GENETICS OF HEMOPHILIA A AND B

An introduction for clinicians

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By

Carol K. Kasper, M.D.
And
Carolyn H. Buzin, Ph.D.
PATTERNS OF INHERITANCE

The nucleus of a human cell contains 46 chromosomes, a set of 23 from the person’s mother and a corresponding set of 23 from the father. Cells with both sets are called diploid cells. Mature eggs and sperm have only 23 chromosomes apiece, haploid, so that, on combination of an egg and a sperm, the new individual will have the full complement of 46 chromosomes. The sex chromosomes, X and Y, determine sex. The other 44 chromosomes are called autosomes.

Chromosomes consist of long strings of genes. A gene is the basic unit of inheritance. It codes for the sequence of amino acids in a protein. Humans have more than 25,000 genes. Each gene influences some aspect of body structure or function. Sometimes, as in hemophilia A or B, a single gene governs a single protein, with little influence from other genes. Sometimes several genes together determine a given characteristic.

Some disorders, including hemophilia, are caused by a change, a mutation, in a single gene. Others, such as diabetes, depend on the interaction of several genes. The patterns of inheritance seen in bleeding disorders include the following:

In autosomal dominant inheritance, a mutant gene on one of a pair of autosomes causes a disorder in a male or a female. The disorder is transmitted from either a father or a mother to either a son or a daughter, who is a heterozygote. Examples are type 2A or 2B or 2M von Willebrand disease. Type 1 VWD also is autosomal dominant but is sometimes hard to trace through a family because the gene is not always fully expressed. Other examples of autosomal dominant disorders include the genetic hyperlipidemias, gastrointestinal polyposis, neurofibromatosis and osteogenesis imperfecta.

Figure 1. Autosomal dominant inheritance, in which a mutant gene on one autosome, indicated in violet, is transmitted equally from fathers and mothers to sons and daughters and is clinically manifested (more or less) in every person bearing the gene (violet-shaded circles and squares.)
In **autosomal recessive** inheritance, the same gene is mutant on both of a pair of autosomes, one from the father and one from the mother, causing the son or daughter to be fully affected. If the mutation on each gene is identical, as often is the case when the parents are related, the affected person is a **homozygote**. If the mutation from one parent is different from the mutation from the other parent, the affected person is a **compound heterozygote** (or double heterozygote). The parent or child of the affected person, with just one mutation (a heterozygote), is likely to be clinically normal or have only a mild protein deficiency. Examples of autosomal recessive bleeding disorders are type 2N and type 3 von Willebrand disease and severe deficiencies of other individual clotting factors (V, VII, X, XI, XII, XIII.) Common recessive single-gene disorders include sickle cell disease which affects about one in 625 black newborns, and cystic fibrosis which affects about one in 2000 white newborns. The prevalence of autosomal recessive disorders is highest wherever consanguineous marriages are commonplace. The rate of consanguineous marriages is about 2% in western countries, about 40% in much of North Africa and the Middle East, and as high as 80% in parts of India.

In **X-linked** inheritance, also called sex-linked, males, with a mutant gene on their only X-chromosome, **hemizygotes**, are fully affected but female heterozygotes with a mutation on only one of their two X-chromosomes usually are unaffected or only mildly affected. Hemophilia is one of over 150 disorders linked to genes on the X chromosome. Others include Duchenne muscular dystrophy, color blindness, and some types of immune deficiency.

**Figure 2.** Autosomal recessive inheritance, in which a mutant gene on an autosome, indicated in orange, is transmitted equally from fathers and mothers to sons and daughters but is fully manifested only in homozygotes (filled-in orange circle). Heterozygotes (half-filled symbols) usually are unaffected or only mildly affected.
Figure 3. Sex-linked inheritance, in which an X chromosome with a mutant gene, indicated in red, is transmitted from an affected male to all his daughters but none of his sons. Heterozygous daughters are indicated by half-filled circles.

Figure 4. Sex-linked inheritance, in which an X chromosome with a mutant gene, indicated in red, is transmitted from a heterozygous mother to half her sons and half her daughters, on average.

INCIDENCE AND PREVALENCE

Hemophilia A and hemophilia B, the so-called sex-linked hemophilias, occur in about one of every 5000 male births, when all levels of severity are considered. Severe hemophilia occurs in about one of every 10,000 male births. Hemophilia A (factor VIII deficiency) is about four times as common as hemophilia B (factor IX deficiency). The gene for factor VIII and the gene for factor IX are both located on the X-chromosome but not close together. The effect of a mutant gene is expressed fully in a male, who has only one X chromosome. Every male in a given family who has the same mutant gene will have similarly low levels of the clotting factor and roughly similar clinical problems. Females have two X chromosomes; heterozygous carriers usually have adequate levels of the clotting factor (see page 32.)

The mutant gene causing hemophilia can be passed down through generations. When a boy with hemophilia is born into a family that has included previous persons with hemophilia, he is said to have familial hemophilia. When hemophilia appears in a family for the first time, the term sporadic hemophilia may be used. More precisely, if a single affected boy is born into a family with no history of hemophilia, he is said to be an isolated case. If hemophilic brothers are born into a family with no prior history of hemophilia, they may be termed a sporadic sibship.

Years ago, mutations causing severe hemophilia tended to disappear after a few generations. Few affected men lived long enough to reproduce. The perpetuation of the mutant gene through the offspring of carriers, who reproduced normally, did not make up for the loss of the mutant gene from affected males who did not reproduce (see page 22.) Mutations causing mild hemophilia tended to last for more generations because more affected males survived to adulthood.

About a third of cases of mild and moderate hemophilia A and B, almost half of cases of severe hemophilia B and more than half of cases of severe hemophilia A are sporadic, the first in the family. The causative mutations arose very recently. A new mutations can arise as follows: (1) in oogenesis (formation of eggs) in the mother or maternal grandmother or (2) in spermatogenesis in the maternal grandfather or (3) in a cell of an early embryo, either in the patient himself, or in his mother or a maternal grandparent. Less than 5% of mutations causing sporadic hemophilia originated earlier than the grandparents’ generation.
MORE ABOUT CHROMOSOMES

Chromosomes are best seen in the nucleus when they are in the process of dividing and are somewhat spread out. At that time, the two copies, chromatids, are still attached at the center, the centromere. The tips of the chromosomes are called telomeres. The two chromatids and centromere look like the letter “X”. The sex chromosomes include the X chromosome which is depicted with the shape of the letter X, and the Y, a smaller chromosome something like the letter Y. The 22 pairs of chromosomes that are not sex chromosomes are called autosomes.

The sex chromosomes determine the sex of the person. An X chromosome is always contributed to the new human being by the mother, while the father contributes either an X or a Y. A person with two X chromosomes, XX, is female, whereas a person with one of each, XY, is male. Fathers are responsible for the gender of their offspring.

When a dividing nucleus is stained, the various chromosomes can be identified by the lengths of their arms and by their band-like staining patterns. A picture of the chromosomes can be cut up, in order to re-arrange the chromosomes in neat pairs. One can see whether the pattern is XX or XY and whether there is an extra chromosome or whether a chromosome is missing. This arrangement is a karyotype, a depiction of a person’s chromosomes. The word also is used to describe a person’s chromosome composition briefly, for example “He is a male, karyotype 46 XY”, “Her karyotype is 45 XO, an X chromosome is missing” (Turner’s syndrome), “His karyotype is 47 XXY, an extra X chromosome” (Klinefelter’s syndrome).

When a chromosome is NOT dividing, it is a single long strand of DNA. In this publication, chromosomes are depicted as X-shaped objects, but that shape exists only during replication.

Figure 5. A diagram of a karyotype. Chromosomes are lined up in pairs, according to size and banding pattern. This person has one “X” and one “Y” chromosome, he is a male, karyotype 46 XY.
Figure 6. The “ladder” structure of DNA consists of a sugar-phosphate “backbone” (sugars are represented by pentagons and phosphate by “P” in a circle) with pairs of nucleotides between them, a cytosine always linked to a guanine and a thymine always linked to an adenine.

Figure 7. The “ladder” is twisted to form the familiar double helix shape of DNA.
MORE ABOUT GENES

A gene is made of DNA (deoxyribonucleic acid). The smallest DNA subunits are called nucleotides, or bases, and there are four types, namely, adenine, cytosine, guanine or thymine, abbreviated as A, C, G, T. The nucleotides attach to each other through sugar and phosphate groups to form a long strand. A parallel strand forms almost as a mirror-image. An adenine on one strand always bonds with a thymine on the other strand, and a cytosine on one strand always bonds with a guanine on the other strand, forming a ladder-like structure. A base on one strand attached to a base on the other strand, A-T or C-G, is known as a base pair, “bp”. When twisted, the ladder forms a double helix.

A promoter site outside the gene, but nearby, serves to regulate transcription (making copies of the gene’s message).

Genes consist of exons, with coding information for a protein, and introns, spacers between exons.

The message of the gene is transcribed (copied) into messenger RNA (ribonucleic acid, a line of nucleotides in a single strand, not double like DNA. RNA uses the base uracil in place of thymine.) To copy the gene message, RNA polymerase (an enzyme) partially separates the two strands of DNA so that RNA nucleotides can line up along one strand of DNA to form a complementary copy.

The direction of transcription of a DNA sequence is as follows. Each sugar in the sugar-phosphate backbone of DNA has five carbons, numbered according to their position from 1 to 5. A sequence of DNA has two ends: one end, the 5’ end, has a carbon in position 5 with a phosphate group (PO₄) attached to it; the other end, the 3’ end, has a carbon in position 3 with a hydroxyl group (OH) attached to it. The 5’ end also is known as the amino terminus and the 3’ end as the carboxy terminus. Transcription is from the 5’ end to the 3’ end. “Upstream” refers to the 5’ direction and “downstream” to the 3’ direction. The promoter sequence is located upstream to the first exon.

After the DNA is copied into RNA, the introns are cut out and discarded, and the exons are spliced together to make the mature mRNA. The splice sites at the exon-intron borders must be intact and correct.

The mature RNA transcript (copy), consisting of the spliced-together exons, moves out of the nucleus, into the cytoplasm of the cell. There, the RNA sequence specifies the identity and sequence of whichever of the 20 amino acids that are needed to form the protein (translation).

DNA and corresponding RNA nucleotides are arranged in sets of three, triplets, each set called a codon because it is a code. Given that there are four nucleotides, and each could be first, second or third within a codon (reading in only one direction), there are 64 possible codons. Of these possibilities, 61 call for specific amino acids and three do not correspond to any amino acid. When one of those three occur, they are stop codons: they can’t be translated into any amino acid so the formation of the protein stops at that point. A normal stop codon is positioned at the end of an RNA sequence to signal the end of translation. A mutation may cause a stop codon in the middle of a coding sequence for a protein. Each codon specifies only one amino acid but most of the amino acids can be specified by more than one of the 61 functional codons, as listed in the table on page 7.

RNA transcription is sometimes imperfect. RNA can make mistakes. Most such mistakes result in a few useless or incomplete proteins. Once in a while, a mistake by RNA corrects an abnormal (mutant) message from DNA (see page 15).

After a new protein is translated from the RNA message in the cytoplasm, it may undergo post-translational modification. Biochemical modifications take place under the control of mechanisms in the cytoplasm. Clotting factors undergo many post-translational changes, an issue that becomes important when recombinant clotting factors for concentrates are made in hamster cells; the human gene in the nucleus specifies the sequence of amino acids lining up in the cytoplasm, but the post-translational modification is under the control of hamster cytoplasm mechanisms that differ from human ones.
Figure 8. DNA is first copied into RNA with exons and introns; then the introns are discarded and the exons are spliced together to make a coding message.

Table 1, below, lists the translation of all the possible DNA codons into corresponding amino acids.
Mitochondria are organelles (tiny organs) in the cytoplasm and also have a small amount of DNA (with 37 genes) which is inherited from mother to child (in the cytoplasm of egg cells) but not from father to child. Mitochondrial DNA mutations are associated with some human disorders, but none involving coagulation.

A person’s genome is all his genes, nuclear (over 25,000) and mitochondrial. Less than 10% of human DNA codes for proteins. The remainder is repetitive material of no known function. Some non-functional stretches of DNA are similar to functional genes and are known as “pseudo-genes”. They may be evolutionary remnants.

**CHROMOSOMES DURING CELL DIVISION**

When a single diploid cell (46 chromosomes) divides into two diploid cells, the chromosomes duplicate themselves by the process of mitosis, forming two identical diploid cells, each with 46 chromosomes.

When diploid primary oocytes (egg-forming cells) in the ovary or diploid spermatocytes (sperm-forming cells) in the testis are ready to divide into haploid eggs or sperm (gametes), they undergo meiosis, a process that occurs only for the purpose of forming gametes. The chromosomes line up closely in pairs, so closely that they may exchange one or more of their genes, or, the material between genes. (Exchange of material within genes is uncommon.) That exchange is called crossing over or recombination. In males, the X and Y chromosomes do not pair up and do not exchange genes. The solitary X, not braced-up by another chromosome, is prone to damage (see “inversion”, pg 16.) After the opportunity for recombination, the diploid cell divides.

Mitochondria also is called “wild-type”. A specific sequence of three nucleotides within each codon calls for a specific amino acid in the protein being formed.

Sperm-forming cells undergo a great many divisions by mitosis, some 30 to 500, depending on a man’s age, in order to produce the 20 million to 900 million sperm needed for each ejaculation. At maturation, meiosis produces secondary spermatocytes with half the chromosomes, a set of 23, each still consisting of two chromatids attached at the centromere. The secondary spermatocyte divides once more, into sperm, each of which has a set of 23, consisting of just one chromatid.
Eggs undergo much less cell division. Females have their lifetime’s supply of oocytes as embryos. The primary oocyte, with 46 chromosomes, starts to enter meiosis while the female is still an embryo, then remains in a sort of maturation arrest for many years until ovulation. At ovulation, meiosis continues, with more opportunities for crossover. The primary oocyte then divides into the secondary oocyte and a smaller cell, a polar body, a bubble on the surface of the egg. Both the secondary oocyte and the polar body contain a set of 23 chromosomes consisting of two chromatids attached at the centromere. Upon the stimulation of fertilization by a sperm, the secondary oocyte divides once more, into the mature egg and another polar body, each with a set of 23 single chromatids. The polar bodies disintegrate spontaneously.

Meiosis is prone to errors. The resulting gamete may contain additional chromosomes, or may lack a chromosome. Occasionally, genetic material may be transferred from one chromosome to another belonging to a different pair, a chromosome where the gene is not supposed to be located (transposition).

The result of the union of a haploid egg and a haploid sperm is the first diploid cell of the new individual, the zygote which then grows and divides into many daughter cells.
Sometimes, during cell division, a chromosome is lost from one cell and is added to another cell or gets lost altogether. Those cells now don’t have the correct number of chromosomes. Euploidy refers to the correct number of chromosomes per nucleus, aneuploidy to an incorrect number. The best-known example of aneuploidy is Down’s syndrome, caused by the presence of an extra chromosome 21. Aneuploidy is most likely to develop during meiosis in oocytes. It also can occur in an early embryo. If some embryonic cells persist with the normal number of chromosomes and others develop aneuploidy, the resulting individual is a mosaic of euploidy and aneuploidy. A mosaic embryo is more likely to survive and more likely to function fairly well than an embryo with aneuploidy in all its cells.

The cells in the ovary and testis that give rise to mature eggs and sperm are called the germline. The rest of the cells in the body are known as somatic cells.

**GENETIC VARIATION**

The human species has a great deal of genetic variation. There are an estimated 3 million differences in the DNA sequence between random pairs of humans. Most variation is normal (that is, it does not disturb function), such as differences in eye color or in blood groups. Other variation causes disease. All inherited human variation springs from mutation, a change in the DNA sequence.

Mutations can occur because of exposure to radiation or to toxins (“exogenous” causes), but the overwhelming majority of mutations are simply errors in DNA replication (“endogenous”) and are not caused by anything the person did or did not do. Mutations happen commonly in rapidly-replicating cells. There are about 100,000,000,000,000 cell divisions in a person’s lifetime, and each cell division requires the incorporation of about 6 billion new nucleotides. It is estimated that each gene is likely to be a locus for a billion mutations (each in a different cell) over a person’s lifetime. DNA repair mechanisms correct an estimated 99.9% of mutations. Mutations can be helpful, neutral or harmful. Mutations that occur in the germline might be passed to the next generation if the affected egg or sperm becomes a new person.

Most mutations arising in somatic cells are not important. For example, if a mutation arises in the factor VIII gene of a somatic cell, it doesn’t matter, because millions of other cells in the organism have normal factor VIII genes. Occasionally, a mutation arising in a somatic cell may be harmful, such as those causing cancer. Mutations arising in somatic cells are not inherited.

If DNA within or between genes has a different sequence at a given locus (plural loci; = location) in each of a pair of chromosomes, the two different forms are called alleles. When more than one allele is known to exist for a locus, the locus is said to be polymorphic: it has many forms. When the difference at a locus consists of substitution of just one nucleotide for another, it is called a single nucleotide polymorphism (SNP, pronounced “snip.”) Such polymorphisms are useful to trace a particular gene in a family. In common parlance, a “polymorphism” usually is a harmless change, and a “mutation” is a harmful change (although all changes are mutations).

A human being is likely to have a great many polymorphic loci in various genes and chromosomes. We often identify just a few loci on a chromosome that are of interest to us. We refer to the relevant polymorphisms, or alleles, on one chromosome as the haplotype of that chromosome, for the other chromosome of the pair may have a different set of alleles, a different haplotype. For example, if three loci, A, B and C, on a chromosome are each known to have two different alleles, we might refer to those alleles as A1 and A2, B1 and B2, C1 and C2. There are eight possible haplotypes:

A1-B1-C1, A2-B1-C1,
A1-B1-C2, A2-B1-C2,
A1-B2-C1, A2-B2-C1,
# Types of Mutations

(Adapted from “Emery’s Elements of Medical Genetics”, 14th Edition)

<table>
<thead>
<tr>
<th>Substitution: (of one nucleotide for another)</th>
<th>Synonymous (codes for same amino acid)</th>
<th>Silent</th>
<th>Non-synonymous (codes for different amino acid)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Polymorphism, Variant, or Missense</td>
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<td></td>
<td></td>
<td></td>
<td>Nonsense</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Splice site</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Promoter</td>
</tr>
</tbody>
</table>

- **Substitution:**
  - **Synonymous:** codes for the same amino acid
  - **Silent:** the same amino acid in the protein, but change in DNA may have a negative effect, e.g., on splicing?
  - **Non-synonymous:** codes for a different amino acid

- **Deletion:**
  - **Multiple of three:** in-frame deletion of one or more amino acids
  - **Not multiple of three:** frameshift, premature termination
  - **Large deletion:** partial gene deletion, premature termination
  - **Whole gene deletion:** loss of expression

- **Insertion:**
  - **Multiple of three:** in-frame insertion of one or more amino acids
  - **Not multiple of three:** frameshift, premature termination
  - **Large insertion:** partial gene duplication, premature termination
  - **Whole gene duplication:** effect of increased gene dosage

- **Inversion:**
  - **Intron 1, intron 22:** loss of protein secretion into plasma (fragments still in cell)
A person’s **genotype** is his combination of alleles for a particular pair of genes. A given genotype may result in a particular physical attribute, the **phenotype**. The genotype of a specific hemophilia mutation results in approximately the same phenotype in all affected males. In contrast, some genotypes of von Willebrand factor result in different phenotypes in different persons, with von Willebrand factor levels ranging from normal to low, depending on other genetic and environmental influences.

