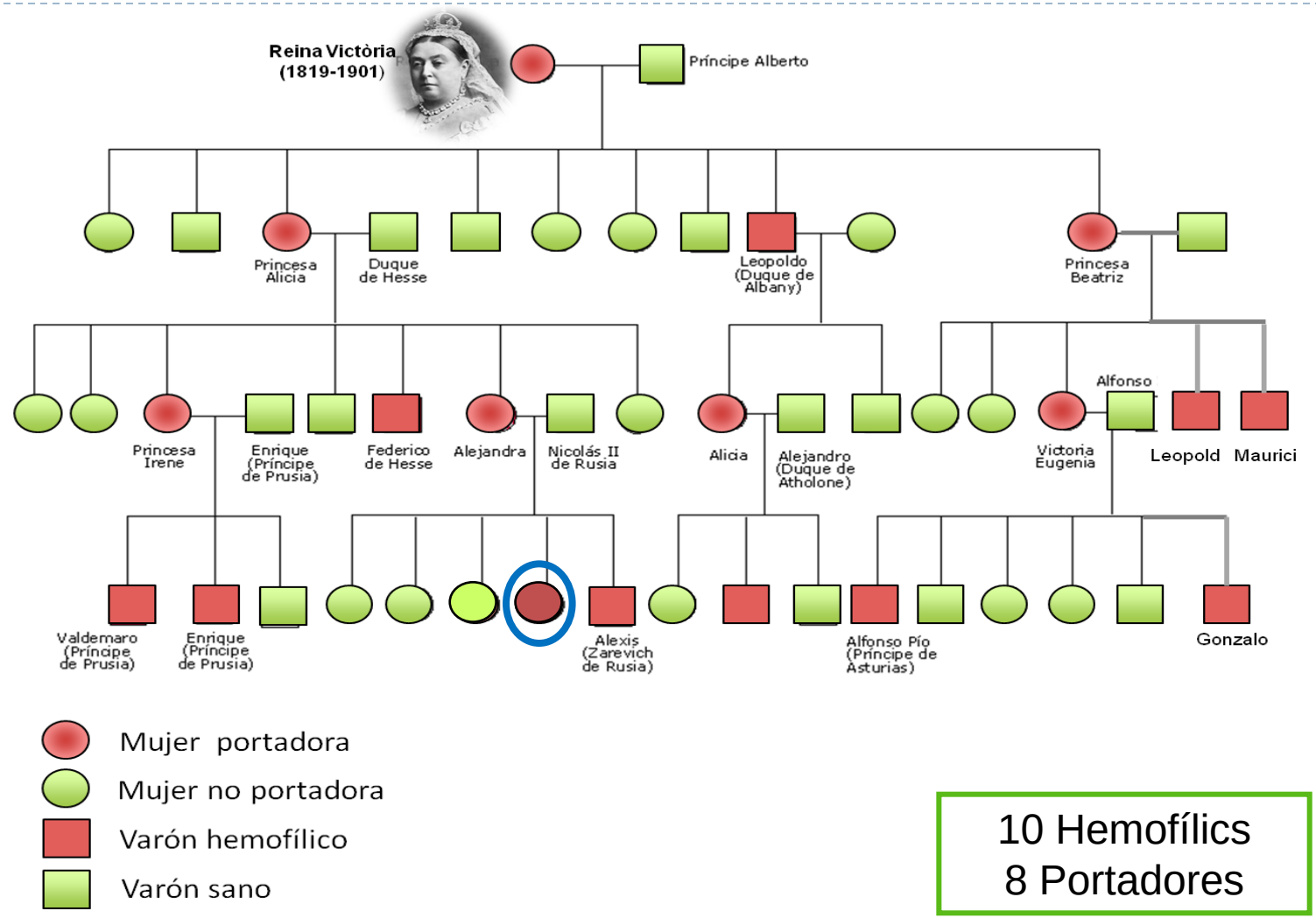
Cause of Death	Number
Operations	25
Circumcision	15
Tooth extraction	6
Vaccination	1
Lanced hematomas	2
Tonsillectomy	1
Trivial injuries (cut lip, bitten tongue, injuries to forehead, finger, scalp, etc.)	23
Internal bleeding	21
Central nervous system bleeding	7
Birth trauma and umbilical bleeding	7
Epistaxis	6
Lung hemorrhage	5
Haematuria	4

20 anys

Hemophilia. By Carroll LaFleur Birch.

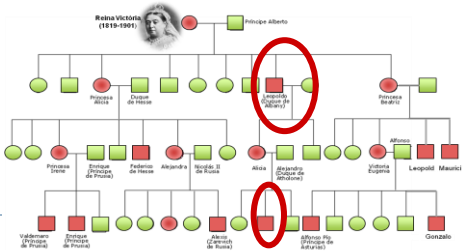
***Am J Dis Child.* 1937;54(4):965. doi:10.1001/archpedi.1937.01980040269021**

Arbre genealògic de la Reina Victòria



10 Hemofílics
8 Portadores

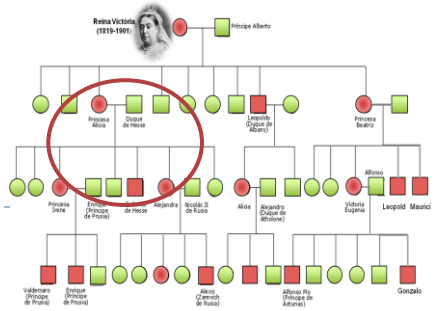
Leopold, duc d'Albany



1907-1928

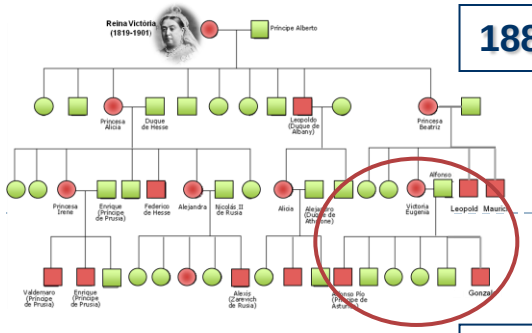
Hemorràgia cerebral 1853-1884

Alícia i el Duc de Hesse



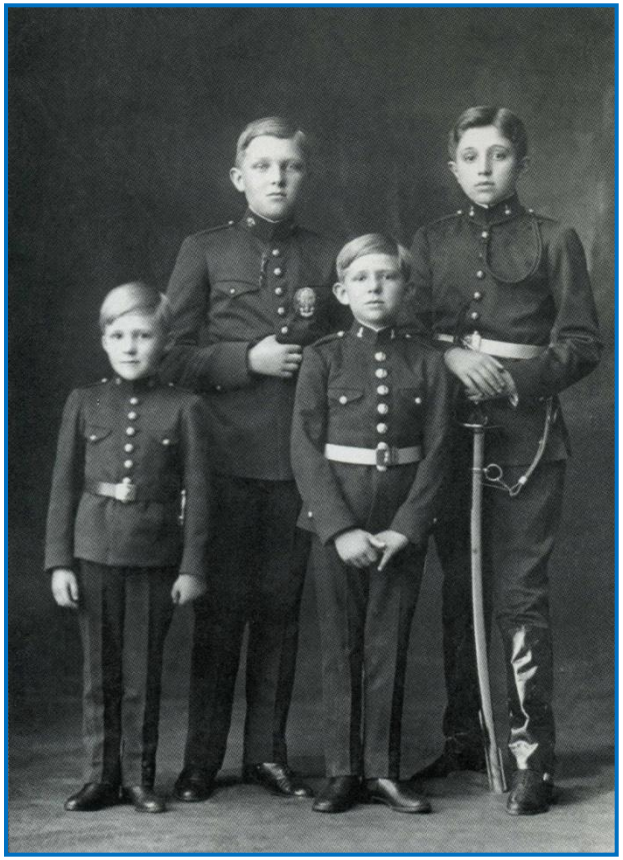
Hemorràgia cerebral

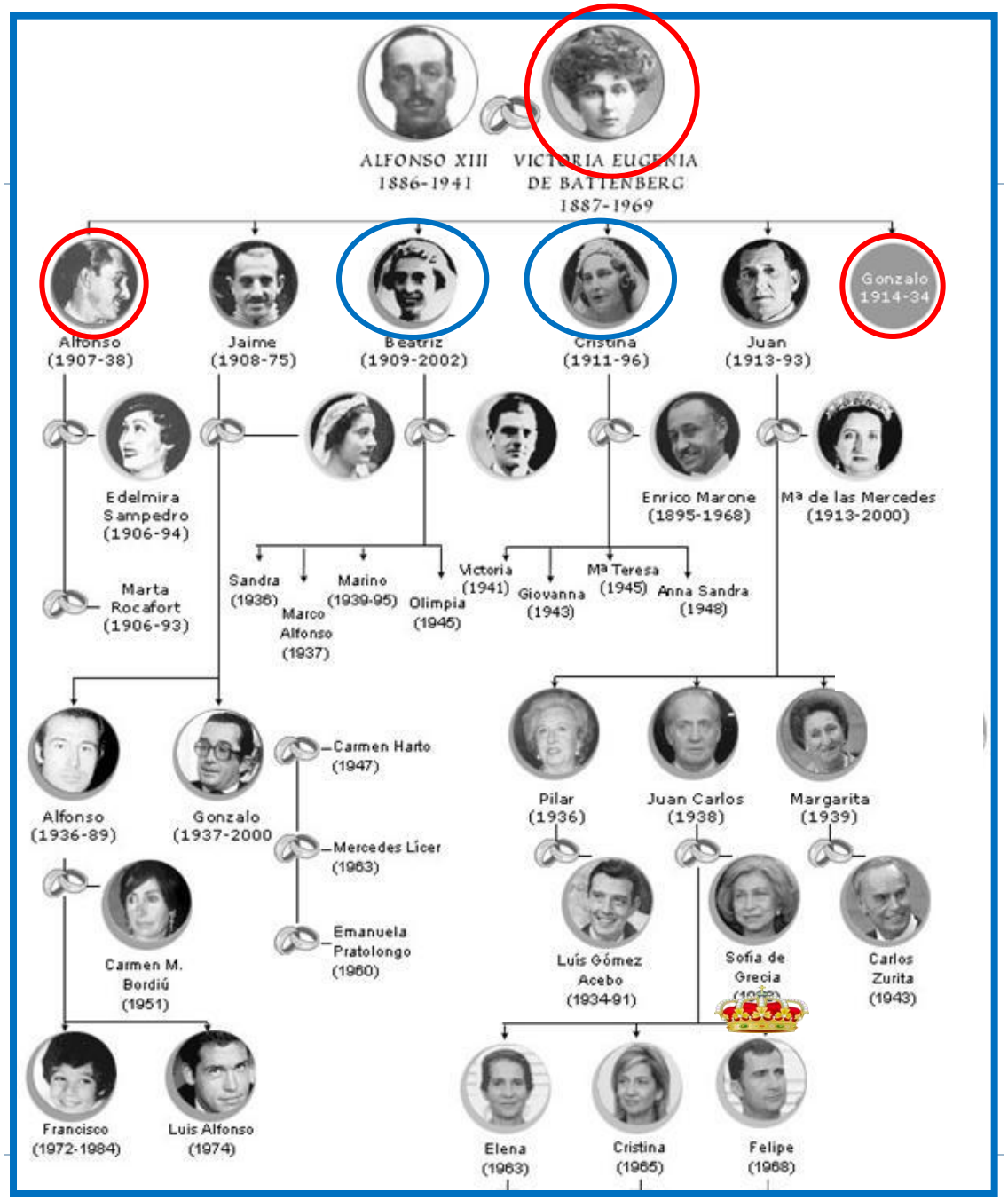
La reina Victòria Eugènia



1889-1922

1890-1914




ALFONSO XIII
1886-1941
VICTORIA EUGENIA
DE BATTENBERG
1887-1969
Alfonso
(1907-38)
Jaime
(1908-75)
Beatriz
(1909-2002)
Cristina
(1911-96)
Juan
(1913-93)
Gonzalo
1914-34
Edelmira
Sampedro
(1906-94)
Marta
Rocafort
(1906-93)
Enrico Marone
(1895-1968)
Mª de las Mercedes
(1913-2000)
Sandra
(1936)
Marco
Alfonso
(1937)
Marino
(1939-95)
Olimpia
(1945)
Victoria
(1941)
Giovanna
(1943)
Mª Teresa
(1945)
Anna Sandra
(1948)
Alfonso
(1936-89)
Gonzalo
(1937-2000)
Carmen Harto
(1947)
Mercedes Licer
(1963)
Emanuela
Pratolongo
(1960)
Carmen M.
Bordiú
(1951)
Francisco
(1972-1984)
Luis Alfonso
(1974)
Pilar
(1936)
Juan Carlos
(1938)
Margarita
(1939)
Luís Gómez
Acebo
(1934-91)
Sofía de
Grecia
Carlos
Zurita
(1943)
Elena
(1963)
Cristina
(1965)
Felipe
(1968)

Carlos Elósegui Sarasola

450 El Restaurador Farmacéutico

BIBLIOGRAFIA

Vitaminas y hemofilia, por *Carlos Elósegui*, del Laboratorio de Investigaciones clínicas, y *Felipe Llopis*, Farmacéuttco. (Trabajo efectuado en el Laboratorio Central de Investigaciones Clínicas de la Facultad de Medicina de Madrid, del que es Director el profesor *G. Pittaluga*).

