Challenges in the Diagnosis and Management of Hemophilia

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March 2014
Null Alleles

Lethal
Null Alleles

Bleeding
Isolation and characterization of a cDNA coding for human factor IX

(cDNA hybridization/DNA sequence analysis/blood coagulation)

KOTOKU KURACHI AND EARL W. DAVIE
Department of Biochemistry, University of Washington, Seattle, Washington 98195
Contributed by Earl W. Davie, July 29, 1982

Factor IX Gene Cloned in Nov 1982
The complete 186,000 base-pair (bp) human factor VIII gene has been isolated and consists of 26 exons ranging in size from 69 to 3,106 bp and introns as large as 32.4 kilobases (kb). Nine kb of mRNA and protein-coding DNA has been sequenced and the mRNA termini have been mapped. The relationship between internal duplications in factor VIII and evolution of the gene is discussed.
Haemophilia

Benefits of cloning genes for clotting factors

from A.L. Bloom

Pathogenesis  Diagnosis  Therapy
Intrinsic Tenase Complex

FXa

FX

Ca²⁺ Ca²⁺

FVIIIa cofactor

10⁹-fold enhanced

Intrinsic Tenase Complex
Thrombin Generation Dynamics

Intrinsic Pathway Amplification

Threshold of activation

Initiation

CT

4.7 ± 0.2

Time (min)

TAT (nM)

Propagation

n=35

Thrombin Generation Dynamics
Thrombin Generation Dynamics

Intrinsic Pathway Amplification

Threshold of activation

Initiation

CT 4.7 +/- 0.2

Time (min)

TAT (nM)

n=35

Propagation
Factor VIII Gene 184 kb

FVIII mRNA 8.5 kb

F8A

F8B
chromosomal flexibility + repetitive elements = intrachromosomal recombination
Factor VIII Intron 22 Inversion
45% of Severe Hemophilia A
Factor VIII Intron 22 Inversion Mutation

Alternative—Long-range PCR/Reverse PCR
34 kb Genomic Sequence - Xq27

F.IX mRNA - 1.4 kbp

Factor IX Gene
Tsarevich Alexei and the Romanov Family
The Royal Hemophilia Mutation
Rogaev et al. Science October 2009

CTCAAAAG ATC

G
**F9 WT**

**EXON 3**

- **Gene**:
  - ...AAG CAG TAT GTT G...gtaagca...ctatctcaAag
  - WT: AT GGA GAT CAG TGT GAG TCC AAT CCA

- **cDNA**:
  - ...AAG CAG TAT GTT GAT GGA GAT CAG TGT GAG TCC AAT CCA TGT TTA...

- **Protein**:
  - ...K Q Y V D G D Q C E S N P C L...

**EXON 4**

**F9 MUT**

**EXON 3**

- **Gene**:
  - ...AAG CAG TAT GTT G...gtaagca...ctatctcaG
  - MUT: AG ATG GAG ATC AGT GTG AGT CCA ATC CAT

- **cDNA**:
  - ...AAG CAG TAT GTT GAG ATG GAG ATC AGT GTG AGT CCA ATC CAT GTT TAA...

- **Protein**:
  - ...K Q Y V...EME SI S V S P I H V STOP
CHRISTMAS DISEASE
A CONDITION PREVIOUSLY MISTAKEN FOR HAEMOPHILIA

BY

ROSEMARY BIGGS, M.D.
A. S. DOUGLAS, M.R.C.P.
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AND
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South Devon and East Cornwall Hospital, Plymouth

Case Reports

Case 1.—The patient was a boy named Christmas, aged 5 years. There was no history of haemorrhage in other members of the family. He had numerous episodes of haemorrhage dating from the age of 20 months, mostly resulting from injuries during play. He was transfused on numerous occasions: each transfusion resulted in abrupt cessation of bleeding.

Characterization of the Original Christmas Disease Mutation (Cysteine 206 to Serine) From Clinical Recognition to Molecular Pathogenesis

Taylor S.A.M., Duffin J., Cameron C., Teitel J., Garvey B., Lillicrap D.