In Von Willebrand disease, a given genotype does not always result in the same phenotype; there often is **variable penetrance**. The reverse can be true. A given phenotype may arise from more than one genotype. A male with the phenotype of bleeding into joints may have the (hemizygous) genotype of any of a variety of mutations causing severe hemophilia A or B or a (a homozygous or compound heterozygous) genotype causing another clotting factor deficiency such as severe factor VII deficiency.

**MUTATIONS**

The factor VIII gene and the factor IX gene have relatively little variation from one normal person to the next, but many different mutations can cause hemophilia. Over 2000 distinct mutations have been reported in different patients with hemophilia A, and over 1000 in hemophilia B.

The major categories of mutation causing hemophilia include substitution, deletion, insertion and inversion, as described on page 11.

**Missense mutation**

One nucleotide is substituted for another, but a protein still is formed. Sometimes the codon containing the substituted nucleotide still codes for the same amino acid (silent, or synonymous), so the protein is unchanged. Some such mutations cause mild hemophilia, by mechanisms not yet understood. Usually, the codon containing the substituted nucleotide codes for another amino acid. The resulting protein may be functionally normal, a normal variant. Or, a mild to severe functional deficiency may result. Nearly all mild hemophilia results from missense mutations, but some missense mutations cause severe hemophilia. One missense mutation in the factor IX gene results in a factor IX protein with greatly increased activity.

When the total amount of the protein is measured as **antigen** in an immunological test using an antibody, we may learn that more of the protein is present than is suggested by an assay measuring function. The abnormal low-functioning protein detected as antigen in an immunologic test is called **cross-reacting material** or **CRM** and if the level of antigen is notably higher than the level of functional activity, the hemophilia (or other disorder) is called **CRM-positive** or **CRM+**. If the abnormal protein is present, but at a subnormal concentration, the term **CRM-reduced** may be used.

**Nonsense mutation**

If a nucleotide substitution creates a codon that does not code for any amino acid, that codon is called a **“stop codon”**. When the protein is being formed, translation stops at that point. Part of the protein is still made. Nonsense mutations typically cause severe disease. However, if the mutation is located towards the end of the protein, allowing most of the protein to be made, it may result in mild disease, especially if the lost amino acids are not evolutionarily conserved.

A cellular mechanism known as “**nonsense-mediated decay**” causes rapid degradation of mRNA containing truncated messages.

Some drugs, in particular aminoglycosides, promote RNA misreading in experimental animals. RNA polymerase may bypass a stop codon and insert an amino acid at that site so that a protein is formed (although it is not fully normal). “**Translational bypass therapy**” with such drugs has been tried as a therapeutic approach for patients with suitable mutations but has not yet been successful.
Figure 13 represents a missense mutation. A change from thymine to adenine in the second codon results in a change in amino acids from cysteine to serine.

Figure 14 represents a nonsense mutation. A change from a codon for cysteine to a codon that does not have a translation stops the formation of the protein at that point.
**Insertions, deletions**

One or more nucleotides may be inserted or deleted from the coding sequence. The deletion or insertion of a single nucleotide alters the triplet reading frame, often creating a stop codon downstream. Disruption of the triplet structure is a frameshift mutation, usually associated with severe disease if located in the early or middle part of the protein, but sometimes less harmful near the carboxy terminus of the protein. On the other hand, if the insertion or deletion consists of three nucleotides, or a multiple of three, so that the reading frame is not affected, less harm may be done. Large deletions or insertions are associated with severe disease.

**Splice site mutations**

Changes in the sites where exons are to be joined together can result in loss of the coding sequence or retention of intron sequences or frameshifts. The change may be in an exon or the adjacent portion of an intron. These mutations usually are associated with severe disease.
Inversions

Reversal of the orientation of a segment of a gene can result when breaks occur at two locations in the gene and the broken-out segment is re-inserted backwards.

The factor VIII gene and the factor IX gene both are on the long arm of the X chromosome. The factor IX gene is closer to the centromere. The factor VIII gene is near the tip, the telomere, a vulnerable position during meiosis in a male, for the long arm of the unpaired X chromosome may bend up on itself.

The factor VIII gene has 25 introns (intron 1 being nearest the telomere). Outside the factor VIII gene, towards the telomere, there are two tiny genes, F8A’ and F8A”. When the long arm bends up, it may break and the fragment from intron 1 to intron 22 may re-combine with either F8A’ or F8A”. The fragment is then in a backwards or inverted position.

With the intron 22 inversion, most of the factor VIII protein still is produced intracellularly, in two fragments, but is not excreted into the plasma.

Inversions because of breakage in intron 22 are the cause of 40% of cases of severe hemophilia A. Inversions because of breakage at another site, in intron 1, are responsible for about 2% of cases of severe hemophilia A.

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**RNA mistakes improved the phenotype of a DNA mutation!**

Young et alia (Am J Hum Genet 1997; 60:565) described a frameshift mutation, expected to cause severe hemophilia A. Instead, factor VIII levels were 4 to 6%, as in moderate hemophilia, because RNA sometimes mis-read the DNA message.

**DNA sequences:**
- Wild-type: ...AGC CAA AAA AAA TAA CTT...(A8 T A2)
- Mutant: ......AGC CAA AAA AAA AA CTT.... (A10)

100 RNA clones were cultured from each of the above sequences. The distribution of RNA sequences was as follows:

<table>
<thead>
<tr>
<th>Wild-type A8 T A2</th>
<th>Mutant A10</th>
</tr>
</thead>
<tbody>
<tr>
<td>87 A 8 T A2</td>
<td>61 A10</td>
</tr>
<tr>
<td>7 A 9 T A2</td>
<td>31 A 9</td>
</tr>
<tr>
<td>3 A10 T A2</td>
<td>4 A11*</td>
</tr>
<tr>
<td>3 A T A2</td>
<td>3 A 8*</td>
</tr>
<tr>
<td></td>
<td>1 A 7</td>
</tr>
</tbody>
</table>

* These sequences restore the triplet reading frame.
Figure 16, right. During duplication in meiosis in males, the X chromosome is not braced, not being in a pair. The long arm can flip up, break, and re-attach backwards.

Figure 17.

Top: the normal position of the factor VIII gene, with intron 22 in solid red. The gene-within-a-gene, indicated as A, is similar to A’ and A” towards the telomere.

Bottom: If the tip flips and breaks during meiosis, a large part of the factor VIII gene from intron 1 to intron 22 may reattach at the site of A’ (or A”). When re-attached, the factor VIII gene is positioned backwards. The two fragments of the factor VIII gene are produced inside the cell but not excreted into the plasma.
Male vs. female origins

Overall, mutations in the germline are more likely to occur in males than in females. Among all known mutations for all disorders, inversion in the factor VIII gene is the most skewed towards occurrence in the male germline. No relationship has been proven between the age of the parent and the likelihood of a hemophilia mutation in the germline. Chromosomal abnormalities, on the other hand, are skewed towards occurrence during meiosis in oocytes and increase in frequency with maternal age.

Some locations in the gene are prone to mutation, and are known as hotspots. One hotspot is exon 14 of the factor VIII gene, containing two adenine runs (repetitions of the nucleotide adenine.) About 25% of all small deletions occur there. Another hotspot is the nucleotide sequence of $\text{CpG}$, a cytosine in the 5’ direction linked by a phosphate group to a guanine in the 3’ direction, both on the same strand of DNA. A CpG site is about twelve times as vulnerable to mutation (a change of cytosine to thymine) than are other nucleotide sequences and mutations in that location are especially likely in male mitosis.

Loss-of-Function, Gain-of-Function

Most single-gene disorders are caused by loss-of-function mutations, in which there is a decreased amount, or decreased activity, or decreased stability of the resulting protein. Hemophilia and most recessive disorders are due to loss-of-function mutations. When no protein is formed at all, the terms null mutation or null allele are used. A heterozygote for a loss-of-function mutation on an autosome typically has half-normal levels of the protein, and that is usually enough for adequate function. Examples are deficiencies of factor V, VII, X, or XI. (Female heterozygotes for X-linked disorders have more variable levels of the protein, according to X-chromosome inactivation patterns (see page 24). In a few diseases, such as familial hypercholesterolemia, heterozygotes are clinically affected (and homozygotes very severely affected). The term haplo-insufficiency refers to situations in which half-normal levels of the gene product are not enough for reasonable normality.

A particular kind of loss-of-function is called dominant-negative. The phenotype is more severe than would be expected in a heterozygote. The product of the mutant gene interferes with the function of the product of the corresponding normal gene. This kind of interference is especially likely if the gene product forms multimers. Von Willebrand factor is multimeric and some mutations causing type 1 or 2 von Willebrand disease are dominant-negative. A non-hematologic example is osteogenesis imperfecta.

A few disorders are caused by gain-of-function mutations. There is over-expression expression of the gene product, or an increase in its activity. An example is von Willebrand disease type 2B, in which von Willebrand factor binds to platelets too eagerly. A non-hematologic example is Huntington’s chorea.

Genotype-Phenotype Correlations

The most obvious correlation of genotype and phenotype is between a hemophilia mutation and the severity of the bleeding disorder in the patient. This correlation usually is close; the genotype predicts the phenotype fairly accurately.

Discrepancies in factor VIII assay levels

In some patients with mild hemophilia A, the apparent level of factor VIII differs depending on the assay method. The patients appear to have normal levels of factor VIII when the test is performed by the common “one-stage” assay used in most clinical laboratories in the world, but appear to have deficient factor levels when tested by the more complicated “two-stage” assay, used only in a few specialized laboratories. Missense mutations in certain regions of the gene, especially the A3 domain, are responsible.

Inhibitor development

The likelihood of developing an inhibitor is correlated with the type of mutation. The highest likelihood of inhibitor formation in hemophilia A and B is associated with deletions of more than one exon. The type of mutation least likely to be associated with inhibitor formation is a missense mu-
tation, in which a clotting factor still is produced. (A few specific missense mutations confer an increased inhibitor risk.)

The mutations associated with an increased likelihood of inhibitor formation are those that prevent or truncate production of the protein. The fetus learns to recognize its own proteins as belonging to itself and it is unlikely to develop antibodies against them in later life. The fetus who learns to recognize his own almost-normal clotting factor as “self” will later consider infused therapeutic clotting factor as self, and the fetus who is exposed only to a truncated clotting factor is likely later to react to infused factor as non-self.

Recently, attention has been called to normal variation in factor VIII structure (different haplotypes), especially in black Africans who have more genetic variation than other races. In one study, inhibitor rates were higher in hemophilia A patients whose basic haplotypes were the black-restricted ones (a large minority of black patients), perhaps because they use clotting factor concentrates of predominantly or solely non-black haplotypes of factor VIII. This observation has not yet been verified in further studies.

Improvement at puberty

A fascinating form of hemophilia B was described first in the city of Leyden, The Netherlands (therefore called hemophilia B Leyden). Factor IX levels are severely or moderately deficient in childhood but rise at puberty. Several unrelated families with this type of hemophilia B have been found in different parts of the world. The causal mutations are in the promoter for the factor IX gene. The transcription of factor IX is hindered until puberty, when a different promoter, testosterone-dependent, becomes active.

HEMOPHILIA ORIGIN AND PERPETUATION

A previous family history of hemophilia is known by some 70% of patients with mild hemophilia A or B, 57% of those with severe hemophilia B, and 45% of those with severe hemophilia A, according to our survey in California.

What about patients with no family history? Once in a great while, a mutant gene may be passed through several generations of women without being inherited by a male, especially in families with few offspring per generation. But that is unusual. Nearly all seemingly-new cases of hemophilia in a family are due to recent mutations.

If a mutation arises in the factor VIII or IX gene on an X-chromosome in an egg, and the egg is fertilized by a Y-bearing sperm, then the resulting son will have hemophilia; if the egg is fertilized by an X-bearing sperm, the resulting daughter will be a carrier. If a mutation arises in an X-bearing sperm, then the resulting daughter will be a carrier. A large majority of hemophilia mutations arise during spermatogenesis. The sperm of the maternal grandfather of the sporadic patient is the most likely origin of the mutation. (This observation is useful in counseling families who have been blaming the mother, for it turns such accusations towards her father. As a male, he is less likely to be castigated. Of course, he did nothing to cause the mutation.)

Mutations are most likely to happen in rapidly-reproducing cells, notoriously during spermatogenesis. A mutation also can arise in one of the rapidly-replicating cells of a very early embryo. If the event is early enough, then the mutant daughter cells are likely to be part of all the organs. If the event is a little later, the mutant daughter cells may contribute to one or more organs but not to all organs. If the affected organ is not important for clotting factor production, then no-one may ever know that the mutation happened. If the affected organ is important for clotting factor production, such as the liver, then the mature individual may be deficient in the factor (usually mildly deficient). If the germline is affected, then some eggs or sperm may be mutant.

Mutations in early embryos usually create a mosaic individual, mutant in some but not all cells.

Women who have had just one son with hemophilia often ask, “Could the mutation have arisen in just that one egg?” The answer is, yes, but we can’t tell whether the mutation was in just one egg or is also present in other eggs.
Figure 18. A mutation strikes the factor VIII or IX gene on an X-chromosome in an egg. If the egg is fertilized by a Y-bearing sperm, it will become a male with hemophilia. If it is fertilized with an X-bearing sperm, it will become a carrier.

Figure 19. A mutation strikes the factor VIII or IX gene in an X chromosome in a cell developing into sperm. If the sperm fertilizes an egg, which has only an X chromosome, it will become a carrier.
Figure 20. A mutation strikes a factor VIII or IX gene in one cell in an early embryo. The result of that event depends on the fate of that cell. If it happens very early, then the cell is likely to grow into part of all organs, including organs producing clotting factor and organs producing eggs or sperm. If it happens later, the affected cell may already be destined to contribute to the liver, for example, or to the testis or ovary. Or the affected cell may be destined to become an organ that isn’t essential for either clotting factor production or for reproduction, such as the skin.

Figure 21. If a mutation hitting an embryo has affected some of the cells of the testis or ovary, or some of the cells of all the organs, then the person is a mosaic. His sperm or her eggs could carry the mutation. A mosaic woman (checkerboard circle, upper left) could have a carrier daughter or a hemophilic son. A mosaic man (checkerboard square, upper right) could have a carrier daughter.
Figure 22. This family came to our attention because the boy in the youngest generation had severe hemophilia B. His mother had the mutant gene and a typical carrier level of factor IX. The white blood cells in his maternal grandfather were mosaic, some had the boy’s mutation and some were normal. The grandfather’s factor IX level of 36%, slightly below the normal range, suggested that his liver might also be mosaic.

Figure 23. This family came to our attention while trying to determine whether the girls in the younger generation were carriers. One carried the same mutation as her hemophilic brother, but their mother’s corresponding gene (identified by linkage analysis) in her white blood cells was not mutant. We presume that the mother is a mosaic and that a hemophilia mutation affects part or all of her ovaries but not her white blood cells.
FOUNDERS OF MILD HEMOPHILIA FAMILIES

Once a new mutation occurs, it can be passed to descendants. Mild hemophilia is not always very deleterious. Some males with mild hemophilia, born generations ago, survived into adulthood, had children, and have many descendants with hemophilia. This phenomenon is called a "founder effect". Sometimes the founding ancestor is known, especially when the descendants continue to live in a restricted geographic area. Sometimes the fact that many persons with mild hemophilia, who did not know that they were related to each other, had a common ancestor is discovered because they all have the same mutation and the same allele of the gene (with the same gene markers, e.g. RFLPs). Members of one family, descended from a single village in Greece, account for 21% of that country’s patients with mild hemophilia A. Another group of related persons with mild hemophilia A lives in rural Newfoundland. Three founders are believed to be the ancestors of about 28% of American patients with mild hemophilia B. Another three founders account of 83% of patients with mild hemophilia B in Ireland.

Figure 24. In the family above, the affected male in the younger generation had severe hemophilia B due to a missense mutation. His mother, two sisters and a normal brother had the same gene (identified by linkage analysis) but in those three persons, the gene was not mutant. Nonetheless, we could not rule out the possibility that the mother was a mosaic, either within an ovary or in multiple organs, and passed the mutant gene to only one of these offspring. Perhaps the mutation affected just one ovum. (This family was analyzed by S. Paul Bajaj, Ph.D., and the mutation and its consequences were described by him and others in J Biol Chem 1990, 265:2956-2961.)

MALE REPRODUCTIVE FITNESS

As the survival of persons with severe hemophilia improves, their reproduction improves. The ability of persons affected by a particular disorder to reproduce is called their reproductive fitness. If no-one with a particular disorder survives to reproductive age, then fitness is zero. If everyone with a particular disorder survives and reproduces as well as members of the general population, then fitness is 100%. In the 19th century, reproductive fitness for severe hemophilia was about 10%, or less. In southern California in the 1960’s, it approached 40%. At the present time, it is approaching 80%. In sex-linked disorders, male fitness has a major impact on the perpetuation of a mutant gene through the contribution of obligatory carrier daughters. The incidence of hemophilia may increase in coming years (see figures on next page.)
Figure 26 a. Hypothetical survival of a de novo mutation for severe hemophilia, if male reproductive fitness is zero. Four de novo carriers are shown in the first generation. Each person who reproduces has one son and one daughter. Each carrier gives her mutant gene to half her sons (who have hemophilia) and half her daughters (who are carriers). The sons with hemophilia do not reproduce, the daughters who are carriers again give the mutant gene to half their daughters and half their sons. The condition dies out in a few generations.

Figure 26 b. Hypothetical survival of a de novo hemophilia mutation in four carriers in the first generation, if the fitness of affected males is 100%, the same as that of unaffected males, a rate being approached in the early 21st century. The perpetuation of hemophilia is strongly dependent on the level of male reproductive fitness.
X-LINKED DISORDERS AND FEMALES

X-chromosome inactivation

At an early stage, when a female embryo consists of only a few cells, each cell chooses one of the two X chromosomes for inactivation. All the daughter cells, for the life of the person, keep the same X chromosome inactivated. In stained tissue under the microscope, the inactive X chromosome can be seen at the edge of the nucleus as a small dense mass (the “Barr body”). Thus, females are typically mosaic in their somatic cells, because some cells have the paternal X chromosome active and others have the maternal X chromosome active. Inactivation usually is random, so, on average, half the cells choose the paternal and half the maternal X chromosome. Random processes, like tossing dice, occasionally may lead to extremes, such as most of one X chromosome inactivated in all or nearly all cells. No matter what the pattern of X-chromosome inactivation in diploid cells, half of the mature haploid oocytes contain the paternal X chromosome and half the maternal X chromosome.

It is not yet clear just how one X chromosome is chosen for inactivation. On the chromosome to be inactivated, a gene near the centromere called XIST sends out inactivation signals in the form of RNA that travels up and down the chromosome, coating it and causing a biochemical change called “methylation”. (Tests to detect methylation have helped figure out inactivation patterns.)