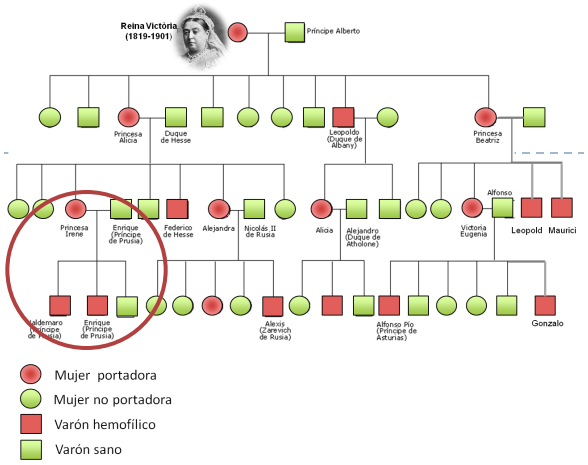
Hemos recibido el folleto cuyo título encabeza estas líneas, y que contiene el notable trabajo de los Sres. Elósegui y Llopis, publicado en *Archivos de Cardiología y Hematología*, en 8 de Agosto de 1926.

Archivos de Cardiología y Hematología (1920-1936)

Segons el *Diccionario Español de Especialidades Farmacéuticas (*San Sebastián: DEDEF, 1946, pàg. 564) és un '*complejo vitamínico antihemorrágico'.*

Irene i Enric de Prússia

Waldemar (1889-1945)

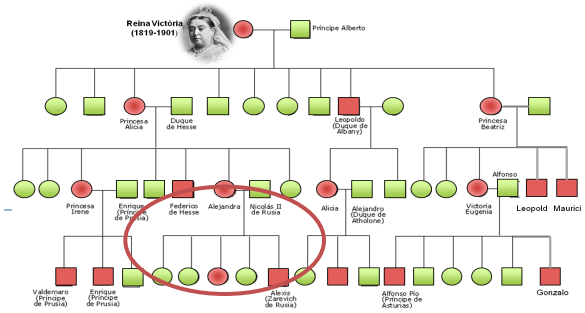


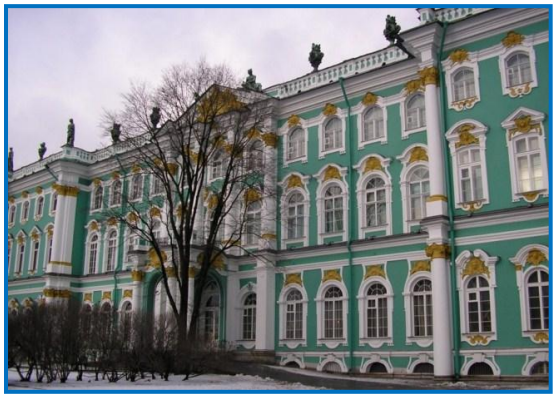
Biggs R et al.
Christmas disease: a condition previously mistaken for haemophilia.
Br Med J. 1952; 2 (4.799): 1.378-1.382

56 anys!

Segona Guerra Mundial

El tsarévitx Alexei





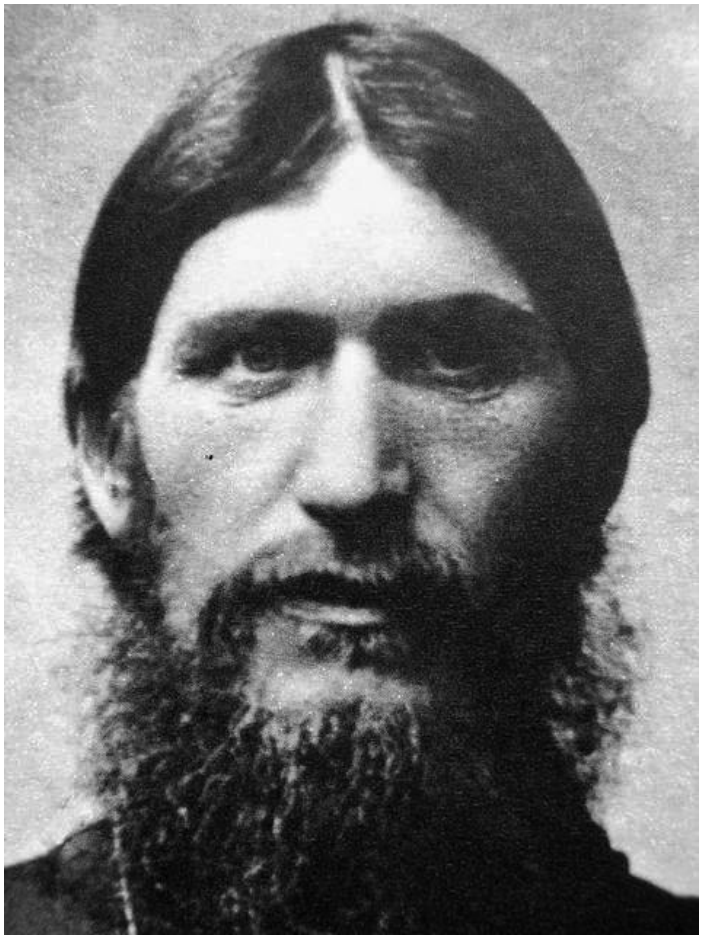
El tsarévitx Alexei

1904

El tsarévitx Alexei

El tsarévitx Alexei

Gregori Yefimovich Rasputin



1869-1918

La revolució russa

2-mar-1917

2-08-1917

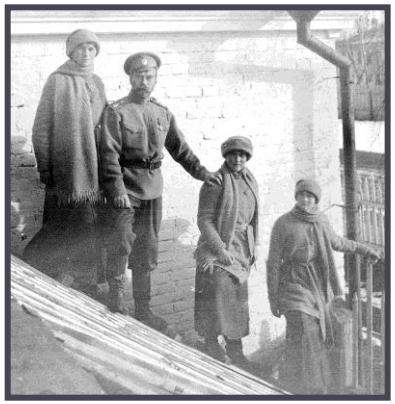
30-04-1918

Tobolsk

Yekaterinburg

Casa Ipatiev

Juliol 1918



Yakov Yurosky

Orientació diagnòstica

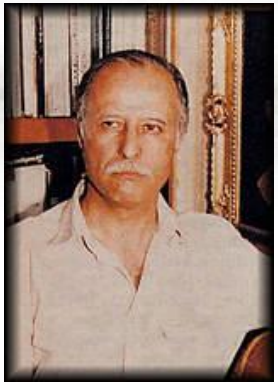
- Tipus d'hemofília
  - A 1/5.000
  - B 1/30.000

Hemofília A greu-moderada

- Gravetat
  - Greu
  - Moderada

Primer descobriment (1979)