Thrombosis and Haemostasis 67:63-65, 1992
Post-Pubertal Changes to F.IX in Normal Subjects and Hemophilia B Leiden Patients

![Graph showing FIX levels over age]

- **FIX**:
  - 100%
  - 75%
  - 50%
  - 25%

- **Age (years)**: 5, 10, 15, 20, 25, 30, 35, 40

- **Normal FIX Levels**
Post-Pubertal Changes to F.IX in Normal Subjects and Hemophilia B Leiden Patients

![Graph showing changes in FIX levels over age for normal and Hemophilia B Leiden patients.](image-url)
Hemophilia B Leiden Mutations
Canadian Hemophilia B Genotyping

Samples referred from 28 centers

282 samples referred for testing
271 reports generated

- 138 missense mutations (24 not in HMB database)
- 17 nonsense mutations (3 not in HMB database)
- 13 frameshift mutations (8 not in HMB database)
- 9 splice site mutations (1 not in HMB database)
- 6 Leyden promoter mutations
- 4 large deletions (most / all of gene)
Spectrum of Hemophilia Mutations

> 2,100 different F8 mutations
http://hadb.org.uk/WebPages/PublicFiles/MutationSummary.htm

> 1,100 different F9 mutations
http://www.factorix.org/
Canadian National Hemophilia Genotyping Laboratory

2000 –

Dept. Pathology & Molecular Medicine, Queen’s University

48% of registered hemophilia A pts genotyped
(91% mutation detection rate)

47% of registered hemophilia B pts genotyped
(92% mutation detection rate)
February 26th 2013

Dear Dr. Lillicrap,

I am a clinical and molecular geneticist currently working in clinical research in Dr. X’s group in ……… Medical Genetics. I am contacting you as we just found a known hemophilia B mutation (sequence variant) as an incidental finding in one of our families which we currently studying for other reasons using exome sequencing (the mutation is highly likely to be a true positive because of the very high coverage, several family members having it etc). Coincidentally the mother just phoned us because she is again pregnant.
Mortality of non-HIV infected Hemophiliacs - UK 1999
1960
- cryoprecipitate
- pd-concentrates

1985
- recombinant concentrates

2011
- nucleic acid-based therapies
- antibody-mediated therapies
- modified recombinant concentrates
Current Hemophilia Therapy

Safe and Effective

but....
Limitations to Current Hemophilia Treatment

• Inconvenient
  - repeated intravenous infusions

• Immunogenic
  - 25% inhibitor incidence in hemophilia A

• Costly

• Only available to ~30% of all hemophiliacs
Normal joints

Early evidence of chronic arthropathy

Moderate to severe chronic arthropathy

Years

10  20  30  40  50
Chronic Hemophilic Arthropathy
Intracranial hemorrhage in hemophilia

Severe Hemophilia

\[
\text{5/1,000/yr < 5 yrs}
\]

\[
\text{1-2\%/yr >55 yrs}
\]

Intracranial hemorrhage in hemophilia
Annual Clotting Factor Concentrate Budget for Canada

~$150 million

860 severe hemophiliacs - ~$175,000/pt/yr
Enhanced Commercial Opportunity

a) Intellectual property/Patent expiration

b) Opening of a global market
Hemophilia Therapy in 2014
For Severe Hemophiliacs <1% FVIII/FIX

On-demand therapy
~2-6 infusions/month  -  Chronic hemophilic arthropathy

Prophylactic therapy
1-3 infusions/week  -  Long-term musculoskeletal benefit
Benefit of Coagulation Concentrate Prophylaxis

Liesner at al
Br.J.Haem. 1996
<table>
<thead>
<tr>
<th></th>
<th>Prophylaxis</th>
<th>On Demand</th>
</tr>
</thead>
<tbody>
<tr>
<td># FVIII units infused</td>
<td>352,793</td>
<td>113,237</td>
</tr>
<tr>
<td>Joint Hemorrhages</td>
<td>0.63</td>
<td>4.89</td>
</tr>
<tr>
<td>Total Hemorrhages</td>
<td>3.27</td>
<td>17.69</td>
</tr>
</tbody>
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Manco-Johnson et al. NEJM 2007
Coagulation Factor Half-lives

Factor VIII  ~12 hrs  (x3/week)

Factor IX   ~24 hrs  (x2/week)

Therapeutic Goal  -  Weekly Prophylactic Infusions
500 units Daily

25 units/kg Q 2 days

50 units/kg Twice/week

50 units/kg Weekly
Strategies for Improved Hemophilia Therapies

Improved Factor Replacement

a) Enhanced proteins

a) Gene transfer
“Slow-release” delivery vehicle (eg liposomal factor)

Prolonged Protein Half-life

Hydrophilic Polymer Conjugation (eg PEGylation)

Variant Protein Generation (eg fusion factors)
Chemical Modification

Polyethylene glycol conjugation (PEGylation)

a) Linear vs branched PEGs

b) Random vs Site-specific

Other hydrophilic polymers (hydroxyethyl starch)
Random Chemical Modification

i) Heterogeneous product
ii) Interference with cofactor activity
iii) FVIII Assay interference
Site-Specific Chemical Modification

23 surface exposed sites – mutated to cysteine
Conjugated with PEG-maleimide
1.5 to 2-fold Increase of FVIII Half-life
Factor IX/FVIII Fusion Proteins