Some women have non-random X-chromosome inactivation, that is, something predisposes the inactivation of one or the other X chromosome. Possible causes are as follows:

1. XIST on one chromosome may be defective.
2. Another gene affecting XIST may be mutant.
3. One X chromosome may be damaged.
4. X-inactivation is random at the outset, but looks non-random in the mature organism, e.g. one X chromosome may have a deleterious gene that slows cell replication. Cells with the other, healthier X chromosome replicate more rapidly and become predominant.

X-chromosome inactivation was described by the scientist Mary Lyon. Inactivation is sometimes called “Lyonization”.

Figure 25. An early female embryo chooses, at random, to inactivate paternal X chromosomes (blue, normal) in some cells and maternal ones (red, with a hemophilia A mutation) in others. On average (middle right) half the cells retain the ability to make factor VIII normally and half do not. Occasionally, most cells retain the ability to make factor VIII (top right) or lose that ability (bottom right).
Figure 27. In this family, reported by Mannucci et alia, 1978, affected males had severe hemophilia A. Identical twin girls in the youngest generation have different levels of factor VIII, suggesting that X-inactivation occurred after the zygote divided into two embryos.

Figure 28. In this family, reported by Kitchens et alia, 1987, a man with moderately severe CRM+ hemophilia B had identical twin daughters with different levels of factor IX, suggesting that X-chromosome inactivation occurred after the zygote divided into two embryos.
Figure 29. X-chromosome inactivation sometimes appears to be non-random because of selective pressure on cell survival. The maternal side of this family carried a gene on the X chromosome for incontinentia pigmenti (half-blue circles), a disorder of skin and nervous system that is lethal in hemizygous male fetuses but expressed only mildly in heterozygous females. In female carriers, cells in which that chromosome is activated do not reproduce well. The paternal side of this family carried a gene for mild hemophilia A (red). In the daughter who was a carrier of both conditions (half-red, half-blue circle) cells in which the chromosome with the hemophilia gene was activated proliferated well and therefore became predominant. Thus, the double carrier had a phenotype (factor VIII level) similar to her father’s. (Coleman et alia, J Med Genet 1993, 30:497.)

A person can be a MOSAIC if his or her somatic cells differ in:

- The number of chromosomes
- The presence/absence of a mutation
- Which X chromosome is active (in females)
The average carrier of hemophilia A has half the average normal level of factor VIII, and the average carrier of hemophilia B has half the average-normal level of factor IX. Some carriers have higher-than-normal levels of the factor and some have lower-than-normal levels. It depends on the pattern of X-chromosome inactivation. Did most of the cells in the early embryo inactivate the X-chromosome with the normal factor gene? Then the woman’s factor level is apt to be low. In our clinic, one out of five proven carriers had a factor level below 30% of normal, that is, they had levels in the same range as males with mild hemophilia. Some of them had mild bleeding symptoms.

Random X-chromosome inactivation does not account for the number of females who have severe hemophilia. We have seen several such persons. Examples are on the next pages. (They are our patients unless otherwise indicated.)

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**Table 2. WAYS THAT A FEMALE CAN HAVE HEMOPHILIA**

- **Two mutant genes, homozygous or compound heterozygous**
  - hemophilic father and carrier mother
  - hemophilic father and mother with a new germline mutation
  - carrier mother and normal father with a new germline mutation
  - normal parents, both with new germline mutations

- **One mutant gene plus extreme or non-random X chromosome inactivation**
  - a hemophilic father or a carrier mother
  - a mutation in the germline of either parent
  - a mutation in the zygote (early embryo) herself

- **One mutant gene plus XY genotype manifesting as a female**

- **Chromosomal abnormality**
  - deletion of an X chromosome with or without mosaicism for that deletion
  - partial deletion of that part of the chromosome with the relevant gene
  - transposition of relevant gene to an autosomal chromosome

- **Autosomal condition mistaken for sex-linked hemophilia**
  - severe (type 3) von Willebrand disease
  - type 2N von Willebrand disease (but FVIII deficiency is mild-moderate)
  - combined deficiency of factors V and VIII (but FVIII deficiency is mild-moderate)

- **Autosomal condition, above, plus heterozygous hemophilia carrier status**
Figure 30. Female hemophilia due to homozygosity. In this family, males had mild to moderate hemophilia B. The two homozygous females in the youngest generation have lower factor IX levels than their heterozygous mother and eldest sister.

Figure 32. Two instances of female hemophilia due to mutation plus skewed X-chromosome inactivation. On the left, our male patient with severe familial hemophilia A due to a missense mutation had a daughter with severe hemophilia A. She had only one mutation (her father’s missense mutation) and apparent inactivation of the maternal X chromosome. The mother was normal; no factor VIII mutation could be found in her.

On the right, a female had moderate hemophilia A, factor VIII 4% and normal VWF functional and antigenic tests. Her parents were unaffected; no mutations could be found in them. The patient had a de novo inversion mutation together with skewed inactivation of the non-mutant chromosome.
Figure 33. Severe female hemophilia due to deletion of the normal X chromosome (karyotype 45 X0, known as Turner’s syndrome.)

Figure 34. Severe female hemophilia B in a carrier due to deletion of part of the X chromosome bearing the normal factor IX gene. Redrawn from Nisen P et alia, New Engl J Med 1986; 315:1139-1142.

Figure 35. Female hemophilia due to mosaicism for deletion of an X chromosome. The woman with 4% factor VIII, who bled moderately heavily, had a normal karyotype, 46 XX, in her white blood cells. She had no stigmata of Turner’s syndrome. After publication of the initial description (cited below), biopsies of mucosa and fibroblasts revealed that she was a mosaic of 46 XX, 45 XO cells. Her factor-VIII-producing tissues must have been mostly XO. This woman was CKK’s patient during her Fellowship and initiated her interest in genetics. (Whissell DY, Hoag MS, Aggeler PM, Kropatkin M, Garner E. Am J Med 1965; 38:119-29.)
Obligatory carrier | The daughter of a man with hemophilia.
---|---
Proven carrier | A woman with both an antecedent and a descendant relative with hemophilia. The gene must have passed through her.
Probable carrier or possible carrier | The mother of an isolated case of hemophilia.
Potential carrier | A female whose position in a family suggests that she might have inherited the mutant gene, e.g. the sister of a male with hemophilia.

**CARRIER TESTING**

Commonly-used terms in southern California for female relatives of a male with hemophilia are listed above. These terms may be used differently elsewhere. Note that there is no clear term for the mother of a sporadic sibship, that is, the first set of brothers with hemophilia.

**Mutation analysis**

*The best way to determine whether a female is a carrier is to see whether her DNA has the same mutation as her hemophilic relative.* DNA is obtained from white blood cells of the hemophilic male and the potential carrier females. Laboratories with a strong focus on hemophilia (that is, lots of experience) and that can do thorough gene sequencing are recommended. (The Appendix lists some laboratories offering hemophilia gene analysis). The causative mutation can be identified in over 95% of affected males. The results are clear-cut. The woman is, or is not, a carrier. It is advisable to have the mutation established (or DNA stored) for every family with hemophilia, especially if there is only one affected male, lest the opportunity for diagnosis be lost if he dies. If no affected male is available, a proven carrier can be tested.

Other, older methods of carrier diagnosis are described in the next sections, because they are sometimes invoked, for instance, when the causative mutation cannot be found in the affected male. Or, a woman may quote results of earlier tests. However, **mutation analysis is the gold standard!**

*Table 3, right, shows the results of a family’s mutation analysis by Dr. Steve Sommer. The mutation in the patient (H) with hemophilia B is defined. One mutant gene and one normal gene are found in his mother (M) and two sisters (S2, S3) who are thus carriers, but two other sisters (S1, S4) and a niece (S2D1) have only normal genes and are not carriers of this mutation.*

<table>
<thead>
<tr>
<th>Sequence change at bp 17810</th>
<th>H</th>
<th>M</th>
<th>S1</th>
<th>S2</th>
<th>S3</th>
<th>S4</th>
<th>S2D1</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
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<td>X</td>
<td>X</td>
<td>X</td>
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<td>X</td>
<td>XX</td>
<td>X</td>
<td>X</td>
<td>XX</td>
<td>XX</td>
<td></td>
</tr>
</tbody>
</table>
**Linkage analysis (RFLPs)**

A gene may be tracked through a kindred using markers, such as normal small polymorphisms in or near the gene. A “Restriction” enzyme cuts DNA when it recognizes a certain sequence of nucleotides, creating “Fragments” of DNA of various “Lengths”, short if the sequence was present and the DNA was cut, long if the sequence was not present and the DNA was not cut. The marker is useful if the number of people who have the sequence and the number who do not is fairly well balanced in the general population and within the family (Polymorphic), that is, everybody is not the same. Hence the name, restriction fragment length polymorphism.

Linkage analysis became available in the mid-1980’s for tracking genes. In affluent countries, it is a secondary tool for carrier detection nowadays. It may be used to determine which ancestor the mutation came from. Linkage analysis is sometimes a primary method of carrier detection in less-affluent countries, as a less-expensive but still definitive DNA-based test.

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**Figure 36. RFLP analysis in our kindred with familial hemophilia B.** Both males with hemophilia (red squares) were dead. The consultand (female with question mark) wanted to know her carrier status. Several gene markers were used to define the normal factor IX genes in her father and in her normal brother (open box in younger generation). Her mother (half-red circle) was polymorphic at two sites, one intragenic and one extragenic, thus, the mother’s normal gene could be distinguished from her presumed hemophilia gene. The consultand has one gene with her father’s pattern of gene markers and one with the presumed hemophilia gene; thus, she is probably a carrier.
Many carriers of hemophilia A with below-normal factor VIII levels and mild bleeding problems, especially those whose levels were measured in the 1950’s and 1960’s, were told that they had Von Willebrand disease. (At that time, tests for Von Willebrand factor had not yet been developed.) Sometimes that diagnosis persisted well after the identification of sex-linked hemophilia A in related affected males.

Factor levels alone are rarely used nowadays to predict whether a woman is a carrier. In earlier years, a woman’s factor level, together with the closeness of her relationship to a known carrier, was used to calculate the probability that she was truly a carrier. Unfortunately, the method was good at telling a few women that they were carriers, but it could not assure any given woman that she was not a carrier.

All carriers and potential carriers should be tested for the relevant factor level in order to identify those with low levels who should receive special attention when injured or when having surgery. The average factor level and the range of factor levels in carriers of mild hemophilia are only slightly higher than those in carriers of severe hemophilia.

Figure 38. Distribution of factor VIII levels among proven or obligatory carriers versus normal women in one survey in our laboratory. Note that some carriers have levels well below the normal range.
when they had been told only that their risk was low. Unfortunately, some laboratories that took up the method did not undergo the level of standardization characteristic of the original laboratories. Results of these tests done by this method should not be regarded as definitive. Nowadays, DNA methods should be used.

Factor IX activity and antigen

In a third of families with hemophilia B, affected males make a normal or near-normal amount of factor IX molecules that don’t function normally, so the assay for factor IX activity is low. Unfortunately, some laboratories that took up the method did not undergo the level of standardization characteristic of the original laboratories. Results of these tests done by this method should not be regarded as definitive. Nowadays, DNA methods should be used.

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Factor VIII and VWF Antigen

The utility of the factor VIII assay can be supplemented with another test. Hemophilia A affects the production of factor VIII but not of its transport protein, von Willebrand factor (VWF). Levels of VWF are normal in males with hemophilia A and in carriers. Most carriers produce more VWF than factor VIII (figure 40). Levels of VWF are measured with an antibody to VWF. (VWF measured that way is called "VWF antigen," or VWF:Ag; older papers use an outdated term, factor VIII related antigen, or FVIIIIR:Ag.)

Measurement of both factor VIII and VWF:Ag, in a few sufficiently well-standardized laboratories, slightly improved the ability to determine that certain women were carriers. If a woman had a low-normal factor VIII level and much higher levels of VWF:Ag, then she was likely to be a carrier.

Unfortunately, the method never could assure any given woman that she was not a carrier. Unfortunately, many women were told their test results in convoluted language and believed that they had been told that they were not carriers when they had been told only that their risk was low. Unfortunately, some laboratories that took up the method did not undergo the level of standardization characteristic of the original laboratories. Results of these tests done by this method should not be regarded as definitive. Nowadays, DNA methods should be used.

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Figure 40. Relationship of factor VIII levels to those of von Willebrand factor (VWF) in normal women (green) versus carriers of severe hemophilia A. Carriers tended to have excess VWF. If a potential carrier had an excess of VWF, definitely out of the normal range, she was presumed to be a carrier. Note that data on a few carriers fell within the normal range and that a few more were not far from the normal range.

Figure 41. An abbreviated family tree of our patient with CRM+ hemophilia B, whose sister and maternal aunt were strongly suspected of being carriers based on low factor IX activity. They had normal levels of antigen (F.IX Ag). The aunt’s elder daughter, with a borderline level of factor IX activity and a normal level of antigen, was suspected of being a carrier. She later had a child with hemophilia. The aunt’s younger daughter had normal and almost-equal levels of factor IX activity and antigen and later had unaffected children.
Mother of isolated or sporadic case

If a woman is the mother of just one son with hemophilia, the first in the family, an isolated case, it is possible that the mutation arose in just one egg, or in the boy as a very early embryo. Or, the woman may have had a germline mutation that affected more than one egg, or she may have had a mutation as an embryo and became a mosaic, with mutant genes in some of her cells including some of her oogonia (egg-producing cells). Or she may be a mutant in all her cells, somatic and germine, inheriting the disorder from her father’s mutant sperm or her mother’s mutant egg. When we tested white blood cells of mothers of isolated cases, we found the mutation in 85% of them.

If the mutation is not in the mother’s white blood cells, she might still be a mosaic carrier, with the mutation in some ovarian tissue and possibly in other tissues but not in the white blood cells. The mother of an isolated case should presume that she is a carrier and that she might have another hemophilic child with another pregnancy.

If a mother has two sons with hemophilia, or a hemophilic son and carrier daughter, who are the first in the family (a “sporadic sibship”), then she is highly likely to be a somatic mutant and to have the mutation in her white blood cells. Alternatively, she may be a mosaic, with a mutation affecting her ovaries but not her white blood cells (see Figure 23).

Female with low factor level, no family history

With all these mutations going on in spermatogenesis, creating new carriers, you’d expect to encounter occasional women with low factor levels, typical of some carriers, but with no family history of hemophilia. A woman does turn up from time to time with a low factor level. Perhaps a low level of factor VIII is paired with a normal level of von Willebrand antigen. Is she really a carrier? Did the laboratory make an error? Is there an undiagnosed male with hemophilia in her family? The first step, of course, is to repeat the assays in a highly-reliable laboratory. Another step is to see whether familial hemophilia can be ruled out.

We have encountered two undiagnosed hemophilic fathers through their daughters. In one instance, two sisters in their late teens with menorrhagia and low factor VIII levels proved to have an asymptomatic father, in his late 40’s, a middle-class American-born man, with previously-undiagnosed mild hemophilia A. In another instance, our infant patient with severe hemophilia A had a muscular, hard-working 60-year-old maternal grandfather, another middle-class American-born man, who admitted to “rheumatism” and turned out to have previously-undiagnosed severe hemophilia A.

When the factor VIII level is low, one must also consider the possibility of autosomal disorders including von Willebrand disease, especially type 1 (fairly common), or type 2N (uncommon), or combined factor VIII and factor V deficiency (rare). If, after all the investigation, the suspicion strongly remains that the woman is a carrier, a molecular genetics laboratory can be asked to see if a gene mutation can be found. If they find a mutation, the situation may be clarified, but if they do not, it does not rule out the possibility that she is a carrier of a novel, occult mutation.

Pregnancy in carriers

Factor VIII levels rise during pregnancy, typically reaching double the baseline by term. Carriers whose baseline factor VIII levels are below normal may reach a normal level by term. If factor VIII does not reach a normal level, or if the woman expects to undergo an invasive procedure during pregnancy such as prenatal diagnosis, she should be under the care of a coagulation specialist. Reports of levels of factor IX in pregnancy are variable.

A newborn baby with severe hemophilia has less than one percent factor VIII or factor IX activity. The clotting factors do not cross the placenta from the mother to the baby. The normal newborn’s liver does not yet make normal levels of factor IX so a mild deficiency should not be interpreted as evidence of hemophilia. The newborn
has a normal or low-normal level of factor VIII.

In planning the delivery of an infant who has hemophilia, or who may have hemophilia, a scheduled caesarian section is often elected, because the rate of intracranial bleeding at birth is lower than with spontaneous or assisted vaginal deliveries. Ventouse (vacuum) extractions and scalp needles are especially condemned. Circumcision is delayed until the diagnosis of hemophilia is excluded or until plans are made for appropriate therapy of a hemophilic infant.

**Sex selection**

A few couples consider selecting a specific sex to avoid having an affected child. In most instances, a carrier chooses to have a girl (who might be a carrier) instead of a boy (who might have hemophilia). A male with hemophilia who chooses to have a family of boys avoids passing his mutant gene to obligatory-carrier daughters. Gender selection is sometimes attempted by sorting sperm into mostly X-bearing and mostly Y-bearing before insemination. Gender selection can be done with greater accuracy using the techniques of *in vitro* fertilization.

**Prenatal diagnosis**

Nowadays, prenatal diagnosis is made by obtaining fetal cells to look for the hemophilia mutation already identified in a relative with hemophilia. Sometimes, when the mutation is not known but gene markers (e.g. RFLP) are known, they may be used. The fetal cells may be skin cells shed into the amniotic fluid obtained by *amniocentesis*, typically in the 16th week of gestation, or *chorionic villus sampling* (CVS), a biopsy of the placenta (which is fetal tissue), in the 11th or 12 week of gestation.

For amniocentesis, a needle is put through the midline abdominal wall and uterine wall into the fetal sac and a sample of fluid is withdrawn. CVS is performed either by putting a needle through the abdominal and uterine walls or by putting a catheter through the cervix of the uterus. In early years of CVS, some very early pregnancies of less than 10 weeks gestation were tested. Occasionally, injuries to the limbs of the fetus resulted. This complication has not been seen with CVS performed at later fetal ages.

Carriers with low clotting-factor levels often are advised to avoid CVS and undergo amniocentesis instead. The latter procedure punctures less vascular tissue than does CVS. Amniocentesis is performed later in gestation, when the factor levels have started to rise.

In the 1970’s and 80’s, before DNA-based diagnosis was available, prenatal diagnosis was based on assays of clotting factors in fetal blood in the fourth month of gestation, obtained by fetoscopy. A fetoscope (a skinny tube) was inserted through the abdominal and uterine walls to sample fetal blood, usually from the umbilicus at its junction with the placenta. At that stage in a normal fetus, the factor VIII level is around 50% and factor IX around 10%, so a normal fetus could be distinguished from one with severe hemophilia.

A possible consequence of any of these invasive procedures is an unintended spontaneous miscarriage, up to 1% with amniocentesis and 1 to 2% with CVS. The rate of complications is lowest in the most experienced hands.

A few fetal cells can be found in the mother’s circulation. Techniques to isolate them are improving rapidly. At the present, it is possible to use them to determine sex. Such cells may be useful in the future for prenatal diagnosis.