1991



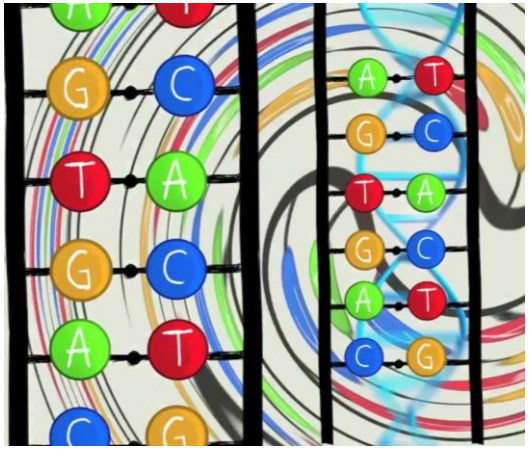
Gueli Riaov

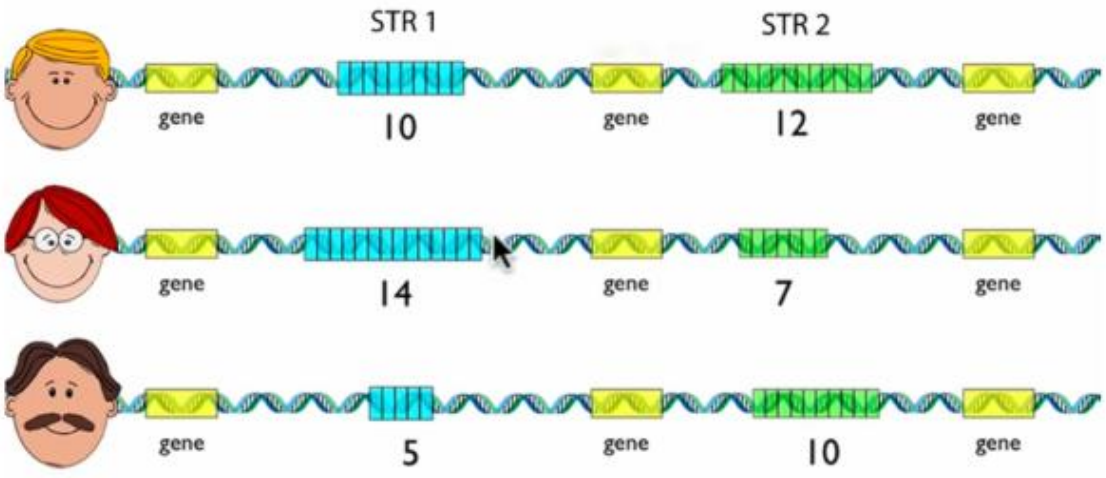
Alexander Avdonin

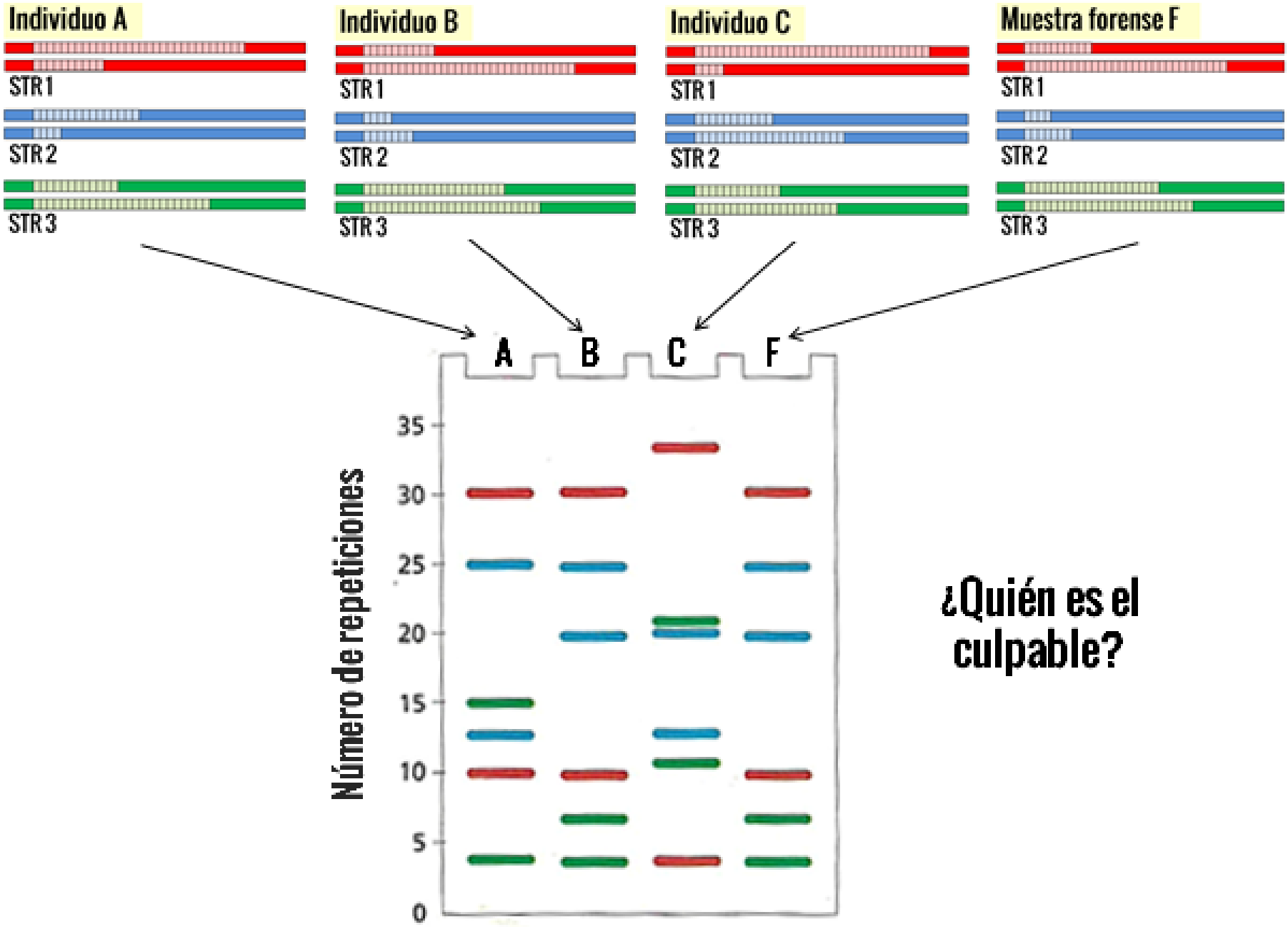
Identificació

Microsatèlits: *short tandem repeat*

- Seqüències d'un fragment d'ADN de 1-6 nucleòtids. La variació en el nombre de repeticions crea diferents al·lels de longituds diferents






Individuo A
STR 1
STR 2
STR 3
Individuo B
STR 1
STR 2
STR 3
Individuo C
STR 1
STR 2
STR 3
Muestra forense F
STR 1
STR 2
STR 3
A
B
C
F
Número de repeticiones
35
30
25
20
15
10
5
0
¿Quién es el culpable?

Short tandem repeats

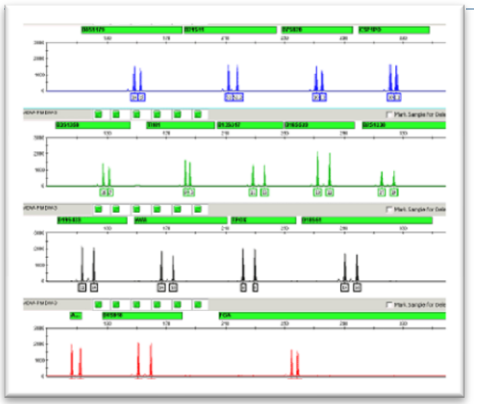
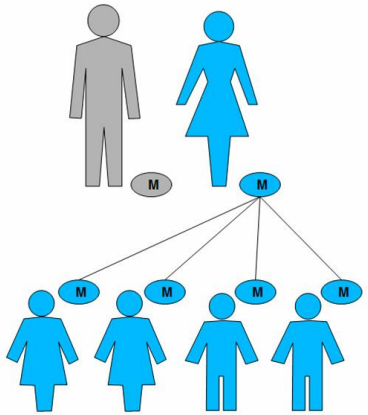


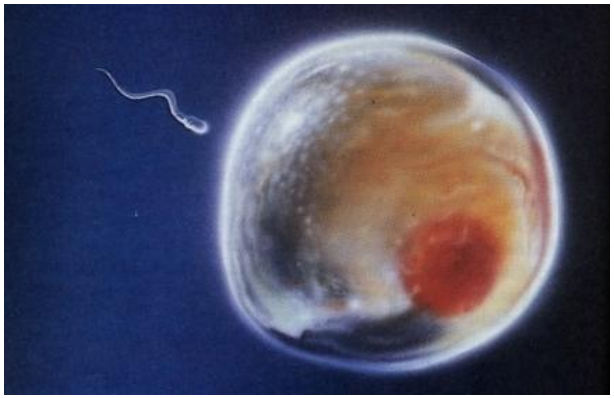
Table 1 STR genotypes[a] for the nine skeletons

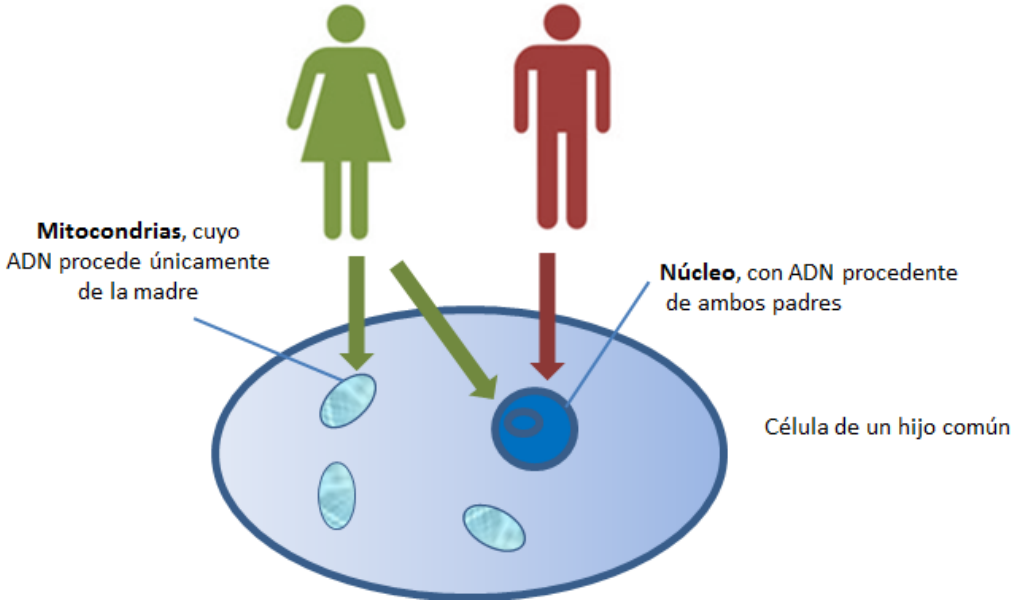
Skeleton	HUMVWA/31	HUMTH01	HUMF13A1	HUMFES/FPS	HUMACTBP2
1 (servant)	14,20	9,10	6,16	10,11	ND
2 (doctor)	17,17	6,10	5,7	10,11	11,30
3 (child)	15,16	8,10	5,7	12,13	11,32
4 (Tsar)	15,16	7,10	7,7	12,12	11,32
5 (child)	15,16	7,8	5,7	12,13	11,36
6 (child)	15,16	8,10	3,7	12,13	32,36
7 (Tsarina)	15,16	8,8	3,5	12,13	32,36
8 (servant)	15,17	6,9	5,7	8,10	ND
9 (servant)	16,17	6,6	6,7	11,12	ND

DNA mitocondrial en l'anàlisi forense

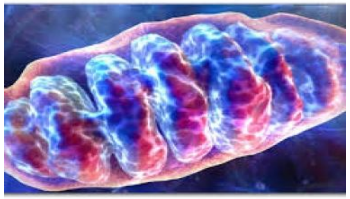


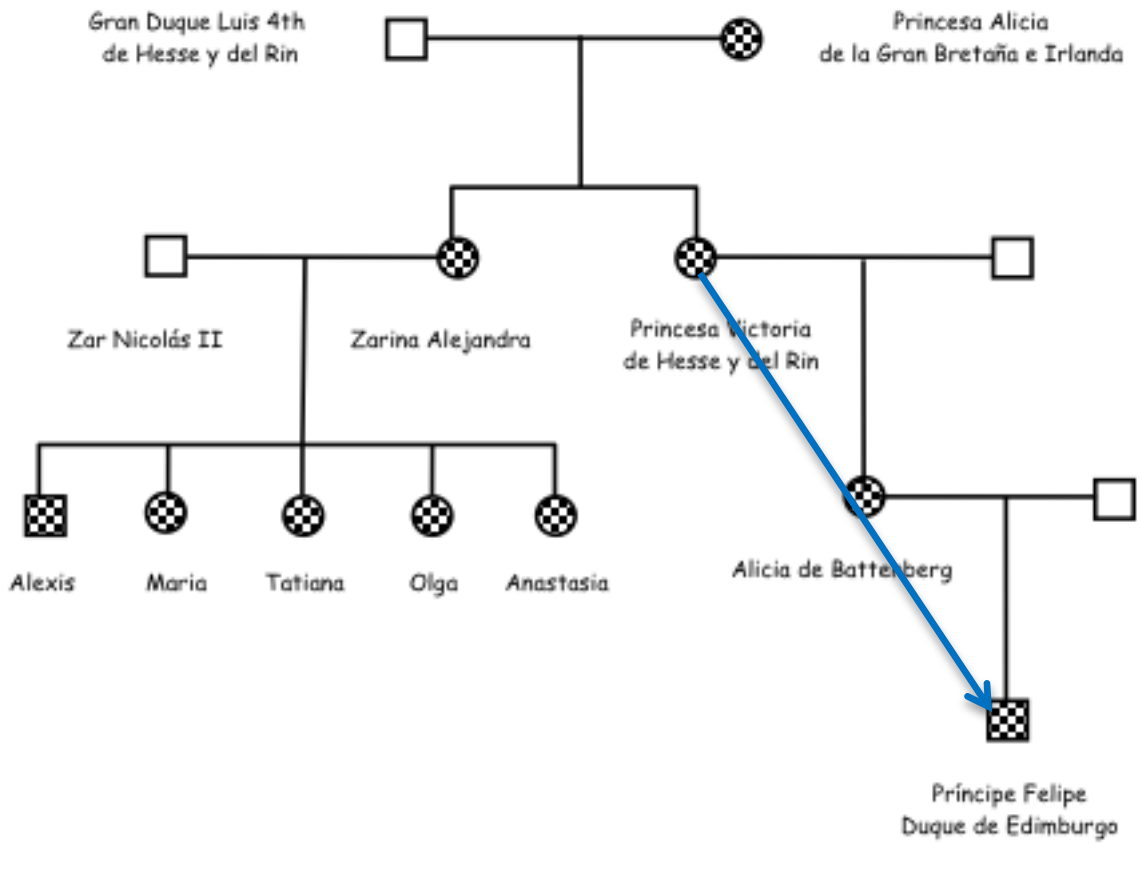
- Característiques
  - centenars o milers de còpies per cèl·lula
  - útil per a estudiar material escàs o degradat
  - herència materna

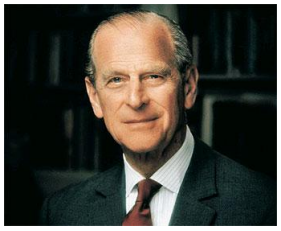




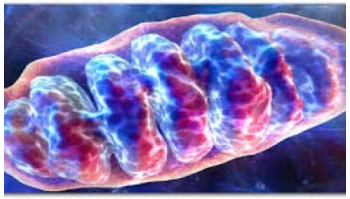
DNA mitocondrial de la tsarina Alexandra

