Bleeding disorders have been recognized since ancient times…

• The Talmud (2nd century AD) states that male babies do not have to be circumcised if two brothers have died from the procedure

• In 12th century Albucasis, an Arab physician, wrote about a family in which males died of excessive bleeding from minor injuries

• In 1803, Dr. John Otto, Philadelphia, wrote about an inherited hemorrhagic disposition affecting males

• In 1828 at the University of Zurich, “hemophilia” was first used to describe a bleeding disorder
A “Royal Disease”

Queen Victoria (1837 to 1901) passed hemophilia on to German, Russian and Spanish royal families. Her son, Leopold, had frequent hemorrhages (British Medical Journal, 1868) and died of a brain hemorrhage at 31. His grandson also died of a brain hemorrhage in 1928.
Types of Bleeding Disorders

*von Willebrand disease*
Factor I deficiency
Factor II deficiency
*Factor V deficiency*
Factor VII deficiency
Factor VIII deficiency (Hemophilia A)
Factor IX deficiency (Hemophilia B)
Factor X deficiency
Factor XI deficiency
Factor XII deficiency
Factor XIII deficiency
Molecular Genetics of Hemophilia

- Hemophilia A
  - Factor VIII deficiency
- Other Genetic Disorders with low Factor VIII
  - Von Willebrand Disease (Type 2N)
  - Combined Deficiency of Factor V and Factor VIII
- Hemophilia B
  - Factor IX deficiency
Hemophilia A

• Incidence 1:5,000 - 1:10,000 males
  – about as rare as the birth of triplets
  – ~ 1 in 5,000 live male births are affected.
  – ~ 15,000 to 20,000 people with hemophilia in the US

• Hemarthroses, post-traumatic and post-surgical bleeding

• Severity related to factor VIII level
  – <1% = severe
  – 1-5% = moderate
  – 5-15% = mild

• Inhibitors develop in ~10-20% of severe patients

Symptoms of hemophilia include...

Primary: Bruising and Bleeding

Minor bleeds:
• early joint and muscle bleeds
• bleeding in the mouth and gums
• epistaxis (nosebleed),
• hematuria (blood in the urine)

Major bleeds
• central nervous system
• severe injury
• neck/throat, eye, gastrointestinal, hip, iliopsoas, late joint and muscle, testicles, and retroperitoneum bleeds
Secondary:

- Chronic joint deformities from recurrent bleeding
- Antibodies to transfused factor VIII (inhibitors develop only in 20-30% of severe patients, not in mild-moderate)
- AIDS - Over 60% of persons with hemophilia treated with plasma concentrates in the early 1980s became HIV+

Mild hemophilia patients (factor levels >5% and <50%)
- usually bleed only after injury or surgery
- some never have a major bleed, others have several episodes depending on functional factor levels
- carriers of hemophilia may fall in the mild range

Moderate hemophilia patients (factor levels 2% to 5%)
- bleed about one a month, usually after trauma, surgery, or exertion.
- once a bleeding history is established in an area, may have spontaneous bleeding episodes into those areas

Severe hemophilia patients (factor levels <1%)
- bleed very easily, sometimes spontaneously with no warning and for no apparent reason, usually targeting the joints but potentially in any area
Hemophilia A: Genetics

- X-linked inheritance
  - ~1/3 patients represent new mutations (Haldane hypothesis)
- Germinal mosaicism
- Low FVIII in female consider:
  - skewed X-inactivation
  - chromosomal abnormality (normal X inactivated)
  - VWD (particularly type 2N)

X-Linked Recessive Inheritance

- Affected males (XY):
  - sons unaffected (no male to male transmission)
  - daughters obligate carriers
- Carrier female (XX):
  - _ sons affected; _ daughters carriers
- Affected females: very rare.
Is this woman a hemophilia carrier of hemophilia?

- A biological daughter of a man with hemophilia
- A biological mother of one son with hemophilia
- A biological mother of more than one son with hemophilia
- A biological mother of one hemophilic son and has at least one other blood relative with hemophilia
- A sister of a male with hemophilia

Germline/Gonadal Mosaicism

46, XX

46, XY

Factor VIII allele - normal
Mutant VIII allele - normal

ovary
testes
Factor VIII

- Factor VIII gene
  - X-chromosome (Xq28), 186 kb, 26 exons
- 300 kDa protein:

  \[
  \text{A1} \quad \text{A2} \quad \text{B} \quad \text{A3} \quad \text{C1} \quad \text{C2}
  \]

- Biosynthesis: ?liver, ?lymphocytes, ?subset of ECs
- Low concentration (100 ng/ml), bound to VWF

Molecular Defects in Hemophilia A

- >500 specific mutations identified:
  - [http://europium.csc.mrc.ac.uk](http://europium.csc.mrc.ac.uk)
  - 1/3 new mutations (Haldane hypothesis)
  - CpG dinucleotides = hot spot (~25% of point mutations)
- L1 insertion
- Severe hemophilia A (FVIII<1%)
  - gene deletions (5%)
  - intron 22 inversion (45%)
  - point mutations (50%)
- ? Genetic modifiers -- VWF, FV Leiden
FVIII Gene Inversion (Intron 22)

- 45% of severe hemophilia A patients
- Region particularly prone to rearrangement
  - recurrent mutation event
  - recombination between repeated elements (gene A)
- Only occurs during male meiosis
  - Mother of “new” patient is generally a carrier
Genetic Diagnosis for Hemophilia A

- Prenatal diagnosis
  - genetic consultation
  - CVS, amniocentesis, cord blood sampling
- Screen for intron 22 inversion
  - only in severe patients (FVIII <1%)
- Mutation screening
  - available through specialized DNA diagnostic labs
- Linkage analysis
  - informative in >90% of families
  - requires other family members
  - potential for incorrect diagnosis (recombination)