Prenatal diagnosis is used heavily nowadays in developing countries that accept abortion and have adequate DNA technology but can provide only modest levels of medical care for hemophilia and little or no public subsidy of its expense.

In the USA nowadays, the predominant reason for prenatal diagnosis is to plan the management of the delivery (see “Pregnancy”). Some women also want more time to adjust psychologically to the imminent arrival of a hemophilic son. Sometimes a woman who originally intended to abort an affected fetus changes her mind when the diagnosis arrives, especially when the pregnancy already is in the second trimester.
Preimplantation genetic diagnosis (PGD)

(See figures next page)

In vitro fertilization (IVF) first resulted in the birth of a child in 1978. A decade later, the process was refined to allow diagnosis of some chromosomal or genetic disorders. In 1995, PGD first succeeded in giving an unaffected child, a boy, to a hemophilia carrier.

For IVF, ovulation is stimulated and the eggs are aspirated from the pelvic cavity. The eggs are placed on a culture plate in a laboratory. At this stage, the egg is not quite mature. It undergoes meiosis and discards a set of 23 chromosomes into a first polar body. When a sperm begins to penetrate the egg, the egg’s nucleus continues meiosis and discards the excess 23 chromatids into a second polar body. Analysis of both of the polar bodies, easily sucked off the egg, tells whether the mutant gene of interest is in the discarded chromosomes or whether, by inference, the gene is still in the egg’s nucleus. Analysis of polar bodies sometimes is used alone for X-linked disorders, because X-linked recessive disorders are transmitted by eggs.

After fertilization, the combined egg and sperm, the zygote, grows and divides into more cells. At about 48 hours after fertilization, when about eight cells are present, one cell can be taken for genetic analysis. Fertility specialists prefer to take this cell, in addition to the polar bodies, for confirmation of the polar-body diagnosis, and to make sure the zygote has the right number of chromosomes (euploidy).

If the woman is at a distance from the expert diagnostic laboratory, the polar bodies and the cell from the zygote are sent by rapid courier to the laboratory. The analysis is completed within one or two days while the zygotes still grow in the culture dish. Then one or two healthy-looking zygotes, or embryos, diagnosed as free of the relevant disorder, are introduced into the uterus.

The diagnostic laboratory has the responsibility of providing swift and accurate diagnosis. The obstetrician tries to achieve a good probability of pregnancy by choosing healthy-looking embryos. It’s tempting to insert several embryos, because they don’t all implant (stick to the endometrium, the lining of the uterus, and grow.) That’s a temptation best avoided, because more than one might implant. The greatest threat to the baby’s health is prematurity, which is more common with twins and other multiple births than with singletons. Unused embryos can be frozen for a second attempt or for a sibling. Expert fertility centers have the highest rates of success. For young parents with a single-gene disorder such as hemophilia, the chance of achieving a pregnancy in one menstrual cycle with IVF and PGD is about 30%, but it approaches 50% in the most expert hands.

IVF and PGD are closely regulated in the UK but are virtually unregulated in the USA. A few countries do not allow PGD, or restrict it severely.

Insurance coverage for PGD for carriers of hemophilia improved suddenly in the past few years, as major insurers recognized that its cost, although high, is a small fraction of the cost of caring for a child with hemophilia.

Genetic counseling

Information about the inheritance of hemophilia can and should be made available to patients and their relatives whether or not they choose to have carrier testing or intervention with pregnancies. Professional counselors present information in a non-judgmental manner, that is, without suggesting what the listener should do with the information. Sometimes genetic information is not welcome. Some men with hemophilia are uncomfortable with the implication that genetic information might have prevented their own births, or that a boy with hemophilia is less desirable than an unaffected boy. They sometimes say, “Coping with hemophilia has strengthened me.”

Women tend to accept genetic information very readily; they worry about the responsibility and worry of raising a boy with hemophilia. Some carriers may worry about being stigmatized by potential spouses. Some feel that they carry an unwelcome burden of responsibility for decisions about future pregnancies.
Figure 42. PGD begins with in vitro fertilization of eggs by sperm in a laboratory dish.

Figure 43. The polar bodies are removed from the newly-ferertilized egg. A holding pipette, left, applies gentle suction to hold the egg. The biopsy pipette, right, breaks through the zona pellucida and sucks off the polar bodies, shown on top of the egg.

Figure 44. A single cell is removed from a two-day zygote consisting of about eight cells for analysis for a mutation and for euploidy. The pipette on the left holds the embryo steady as the one on the right sucks off one cell.

Figure 45. The sex of each early embryo and the presence or absence of the relevant mutation is known at age five days. Healthy embryos are selected for implantation in the uterus.
The discussion of reproduction provokes a lot of anxiety. Explanations of risks and possible actions involves the use of concepts and vocabulary that may be new to the family. They may not understand it all at once. They may reject, deny or forget distressing information. Genetic counseling takes time, empathy and repetition.

Persons in whom the mutation may have originated may feel guilt. They should be reassured emphatically that nothing they did, or did not do, caused the mutation.

The counselor should remember that coincidences happen. Hemophilia is not rare. A woman who does not bear her brother’s hemophilia mutation should be told just that, for we can’t be sure that she doesn’t have another, de novo mutation. A hemophilic man may unwittingly marry a carrier (see Williams et alia, J Thomb Haemost 2007; 5:210). Or his son may do so, see Figure 46. A carrier may be told that, on average, half her sons are likely to have hemophilia, but each pregnancy is a fresh toss of the dice. Thus, she might have a streak of offspring with the mutant gene or a streak of offspring without it.

### Historical Aspects & the Royal Hemophilia

The practice of circumcision may have made early Jewish authorities more aware of the inheritance pattern of hemophilia than other contemporaries. The oldest rabbinical view is attributed to Judah the Patriarch, who, in the second century, said that if a woman’s first and second son had died of bleeding from circumcision then her third son should not be circumcised. In the 12th century, Moses Maimonides expanded the ruling, saying that the ruling remained valid even if the woman’s third son had a different father; thus, he apparently understood that the disorder was transmitted through females.

There was a report in early 19-century American literature by John Otto, later enlarged by John Hay, of a large kindred with mild to moderate hemophilia, transmitted by females but expressed in males. Otto mentioned knowing of other families with similar histories. The inheritance pattern of hemophilia was also described in Germany in the mid-19th century. General knowledge of the inheritance pattern spread gradually and was well-understood by the beginning of the 20th century.
There was no history of hemophilia in the royal families of Europe before Queen Victoria’s time. Questions have arisen about the several deaths of male babies and toddlers on her mother's side of the family, but, given the high death rate in young children at the time, not much can be made of the story.

Victoria was born in 1819 and, at age 18, in 1837, ascended to the throne of Great Britain. In 1840, she married her first cousin, Prince Albert of Saxe-Coburg-Gotha, a German principality. Her eldest son, later King Edward VII, did not have hemophilia, thus, his descendants, the current British royal family, are unaffected.

The eighth of Victoria’s nine children, Prince Leopold, born in 1853, had hemophilia. Of Victoria’s five daughters, four had children, and two proved to be carriers. Her daughter Alice, who married the Duke of another German principality, Hesse-Darmstadt, bore Victoria’s first hemophilic grandson in 1870. Victoria’s youngest daughter Beatrice, married to Prince Henry of Battenberg, provided another hemophilic grandson in 1889. Beatrice’s youngest son Maurice, born in 1891, usually is described as having had hemophilia but the diagnosis has been challenged because he died on active service in World War I.

Victoria’s granddaughters carried the gene into other dynasties. Alice’s daughter Alix, or Alexandra, married Tsar Nicholas of Russia in 1894 and bore the hemophilic Tsarevitch Alexis in 1904. Beatrice’s daughter Victoria Eugenie (“Ena”) married King Alfonso XIII of Spain in 1906. By that time, the inheritance pattern was widely understood, but the headstrong 20-year-old King disparaged warnings that Ena might be a carrier. Their first son, Alfonso, born in 1907, had hemophilia, as did their fifth son, Gonzalo. Their fourth son, Juan, who was unaffected, was the father of King Juan Carlos of Spain.
Figure 48. Victoria’s daughter Alice had a hemophilic son, an unaffected son, two proven carrier daughters and three other daughters. Alice also had three hemophilic grandsons.

Figure 49. Victoria’s youngest daughter Beatrice had one or two hemophilic sons (Maurice’s status is questioned) and an unaffected son. Her only daughter Ena had two hemophilic sons and three unaffected ones.
Three other great-grandsons of Victoria also had hemophilia but no later descendants were afflicted. (Her descendants number over 800 and continue to be tracked.)

The hemophilia was severe, with joint and muscle bleeding. Two of Victoria’s hemophilic descendants died as small boys, one (Alexis) was murdered as a teen-ager, three died in their early 20’s (including Maurice, whose status is questioned), three died in their early 30’s and one, the last survivor, died at the age of 56, in 1945.

Victoria probably was a mutant thanks to a recent mutation in the germline of her mother or father. Much has been made of her father’s age, 52, at her birth, but older age is not clearly associated with a higher probability of hemophilia mutations in the male germline. Recently, studies of DNA from exhumed skeletons of the Russian royal family revealed a factor IX mutation. Thus, the royal hemophilia was hemophilia B.

Victoria, like many parents today, was shocked to learn of her son’s bleeding tendency and challenged to care for him. Like many a mother, Victoria tried to protect her hemophilic son and keep him safe at home, and, like many a son, he struggled to escape. Leopold was notably intelligent and persevering. His life story is reminiscent of that of many men with hemophilia born a century later. He was frequently bedridden and became an avid reader. He served as the Queen’s personal secretary for a while but, breaking loose, he enjoyed a few years of study and social life at Oxford University. Determined to set up his own independent household, he traveled to the continent and chose a German princess to be his wife. He fathered a daughter, another Alice, and a son Charles before dying at age 31 of the consequences of a fall. He has a great many descendants, including, through Charles, the present King of Sweden. Hemophilia appeared only in Alice’s son, Rupert. Leopold was the only hemophilic descendant of Victoria to have children.

Figure 50. Victoria’s hemophilic son Leopold had a daughter, Alice, a carrier, and an unaffected son.
MUTATION REGISTRIES

The International Society on Thrombosis and Hemostasis, in association with the European Association for Haemophilia and Allied Disorders, has centralized mutation data bases for coagulation disorders at:

Www.eahad-db.org

GENE SEQUENCING LABORATORIES
(USA and Canada, academic)

Factor VIII, Factor IX and full von Willebrand factor

Blood Center of Wisconsin
Stefanie Dugan, Genetic Counselor
638 North 18th Street
Milwaukee, WI 53233-2121
Email: Stefanie.Dugan@BCW.edu

The above laboratory takes a research interest in VWD and is capable of full-gene sequencing in addition to specific tests for various subtypes of VWD.

Factor VIII, Factor IX, partial von Willebrand factor

Most labs test only for type 2 VWD.

Clinical Molecular Diagnostic Laboratory
Fox South Second Floor
City of Hope National Medical Center,
1500 East Duarte Road
Duarte, CA 91010, USA
Email: mdl@coh.org

Barbara A. Konkle, M.D; Gayle Teramura
Hemostasis Reference Laboratory
Puget Sound Blood Center
921 Terry Avenue
Seattle, WA 98104, USA
Email: GayleT@psbc.org; website: www.psbc.org

ARUP Laboratories (associated with Univ. of Utah)
500 S. Chipeta Way
Salt Lake City, Utah 84108
Www.aruplab.com

Dr. David Lillicrap
Molecular Hemostasis Laboratory
Department of Pathology and Molecular Medicine
Richardson Laboratory, Room 201
Queen’s University, Kingston, Ontario K7L 3N6,
CANADA
Email: nphmt@clinlabs.path.queensu.ca

Factor VIII and factor IX genes

Arupa Ganguly, PhD
Jessica Ebrahimzadeh, Genetic Counselor
Genetic Diagnostic Laboratory,
University of Pennsylvania
Room 415 Anatomy-Chemistry Building
3620 Hamilton Walk,
Philadelphia, PA 19104, USA
Email: gdllab@mail.med.upenn.edu

Dr. Rajiv Pruthi, Dr. Dong Chen, Dr. Rong He
Lea Coon, M.S., Genetic Counselor
Mayo Medical Laboratories
Special Coagulation DNA Diagnostic Laboratory
3050 Superior Drive NW
Rochester, MN 55905, USA
Mayo Clinic, Rochester, Minnesota 55905, USA
Email: mml@mayo.edu; coon.lea@mayo.edu

PRE-IMPLANTATION GENETIC DIAGNOSIS

Women commonly attend local fertility clinics to create embryos. Biopsies are diagnosed at reference laboratories such as those listed below.

Dr. Santiago Munné
Reprogenetics
3 Regent Street
Livingston, NJ 07039
973-436-5000
www.reprogenetics.com

Dr. Svetlana Rechitsky, Dr. Anver Kuliev
Reproductive Genetic Innovations, LLC
2910 MacArthur Boulevard
Northbrook, IL 60062
www.rgipgd.com

Dr. Mark Hughes
Genesis Genetics Institute,
705 South Main Street
Plymouth, MI 48170
www.genesisgenetics.com

APPENDIX
GLOSSARY

Alleles: alternative forms of a gene or DNA sequence found at the same locus on homologous (paired) chromosomes.

Amino acids: the major building blocks of polypeptides; organic compounds containing both carboxyl (—COOH) and amino (—NH₂) groups.

Amino terminus: the 5’ end of a DNA sequence, the end from which transcription starts.

Amniocentesis: prenatal diagnostic technique in which a small amount of amniotic fluid is withdrawn from the uterus for analysis of shed fetal cells.

Aneuploidy: Not-euploid, a set of chromosomes with one or more chromosomes extra or missing, instead of the normal euploid set of 46 in humans.

Antibody: molecule produced by plasma cells that binds to antigens.

Antigen: a substance that provokes antibody formation (from antibody generator.)

Autosome: any chromosome other than the sex chromosomes X and Y.

Autosomal dominant: an inheritance pattern in which a gene on one of the autosomes is fully manifested in a heterozygote.

Autosomal recessive: an inheritance pattern in which genes at a given locus on paired autosomes fully manifest themselves only if both code for the same message.

Barr body: the inactive X chromosome, seen as densely staining material in nuclei of somatic cells in females.

Base: short for one of the four nitrogenous bases that make up DNA; a base with a phosphate and a pentose sugar is a nucleotide.

Base pair (bp): a unit of complementary DNA bases in a double-stranded DNA molecule. (A-T or C-G)

Blastocyst: a very early stage in embryonic development when an embryo consists of a hollow ball of cells.

Carrier: person heterozygous for a recessive gene; male or female for autosomal genes, female for X-linked genes.

Carboxy terminus: The 3’ end of a DNA sequence, the end at which transcription finishes.

Centromere: the narrowest part of a chromosome, the central point between the short arm and the long arm; the point at which the two chromatids are attached during replication.

Chorionic villus sampling (CVS): A prenatal diagnostic technique in which a small sample of chorionic villi is aspirated from the placenta (which is fetal tissue).

Chromatid: one of two identical strands formed from a chromosome during cell division. Each chromosome divides longitudinally into two “sister” chromatid strands held together by the centromere.

Chromosome: Strand of DNA containing genes, located in the cell nuclei.

Codon: a sequence of three adjacent nucleotides, a “triplet”, that codes for a specific amino acid or for chain termination.

Compound heterozygote: a person with an autosomal recessive condition who has two different mutations in the (paired) (homologous) genes at a given locus.

Concordant: having the same condition, as in, related persons with the same genotype and the same phenotype. (Discordant, having different phenotypes.)

Congenital: any abnormality present at birth, genetic or otherwise.

Consanguineous marriage: a marriage between blood relatives; in much of the world, the term denotes marriage of cousins; in some areas, marriage between uncles and nieces is commonplace. (The term “incest” refers to mating of parent-child or sibling-sibling.)

Conservation: preservation of highly similar DNA sequences among different species. Conserved sequences usually are found in functional genes. A highly-conserved sequences is presumed to be vital.
Consultand: the person asking for genetic advice

CpG dinucleotides: The sequence 5’ CG 3’ (that is, an upstream cytosine adjacent to a downstream guanine) within a longer DNA molecule, which is associated with a high frequency of conversion of cytosine to thymine as a mechanism of mutation.

Cross-reaction: binding of an antibody to an antigen that is similar to, but not exactly the same as, the one that originally stimulated formation of the antibody; cross-reacting material, the similar antigen to which the antibody reacts.

Crossing over: exchange of DNA between paired chromosomes during meiosis. The result is “recombination”.

Cryptic splice site: mutation in a splice site that results in abnormal splicing of the mRNA.

Daughter cells: cells that result from the division of a cell.

Deletion: a mutation in which there is loss of one or more nucleotides, or part of a chromosome.

De novo: (“from new”), a newly-occurring mutation or other phenomenon, rather than a familial one.

Diploid: having two copies of each type of chromosome, the normal constitution of human somatic cells.

Discordant: having different conditions, as in, related persons with the same genotype having a different phenotype.

Dizygotic twins: (= fraternal twins) twins produced by fertilization of two ova by two sperm.

DNA (deoxyribonucleic acid): A double-helix molecule consisting of a sugar-phosphate backbone and four nucleotides; the nucleic acid in chromosomes in which genetic information is coded.

Dominant: a trait that is expressed strongly in a heterozygote.

Dominant-negative mutation: in the heterozygous state, a mutant gene whose dysfunctional product interferes with the normal gene product of the corresponding normal gene on the paired chromosome.

Double heterozygote: a person with a different mutant gene at the same locus in both of a pair of chromosomes. The resulting phenotype may be similar to that of a homozygote for one of the mutations.

Epigenetics: Study of the transmission of modifications of genes or chromosomes, without a change in the DNA sequence, from one somatic cell to the next generation of its daughter cells, usually persisting over several generations of somatic cells. Examples of epigenetic modification include X-chromosome inactivation, gene silencing and imprinting of specific genes, all involving DNA methylation. A few reports have suggested that occasionally an epigenetic change may be transmitted from one generation of persons to another, e.g. from mother to son.

Euploidy: a normal number or set of 46 chromosomes with none missing or extra, the normal situation.

Exon: (from expressed sequence) a segment of a gene that is represented in the final RNA, usually coding for a segment of a protein.

Fetoscopy: a technique for visualizing the fetus in which an endoscope is inserted through the abdominal wall.

Fitness, or, reproductive fitness: in population genetics, a measure of the success in transmitting genotypes to the next generation (ranging from zero for no transmission to one, or 100%, for normal transmission.) Conditions leading to death before sexual maturity, or to the inability to reproduce, have zero fitness.

Founder effect: higher-than-expected frequency of a mutation in a population because an ancestor who carried that gene had many descendants.

Frameshift mutation: A mutation in which an insertion or deletion in the DNA sequence is not a multiple of three base pairs, thus disrupting the triplet reading-frame.

Gain-of-function mutation: a mutation that intensifies the function of a gene product, or adds a new function. The mutation causing von Willebrand disease type 2B is a gain-of-function mutation, intensifying the avidity of von Willebrand factor for platelet binding sites.

Gametes: sperm cells and egg cells.