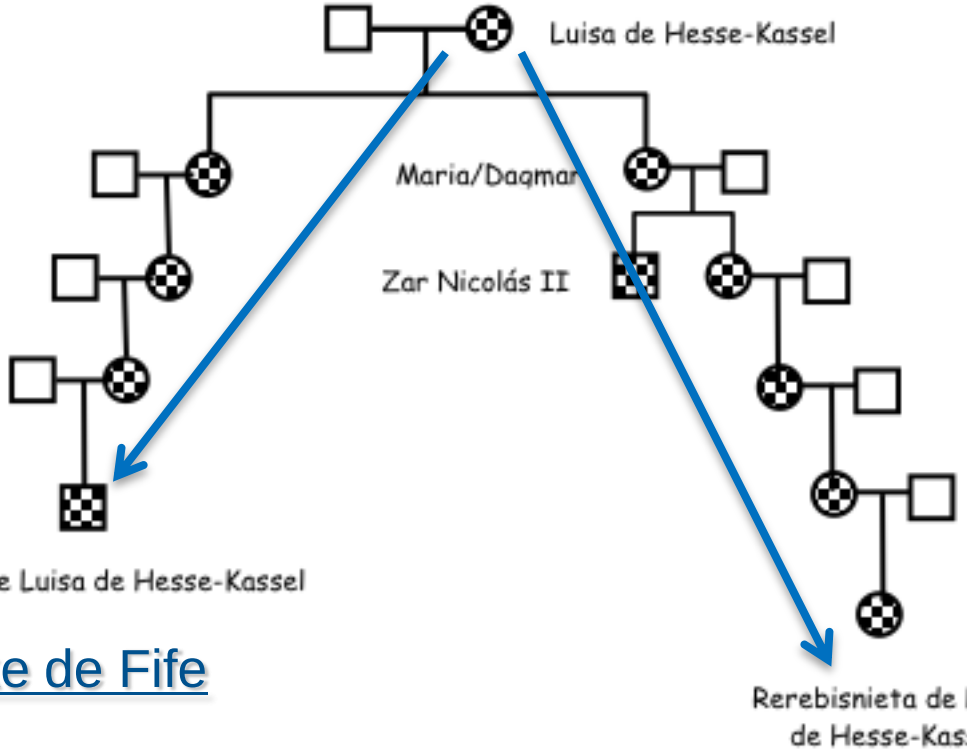


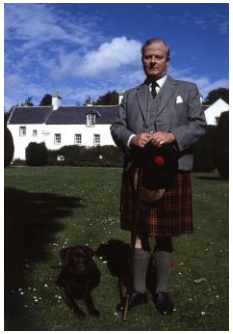




DNA mitocondrial del tsar Nicolás II



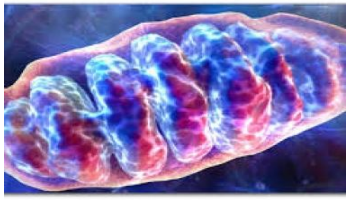




Duke de Fife

Xenia Cheremeteff-Sfiri

Anàlisi del DNA mitocondrial



	1	6	9	1	4	8	3	4	6	4	1	7
	1	2	6	6	6	7	9	9	9	0	1	5
	1	1	1	2	2	2	2	2	2	3	3	3
	6	6	6	6	6	6	6	6	6	6	6	6
	1	1	1	1	1	1	1	1	1	1	1	1
Secuencia de referencia	C	T	C	C	C	C	A	C	C	T	T	T
Sirviente 1 (?)	.	.	.	.	.	.	.	.	.	C	.	.
Sirviente 2 (?)	.	.	.	.	.	.	.	.	.	.	C	.
Sirviente 3 (?)	.	.	.	T	.	T	G	.	.	.	C	.
Dr. Botkin (?)	.	.	.	.	T	.	.	.	.	.	.	.
Zarina Alejandra (?)	T	.	.	.	.	.	.	.	.	.	.	C
Gran duquesa 1 (?)	T	.	.	.	.	.	.	.	.	.	.	C
Gran duquesa 2 (?)	T	.	.	.	.	.	.	.	.	.	.	C
Gran duquesa 3 (?)	T	.	.	.	.	.	.	.	.	.	.	C
Duque de Edimburgo	T	.	.	.	.	.	.	.	.	.	.	C
Zar Nicolás (?)	.	C	Y	.	.	.	.	T	T	.	.	.
Rebisnieto de L. Hesse-Kassel	.	C	T	.	.	.	.	T	T	.	.	.
Rebisnieta de L. Hesse-Kassel	.	C	T	.	.	.	.	T	T	.	.	.

Identification of the remains of the Romanov family by DNA analysis

Peter Gill[1], Pavel L. Ivanov[2], Colin Kimpton[1], Romelle Piercy[1], Nicola Benson[1], Gillian Tully[1], Ian Evett[1], Erika Hagelberg[3] & Kevin Sullivan[1]

Nine skeletons found in a shallow grave in Ekaterinburg, Russia, in July 1991, were tentatively identified by Russian forensic authorities as the remains of the last Tsar, Tsarina, three of their five children, the Royal Physician and three servants. We have performed DNA based sex testing and short tandem repeat (STR) analysis and confirm that a family group was present in the grave. Analysis of mitochondrial (mt) DNA reveals an exact sequence match between the putative Tsarina and the three children with a living maternal relative. Amplified mtDNA extracted from the remains of the putative Tsar has been cloned to demonstrate heteroplasmy at a single base within the mtDNA control region. One of these sequences matches two living maternal relatives of the Tsar. We conclude that the DNA evidence supports the hypothesis that the remains are those of the Romanov family.

Nature Genetics volume 6 february 1994

Establishing the identity of Anna Anderson Manahan

Nature Genetics volume 9 january 1995

Mark Stoneking
Terry Melton
Department of Anthropology,
Pennsylvania State University,
Pennsylvania 16802, USA

Julian Nott
Peninsula Films Ltd., 222 Kensal Rd.,
London W10 5BN, UK

Table 2 Mitochondrial DNA sequences

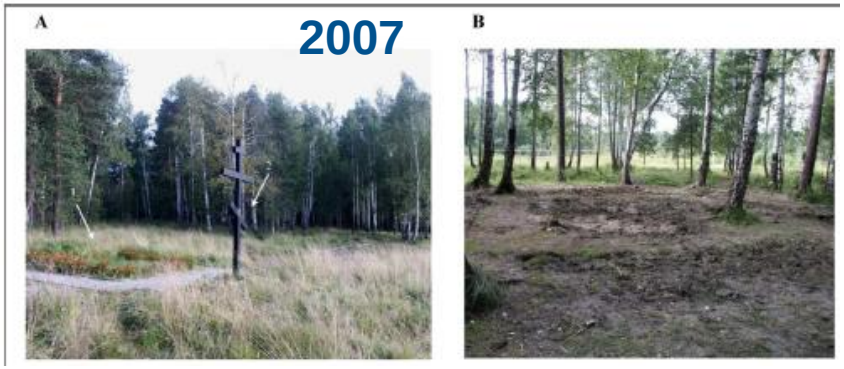
Origin of sample	DNA source	Length sequenced (bp)	Position within the non-coding region (ref. 11)					
			C 16111	T 16126	C 16266	C 16294	T 16304	T 16357
Reference sequence	–	–	C	T	C	C	T	T
Duke of Edinburgh (Great nephew of Tsarina)	Blood sample	403	T	.	.	.	.	C
Anna Anderson	Intestine sample	403	.	C	T	T	C	.
Anna Anderson	Hair sample	344–362 (3 hairs)	.	C	T	T	C	.
C. Maucher (Great nephew of Schanzkowska	Blood sample	380	.	C	T	T	C	.

	VWA	TH01	F13A1	FES/FPS	ACTBP2	AMELOGENIN
Tsar (Skeleton 4)[a]	15,16	7,9,3[b]	7,7	12,12	11,32	X,Y
Tsarina (Skeleton 7)[a]	15,16	8,8	3.2[c],5	12,13	32,36	X,X
Anna Anderson (intestine sample)	14,16	7,9.3	3.2,7	11,12	15,18	X,X

Genomic identification in the historical case of the Nicholas II royal family

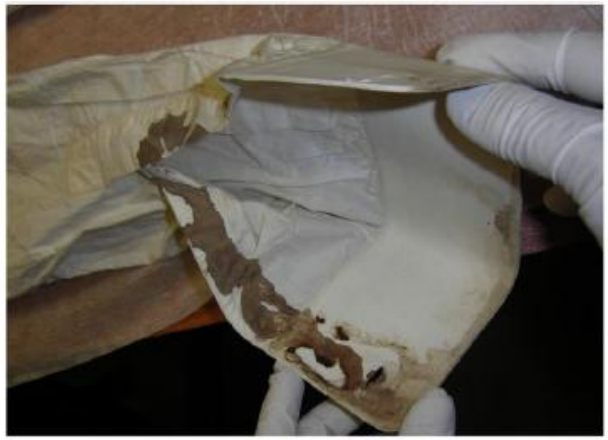
Evgeny I. Rogaev[a,b,c,d,1], Anastasia P. Grigorenko[b,d], Yuri K. Moliaka[b], Gulnaz Faskhutdinova[b], Andrey Goltsov[d], Arlene Lahti[e], Curtis Hildebrandt[e], Ellen L. W. Kittler[f], and Irina Morozova[a]

5258–5263 | PNAS | March 31, 2009 | vol. 106 | no. 13

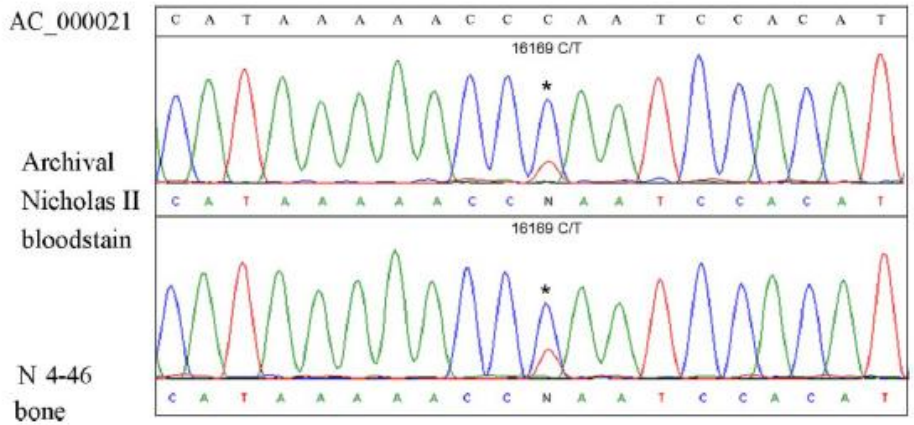


s. Results of genotypic analyses of damaged historical specimens were evaluated alongside samples from descendants of both paternal and maternal lineages of the European Royal families, and the results conclusively demonstrate that the recently found remains belong to children of Nicholas II: Prince Alexei and his sister. The results of our studies provide unequivocal evidence that the remains of Nicholas II and his entire family, including all 5 children, have been identified