- FIX or FVII
- Albumin
- FXIIa cleavage sequence
- FIX or FVIII
- IgFc
Albumin and IgFc as Fusion Partners

• Present in plasma at high concentrations
  IgG 12 g/L    Albumin  42 g/L

• Half-life ~25 days

• Same mechanism of rescue (FcRn receptor)
  - present in endothelial endosomes
Other Properties of Fc Fusion Proteins

(Mediated by FcRn receptor)

a) Transplacental transport

b) Mucosal epithelial transport
Hemophilia A

Ca²⁺ Ca²⁺
Hemophilia A

FIX variants with enhanced proteolytic capability

FIXa

Ca$^{2+}$

FX

Ca$^{2+}$

Hemophilia A
Hemophilia A

FVIIIa Mimetic
Bispecific Antibody therapy

Hemophilia A
REALISTIC HOPE FOR EVEN BETTER HEMOPHILIA THERAPIES

23rd March 1994

Realistic hope for even better hemophilia therapies
Hemophilia Gene Therapy

Viral Gene Transfer
- retrovirus
- adenovirus
- adeno-associated Virus

Cell-based Gene Therapy
- embryonic stem cells
- adult stem cells
- iPS cells

Mutation Repair
- zinc finger nucleases
- TALENs
- Crisp nuclease system

Non-Viral Gene Transfer
- hydrodynamic delivery
- oral chitosan nanoparticles
- targeted nanoparticle

Cell-based Gene Therapy
- embryonic stem cells
- adult stem cells
- iPS cells

Mutation Repair
- zinc finger nucleases
- TALENs
- Crisp nuclease system

Non-Viral Gene Transfer
- hydrodynamic delivery
- oral chitosan nanoparticles
- targeted nanoparticle

Viral Gene Transfer
- retrovirus
- adenovirus
- adeno-associated Virus
In Vivo Gene Transfer

Harvest/isolate autologous, long-lived progenitor cells

Deliver normal clotting factor gene

Expand cell numbers

Ex Vivo Gene Transfer
Hemophilia Gene Transfer: Pre-2011

Small Phase I/II Clinical Trials Involving a total of ~50 patients

- Ex vivo retroviral vector into autologous fibroblasts (FIX)
- Ex vivo electroporation into autologous fibroblasts (FVIII)
- IV retroviral vector (FVIII)
- IV adenoviral vector (FVIII)
- IM AAV vector (FIX)
- Hepatic artery AAV vector (FIX)
Adeno-associated Virus
Adeno-associated Viruses

- Non-pathogenic human parvoviruses
- Many serotypes
- Require helper virus to replicate
- Pre-existing immunity in 20-80% of humans
AAV Factor IX Liver Trial

Avigen-sponsored AAV2 liver-directed trial

AAV2 administration to liver via hepatic artery
Patient “E” – Best Result Yet
Pre-Existing AAV Immunity

1. Anti-AAV Abs - prevent transduction (bind to and clear infused viral vectors)

2. Anti-AAV T cells - transduced cell cytotoxicity

Anti-AAV2 Abs ~60%

Anti AAV8 Abs ~30%
University College, London/St. Jude, Memphis

Ted Tuddenham, Amit Nathwani (UCL)
Andrew Davidoff (St. Jude)

Phase I/II Study of Systemic AAV8 FIX Gene Transfer

• Peripheral vein infusion

• Hepatotropic AAV8 (codon-optimized FIX cDNA)

• Three dose escalations - 2 pts/dose

• No immunosuppression
Subject 8; $2 \times 10^{12}$ vg/kg

![Graph showing hFIX and ALT levels over weeks post vector infusion](image-url)
Ongoing Hemophilia Gene Therapy Trials

1. University College London/St. Jude - Biomarin (FVIII) (Nathwani/Tuddenham/Davidoff)
   - scAAV8/ WT FIX

2. Children’s Hospital of Philadelphia (High)
   - AAV8/ WT FIX

3. University of North Carolina/Asklepios/Chatham (Samulski/Monahan)
   - scAAV8/ FIX Padua
Challenges to Hemophilia Treatment Innovation

• Current high patient satisfaction
• Clinical trial protocols
• Global research involvement
• Regulatory agency facilitation
• Potential for neo-immunogenecity
• Cost
Severe Hemophilia A Therapy 2015-2020

Plasma-derived FVIII

Recombinant FVIII

Recombinant FVIII + Recombinant VWF

FVIII Conjugates (eg. PEG)

FVIII Gene Transfer

Novel Intrinsic tenase

Modified FVIII (eg. fusion proteins)

Novel Adjunctive Therapies ie. anti-fibrinolysis, TFPI/PC/AT inhibition
Queen’s University in Kingston