Gene: a DNA sequence directing the synthesis of a specific polypeptide chain.
**Genome**: The total DNA found in the cells of an organism; also defined as, all the genes of an organism.

**Genotype**: the genetic constitution of an individual; usually used to refer to his alleles at a specific locus.

**Germ cells**: sperm cells and egg cells.

**Germline**: the germ cells and those cells giving rise to them (as opposed to somatic cells).

**Germline mosaic**: an individual with a subset of germ-line cells carrying a mutation that is not found in other germ-line cells.

**Gonad**: a gamete-producing organ; the testis or ovary.

**Haploid**: having only a single copy of each chromosome, that is, the 23 chromosomes in a sperm or an ovum.

**Haplotype**: the alleles found at different sites on a single chromosome; usually, refers to sites close together.

**Haplodinsufficiency**: a situation in which half of the normal level of gene expression in a heterozygote is not sufficient for normal function.

**Hemizygote**: person with only one copy of a gene in diploid cells. Males are hemizygous for genes on the X chromosome.

**Heterozygote**: a person with two different alleles at a particular locus.

**Heterozygote advantage**: an increase in biological fitness seen in unaffected heterozygotes, although homozygous have decreased fitness. *The most common example is sickle-cell trait, which enables resistance to malaria.*

**Heritability**: the proportion of variation among persons that can be attributed to genetic factors.

**Homologous chromosomes**: the two members of a pair of chromosomes, one derived from the father and one from the mother; they pair during meiosis.

**Homozygote**: person with two identical alleles at a particular locus on each of a pair of chromosomes.

**Hotspot**: a DNA sequence associated with an abnormally high frequency of recombination or mutation.

**Indel mutation**: A deletion and insertion at a particular site, occurring as one event, in which the number of deleted nucleotides is not the same as the number of inserted ones. The term is also used as a collective word for insertions and deletions.

**Index case**: first person in a family to be identified with a particular disorder; = proband, = propositus. (he or she may not be the first afflicted person, but is the first to come to the attention of the counselor.)

**Intron**: (from intervening sequence) noncoding DNA which separates neighboring exons in a gene; it is spliced out and discarded during transcription of DNA by RNA.

**Inversion**: a structural rearrangement of a chromosome, in which two breaks occur and the broken-off segment is re-inserted in reversed order.

**In vitro** ("in glass"): in the laboratory

**In vivo** ("in life"): in the living cell or organism.

**Karyotype**: (1) A picture of the chromosomes from one individual, arranged in pairs according to size and appearance; (2) a summary of the chromosome constitution of a cell or a person, such as, “46,XY” (meaning, 46 chromosomes, with one X and one Y chromosome, a male.).

**Kilobase (kb)**: One thousand DNA nucleotide pairs.

**Lethal mutation**: a mutation leading to the premature death of an individual, usually used to mean death as a fetus or a newborn.

**Linkage**: the tendency of two alleles to travel together in a pedigree because they are close together on a chromosome; their probability of crossing-over (recombination) is low.

**Linkage analysis**: tracking a gene through a family by means of RFLPs or VNTRs or other markers.

**Linkage equilibrium**: a lack of association between particular alleles at different loci. *In population studies, presume that allele A or allele B is at locus one; allele C or allele D is at locus two. The finding of A and C on one chromosome is as likely as the finding of A and D; the combination of B and C is as likely as B and D. Identifying the allele at both sites helps in tracking genes.*
**Linkage disequilibrium**: an association between particular alleles at different loci; in population studies, the two loci tend to have the same set of alleles, perhaps because of a common ancestral haplotype. *Presume that allele A or B is at locus one; allele C or D are at locus two. Alleles A and C tend to be found together; alleles B and D tend to be found together. Looking at the second locus does not add much to the tracking information gained by looking at the first locus.*

**Locus** (plural, loci): the site of a gene or DNA sequence on a chromosome.

**Loss-of-function mutation**: Mutation causing lost or decreased activity of the gene product. *The mutation causing von Willebrand disease type 2A is a loss-of-function mutation. Many more mutations are loss-of-function than are gain-of-function.*

**Lyonization** (sometimes misspelled "lionization" by those not understanding the origin of the term): The process of inactivation of one of the X chromosomes in females, originally proposed by the scientist Mary Lyon.

**Manifesting heterozygote**: a female carrier of an X-linked condition who shows some clinical symptoms.

**Markers**: polymorphisms that are linked to a disease locus.

**Meiosis**: cell division process in which haploid gametes are formed from diploid germ cells.

**Messenger RNA (mRNA)**: RNA molecule formed from the transcription of DNA. Before intron splicing, mRNA is termed a primary transcript. After splicing, the mature transcript travels to the cytoplasm where it is translated into an amino acid sequence.

**Methylation**: the attachment of methyl groups, in genetics, the addition of methyl groups to cytosine bases. Methylation is associated with reduced transcription of genes. The inactivated X-chromosome is methylated.

**Microsatellite**: a small run of repeats of a simple DNA sequence, also called tandem repeats. See also VNTR, variable number tandem repeats, a form of polymorphism useful for gene tracking.

**Missense mutation**: a nucleotide substitution that results in an amino acid change in the resulting protein.

**Mitochondrial DNA (mtDNA)**: Genetic material in mitochondria that codes for enzymes involved in energy production. Mitochondrial DNA is transmitted in the cytoplasm of eggs, from mothers to their offspring, and is not inherited from fathers.

**Molecular genetics**: study of the structure and function of genes at the molecular level.

**Monozygotic twins**: identical twins, twins derived from a single fertilized ovum that splits into two.

**Mosaic**: a person who has two or more genetically different cell lines derived from a single zygote. The differences may be in chromosomes or in genes.

**Mutation**: a change in genetic material, either of a single gene, or in the number or structure of the chromosomes. A mutation occurring in a gamete is inherited whereas a mutation in a somatic cell is not.

**Mutation rate**: the number of mutations at any one particular locus which occur per gamete per generation.

**Non-penetrance**: a situation in which a person carrying a gene that usually causes a dominant phenotype does not show that phenotype.

**Nonsense mutation** ("non-sense", not making sense): a mutation that occurs within a codon and changes it to a stop codon.

**Nonsense-mediated decay**: a cellular mechanism that rapidly degrades mRNA bearing truncated messages (due to mutations causing premature stop codons); it has the effect of reducing the expression of truncated proteins.

**Nucleotide**: a basic unit of DNA or RNA, consisting of one deoxyribose (or ribose in the case of RNA) with one phosphate group and one nitrogenous base.

**Null allele**: a mutant allele that produces no product.

**Oogenesis**: the process by which ova (eggs) are produced.

**Pedigree**: A family tree, a diagram that describes family relationships, gender, disease status and so on.

**Penetrance**: the frequency with which a genotype manifests itself as a given phenotype. If the frequency of the given phenotype is 1.0, the genotype is completely penetrant (as is the case with males with he-
mophilia A or B). If the frequency of the given phenotype is less than 1.0 (as is sometimes the case in von Willebrand disease) the genotype has reduced or incomplete penetrance.

**Peptide:** an amino acid. **Polypeptide:** a compound of three or more amino acids.

**Pharmacogenetics:** the study of genetic variation in the metabolism or function of drugs.

**Phenocopy:** A phenotype that resembles the phenotype produced by a specific gene but is caused instead by different genetic or environmental influences. *Some persons with borderline-low levels of von Willebrand factor, consistent with mild type 1 von Willebrand disease, do not have any mutation in the von Willebrand factor gene but may have other genetic or environmental factors depressing the factor level, such as type O blood group or hypothyroidism.*

**Phenotype:** the observable characteristics of an organism produced by the interaction of genes and environment. In hemophilia, the phenotype includes the characteristic history, physical findings and coagulation test findings.

**Point mutation:** the alteration of a single nucleotide to a different nucleotide. (If a point mutation in a codon still codes for some amino acid, it is a missense mutation, but if it codes for no amino acid, it is a nonsense mutation.) This term is used infrequently for any change, such as a deletion, of a single nucleotide.

**Polar bodies:** the small daughter cells of division of the primary and secondary oocytes in meiosis that do not go on to become mature ova. A polar body has a nucleus but very little cytoplasm.

**Polymorphism:** Any non-pathogenic DNA sequence variant, present in more than one percent of a population. (There are several definitions, this seems to be the one most commonly meant.)

**Polypeptide:** a series of amino acids linked together by peptide bonds; an early stage of a protein.

**Post-translational modification:** various types of additions and modifications of a polypeptide (an amino acid sequence) that take place after the mature mRNA transcript is translated into a polypeptide (e.g., hydroxylation, glycosylation, cleavage of portions of the polypeptide. Problems in post-translational modification may arise in production of recombinant proteins in animal cells with different cytoplasm mechanisms than human cells.)

**Prenatal diagnosis:** testing during pregnancy to determine whether the fetus is affected with a particular disorder.

**Preimplantation genetic diagnosis:** the detection of the presence of an inherited disorder in an *in vitro* fertilized zygote before implantation.

**Prior probability:** in hemophilia genetics, the probability that a female is a carrier, based on her position in the family tree, before laboratory testing; the daughter of a carrier has a 50% chance of being a carrier and her daughter has a 25% chance, and so on.

**Proband:** the first affected patient in a kindred coming to medical attention, the index patient.

**Probe:** in genetics, a labeled substance, such as a segment of DNA with a fluorescent or radioactive tag, that is used to identify a gene or RNA or a gene product.

**Promoter:** a short sequence of DNA adjacent to a gene, to which the enzyme “RNA polymerase” binds to initiate transcription of the gene.

**Pseudogene:** a non-functional gene that is highly similar in its DNA sequence to another, functional gene. The pseudogene has been rendered inactive over the course of evolution by mutations interfering with its transcription or translation, but it remains in the genome.

**Recessive:** a characteristic is recessive if it is fully manifested only in homozygotes or hemizygotes and not in heterozygotes. A recessive allele is masked by a dominant allele in a heterozygote.

**Recombination:** cross-over between two linked loci, usually during meiosis.

**Recombinant DNA:** A DNA molecule that consists of components from more than one parent molecule. *To make recombinant human factor VIII, the human factor VIII gene, with promoter or other facilitating genes are inserted into hamster cell nuclei (re-combined with hamster DNA) so that the hamster cell will produce human factor VIII.*

**Replication:** the process of copying the double-stranded DNA molecule.
Restriction fragment length polymorphism (RFLP): a genetic marker, a single nucleotide polymorphism that, depending on the allele, is or is not subject to cutting with restriction enzymes. The length of resulting DNA fragments indicates whether or not the site is present.

Restriction site: a DNA sequence that is cleaved by a specific restriction enzyme.

Ring chromosome: an abnormal chromosome formed when both ends are lost and the new ends fuse together.

Segregation analysis: study of the way in which a disorder is transmitted in families to establish the mode of inheritance.

Sibling, sib: brother or sister

Sibship: set of brothers and sisters, children of the same parents or, in some instances, the same parent.

Silent mutation: a missense mutation that still results in the same amino acid sequence in the protein.

Single gene disorder: a disease caused by a single gene (such as hemophilia), as opposed to a disease caused by the interaction of multiple genes (such as diabetes).

Single nucleotide polymorphism: any polymorphic variation at a single nucleotide site.

Somatic cell: any cell in the body except the gametes (i.e. except for the egg and sperm)

Splice site mutation: alterations in DNA sequence at the sites where exons are to be spliced together.

Splicing: RNA sequences transcribed from exons are spliced together in the same linear order as the exons in the gene, while the intron sequences are discarded

Spontaneous mutation: a mutation that arises de novo, apparently not due to environmental factors, e.g. toxins.

Sporadic: occurrence of a disease in a family with no previous history of it (usually as a result of a new mutation)

Stop codon: A nucleotide codon that specifies the point at which translation of RNA halts. A nonsense mutation produces a stop codon.

Telomere: the tip of a chromosome. In humans, it consists of a closed loop that protects the chromosome ends.

Transition: a missense mutation in which a purine (adenine or guanine) is substituted for a purine or a pyrimidine (cytosine or thymine) for a pyrimidine.

Transcription: in genetics, the process by which an mRNA sequence is synthesized from DNA.

Translocation: transfer of DNA sequences from one chromosome to another, not of the same pair.

Transversion: a missense mutation in which a purine (adenine or guanine) is substituted for a pyrimidine (cytosine or thymine) or vice-versa.

Triplet code: a series of three bases in the DNA or RNA molecule that codes for a specific amino acid.

Variable expression: Varying extent or intensity of phenotypic signs among persons with a given genotype. This concept is related to penetrance. (Variable expression is sometimes found in von Willebrand disease, but not in hemophilia.)

Variable number tandem repeat (VNTR) polymorphism: arrays of repeated sequences that vary among persons in the number of repeat units (gene “stuttering”). The differences in the length of the repeats can be used as gene markers.

X inactivation (Lyonization): the process of inactivation of one of the two X chromosomes in the cells of female embryos.

XIST: gene on the X chromosome that sends out an inactivation signal.

X-linked: Caused by genes on the X chromosome.

Wild-type: a normal gene or genotype

Zygote: the diploid fertilized egg cell.
REFERENCES

Principles of Genetics


Li WH, Yi S, Makova K. Male driven evolution. Curr Opin Genet Dev 2002; 12:650-656. *This paper is frequently cited, perhaps because of its catchy title.* Mutations occur 5.3 times as often in the male germ-line as in the female germ-line. Causes are discussed.


Nomenclature

Peake I, Tuddenham E. A standard nomenclature for factor VIII and factor IX gene mutations and associated amino acid alterations. (On behalf of the ISTH SSC Subcommittee on Factor VIII and Factor IX.) Thromb Haemost 1994; 72: 475-476. *This numbering system, now called “legacy”, has been superceded, see below.*


Incidence and Prevalence


A study of six scattered states within the USA during 1993-5 identified 2743 patients with hemophilia A or B (defined as less than 30% FVIII or IX); 79% had hemophilia A. Of those with hemophilia A, 43% had <1% FVIII, 26% had 1-5 % FVIII and 31% had 6-30% FVIII. The median age was 23 years. The prevalence, as of 1994, was 10.5 cases of hemophilia A per 100,000 males and 2.9 cases of hemophilia B per 100,000 males. Prevalence among white, African American and Hispanic males was similar. The estimated national population was 13,320 cases of hemophilia A and 3,640 of hemophilia B. For the 10-year period 1982-91, the average incidence of hemophilia A and B was one in 5,032 live male births.


In 804 well-documented pedigrees, a positive family history was found in 70% of instances of mild or moderate hemophilia A or B, 57% of severe hemophilia B and 45% of severe hemophilia A. Others were the first case or first affected sibship. All 18 tested mothers of sporadic sibships bore the mutation as did 56 / 66 mothers of isolated cases and 2 / 19 maternal grandmothers of isolated cases.

Reviews


The Canadian national genotyping laboratory had tested 1177 hemophilia patients, and found mutations in 91% of those with hemophilia A and 94% of those with hemophilia B. *Some mutations are occult.*

Oldenburg J, Ananyeva NM, Saenko EL. Molecular basis of haemophilia A. Haemophilia 2004; 10 (suppl 4) 133-139. *A review.*

Mutations in the factor VIII gene were identified in 874 (89%) of families with sporadic severe hemophilia, 146 (89%) with moderate and 133 (94%) with mild hemophilia A. Some mutations are occult.

**Hemophilia A**

**Origin**


In a survey of 1034 cases, no effect could be seen of maternal age at birth of the proband or of maternal grandfather’s age at birth of the mother on the rate of mutation causing hemophilia A.


Factor VIII levels in 56 mothers and 47 maternal grandmothers of isolated cases of severe hemophilia A were compared to those in 134 normal women and those in 61 proven carriers. Mean factor levels in mothers and grandmothers of isolated cases were slightly higher than in age-group-matched proven carriers. The author calculated that about 86% of mothers and about 28% of maternal grandmothers of isolated cases are true (somatic) carriers. His predictions of almost 50 years ago, based on careful analysis of factor VIII levels only, were borne out later in studies based on DNA analysis.


Assays of factor VIII activity and of von Willebrand factor antigen in 82 proven or obligatory carriers yielded a mean ratio of FVIII/ VWF:Ag of 0.53. In 41 mothers of isolated cases of hemophilia, the mean FVIII/VWFAg ratio was 0.46, with four subjects having normal ratios. The authors estimated that 39 of the 41 mothers of isolated cases were true carriers. "... Mothers of single cases of hemophilia should for practical purposes all be regarded as carriers of haemophilia."


In patients with severe sporadic hemophilia A, male germelines were 3.6 times as likely to be the source of mutations as female germelines. Three of 16 mothers who were the source of de novo mutations were somatic mosaics.


In 29 families with sporadic hemophilia, 21 mothers were carriers of the mutation which was of paternal origin in 15 cases and maternal in one.


Hot spots for mutation include not only inversions at introns 1 and 22 but also CpG sites (where about 40% of point mutations occur) and adenine runs in exon 14. In that exon, the vast majority of mutations are in a few hundred base-pairs around the functionally-important thrombin cleavage sites.

Inversions are more than 15 times more likely to occur in the male germline than in the female one, and point mutations are more than five times more likely in the male germline, but deletions predominantly occur in the female germline.

**Founder effects**


In the village of Aiani in northern Greece, one in 30 male inhabitants has mild hemophilia A (factor VIII levels of 5 to 25%), caused by the same missense mutation. They represent 21% of all Greek patients with mild hemophilia A. The hemophilia has been traced for seven generations to a common ancestress.


Mild hemophilia A was present in 44 of 3300 males in an isolated population in rural Newfoundland. The affected kindred could be traced back ten generations.
Intron 22 inversion occurs predominantly during meiosis in males. In this instance, however, a mother of a male with *de novo* severe hemophilia A was a somatic mosaic for intron 22 inversion, with the mutation in about half her lymphocytes and fibroblasts. The mutation probably happened during mitosis in a somatic cell when she was an early embryo.

**Intron 22 inversion**


Studying messenger RNA (mRNA), they found that a mutation of some undefined kind, involving the intron 22 region, led to defective joining of exons 22 and 23 in mRNA and caused hemophilia in 10 of 24 severely-affected UK patients. (*This observation led to further discoveries, below.*)

Lakich D, Kazazian HH, Antonarakis SE, Gitschier J. Inversions disrupting the factor VIII gene are a common cause of severe haemophilia A. Nature Genetics 1993; 5:236-241.

During meiosis, as the tip of the X chromosome bends up towards the centromere, a region ("gene A", *called F8A in this monograph*) in intron 22 can pair inappropriately with one of the two homologous (similar) regions upstream on the same chromosome. The pairing is followed by re-attachment, resulting in a large inversion disrupting the factor VIII gene.


Inversion at intron 22 of the factor VIII gene was present in 42% of 2,093 patients with severe hemophilia A. 98% of 532 mothers of patients with inversions had the mutation in their white blood cells. The mutation had originated in the germline of only 9 of the 225 mothers of sporadic cases. Maternal grandparents of 70 sporadic cases were tested: the grandfather's germline was the origin in 69 cases and the grandmother's germline in one case.


Levels of F8 mRNA and intracellular FVIII protein in B lymphoblastoid cells and liver biopsies from an individual with the intron 22 inversion were compa-
rable to those in healthy controls. Thus, in the presence of the intron 22 inversion, FVIII is made intracellularly but does not reach the circulation. The presence of this FVIII may help make the fetus tolerant to exogenous normal FVIII.