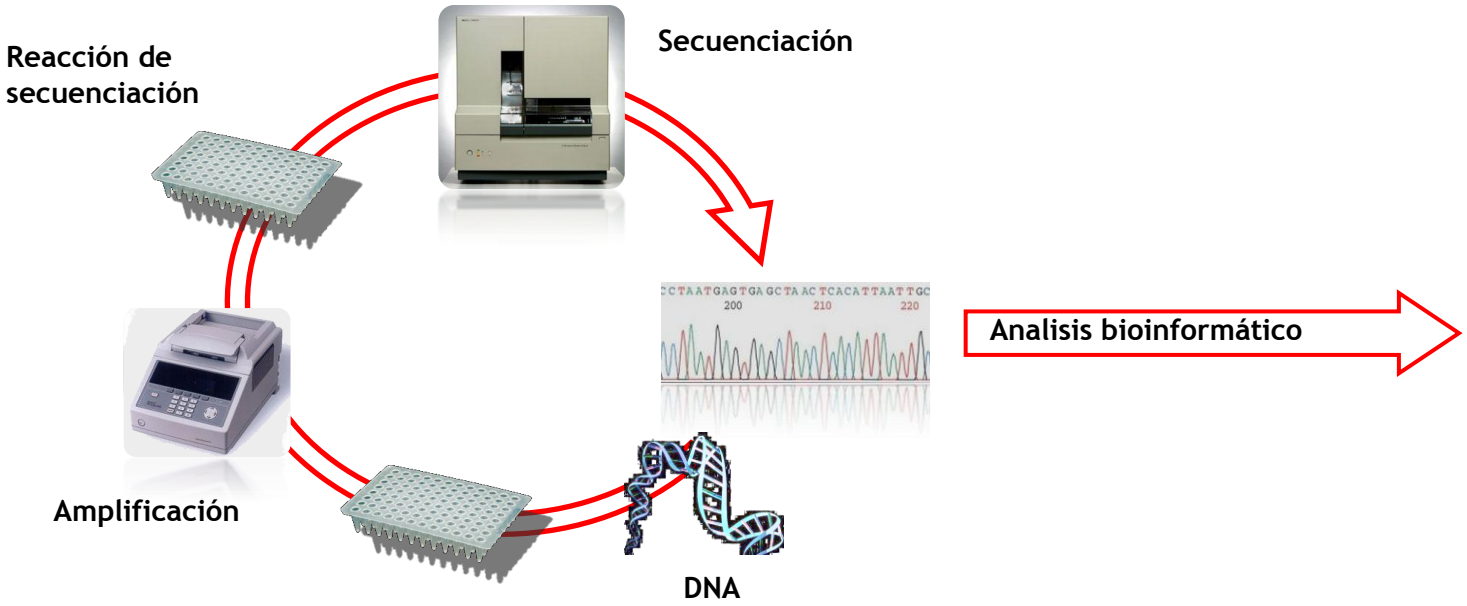
A



B



Identificació de l'hemofília

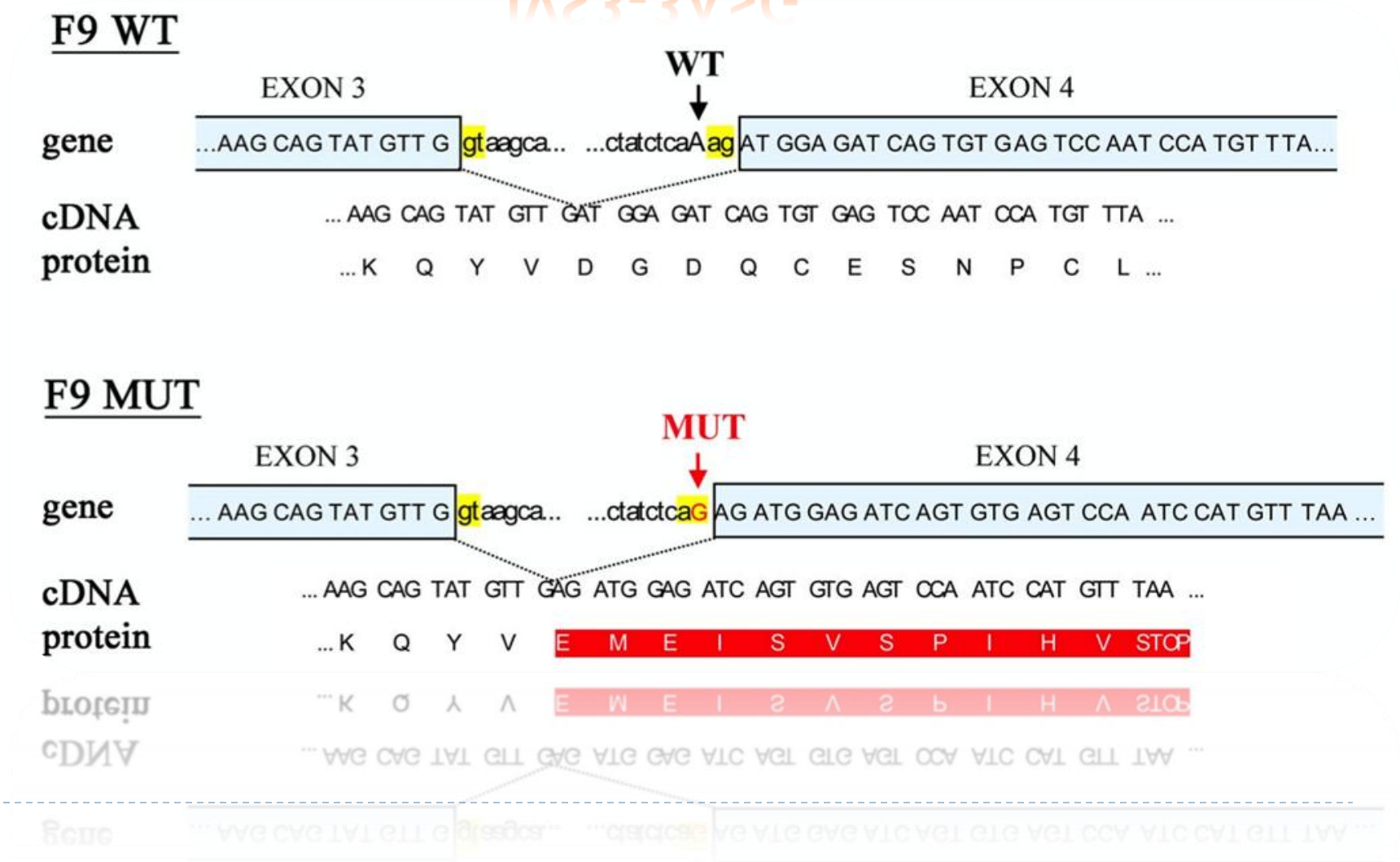


Gen *F8*

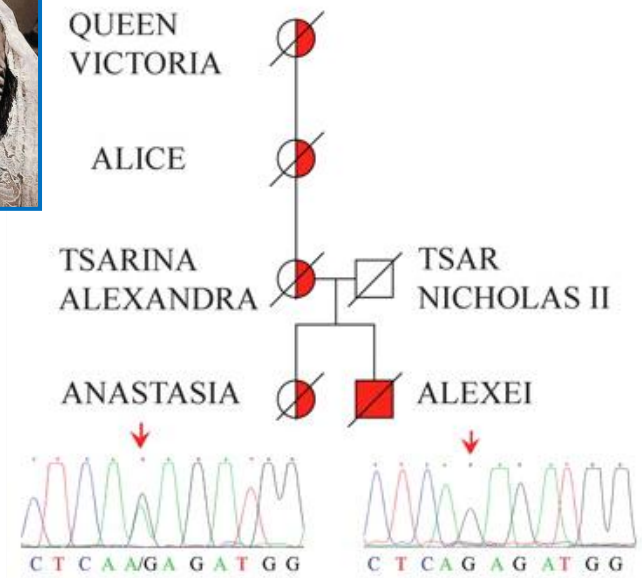
Gen *F9*

Mutació d'acoplament *(splicing)*

IVS3-3A>G



Identificació de portadora



Identificació de l'hemofília

IVS3-3A>G

Genotype Analysis Identifies the Cause of the "Royal Disease"

Evgeny I. Rogaev,[1,2,3,4]*† Anastasia P. Grigorenko,[1,2,3]* Gulnaz Faskhutdinova,[1] Ellen L. W. Kittler,[1] Yuri K. Moliaka[1]

SCIENCE VOL 326 6 NOVEMBER 2009

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*).

Confirmació de la mutació

Factor IX gene sequencing by a simple and sensitive 15-hour procedure for haemophilia B diagnosis: identification of two novel mutations

British Journal of Haematology, 2000, **111**, 549–551

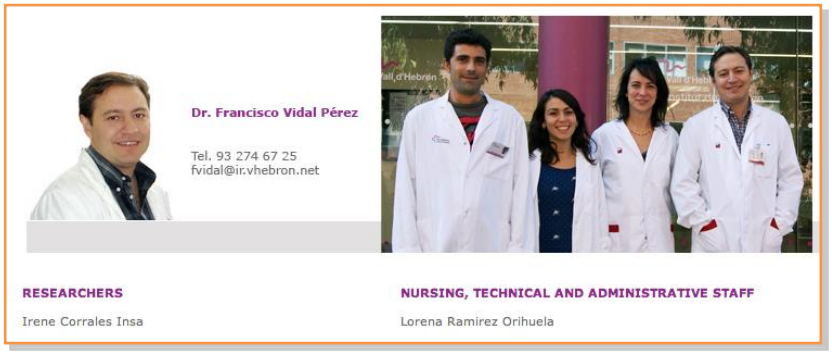
FRANCISCO VIDAL,[1] ELISENDA FARSSAC,[1] CARME ALTISENT,[2] LLUÍS PUIG[2] AND DOMINIQUE GALLARDO[1]
[1] *Unitat de Recerca del Centre de Transfusió i Banc de Teixits de Barcelona, and* [2] *Unitat d'Hemofília de l'Hospital General Vall d'Hebron, Barcelona, Spain*

HB016	Severe	< 1	Intron 3	–	aag/AT→gag/AT	–

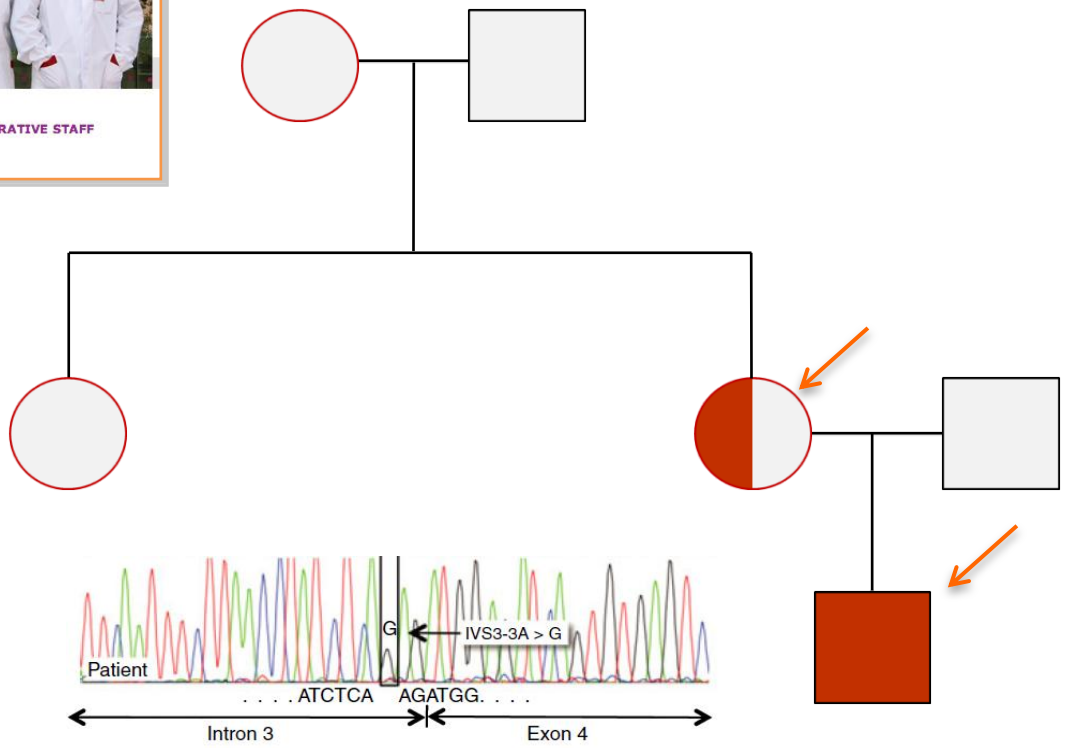
http://www.hemobase.com

IVS3-3A>G

Pacient espanyol amb la mutació real



Hemofília B greu



Informació addicional

The 'royal disease' mutation in a Spanish patient

L. RAMÍREZ,* C. ALTISENT,† R. PARRA*† and F. VIDAL*
*Unitat de Diagnòstic i Teràpia Molecular, Banc de Sang i Teixits; and †Unitat d'Hemofília de l'Hospital General Vall d'Hebron, Barcelona, Spain

To cite this article: Ramírez L, Altisent C, Parra R, Vidal F. The 'royal disease' mutation in a Spanish patient. *J Thromb Haemost* 2010; **8**: 2316–7.

See also Green P. The 'Royal Disease'. This issue, pp 2214–5.

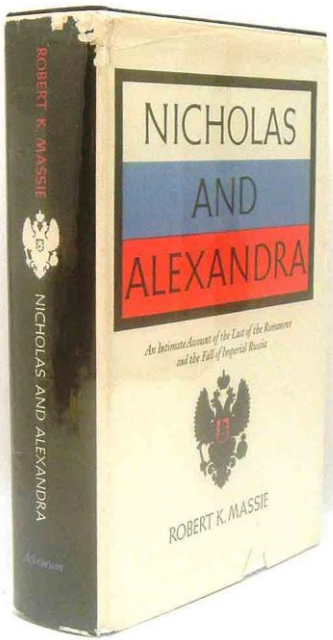
A recent article has unra
'royal disease', a blood
Victoria to European roy
severe form of hemophilia
creates an abnormal spli
authors convey their un
described patients with the
are now no living desc
hemophilia; hence, patient
represent excellent mimics
characteristics of the disea

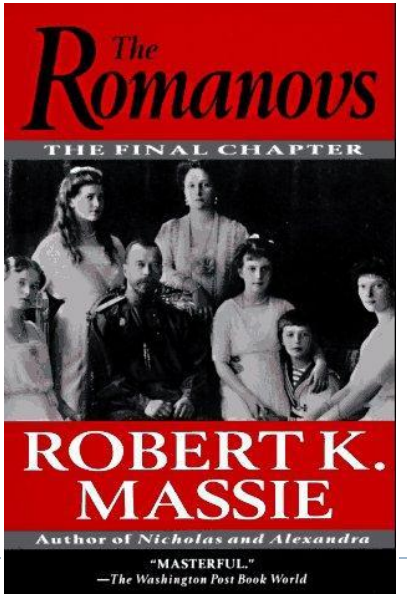
Was the 'Royal Disease' the severe form of hemophilia? Certainly, it would appear so from contemporary reports on spontaneous bleeds in affected individuals. Now that we have a living patient with the same mutation, it can also be seen that this mutation results in < 1% FIX coagulant activity, which fits with the modern definition of severe hemophilia B.