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VWF/Factor VIII Interaction

- VWF necessary for FVIII stability
- Non-covalent complex
- 1-2 FVIII per 100 VWF monomers
- Plasma FVIII and VWF levels proportional in normal and VWD

Type 2N VWD (*VWD Normandy*)

- Mutations in FVIII binding domain of VWF
  - decreased or absent FVIII binding activity
  - normal adhesive function
- Heterozygotes
  - disproportionately low FVIII
  - co-inheritance with type 1 -- ? increased severity
- Homozygotes
  - FVIII ~5-25% (? rare severe mutation)
  - mimics mild/ moderate hemophilia A, but autosomal recessive
  - poor response to FVIII concentrates
- Test plasma VWF for FVIII binding
- DNA testing available for limited set of type 2N VWF mutations

Combined Deficiency of Factors V and VIII

- Rare autosomal recessive
  - Most frequent in Jews of Sephardic and Middle Eastern origin
  - > 100 families worldwide
- Moderate bleeding tendency
  - FV and FVIII antigen and activity ~5-30%
- Mutations in ERGIC-53 (~75% of patients)
Molecular Genetics of Hemophilia

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Hemophilia B

- ~25% of hemophilia (incidence ~1:35,000 males)
- Phenotype indistinguishable from hemophilia A
- Severity related to factor IX level
- Inhibitors correlate with type of mutation
  - deletions > point mutations
- Hemophilia B Leyden
  - Severe hemophilia as children
  - Dramatic improvement at puberty

Factor IX

- Factor IX gene
  - X-chromosome (~ 10 cM from FVIII) 34 kb, 8 exons
- Serine protease (requires FVIII as cofactor: Xase complex)
  - Vitamin K-dependent (γ-carboxylated)
- Biosynthesis: liver
- Plasma concentration ~10 µg/ml
Hemophilia B: Genetics

• X-linked inheritance
  – ~1/3 patients represent new mutations (Haldane hypothesis)
• Germinal mosaicism
• Low FIX in female consider:
  – skewed X-inactivation
  – chromosomal abnormality (normal X inactivated)

Molecular Defects in Hemophilia B

• >680 specific mutations identified:
  – http://www.umds.ac.uk/molgen/
  – 1/3 new mutations (Haldane hypothesis)
  – CpG dinucleotides=hot spot (~1/3 of point mutations)
  – 425 different amino acid substitutions
  – Mutations at 9/12 Gla codons
• Large deletions: increased risk of inhibitor development
• Estimate of human mutation frequency:
  – 2.14 X 10^-8 per base per generation
  – 128 mutations/zygotes (1% detrimental)
• Mutation screening
  – available through specialized DNA diagnostic labs
• Linkage analysis
History of Treatment for Hemophilia

1950s  A basic understanding of coagulation
1960s  Cryoprecipitate
1970s  Freeze-dried concentrates from pooled plasma
       Increase in viral inactivation efforts
1980s  More advanced viral inactivation procedures
       Expanded donor screening/testing
       Increased concentration/purity
1990s  Non–plasma Recombinant DNA concentrates
       More sensitive viral marker screening tests
2000s  Gene therapy?

Dr. Graham Pool
discovered factor VIII-rich cryoprecipitate

Goals of Hemophilia Care

• **Prevention:**
  Education, prophylaxis, and/or physical fitness with injury avoidance.

• **Prompt self-treatment:**
  Patients become quite adept at prompt preventive and emergency self-treatment.

• **Rehabilitation:**
  To limit secondary musculoskeletal and neurologic complications once the bleed subsides.

C. Harris, age 14
Treatment Basics

- Infuse concentrated Factor VIII
  - 80% of patients do at home
  - dose based on weight
    (eg. 2% rise/unit/kg) -
  - don’t need levels of 100%.

- DDAVP (IV/nasal) (mild hemophilia A)
  - releases factor VIII from endothelial cells
  - doubles or triples plasma factor VIII level

Recombinant Factor VIII:

Insertion of human factor VIII DNA into vector system allowing incorporation into non-human mammalian cell lines for continued propagation
HEMOPHILIA THERAPY IMPROVEMENTS:
Volumes (mL) required to obtain a factor VIII dose of 2000 IU:

- Whole Blood >4000 ml
- Whole Plasma = 2000 ml
- Cryoprecipitate = 400 ml
- Early Concentrates = 80 ml
- Today’s Concentrates <20 ml

Jason Burdick, age 12, Green Bay, WI

Financial & Insurance Issues

- > 70% of clotting factor distribution is by for-profit companies
  average cost/yr for human plasma derived or recombinant factor is
  $50,000 - $100,000

- Prophylaxis requires about 150,000 units/yr for a 65-pound child
  costing $85,000 per year

- Prophylaxis is covered by insurance on a case-by-case basis.
Avoid drugs that aggravate bleeding problems:

- Aspirin
- Heparin
- Warfarin
- Nonsteroidal anti-inflammatory drugs

Hemophilia is an ideal disease for gene therapy:

- caused by a single malfunctioning gene
- just small increase in factor level will provide great benefit:

raising factor by 2% will prevent spontaneous hemorrhages into joints, brain and other organs; levels greater than 20% to 30% will prevent bleeding in most injuries
Molecular Genetics of Hemophilia

**Summary**

- **Hemophilia A**: mutations in FVIII gene
  - Intron 22 inversion (~45% of severe)
  - Other deletions and point mutations
- **Other Genetic Disorders with low Factor VIII**
  - Type 2N VWD: mutations in VWD factor VIII binding domain
  - Combined Deficiency of Factor V and Factor VIII
    - ERGIC-53 gene mutations, ?other gene
- **Hemophilia B**: mutations in Factor IX