**Intron 1 inversion**


The frequency of intron one inversion among 209 patients with severe hemophilia A was 5%.


The prevalence of intron 1 inversion among 1127 patients with hemophilia A was 2%. A total of 2910 patients had been studied in this and nine other studies, for an overall prevalence of 2.3%. The inhibitor prevalence with intron 1 inversion was 14.9%.

**Intronic Mutations**


Dr. Goodeve discusses unusual mutations including intronic mutations, duplications of exons and copy-number variations.


In about 10% of families with mild hemophilia A, no mutation is found after complete sequencing of the coding region (exons) of the factor VIII gene. In four such instances, abnormal mRNA was found. Three different intronic variants (two missense, one small deletion) were found. Two were predicted to generate new splice sites and the third to generate a protein with additional amino acids.


Nine patients with mild hemophilia A, in whom no mutations were found in the usual studies of exons of F8 and the VWF gene, were further studied and eight new intronic polymorphisms were found. Two of them created new splice sites that resulted in the introduction of intronic DNA sequences into the mRNA with creation of premature stop codons.

Zimmermann MA, Gehrig A, Oldenburg J, Müller CR, Rost S. Analysis of F8 mRNA in haemophilia a patients with silent mutations or presumptive splice site mutations. Haemophilia 2013; 19:310-7

Mutations which do not result in amino acid substitutions (“synonymous”) and intronic variants located outside the splice site cannot be easily classified as causative for hemophilia. Seven of 11 such mutations were shown to have an effect on F8 mRNA splicing.

**B domain**


Eleven of 19 B-domain variants analysed by expression in HEK293T cells showed normal factor VIII activity but eight other variants resulted in decreased FVIII activity because of reduced secretion or stability.

**Hemophilia B**


A patient with thrombophilia had a gain-of-function missense mutation in the factor IX gene leading to factor IX activity several times the normal. This gene has since been employed in gene therapy.

**Origin**

Thompson AR, Chen SH. Germ line origins of de novo mutations in hemophilia B families. Hum Genet 1994; 94:299-302

In 14 patients with de novo hemophilia B in the Seattle area, the origin could be identified as the germline of the maternal mother or grandmother for five deletions and two point mutations, and the maternal grandfather for five point mutations and one small insertion. One origin was unidentified.

Knobloch O, Zoll B, Zerres K, Brackmann HH, Olek K, Ludwig M. Recurrent mutations in the factor IX gene:
The causative mutation was defined in 103 of 114 unrelated patients with hemophilia B and 84 different mutations were found. Haplotype analysis revealed that the great majority of these mutations arose independently. More than 2/3 of de novo mutations caused severe disease. Family analysis of 45 patients with severe hemophilia revealed that a nearly equal number of mutations had arisen in the male and the female germlines. Of the de novo point mutations, 40% were transitions at CpG dinucleotides.


The dinucleotide CpG is a hotspot for multiple types of mutations and is the site of more than 25% of the independent mutations of the factor IX gene. Transitions at CpG and some missense mutations are more likely to occur in the male germline than in the female, but deletions are equally likely to occur in male and female germlines.


In 424 apparently-unrelated families with hemophilia B in the UK, 412 mutations were found. Fifty mutations were repeated and accounted for 245 families, among whom 19 had an identical mutation believed to derive from a common ancestor and 135 appeared to have independently- arising mutations.

Of the mutations, 13 were in the promoter region, 262 were missense, 48 nonsense, 58 splicing defects, 17 frameshifts, 7 in-frame deletions/insertions (that is, they did not disrupt the triplet structure), and 7 were large deletions.

The relevant mutation was found in the white blood cells of 88 of 110 mothers of isolated cases who were available for testing, and in 8 of 35 maternal grandmothers. Extrapolating, the authors estimate that 222 mothers of males with hemophilia B in the UK are new mutants. There must be additional women in the population who are new mutants but have not had a hemophilic son and go unnoticed.

Grandparents were available from 20 families for studies of the site of origin of mutations. Origins were grandpaternal in 16 and grandmaternal in 4.


The likelihood that a factor IX missense mutation will cause disease correlates with the degree of evolutionary conservation of the amino acid. For nonsense mutations, no such correlation exists. The authors interpret Green’s 1999 UK report (above) to mean that the mutation rate in females is about 2.19 x 10^-6 per gamete per generation, and for males 18.8 x 10^-6 per gamete per generation. The mutation rate average (males plus females) was 7.7 x 10^-5 per gamete per generation. This estimate for the overall rate of de novo mutation in factor IX is three-fold greater than previous estimates for the frequency of cases of hemophilia B due to a de novo mutation, suggesting that in populations, such as the UK, with small family sizes, 2/3 of deleterious mutations in the factor IX gene disappear before causing a hemophilic male.

**Founder effects**

Ketterling RP, Bottema CDK, Koeberl DD, Setsuko Ii, Sommer SS. T 296 → M, a common mutation causing mild hemophilia B in the Amish and others: founder effect, variability in factor IX activity assays, and rapid carrier detection. Hum Genet 1991; 87:333-357.

Five of six patients with the same missense mutation and mild hemophilia B were Amish and had a haplotype suggesting common ancestry although the families had not been known to be related. The pedigree documents at least 50 males with hemophilia and 65 carriers traced over two centuries.


A new founder was identified in a study of 160 consecutive patients with hemophilia B. Fourteen patients had a G→A transition at bp 10430. Haplotype data indicate that 12 of them were likely to have had a common ancestor. Two previously-reported founder mutations were also represented in the series and haplotype data confirmed the founder hypothesis.

In total, these three mutations (including the Amish families described above) were found in 44 of the 160 Caucasian patients with mild hemophilia B who were not previously known to be related. Descendants of these three founders account for about 2/3 of Caucasians with mild hemophilia B in the USA.


Two point mutations accounted for 19% of
mutations found in Mexicans with mild-moderate hemophilia B. Haplotype data suggested founder effects. The same two mutations also are associated with founder effects in the US Caucasian population, but with different haplotypes, indicating independent origins. About one third of mutations causing hemophilia B occur at CpG dinucleotides, a hotspot of mutation.


Three mutations accounted for 83% of Irish kindreds with mild hemophilia B. Haplotype analyses suggest a founder effect. In contrast, each family with moderate or severe hemophilia had a distinct mutation.


Of 77 presumably unrelated Swedish patients with hemophilia B due to missense mutations, 61% had mutations in common with other patients and haplotyping showed that half of these had identical genes. These mutations had estimated ages between two and 23 generations.


Among 226 patients with hemophilia B, 24 missense mutations were recurrent. Three of them accounted for 37% of the patients. Haplotype analysis revealed a founder effect.

Mosaics


A boy, an isolated case of severe hemophilia B, had a small deletion mutation also carried by his mother in her white blood cells. The maternal grandfather was mosaic for the mutation in his white blood cells. The boy's maternal aunt, another daughter of the mosaic grandfather, had the same X chromosome (that is, with the same haplotype) but its factor IX gene was not mutant. Thus, the grandfather was mosaic both in his somatic cells and in his germline.

Translational Bypass Therapy


In an in vivo mouse model for Duchenne muscular dystrophy (absence of the protein dystrophin in muscle cells) caused by a nonsense mutation, a dosage of gentamycin was identified that resulted in the appearance of dystrophin in muscle cell membranes (at levels 10 to 20% of normal), providing some protection against muscular injury. This study showed the utility of an aminoglycoside in a living animal with a protein deficiency due to a nonsense mutation, background for the next reference.


Aminoglycosides, e.g. gentamycin, can cause misreading of the RNA code, sometimes bypassing nonsense mutations, leading to hope for "translational bypass therapy" for hemophilia through use of these or similar drugs. When mice with a nonsense mutation causing severe hemophilia B were given a less toxic aminoglycoside, geneticin, they obtained an average of 8% plasma factor IX activity after two days.

Genotype-phenotype correlations

Hemophilia A

Cross-reacting material


Plasma from only two patients from 27 families with hemophilia A neutralized an acquired inhibitor of factor VIII, that is, these two patients had cross-reacting material at a level similar to that of normal plasma. Patients from the other families had no cross-reacting material. Other authors confirmed this observation in the next year.


Mutations causing CRM+ hemophilia A were defined and the mechanisms of action studied.
**Clinical presentation**

Carcao MD, van den Berg HM, Ljung R, Mancuso ME. Correlation between phenotype and genotype in a large unselected cohort of children with severe hemophilia A. Blood 2013; 12:3926-52

In a study of 621 babies with severe hemophilia A, those with null mutations had their first bleed and first joint bleed at younger median ages than patients with non-null mutations (9.7 vs. 10.9 months and 13.8 vs. 16.1 months). Thus, in babies with non-null mutations, might the start of prophylaxis be delayed slightly?

**Assay method discrepancies**

See also Goodeve and Peake, 2003


In ten families, the factor VIII activity in persons with hemophilia was 2- to 7-fold higher when measured by the one-stage assay than it was when measured by the two-stage assay. Six different nonsense mutations, affecting the A1, A2 or A3 domain of factor VIII, were found in patients with this phenomenon. In a control group of 23 patients with mild hemophilia but no assay discrepancies, none of the above mutations were found, instead, they had missense mutations affecting the A3, C1 and C2 domains.


The molecular basis for one of the mutations (at Arg 531) causing assay method difference in factor VIII levels is an increased rate of A2 subunit dissociation, at about three times the normal rate. That mutation lies at the interface of the A1 and A2 domains and affects the stabilization of their interaction. Patients with mutations either directly contacting Arg 531 or closely adjacent to the A1-A2 interface have the same phenotype of assay discrepancy.


Among 99 patients with discrepancies, levels were higher with the one-stage assay in 16 patients and with the chromogenic assay in 83. Mutations associated with a higher one-stage assay result were in the A1-2-3 area. Most of those with a higher chromogenic assay result were close to or within the thrombin cleavage site or the binding sites for factor IX or VWF.


In a family with the above mutation, APTTs were prolonged and one-stage assay results were below normal at 16, 21, 36 and 36%, leading to a diagnosis of mild hemophilia although there were no bleeding symptoms; the two-stage assays were in the normal range at 53, 63, 65 and 71%.

**Discrepancies due to RNA mistakes**


Errors that occur during copying of DNA, which are especially likely at sites with multiple repeats of the same nucleotide, may allow a low level of protein production, albeit of a slightly abnormal molecule.


In the wake of the above article, the authors studied four patients with frameshift mutations of one or of four nucleotides within or near an A run of exon 14, thus, expected to cause severe hemophilia. All had <1% Factor VIII activity but all had thromboelastogram patterns that were less impaired than those of other patients with severe hemophilia who had stop mutations or inversions. Clinically, patients with these mutations had a less severe bleeding tendency than other patients who had stop or inversion mutations. The mutations described may allow a trace of factor VIII to be formed.

**Inhibitors**


Eight series of reports correlating mutation with inhibitor formation were reviewed. The incidence
of inhibitors in patients with large deletions was 40%, with nonsense mutations was 35%, with inversion mutations was 22%, with frameshift mutations was 15% and with missense mutations causing severe hemophilia was 8%.


Inhibitor prevalence in patients with hemophilia A and various mutations was as follows:

- Inversion, 34%;
- Insertion/deletion of more than one exon, 68.2%;
- Insertion/deletion of a single exon, 11.9%;
- Small insertion, deletion, non-A run, 20.6%;
- Small insertion, deletion, A run, 6%;
- Splicing error, 2.2%;
- Nonsense mutation in general, 34.4%;
- Nonsense in A3, C1, C2 domains, 50%;
- Nonsense elsewhere, 14.3%;
- Missense mutation in general 7.6%;
- Missense in A3, C1, C2 domains, 12%;
- Missense elsewhere, 3.9%.


Data were pooled from 30 studies representing 5383 patients with severe hemophilia A including 1029 inhibitor patients. Using the rate in those with intron 22 inversions as a reference, the risk (odds ratio) in patients with large deletions was 3.6, with nonsense mutations was 1.4, with intron 1 mutations and splice site mutations was equal, with small deletions/insertions was 0.5, and with missense mutations was 0.3. The relative risk for developing high-titer inhibitors followed a similar pattern.


Among 1112 non-severe hemophilia A patients from Europe and Australia, 5.3% developed an inhibitor after 28 exposure days, rising to 13.3% at 100 exposure days. Among these patients, there were 214 different missense mutations, of which 19 were associated with inhibitor development.


The most dangerous missense mutations are those in which the amino acid substitution belongs to another physicochemical class than the original.


Patients with mutations considered low-risk for inhibitors also had a significantly higher success rate with induction of tolerance.


Among 78 black patients with hemophilia A, 24% had one or another single nucleotide polymorphism in their F8 genes, not found in Caucasians. The prevalence of inhibitors among patients with black-restricted haplotypes was 3.6 times that of black patients with haplotypes in common with Caucasians.

Hemophilia B

Cross-reacting material


Two brothers with hemophilia B were found to have prolonged prothrombin times when performed with bovine-brain thromboplastin. The mother and maternal grandmother also had prolonged prothrombin times with that reagent. The prolongation appeared to be due to an inhibitor, which was believed to be an abnormal factor IX molecule. The designation "Bm" came from the surname of the family described.


Factor IX activity levels, factor IX antigen levels and prothrombin times with bovine brain thromboplastin were studied in 92 patients with hemophilia B from 71 kindreds. Those with severe and moderately severe hemophilia were divided into four groups: (1) seven patients with greatly prolonged prothrombin times and normal levels of factor IX antigen (CRM+; Bm); (2) 17 patients with mildly prolonged prothrombin times and factor IX antigen levels between 25% and normal (CRM or CRM reduced, mild Bm); (3) eight patients with normal prothrombin times and factor IX
antigen levels between 25% and normal (CRM+ or CRM reduced); and (4) 48 patients with normal prothrombin times and no excess antigen (CRM negative). None of 12 patients with mild hemophilia B had prolonged prothrombin times but four of them had an excess of factor IX antigen over activity (CRM reduced). In appropriate families, some carriers could be distinguished from normal because of an excess of antigen over activity, or, on the basis of their prolonged prothrombin times.

**Inhibitors**


Mutation analysis in 17 children with hemophilia B and inhibitors to factor IX who had had anaphylaxis or an anaphylactoid reaction on receiving factor IX products showed that 10 had large gene deletions and seven had other major derangements such as frameshift, splice site or stop mutations.


Mutations in 8 unrelated patients who had had anaphylaxis were compared to those of 276 other patients with severe hemophilia B. Complete gene deletions conferred an estimated risk of 26% or more. Anaphylaxis was less likely in patients with partial gene deletions or mutations that truncated the protein. Anaphylaxis had not been seen with missense mutations.

**Immunisation at puberty**


The above report was extended. Affected children had severe factor IX deficiency, with <1% factor IX. Hemorrhages ceased and factor levels began to rise at age 15 to 16 years. Factor levels reached the low-normal range by the late 20’s. Factor IX antigen levels equaled activity levels. *Later papers reported a few more unrelated families with a similar phenotype.*


Mutations in the promoter region of the factor IX gene cause hemophilia B Leyden. One patient with a mutation at a specific locus in the promoter region did not improve at puberty. His mutation disrupted an androgen-responsive element. Promoter mutations that don’t disrupt the androgen-responsive element are associated with improvement at puberty.


Mutations causing hemophilia B Leyden are all in a 40-nucleotide region encompassing the major transcription start site for factor IX. These studies suggest the mechanisms by which these mutations disrupt transcription. The role of androgen is not entirely clear, but it is postulated to increase protein binding to one site, improving the stability of the transcriptional initiation complex.

**Reproductive fitness**


The prevalence of marriage among 250 men with severe hemophilia in southern California was 82.5% of that of age-matched males of the general US population as of 1968. Married patients age 30 or older had an average of 2.1 children each, as of 1971. In the periods 1950-54 and 1960-64, the numbers of children sired by men with hemophilia were 25 and 38 percent, respectively, of the numbers expected of age-matched men of the general US population.


The number of children sired by men with severe hemophilia in 1975-77 was 38% of that of age-matched men in the generation population. Additional births to women who already were mothers of males with hemophilia, compared to the US general population by age and parity, were not significantly different in 1940-49, but began to drop in the 1950’s and dropped further in the early 1960’s (but not quite to a statistically-significant extent.) The effect of parity was analyzed. Further births to mothers of a hemophilic son, if the mother had only one or two children already, were significantly fewer than among the age-
and-parity-matched general population. If the mother had three or more children, however, further births were not significantly fewer than in the general age-and-parity matched population. Among sisters of hemophilic males, births of a first child were significantly fewer than among age-matched women of the general population but births of subsequent children were not.


In a study of 309 patients with hemophilia, 66% of hemophilic men over age 25 had married and 41% had children.


Among men with severe hemophilia A in northwest Hungary, 51% were married compared to 74.5% of unaffected men. The number of children per married man with hemophilia was 0.63 versus 1.82 in normal men, thus, the reproductive fitness was about 29-34%. Reproductive fitness was better in severe hemophilia B, namely 73-86%. Of 57 carrier mothers, 47 said they would have prenatal diagnosis if available and 43 wanted to terminate any future pregnancy with a male fetus. “The perceived burden of hemophilia A has lessened insufficiently in Hungary.”

**X-chromosome inactivation**


In cells of various tissues in female mice, one densely-pigmented chromosome was seen in nuclei and postulated to be an X chromosome.


Mary Lyon built on the above observation. Female mice heterozygous for sex-linked genes affecting the color of their fur often had a coat consisting of patches of different colors, like a calico cat. Her hypothesis was that the dense chromosome was an X chromosome, genetically inactivated, and that it could be either maternal or paternal in origin in different cells of the same animal, and that inactivation occurred early in embryonic development. The retina in males with X-linked ocular albinism lacks pigment, but heterozygous females have patches of retina with pigment and patches without, that is, a mosaic appearance. Monozygotic female twins may vary in the expression of an X-linked trait. Women with the karyotype 45, X0 do not inactivate the single X chromosome and men with the karyotype 47, XXY do inactivate one of their two X chromosomes.

**Twin studies**


In a family with no history of hemophilia, a woman with 28% factor IX gave birth to proven monozygotic twin girls, one of whom had <1% factor IX and the other 15% factor IX. X chromosome inactivation appears to take place after division of the zygote into two persons.


One of the identical twin sisters of a boy with severe hemophilia A had a factor VIII level of 4% and the other had 36%.


A man with CRM+ hemophilia B (factor IX activity 1.6%, antigen 160%) had different levels of factor IX activity had identical twin daughters. One had 2.7% factor IX activity, with 70% antigen, and the other had 30% factor IX activity with 60% antigen.


Identical twin girls with no family history of hemophilia had factor VIII levels of <1 % and 12%, respectively. Both had the same de novo missense mutation, not found in either parent.

**Skewed (non-random) inactivation**


Patterns of X chromosome inactivation were studied in 225 females from 36 three-generation pedigrees. In one family, a mother, her son and all the son’s seven daughters had highly skewed inactivation
of the maternal X chromosome, with 75-96% of their cells having the paternal X active.