Hemophilia is probably one of the best known single-gene disorders in the eyes of the public, not because of its frequency (others are more common), but because it occurred in the royal families of Europe. Queen Victoria was a carrier of the disease and had a son, Prince Leopold, who suffered from it (Fig. 1). At least two of her daughters were also carriers and passed it on to their children. In total, at least nine male descendants of Queen Victoria had hemophilia (there may have been more: the disease was an embarrassment to the monarchy, who generally kept as quiet as possible about it, and some that died in infancy may not have been reported). By far the most well-known hemophiliac is Alexei, only son of Tsar Nicholas II and Tsarina Alexandra, grand-daughter of Queen Victoria. It is

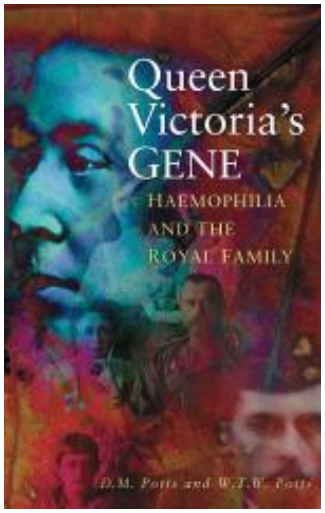
cases are attributable to a large inversion [6], this might be expected to be the prime suspect. This inversion could not be identified during next-generation sequencing protocols carried out by Rogaev *et al.*, as only the exonic regions were sequenced, and the inversion breakpoints are within intronic repeats. In the end, however, it turned out to be a mutation just inside an intron of FIX that creates a novel acceptor splice site, thereby confounding normal splicing. This effect was at first predicted from the sequence, but was difficult to prove, as the FIX gene is expressed almost exclusively in the hepatocytes. The authors sought to confirm that this was indeed the pathogenic mutation: first, they demonstrated that the mutation caused mis-splicing in an exon trap assay (although this was in COS cells); and second, they noted that the mutation

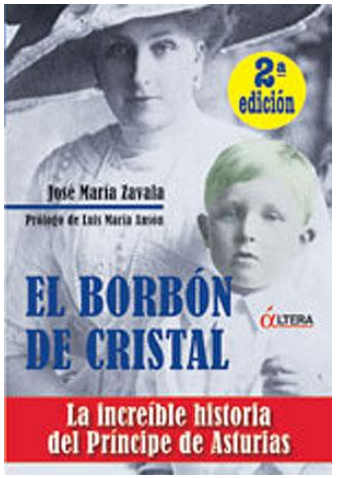
Interès cinematogràfic

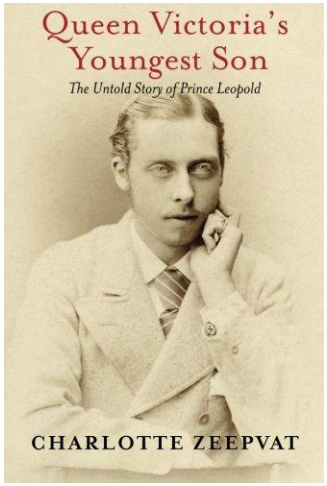


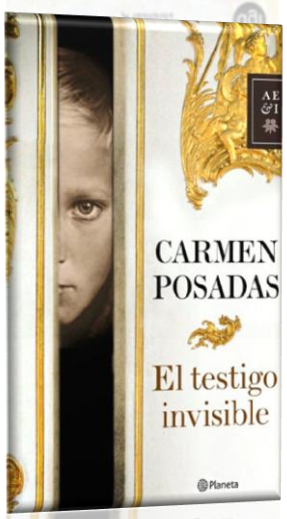


Interès literari

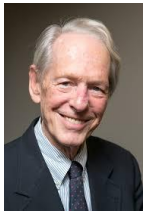


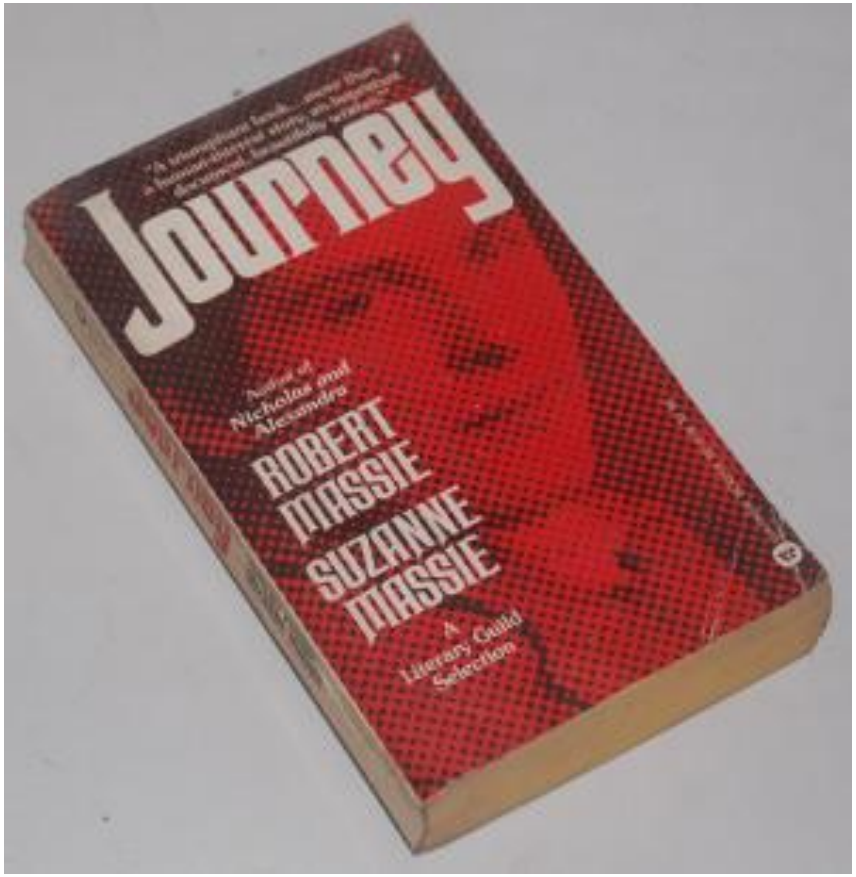


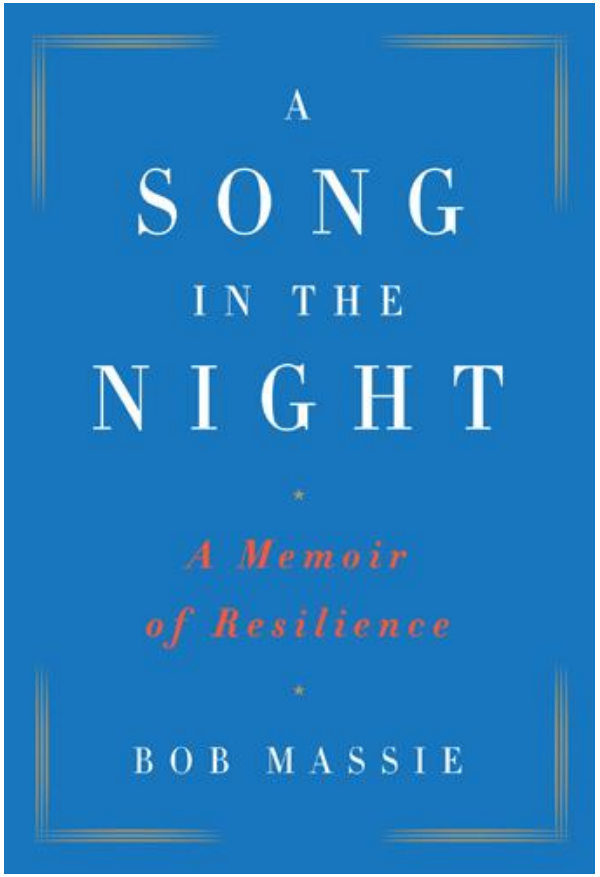




Suzanne – Robert - Bob





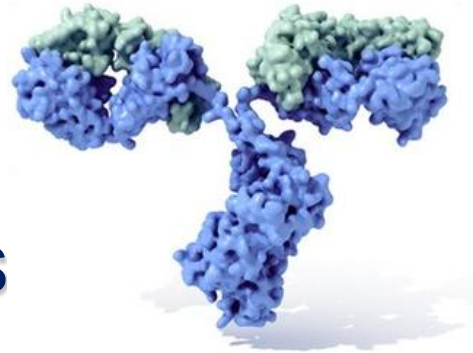



ΜΠΑΚΑΛΑΡΟΣ

Segle XXI. Esperança de vida

Cronologia del tractament

- 1900. . . . . Sense tractament
- 1930. . . . . Sang total
- 1950. . . . . Plasma fresc
- 1960. . . . . Crioprecipitat
  Factor VIII concentrat
- 1980. . . . . Inactivació vírica
  Concentrats d'alta puresa
- 1990. . . . . Recombinant de 1ª generació
- 2000. . . . . Recombinant de 2ª generació
  Recombinant de 3ª generació
  Assajos clínics en teràpia gènica
- 2010 . . . . . Noves molècules

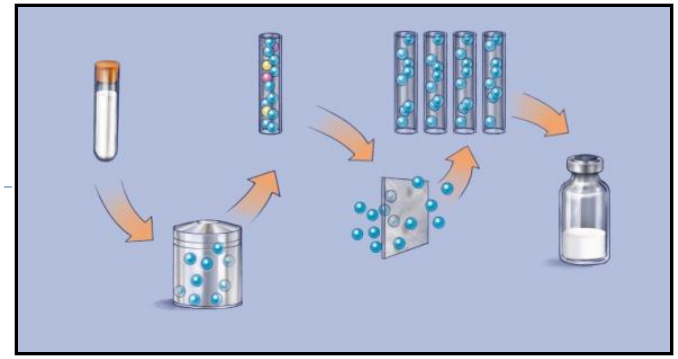


Evolució dels efectes secundaris

Transmissió de virus	+++	+/-
Sobrecarga de volum	++	-
Alèrgia o piròGens	+++	+/-
Inhibidor	+++	+++
Hemòlisi	+	-
Trombosi	-	+/-
Inmunosupressió	++	+/-

Factors recombinants

classificació

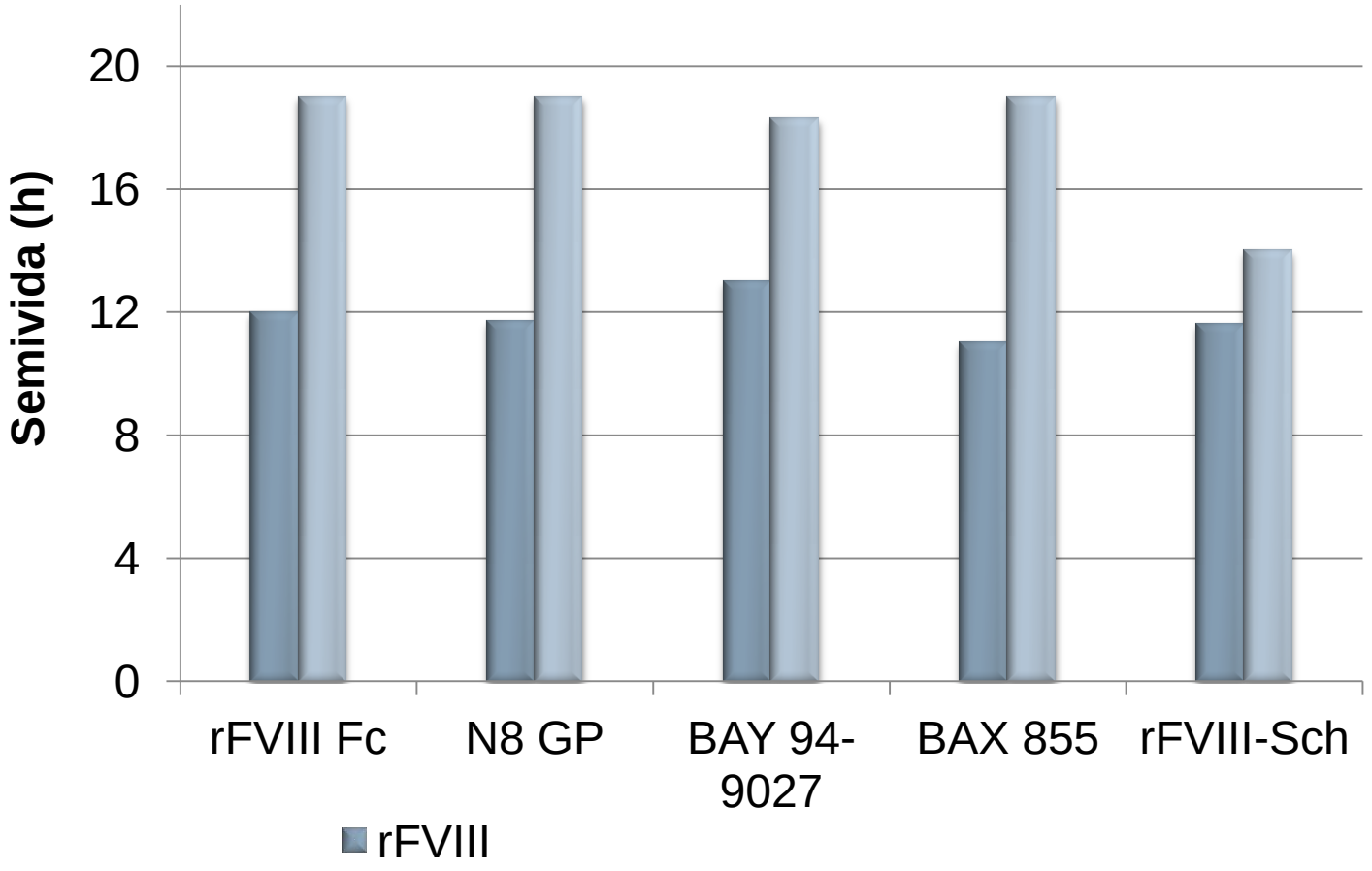


Products	Cell line	Gene	Protein in culture medium	Murine mAbs	Human albumin as stabilizer	Viral inactivation removal	Generation
Recombinate	CHO	VIII VWF	Bovine albumin, insulin, aprotinin	Yes	Yes	No	1
Helixate NexGen Kogenate-Bayer	BHK	VIII	Human albumin	Yes	No	SD and high salt concentration hold step	2
Refacto	CHO	B domain deleted VIII	Human albumin, insulin, aprotinin	Yes	No	SD	2
Advate	CHO	VIII VWF	No	Yes	No	SD	3
NovoSeven	BHK	VII	Foetal calf serum (Bovine)	Yes	No	SD	2
Benefix	CHO	IX	No	No	No	NF	3

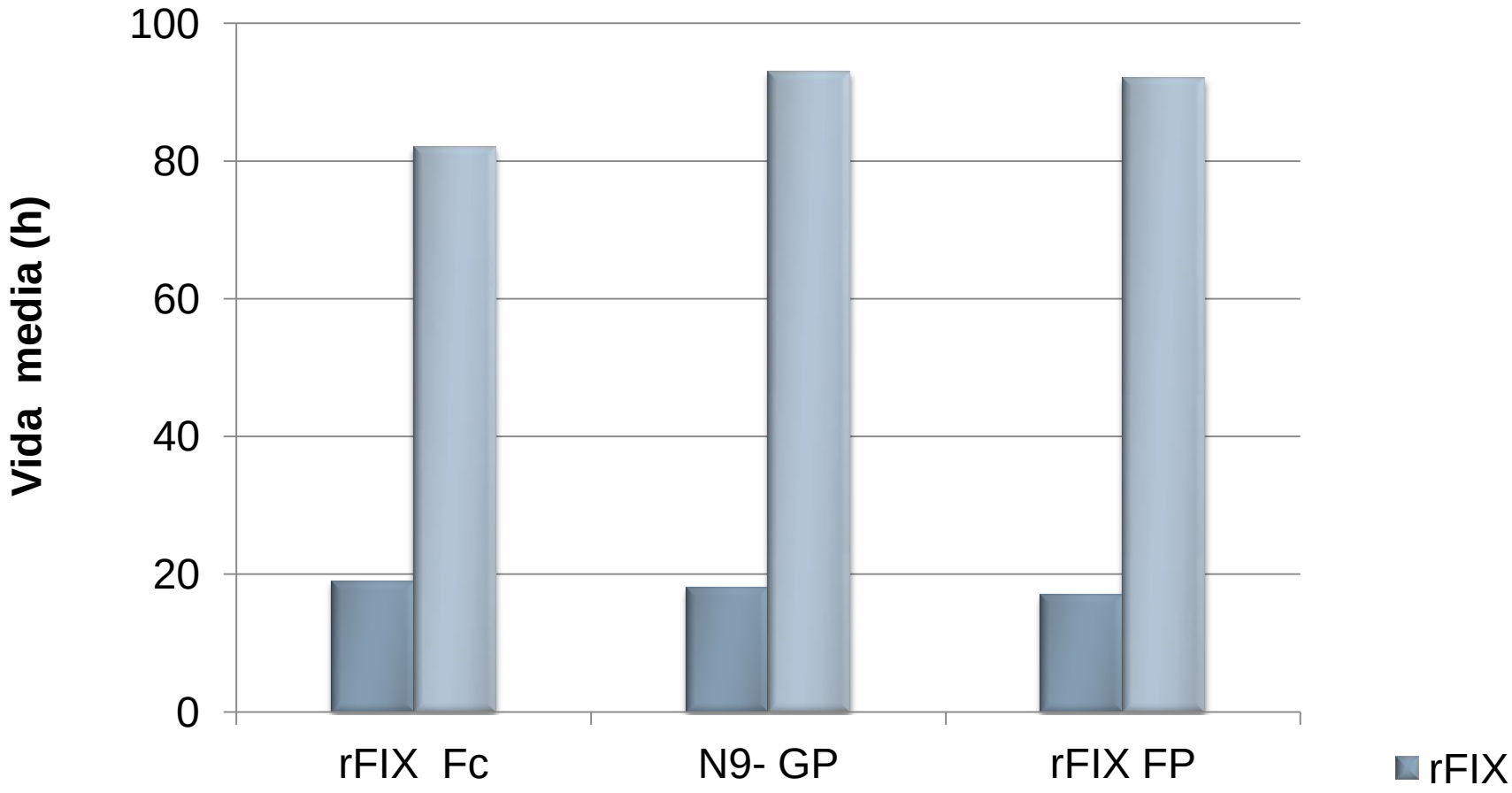
BHK, baby hamster kidney; CHO, Chinese hamster ovary; SD, solvent detergent; NF, nanofiltration; mAbs, monoclonal antibodies.

Refacto AF | **Nuwik** | **Novoeight** | **Rixubis**

Semivida del FVIII



Semivida del FIX



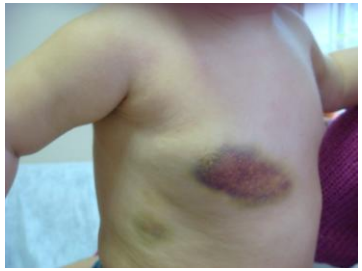
Impacte potencial en la profilaxi

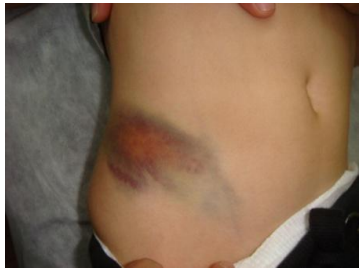
- *Més temps amb nivells de factor >1%*
- *Infusions més espaiades*
- *Menys nombre d'hemorràgies*
- *Conservació de l'estructura i funció de l'articulació*
- *Estil de vida més actiu*

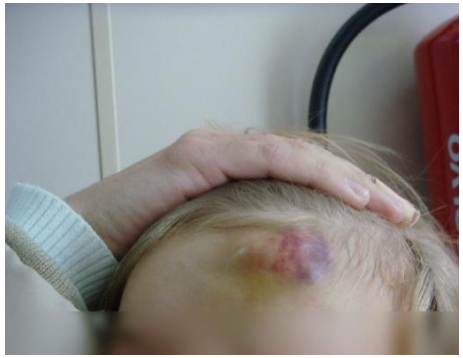
Drugs (2015) 75:1587–1600

Cas clínic

- Part per cesària al 1998
- Hematomes espontanis des dels 6 mesos
- Diagnòstic d'hemofília B greu als 8 mesos
- Primer tractament als 9 mesos
- Indicació: traumatisme cranial

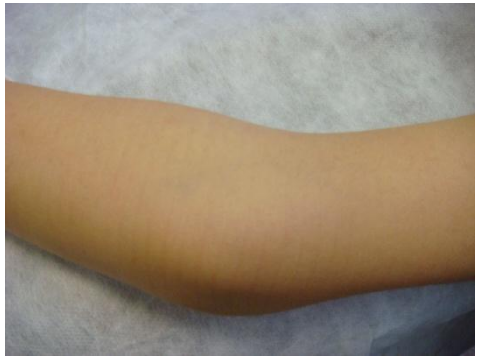


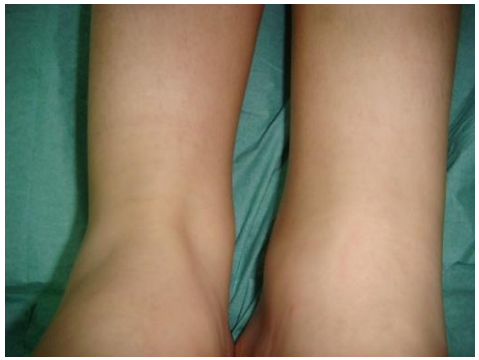




Cas clínic

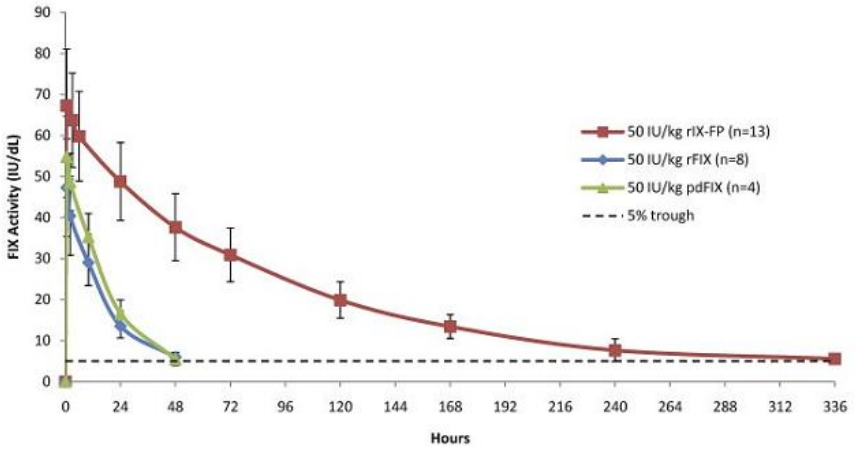
- Primera hemartrosi al colze esquerre als 9 mesos
- Hemartrosi del maluc dret als 3,5 anys
- Profilaxi 2 dies a la setmana
- Hemartrosi del maluc als 4 anys
- Hemartrosi postraumàtica al colze als 7 anys
- Hemartrosi postraumàtica al turmell als 8 anys





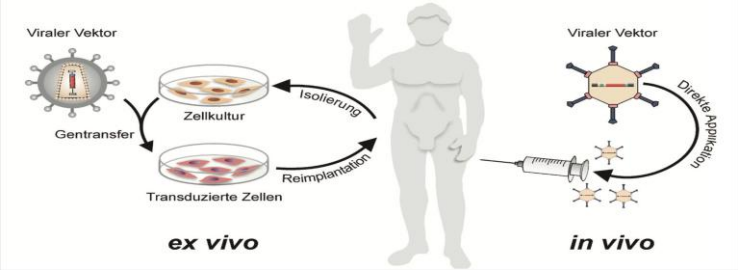
Cas clínic

- Tractament
  - FIX plasmàtic:1998
  - FIX recombinant: 1999
  - FIX de semivida llarga: 2012
- Sense seqüeles articulars
- Escolarització normal