Somewhat skewed inactivation is not unusual: 22% of normal females had the same X chromosome inactivated in 80% or more of their white blood cells.


A rare C → G mutation in the promoter region of XIST led to preferential inactivation of the mutant X chromosome in two females in one family, and in seven females in another family.


Of 50 women in a family with X-linked muscular dystrophy, 16 had skewed X chromosome inactivation, with 95% or more of cells having the (mutant) maternal X chromosome inactivated. The mutation was a large deletion spanning more than one gene.


Females with hemophilia


A survey of 11 hemophilia centers discovered ten women with hemophilia A and six with hemophilia B, levels from severe to mild (all under 10%).


Among seven females with hemophilia, two were homozygotes resulting from consanguineous unions, one was a compound heterozygote with a maternal missense mutation and a de novo large deletion, and four had a mutation on one X-chromosome and non-random inactivation of the other X-chromosome.


Cytogenetic studies were performed on 18 of 22 females with moderate to severe hemophilia A or B; 14 were XX, two had translocations, one had partial deletion of an X chromosome and one had mosaic XX/XO. All of the 14 with satisfactory analysis showed skewed X chromosome inactivation.

**Homozygosity**


A man with moderate hemophilia B, married to his first cousin, had a daughter with hemophilia of a similar severity.


Hemophilia A (FVIII <2%) was observed in a female, the product of a marriage of a hemophilic man and a carrier woman in an isolated, inbred community.

**One established, one de novo mutation**


A girl had 3.4 % factor VIII activity and her father with 6.9% factor VIII activity both had the same missense mutation. The girl had a de novo frameshift mutation on her other X chromosome, but that mutation was not found in either parent.


A girl inherited a FVIII intron 22 inversion from her carrier mother and also a de novo FVIII inversion 22 mutation from her father. She had <1 % factor VIII activity and 95% vWF:Ag. Methylation studies showed that her paternally-derived X chromosome was inactivated in 95% or more of her cells. The girl’s mother also had skewed inactivation of one X chromosome.

**Two de novo mutations**


A girl with <1% factor VIII activity had a de novo type 1 factor VIII gene inversion on her paternal X chromosome. Her maternal X chromosome had a
de novo deletion of a portion of the long arm including the FVIII gene; an abnormality that was not found in the mother’s somatic cells. The gross deletion in the maternal X chromosome probably led to its preferential inactivation.


In a family with no prior hemophilia history, two of three sisters had hemophilia B, one severely (factor IX 1%) and the other mildly (factor IX 7%). Both had a missense mutation, also found in the mother’s white cells in a mosaic pattern. The more severely affected girl also had a different mutation in the factor IX gene on her other X chromosome, presumed to have arisen as a de novo paternal mutation.

Non-random X-chromosome inactivation


A man with 10% factor VIII had two daughters, one with a typical carrier factor level of 40% and the other with a high level, 180%, suggesting skewed inactivation of the paternal X chromosome. The latter daughter had a daughter who had only 9% factor VIII, suggesting skewed inactivation of her paternal X chromosome. Perhaps the two women share a maternally-inherited X chromosome which resists inactivation.


A woman with 2% factor IX had a normal paternal factor IX gene but a large deletion in her maternal factor IX gene. Methylation analysis showed skewed inactivation of the paternal X-chromosome.


The daughter of a carrier of hemophilia A had 5% factor VIII and 80% VWF:Ag. Her paternal X chromosome was inactivated in almost all white cells.


A man with mild hemophilia A (12% FVIII) and his wife, a carrier of incontinentia pigmenti (an X-linked condition causing dermatosis in carriers and death of affected males in utero) had a daughter, a carrier of both conditions. Cells in which the activated X chromosome bears the incontinentia pigmenti gene do not proliferate well, leading to predominance of cells with the other X chromosome. The double carrier had a 12% FVIII level, like her hemophilic father.


A girl inherited the incontinentia pigmenti gene on one X-chromosome and hemophilia A on the other X-chromosome, resulting in her hemophilia. Another girl had a de novo inversion mutation, presumably from the paternal germline, and more than 95% expression of the paternal X chromosome.


A man with mild hemophilia B, factor IX activity 35 %, had varying levels of mosaicism for a missense mutation, with 87% mutant cells in the liver, 91% in white blood cells, 52% in kidney cells and 48% in smooth muscle cells. His mutation was similar to one reported to cause moderately severe hemophilia B. The man’s daughter had 3% factor IX activity, and her daughter (the man’s granddaughter) also had 3% factor IX activity. The authors postulated inherited non-random X-inactivation in two generations.

De novo mutation, non-random X inactivation


A girl with 1% factor VIII activity had a de novo frameshift-stop mutation on her paternal X-chromosome which was active in all or nearly all cells.

A girl born to unrelated parents with no family history of hemophilia had 2% FVIII and 150% VWF:Ag. The mother had 35% FVIII and 100% VWF:Ag. The girl and mother both had an intron 22 inversion mutation and the girl also had a de novo intron 22 inversion mutation in her paternally-inherited X chromosome. Methylation pattern studies showed that the maternal X chromosome was inactivated. The girl’s sister had normal FVIII genes from both mother and father. The authors postulate that genes (other than the factor VIII gene) in the region of the inversion are directly involved in X-inactivation and disturbance may lead to skewed inactivation. This hypothesis is interesting, because the combination of the inversion mutation and skewed inactivation is not unusual.

Chromosomal abnormalities


A girl with 6% factor VIII activity, sister of a boy with 4.5 factor VIII activity, was found to have two X chromosomes in some of her white blood cells and only one in other white blood cells. She had a normal female phenotype. It was suggested that the cells or organ making factor VIII must have predominantly XO cells bearing the (mutant) maternal X chromosome.


The sister of two males with severe hemophilia A due to the intron 22 inversion was born with overt Turner’s syndrome, karyotype XO, and a factor inversion on her only X chromosome.


The sister of a male with severe hemophilia A also had <1% factor VIII. She had some signs of Turner’s syndrome. She was mosaic: some cells had a 45 XO karyotype and some had a 46 XXr karyotype (Xr = an X chromosome in a ring formation.) The ring chromosome was the originally-normal paternal X chromosome; the factor VIII gene was lost in the formation of the ring. An abnormal X chromosome is late to replicate and may thus be lost.


In a hemophilic girl, the long arm of chromosome 17 was transposed onto the X chromosome in exchange for part of the X chromosome transposed onto chromosome 17, at a cryptic break-point. The girl’s other X chromosome was normal, but, in instances of transposition, the normal X chromosome is inactivated.

Carrier detection (see also mutation origins)

Gene sequence analysis


Normal persons can have either a threonine or an alanine at a certain locus in the factor IX gene (that is, a single-nucleotide polymorphism, SNP). The SNP can be identified with an antibody. In certain Caucasian families with hemophilia B, carriers are polymorphic for the SNP and the pattern of SNP transmission can be used to confirm and supplement RFLP testing for carrier status of their daughters. A later paper noted that all the Asian subjects tested had threonine at this site, as did most black subjects.


A young woman with excessive bleeding after a dental extraction was found to have a prolonged APTT and 16% factor IX. Her mother, who also had a history of excessive bleeding, was found to have 23% factor IX. The causative nonsense mutation was associated, in other families, with severe hemophilia B. The factor IX gene haplotype had been inherited from the maternal grandfather, no longer alive, who had no bleeding problems.
Restriction Fragment Length Polymorphisms


The utility of factor VIII and VWF:Ag levels in the diagnosis of hemophilia A, and factor IX activity and factor IX antigen in the diagnosis of hemophilia B carriers, is reviewed, as well as RFLP markers. Infragenic sites are the most reliable, because the likelihood of crossover within the gene, between the marker site and the hemophilia mutation, is very low, estimated at 0.01%. When polymorphism is present at infragenic sites, carrier classification based on those sites is 99.9% accurate.


Use of two extragenic and one infragenic RFLPs allowed a clear diagnosis for 52 of 59 potential carriers and 6 of 7 fetuses. The 7th fetus could not be diagnosed because the mother was not polymorphic.


RFLP polymorphism was seen in 41 of 45 obligatory or proven carriers of hemophilia A. Using RFLPs, 36 of 100 potential carriers were diagnosed as true carriers, 37 were diagnosed as non-carriers and in 27 the carrier status remained uncertain (usually because the sporadic, or because crucial family members were missing.) Prenatal diagnosis of 11 male fetuses showed six to be affected and five to be normal.


The multi-ethnic population of Kuala Lumpur, Malaysia, was studied with regard to the frequency of two RFLP sites (Taq I and XmnI) used to mark the factor IX gene. Heterozygosity was common in Europeans, fairly common in black Americans and less common in persons from India. Malays and Chinese were NOT heterozygous for these markers.


The 1987 study was expanded. Three infragenic and two extragenic factor IX polymorphisms used for carrier diagnosis were studied in five ethnic groups. Europeans were the most polymorphic, Africans were intermediate, and Orientals (East Indians, Chinese, Malays) were least polymorphic. (Thus, for RFLP diagnosis, one must know the polymorphisms of the ethnic group to which the consultant belongs.)


Intra- and extragenic polymorphisms of the factor VIII gene in Japanese subjects were similar to those observed in Caucasians; 79% of 24 carriers were informative with infragenic markers. The remaining carriers were informative with extragenic markers.


Some 70% of European women are polymorphic at one or more of three infragenic RFLP marker sites. Two extragenic sites are used when the infragenic ones are not informative, but there is danger of confusion from cross-over. This paper has some excellent pedigrees showing RFLP analysis including an instance of confusing cross-over. It also analyses the cost of medical care for severe hemophilia in the UK and the potential savings from prevention of more familial hemophilia births.


Using RFLP analysis with infragenic and extragenic markers, 183 female relatives of patients with familial hemophilia A were tested; 66 were carriers, 103 were not carriers, and a diagnosis could not be reached in 14, either because of homozygosity of the mother or absence of relevant family members. RFLPs were used for prenatal diagnosis; 23 male fetuses were affected, 11 were not, and no diagnosis was possible in one. Abortion of an affected fetus was elected by 17 women, three of them twice and one woman three times. Eleven of these women asked for prenatal diagnosis in one or more subsequent
pregnancies. For hemophilia B, some fetal diagnoses were done by RFLP analysis and some by mutation analysis which had just become possible. Three of six male fetuses tested were affected. All affected fetuses had the same mother and were aborted.

**Factor levels**


Factor VIII levels in normal women ranged from 52 to 133%, mean 92%. In obligatory or proven carriers of hemophilia A, levels ranged from 22 to 135%, mean 58%. Approximately half of carriers had levels below 60%. The carrier with the lowest level, 22%, had mild excessive bleeding. Seven carriers with levels between 30 and 40% had no excess bleeding, suggesting that 30% factor VIII is adequate for hemostasis. Normal women had similar levels during each week of the menstrual cycle.


By questionnaire, bleeding symptoms in 135 carriers were compared to 60 age-matched normal females. Carriers were significantly more likely to bruise easily and to have prolonged bleeding form small wounds, tooth extractions, tonsillectomy, surgical operations and deliveries. No significant difference in nosebleeds or menorrhagia was found. The probability of excess bleeding after tooth extraction, surgical operations and tonsillectomy correlated well with lower factor levels. The authors advised that factor levels be measured even in obligatory carriers or persons in whom the carrier status was confirmed by DNA methods, to identify women at risk of bleeding.


Levels of factor VIII and of VWF:Ag rise during pregnancy, and they rise in parallel. The levels were measured in 32 normal women and 25 known carriers of hemophilia A in the second trimester. The abnormal ratio of the two factors indicated the carrier state in 23 / 25 known carriers.

**Von Willebrand Disease: Co-inheritance?**


In five families containing members with VWD and members with hemophilia A, no male had both disorders but two obligate hemophilia carriers also had VWD. *(The two doubly-affected women had laboratory tests reflecting the VWD. Co-existing conditions can confuse the attempt to diagnose the carrier state.)*


Five of 164 patients with hemophilia A or B also had laboratory tests suggestive *(3 cases)* or diag-
nastic (2 cases) of VWD. The authors do not discuss carriers, but the relatively high incidence of VWD suggests that some carriers might be affected.


A woman not related to anyone with hemophilia had 32% factor VIII and 107% VWF:Ag and proved to have a very low level of VWF binding to FVIII characteristic of homozygous or doubly heterozygous type 2N VWD. Two daughters were heterozygotes for type 2N VWD. One had 44% factor VIII with 89% VWF:Ag, the other had 61% factor VIII with 93% VWF:Ag. This report demonstrates the danger of assuming that a woman is a carrier of hemophilia A from her factor levels alone.

Pregnancy in carriers


Factor VIII levels were followed during pregnancy in 10 normal women. Peak levels at delivery were 140 - 480 %. A woman with von Willebrand disease, a baseline factor VIII level of 12% and a very prolonged bleeding time had, at delivery, 47% factor VIII and a bleeding time of 10 minutes, only slightly prolonged.


In normal women, factor VIII levels doubled by term. In five known carriers of hemophilia A, factor VIII levels also rose to double or more the baseline value by term. In cord blood samples from 10 normal term newborns, the mean factor VIII level was 96%, range 60-164%. The cord blood of an infant with severe hemophilia A had 0.5% factor VIII. (Previously, it was thought that maternal factor VIII or IX could cross the placenta and protect the newborn temporarily. That notion was debunked by this and other reports.)


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Notable hemorrhages occurred in only 26 of 61 babies with severe hemophilia who were circumcised within a few days of birth. (We don’t know why some but not all hemophilic babies bleed from circumcision; perhaps pressure on the wound is not always sustained for the prescribed period of time.) In two instances, cord blood samples from newborn hemophilic boys had less than 0.5% factor VIII. Thus, factor VIII did not cross the placenta.


A woman, already the mother of one son with severe hemophilia B, delivered a second hemophilic son whose cord blood contained <1% factor IX activity. A third, non-hemophilic son, one month premature, had 7% factor IX activity in the cord blood. A fourth, non-hemophilic son, born at term, had 27% factor IX activity. (Normal newborns may not yet have normal factor IX levels; their livers are not yet mature.)


In The Netherlands, 36 of 73 pregnant women were aware of their carrier status at delivery. Instrumental deliveries were less frequent in aware carriers (8%) than unaware ones (16%); 22% of aware carriers had caesarian sections versus 8% in unaware carriers. Five of nine instrumental deliveries were associated with intra- or extra-cranial bleeding as were two prolonged spontaneous deliveries. No cranial bleeding was seen after 11 caesarean sections. The first bleeding episode was iatrogenic in 38% of babies of unaware mothers and 9% of babies of aware mothers.

Use of sex-sorted sperm


A method of separating Y-chromosome-bearing sperm by sorting sperm through three layers of albumin resulted in 79% male births. If Clomid was used to stimulate ovulation, however, female births outnumbered male births. (These authors were very adept at this technique. Increasing the probability of male children might be appropriate for hemophilic men who hope to avoid having carrier daughters.)


Attempts at pre-selection of gender-specific sperm to that date are reviewed. The best-documented method was the separation of X and Y sperm with differential centrifugation and with migra-
tion of sperm through progressively denser solutions of bovine albumin. Clinical trials with the Y fraction resulted in 75 to 79% male babies. Other methods used in the 60’s to 80’s, timing of conception and dietary treatment, have not been proven successful.


Separation of X and Y bearing sperm has been improved using flow cytometry with fluorescent staining, and has proven useful clinically.

**Prenatal diagnosis**

**Diagnosis of fetal sex**

*Fetal sex usually can be determined by sonography, which is non-invasive but late. Early sex diagnosis can be used to decide whether or not to proceed to more invasive prenatal diagnostic procedures.*


Blood samples from 196 pregnant women were analyzed for the presence of the sex-determining region of the Y chromosome from fetal DNA. Fetal gender could be identified reliably from the seventh week of gestation. Blood samples from 15 pregnant carriers at seven weeks correctly predicted the gender of the fetus, thus sparing pregnancies with females from CVS or amniocentesis for prenatal diagnosis.

**Fetal blood sampling**


Blood from 40 fetoscopies, performed for reasons other than hemophilia diagnosis, showed that the mean factor levels were: FVIII coagulant activity 45%, FVIII:Ag 23%, VWF:Ag 57%, factor IX coagulant activity 8.3%, factor IX antigen 4.6%. In ten of the blood samples, other factors were measured, with the following results (means): factor II (prothrombin), 17%; factor V, 52%; factor VII, 19%; factor X, 20%. By 1983, 130 male fetuses at risk for hemophilia A had been evaluated by fetoscopy, of whom 44 were affected and 43 aborted; 15 fetuses at risk for hemophilia B were evaluated of whom 3 were affected and aborted. (This London center had the largest experience with the fetoscopy technique for prenatal diagnosis. In contrast to the paper by Golbus et alia, cited below, the London group, in a 1980 paper, reported no difficulties with continuing pregnancies.)

Golbus MS, Mcgonigle K, Goldberg JD, Filly RA, Callen PW, Anderson RL. Fetal tissue sampling: the San Francisco experience with 190 pregnancies. Western J Med 1989; 150:423-430

Fetal blood was sampled in 167 pregnancies using fetoscopy (90 cases) or percutaneous umbilical blood sampling (PUBS, 77 cases.) Of these, 28 procedures were performed for hemophilia diagnosis. In fetoscopy, used between 1977-early 1985, the fetal umbilical cord or placental surface vessels were punctured under direct vision. In PUBS, the cord was punctured directly under ultrasonic guidance. Up to 2 ml fetal blood was aspirated into citrate anticoagulant. With fetoscopy, an adequate sample was obtained on the first attempt in 80% of cases, and pure fetal blood not contaminated with amniotic fluid in 61% of cases. Pure fetal blood was obtained in 97% of instances with PUBS. With fetoscopy, fetal death occurred in 12% of continuing pregnancies. With PUBS, fetal death occurred in 10% of continuing pregnancies; five of six fetal deaths were of abnormal fetuses. In continuing pregnancies, pre-term delivery occurred in 17% after fetoscopy and in 39% after PUBS.


In Paris, fetoscopy was used to obtain blood from the umbilical cord at its insertion into the placenta to determine the status of 55 fetuses at risk for hemophilia. Fourteen fetuses had hemophilia A, with factor VIII activity levels of <1%, and one had hemophilia B, factor IX activity and antigen levels of <1%. For comparison, factor level measurements were performed in 103 fetuses age 18-27 weeks who were being sampled for other disorders and who turned out to be normal. Their mean factor levels were as follows: factor VIII activity 40.5%, factor IX activity 10%, factor IX antigen 9%. In the authors’ experience of more than 500 fetal blood samplings, there was one fetal death, one premature rupture of the amniotic membranes, and no infections.


For prenatal diagnosis, blood was obtained by fetoscopy at 18-21 weeks gestation. Amniotic fluid and blood were aspirated into citrate and tested for factor VIII antigen. Of six male fetuses of carrier women,
Evans MI, Wapner RJ. Invasive prenatal diagnostic procedures ranged from zero to 22.7/1000 births. In centers with less than 500 CVS cases, the rate of limb abnormalities ranged from zero to 2.9/1000 births. In the second trimester, about 1/200, is about the same as with chorionic villus biopsy. There is no increased risk of limb reduction defects following CVS at 10 weeks or older. In the first trimester, CVS is safer.


In Sweden, a sample of mixed fetal blood and amniotic fluid was obtained in the 19th week from the male fetus of a carrier of CRM-negative hemophilia B, and found to have no factor IX antigen; after abortion, he was confirmed to have hemophilia. Factor IX antigen was measured in eight fetuses aborted for other reasons at 16 to 20 weeks and ranged from 1.8 to 10%. Factor IX activity was measured in four fetuses and ranged from 4 to 9%.

**DNA analysis via CVS or amniocentesis**


In the first trimester, a catheter is inserted through the cervix under ultrasound guidance to biopsy the developing placenta. The efficacy and safety of this method in 2278 women was compared to amniocentesis in 671 other women. The rate of combined losses due to spontaneous and missed abortions, termination of abnormal pregnancies, stillbirths and neonatal deaths was 7.2% after chorionic villus biopsy and 5.7% after amniocentesis. In CVS, if the cervical catheter had to be inserted only once, the loss of normal fetuses was 2.9% whereas if the catheter was inserted three of four times, the rate of loss was 10.8%. (A skilled operator can make a big difference.)


In newborns whose mothers had not undergone any invasive procedure, the frequency of limb shortening defects was about 0.5 / 1000 births. In a review of 26 cohort reports, the rate of limb abnormalities after CVS in centers with more than a thousand CVS cases ranged from zero to 2.9 / 1000 births. In centers with less than 500 CVS cases, the rate of limb abnormalities ranged from zero to 22.7/1000 births.


These American authors say that in skilled hands, the risk of inducing miscarriage with amniocentesis, about 1/200, is about the same as with chorionic villus biopsy. There is no increased risk of limb reduction defects following CVS at 10 weeks or older. In the first trimester, CVS is safer.


CVS is the most widely used method, and is performed in gestational weeks 10-12. Earlier CVS is associated with the risk of fetal limb abnormalities. In the second trimester, amniocentesis can be carried out, but its major disadvantage is that it is a late diagnosis, with a more psychologically upsetting abortion.

Dr. Ljung reminds readers that prenatal diagnosis will never eliminate hemophilia, because of the high rate of new mutations. In Sweden, with its permissive attitude to abortion, prenatal diagnosis may be expected to reduce the prevalence of hemophilia. Their statistics show that the annual incidence of births with moderate or severe hemophilia increased from 0.78 / 10,000 births in the 1970’s to 1.34 / 10,000 in the 1980’s and leveled off at 1.31 / 10,000 in the 1990s. Had prenatal diagnosis not been available and practiced, there would have been a higher incidence, 1.83 / 10,000, in the 1990’s.


In New Delhi, CVS samples from 41 families with a history of hemophilia were tested using RFLPs and VNTRs, which were adequate for diagnosis in 88% of families. Of 21 male fetuses, 13 were diagnosed as likely to have hemophilia.


In Mumbai (Bombay), an array of tests, including RFLPs and other markers, and direct test for inversion mutation of factor VIII and gross deletions of factor IX were used for prenatal diagnosis for 438 families. Prenatal diagnosis is a mainstay in countries where technical expertise is sufficient but where hemophilia care is scarce and expensive.


As of 2009, average medical cost/year of he-
mophilia patients in Taiwan was 2.1 million New Taiwan dollars. They estimated that 26 potential carriers had to be tested to prevent one case of hemophilia. At a screening rate of 79%, carrier and prenatal testing would cost 85.9 million NT$ but would be offset by a saving of 203 million NT$ per year in cost of treatment, and over a life expectancy of 70 years, would save NT$ 14.2 billion.


Italy has country-wide mutation data bases. That for hemophilia B includes 373 patients whose mutation is known, and 274 identified carriers of child-bearing age. Sixty-six prenatal diagnoses were performed on 52 carriers, of whom 44 had been studied before pregnancy. Note the efficiency of such a database, and of knowing carrier status before pregnancy.


Blood samples were drawn in the seventh week of pregnancy from 15 carriers of hemophilia A and Y chromosomes were found in 11 of them, allowing determination that the foetus was male. Predictions turned out to be accurate, permitting planning for prenatal diagnosis. If fetal cells could be isolated, and the mutation were known, then perhaps hemophilia could be diagnosed or excluded this early.


In a review of several studies, intracranial hemorrhage occurred in 2/283 newborns with hemophilia delivered by caesarean section, in 5/66 delivered by assisted (e.g. forceps) vaginal delivery and in 22/816 delivered by spontaneous vaginal delivery.


Two to three percent of infants with hemophilia experience an intracranial hemorrhage at birth. Of these, 36% are associated with neurological deficits and 7% are lethal. The risk with vaginal delivery is 6.8 times that of a planned caesarean delivery performed before the onset of labor.

Attitudes of carriers to prenatal testing

Evans DIK, Shaw A. Attitudes of haemophilia carriers to fetoscopy and amniocentesis. Lancet 1979; 2:1371.

The authors interviewed 29 carriers, 27 of whom had affected sons, in the Manchester, UK, area. “Most hemophilia carriers decide either that they want no more children or that they must have another child, regardless of the risk, and will not contemplate terminating a pregnancy even if the baby should prove to be affected.” “For some, the knowledge that at 15-20 weeks the fetus is clearly recognizable as a baby made late abortion unacceptable”. The authors predicted that use of prenatal diagnostic techniques requiring late abortion would be limited.


In New England, 79% of 35 mothers of children with hemophilia realized that their risk of having another affected child was high. While 43% of these women would consider prenatal diagnosis, only 17% would terminate a pregnancy with an affected fetus. The majority of mothers did not view having a child with hemophilia as an insurmountable burden.


Twelve Swedish and ten Danish hemophilia carriers were interviewed 1 to 5 years after a pregnancy in which prenatal diagnosis was carried out by fetoscopy. Of these, 80% experienced the procedure as more trying than anticipated.

Women who had been found to have an unaffected fetus were relieved and happy, and half of them had a positive attitude towards prenatal diagnosis in a future pregnancy. Women whose fetus had been diagnosed as affected had been seriously distressed by the news. However, six of seven women who had prenatal diagnosis once and whose pregnancies ended in abortion or miscarriage were positive towards using the method again.


This thoughtful article from The Netherlands is highly recommended. There is near-universal agreement among women that carrier testing is helpful, but only 49% of potential carriers had been tested.
Women who were married, whose families were affected by severe hemophilia and who were closely related to a male with hemophilia were more likely to have been tested. Thirty-one percent of the women surveyed would favor prenatal diagnosis with the implication of a possible abortion. Acceptance of abortion was greater if the hemophilia in the family was severe, and if the woman had restricted the size of her family because of hemophilia, or intended to do so. Most of the women who objected to prenatal diagnosis did so because they did not consider hemophilia to be a sufficiently serious disorder to justify an abortion.

“Attitudes towards prenatal diagnosis are not only determined by the possibility of diagnostic techniques but also by the perceived burden of the disease. Modern hemophilia treatment renders the disease less serious.” Conflicting values may affect the decision to undergo prenatal diagnosis. “Providing information about the heredity of a disease, carrier testing and reproductive choices to prospective parents means that these parents may feel themselves obliged...this need to make a decision is often experienced as a burden...”

What is the function of carrier testing if it does not lead to prenatal testing? Information dispels misconceptions and unjustified anxiety. “Many women with a family history of mild hemophilia, for instance, are relieved to hear that they cannot be carriers of severe hemophilia.”


The actual reproductive choices of over a quarter of all carriers, age 25-48, of severe or moderate hemophilia in Sweden were compared to those of an age-matched group of women who were not carriers. In general, the 105 carriers had the same number of children as other women of similar age. Carriers who already had a hemophilic child, and who did not choose prenatal diagnosis, often abstained from further pregnancies; they had significantly fewer children than carriers who did not have hemophilic children or than normal women. Carriers choosing prenatal diagnosis tended to have a family history of hemophilia and an accepting attitude towards abortion. Some carriers, however, do not view prenatal diagnosis as an avenue to abortion but view it as helpful in preparing themselves, and spouses and obstetricians, for the birth of a child with hemophilia.


The London hemophilia center sent a questionnaire to obligate and potential hemophilia carriers and received 197 replies. Of these, 160 women had been pregnant at least once, and 36 had had prenatal diagnosis. Of 41 women who had had abortions, hemophilia was the main reason in eleven instances; the remainder were for other medical reasons. Of 106 women who had made a decision not to have a child, or not to have further children, the major reasons cited were as follows: in 47 women, not wanting to pass hemophilia on to a child; in 6 women, not wanting to cope with another hemophilic child; and in 7 women, not wanting the stress of prenatal tests. The remaining women decided not to have children either because their family was complete or because of other restrictions. In their center, between 1985 and 1995, 35% of pregnant carriers opted for prenatal diagnosis, and 50% of affected fetuses were aborted.


On behalf of UK doctors treating hemophilia, this excellent paper lists the genetic services (information, tests and procedures) that ought to be available to affected or at-risk persons. They address legal issues of informed consent and confidentiality, as well as the need for family genetic records and a data-base for the sake of future consultands.


A survey was conducted of hemophilia family members around Melbourne and Sydney, Australia, just prior to the widespread introduction of genetic testing. When hemophilia had been familial, the disorder was often viewed with equanimity, even positively as a mark of being “special” and a member of a unique community. At times, non-hemophilic males were pitied for not being part of the in-group. In contrast, when hemophilia had occurred de novo in a family, the shock of the diagnosis could be overwhelming, and mothers were likely to feel guilty. “People with familial inheritance had the most negative views about genetic testing” in contrast to the more eager attitude in sporadic families.

The survey approved testing for the carrier state, mainly to allow the woman to make informed reproductive choices. Some supported testing in adolescence and others in childhood. Some feared that a
positive result would lead to outside pressures about reproductive choices. The primary advantage of pre-natal testing was viewed as allowing the obstetrician and parents to prepare for the birth of a hemophilic child.


In response to the above report, the authors described eagerness for prenatal diagnosis in India because medical care for hemophilia is limited. Only one of 514 families affected by hemophilia did not want prenatal diagnosis. Only three families intended to keep the child if it was affected. Prenatal sex determination is illegal in India, therefore, families were not told the sex, only whether or not the fetus has the mutation. Most families would not want to keep a female fetus who was a carrier.


Recently, statements from the United Nations and WHO and other professional bodies have argued against genetic testing of children for reasons other than their own immediate medical care. In the UK, among 66 adult carriers and 39 husbands or partners, 64% were in favor of testing girls under age 13 years. A majority of younger parents (under age 40) were in favor of testing daughters at age 9 or younger. Some parents felt that a girl should get used to the information before it began to have an impact on her life. Large majorities felt that testing should not be forced on young teenagers against their will, but, teenagers who wanted testing should not be prevented from having it. Some families fear that a carrier diagnosis will make a desirable marriage arrangement difficult.

Preimplantation diagnosis (PGD)


Laboratory methods and problems are described in detail. At the time, pregnancy rates in single-gene disorders were about 25% per embryo transfer considering all centers. Expert centers now do better. .


The successes and risks of IVF and of PGD are reviewed. The greatest risk, because of implantation of more than one embryo, is multiple gestations with prematurity. There is a slightly increased risk of an adverse outcome in the infant even with singleton pregnancies.


The most common single-gene disorder affecting Caucasians is cystic fibrosis (one in 2500 births). It also is the single-gene disorder for which PGD is most commonly requested. This reports the first success in diagnosing that disorder in an early embryo and achieving the birth of a healthy child. The report comes from a renowned British PGD center.


Dr. Handyside’s success is put in context with the problems of prenatal diagnosis and a review of the potential difficulties of PGD.


This Spanish-language magazine reported the first successful PGD performed for a carrier of severe hemophilia A (due to intron 22 inversion.) The child, a normal son born in 1995, was the result of in vitro fertilization at the Bogotá clinic of Dr. Elkin Lucena and DNA diagnosis by Dr. Mark Hughes of Detroit. This event was reported in English-language abstracts but not in full articles, and was not widely noticed.


After in vitro fertilization of a hemophilia carrier, cells from each of several 4 to 8-cell embryos were analyzed and only those with XX chromosomes were implanted, resulting in the birth of a daughter. This method enabled a carrier to have a female child, and avoiding the risk of a hemophilic male and also avoiding abortion. Choice of female gender by PGD was used for sex-linked disorders before specific choice of an unaffected embryo was possible.

Michaelides K, Tuddenham EG, Turner C, Lavender B, Lavery SA. Live birth following the first mutation

A healthy non-carrier daughter was born to a carrier of a single-nucleotide substitution causing severe haemophilia A. These authors represent one of the foremost genetic medicine groups in the U.K.


The authors diagnosed first polar bodies of eggs by looking for the (previously-defined) family mutation as well as by linkage analysis. Only one of seven diagnosed eggs, when fertilized, developed into embryos, and that one, on transfer to the uterus, did not result in pregnancy. This strategy may get past the moral scruples of some couples against testing embryos, because it allows only unaffected eggs to be fertilized.


A PGD program was established in Germany after the 2011 verdict of the Bundestag legalizing the procedure. The family described had hemophilia plus a chromosomal abnormality. The hemophilia could be diagnose on the polar body but a blastocyst biopsy was needed to rule out the chromosomal abnormality.


Historical observations


The oldest rabbinical view, attributed to Rabbi Judah the Patriarch of the second century, was that if a woman’s first and second son died as a result of bleeding from circumcision, her third son must not be circumcised. Moses Maimonides in the 12th century stated that the ruling should be observed even if the third son had a different father than the first sons, suggesting that he understood that the disorder was transmitted through mothers.


A brief biography of Dr. John Otto is provided, together with a facsimile of his 1803 report. He noted that females carried the disorder but were not subject to it. He mentions three other similar families known to other physicians in the eastern United States.


In 1813, John Hay elaborated John Otto’s description of a family affected by (apparently mild to moderate) hemophilia A which he traced back to an Oliver Appleton born in 1677. Appleton lived to age 82. His 14 children included four daughters, two of whom had large families. The descendants of one of these daughters could be traced over 350 years. The pedigree contained at least 25 hemophilic males and 27 carriers in 11 generations. Age at death was known for 20 males, and was bimodal, with 11 deaths by age 29 (all from bleeding), one in the range of 30 to 59 years, and eight at 60-plus years. The disorder was almost extinct by the 1960’s, when this paper was written. Its long persistence may be due, in part, to the family’s high fecundity.


This insightful biography chronicles the challenges of Leopold’s life in the 19th century: frequent periods of enforced inactivity, attempts to escape a protective mother, a refuge in scholarship, and struggles as a young man for useful employment and for marriage.


When Alphonso XIII of Spain courted Queen Victoria’s granddaughter “Ena” in 1906, the inheritance pattern of hemophilia was well-understood, but the King was indifferent to advice that she might be a
carrier of hemophilia. The birth of hemophilic sons burdened their personal relationship and destabilized the political situation.


Dr. Green reviews the analysis of traces of genomic DNA found in bone remains of the family of the last Tsar. A mutation, creating a novel splice site, was found just inside an intron of the factor IX gene. This mutation has been reported in three other unrelated families with severe hemophilia B.

Other plasma clotting factor deficiencies


The autosomal recessive disorder of mild-moderate combined deficiency of factors VIII and V (which could be confused for mild-moderate hemophilia A) was found to be due to a mutation in a gene controlling “ERGIC 53”, a protein that chaperones these factors from place to place in the endoplasm. (also described in the journal Haemophilia, 1998, 4:677-82.)


This article describes not only combined deficiency of factors V and VIII, but also combined deficiencies of vitamin K-dependent clotting factors. The latter may be due to mutations either in the gamma-carboxylase gene or in the gene for vitamin K epoxide reductase. Deficiency in either enzyme leads to reduced activities of all vitamin K-dependent proteins.


In many regions of Asia and Africa, consanguineous marriages currently account for approximately 20 to 50% of all unions.


Factor levels in heterozygotes for rare clotting factor deficiencies are reviewed. The mean levels were as follows: in prothrombin deficiency heterozygotes, 56% (n=16); in factor V heterozygotes, 51% (n=49); in factor VII heterozygotes, 45% (n=163); in factor X heterozygotes, 50% (n=43); in factor XI heterozygotes, 49% (n=35). The distribution of factor levels in heterozygotes for factor XII deficiency was bimodal: the predominant group had a mean level of 60% and a smaller group a mean level of 23%. They presume that there are two major normal alleles for factor XII.


Von Willebrand disease (VWD)


Type 3 VWD


The VWF gene was analyzed in 32 patients with severe VWD from 28 German families. A variety of mutations were found. There was little evidence of common ancestry. Complete deletions of the gene and nonsense mutations in the pro-sequence were correlated with recessive inheritance, that is, with asymptomatic heterozygotes, whereas frameshift and nonsense mutations in the sequence corresponding to the mature subunit tended to result in a type 1 VWD phenotype in heterozygotes.

In a total of 40 patients with type 3 VWD from Italy, Iran and India, 50 mutations were identified. The mutations were scattered throughout the gene. No founder effect was seen in these countries.

**Type 2 VWD**


**Type 1 VWD**

The genetics of type 1 VWD are not straight-forward. Some persons may have been mis-diagnosed based on borderline laboratory test results. They may have had a mild tendency to bleed, perhaps partly due to a borderline level of VWF and, in some instances, partly to mild platelet disorders.


In 31 Swedish families with type 1 VWD, stringently diagnosed, the authors looked for linkage *(that is, did everyone in a family who appeared to have VWD actually inherit the same gene?)*. Linkage to the VWF gene was found in 27 families (87%). A possible common mutation was found in 6 / 27 families with genetic linkage. Blood group O was over-represented in the study group. *(Factor VIII and von Willebrand factor levels are lower in blood group O persons compared to persons of other blood groups, and may have led to increased diagnosis of VWD in persons with borderline-low levels of those factors.)*


A causative mutation was found in 53% of 32 UK families with the phenotype of type 1 VWD. Linkage between the VWF gene and the type 1 phenotype was found in 13 / 32 families.

Goodeve A and many others. Phenotype and genotype of a cohort of families historically diagnosed with type 1 von Willebrand disease in the European study, Molecular and Clinical Markers for the Diagnosis and Management of Type 1 von Willebrand Disease. Blood 2007, 109:112-121.

Mutations were defined in 70% of index patients previously identified as having type 1 VWD. A group of 37% of index cases with abnormal multimer patterns, seen on a sensitive test, had a high prevalence (95%) of gene mutations. Among those with normal multimers, only 55% had gene mutations. A third of the index cases might actually have type 2 VWD.


*This report covers the same study group as that directly above. Segregation analysis in 143 families with previously-diagnosed VWD suggested linkage in 70%. After exclusion of families with abnormal multimer patterns (as defined in the previous paper) the proportion of the remainder (those with normal multimers) in whom linkage could be demonstrated was only 46%. In other words, in some 54% of these families, VWD was not related to a specific gene.*


Mutations in the VWF gene were found in 63% of 123 patients previously diagnosed as having type 1 VWD in Canada. Thus, 37% had no identified mutation. Mutations were more likely to be found in patients with lower VWF levels. The mutations found were of a variety of types (62% missense), at various locations in the gene.


The results of both multicenter studies are reviewed and evaluated.