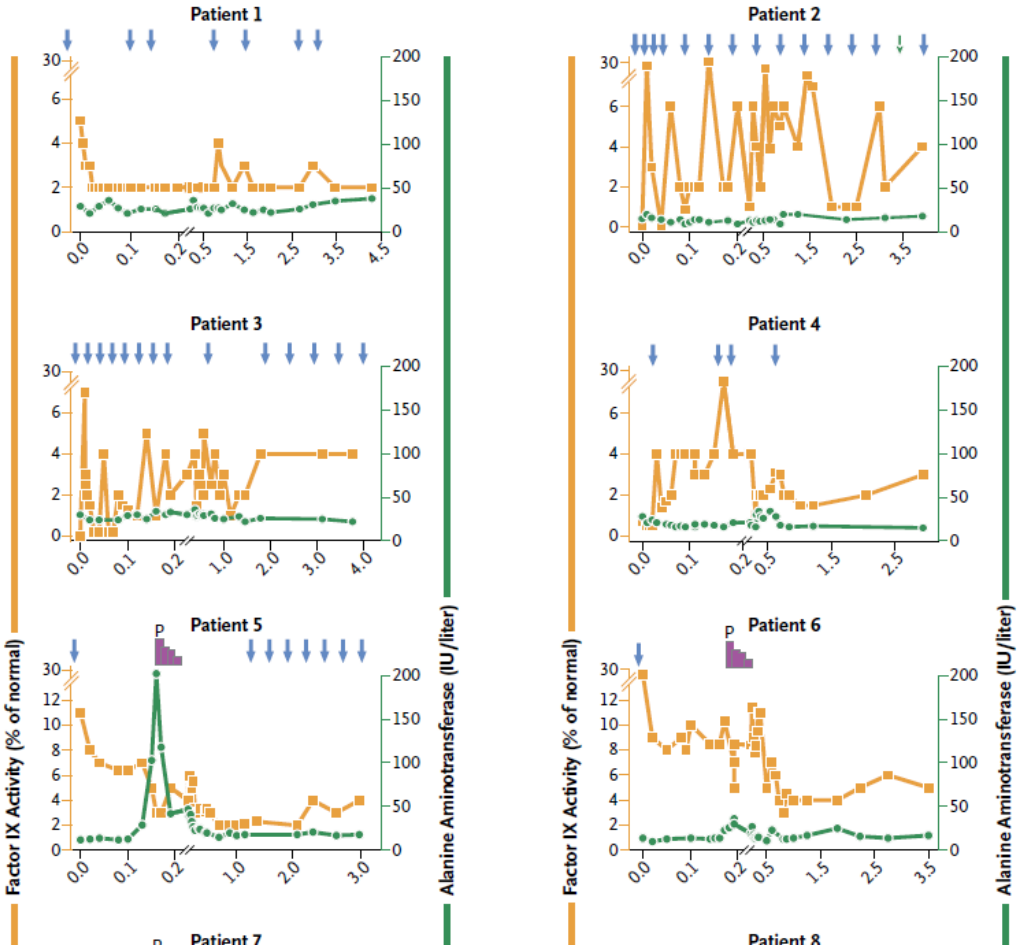
14 dies

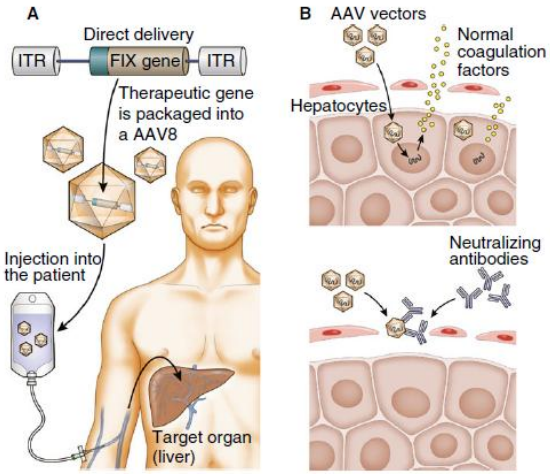
Teràpia gènica

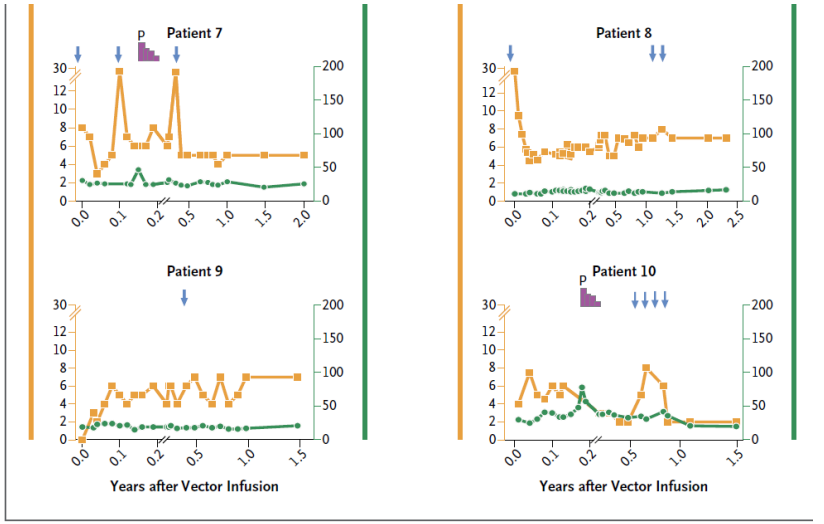


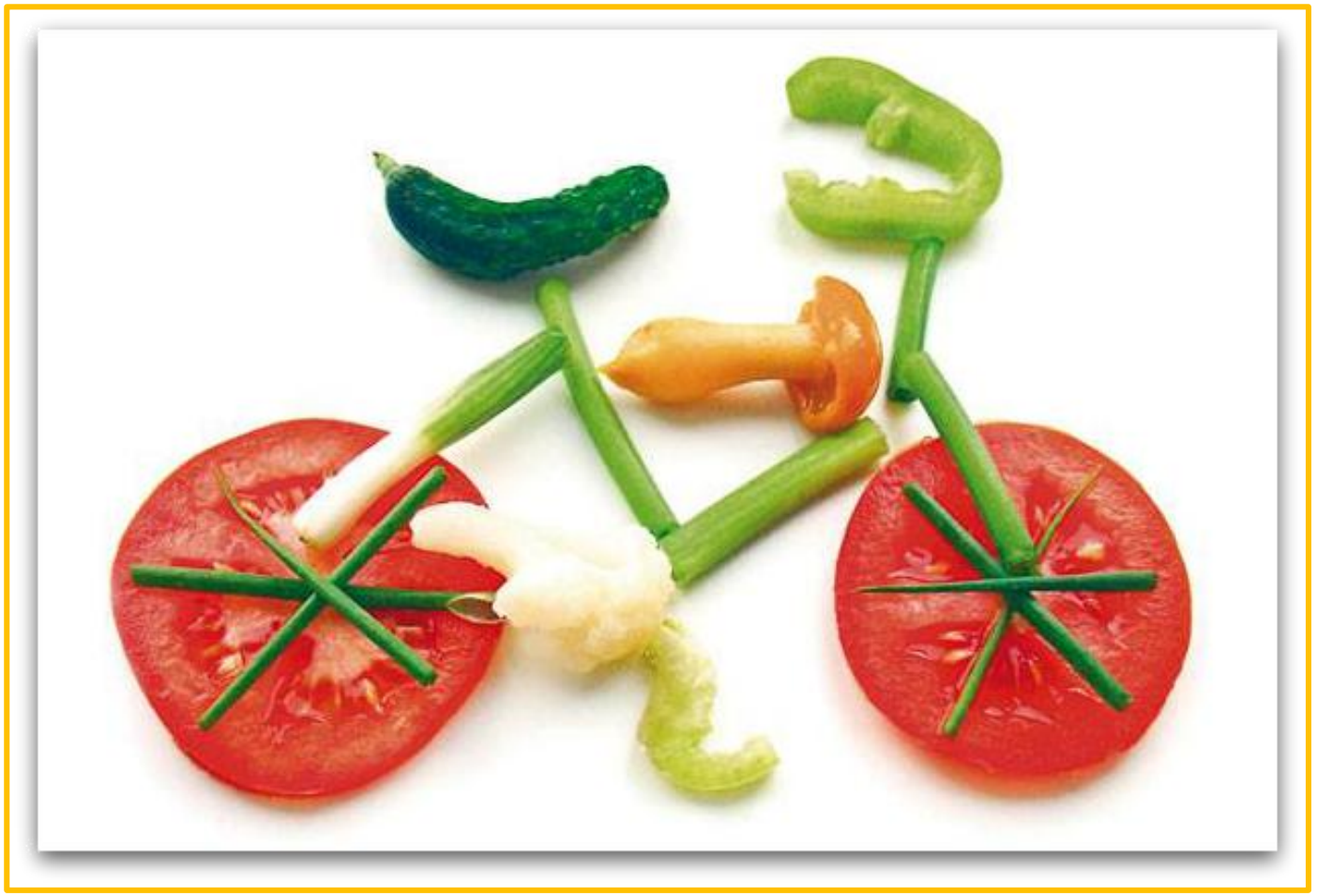
Laboratori farmacèutic	Indicació	Vector	Construcción gènica	Nombre de malalts
Baxter/Asklepios Bio	Hemofília B	AAV8	FIX Padua	7
Pfizer/Spark	Hemofília B	AAV8 modificat (AAV8-hFIX 19)	FIX Padua	4
UniQure BV/Chiesi	Hemofília B	AMT-060 (AAV5-hFIX)	wt FIX	5
St Jude	Hemofília B	scAAV 2/8-LPI-hFIXco	wt FIX	10
BioMarin/St Jude/UCL	**Hemofíia A**	**BMN270 (hFVIII)**	**BDD FVIII**	**8**

Long-Term Safety and Efficacy of Factor IX Gene Therapy in Hemophilia B

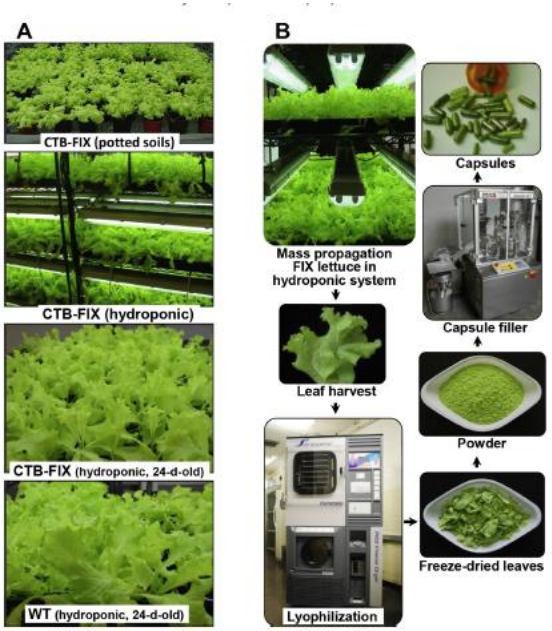








Proteïnes vegetals



Oral Tolerance Induction in Hemophilia B Dogs Fed with Transplastomic Lettuce

Roland W. Herzog,[3,5] Timothy C. Nichols,[2,5] Jin Su,[1,5] Bei Zhang,[1] Alexandra Sherman,[3] Elizabeth P. Merricks,[2] Robin Raymer,[2] George Q. Perrin,[3] Mattias Häger,[4] Bo Wiinberg,[4] and Henry Daniell[1]

[1]Department of Biochemistry, School of Dental Medicine, University of Pennsylvania, Philadelphia, PA 19104, USA; [2]Department of Pathology and Laboratory Medicine, The University of North Carolina, Chapel Hill, Chapel Hill, NC 25716, USA; [3]Department of Pediatrics, College of Medicine, University of Florida, Gainesville, FL 32610, USA; [4]Global Research, Novo Nordisk A/S, Måløv 2760, Denmark

Molecular Therapy Vol. 25 No 2 February 2017

Conclusió

- El segle XXI ha començat oferint múltiples opcions terapèutiques, les quals prometen atendre les necessitats dels malalts amb hemofília, fins i tot la possible curació

- Per aconseguir aquest repte, és necessària la col·laboració dels malalts, dels metges i dels laboratoris farmacèutics

- L'hemofília, una malaltia amb llums i ombres ha assolit una esperança de vida comparable a la de la població general