Welcome to Mayo Medical Laboratories Profiles in Genetics. These presentations provide short discussion of current genetics topics and may be helpful to you in your practice. This presentation discusses the various options for prenatal diagnostic testing.
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Our speaker for this program is Lea Coon, a Genetic Counselor in the special Coagulation DNA Laboratory at Mayo Clinic, Rochester, Minnesota. In this presentation Lea discusses when to test for, and the appropriate tests for, hemophilia A and B.

Welcome, Lea.
Disclosure

- None

Thank you for the introduction. I have nothing to disclose.
The purpose of this presentation is to tell you how to order genetic testing for hemophilia.

Testing for hemophilia is not entirely straightforward, for reasons we’ll get into soon. I also work in a laboratory that performs this diagnostic testing, and in my experience, about a quarter of the orders we receive for a test specific to severe hemophilia A are cancelled because we find out the test was misordered or misunderstood and it would be wasteful to perform.

Granted, these tests are often misordered for a wide variety of reasons, but in part it is because many people have no idea what they are doing when they are ordering a genetic test for hemophilia.

In this talk and the next, I will cover just 2 things... In the first part of this talk, I’ll go over when it is appropriate to test for hemophilia.

In part 2, I’ll discuss the 2 pieces of information you need to choose the correct genetic test. I’ve simplified this information for anyone who is not a practicing hematologist or geneticist, but a referral to either of these specialized fields should be a consideration when evaluating someone with an apparent bleeding disorder because bleeding is a very nonspecific symptom that can be caused by a great number of things besides hemophilia.
But thankfully, there are basically just 2 specific situations in which you should start considering genetic testing for hemophilia. If you don’t have either situation, you probably do not need to order a genetic test for this patient and should consider other options.
Situation 1 is where you have a male patient who is showing signs of soft tissue bleeding and articular hemorrhage, such as joint bleeds, deep-muscle bleeding, bleeding inside the skull, oozing after surgery, and so on.

In severe hemophilia, affected males typically present with these symptoms within the first 2 years of life. In moderate hemophilia, affected males will usually present in their toddler years. Mild hemophiliacs are typically diagnosed later in life and sometimes well into adulthood. When you see these symptoms, it’s a very good idea to perform some routine coagulation studies to cast the net pretty broadly in your workup, including measurements of prothrombin time (or PT as it is abbreviated), platelet counts, and activated partial thromboplastin time, or aPTT for short.

If PT and platelet counts are normal, but aPPT is prolonged, hemophilia should definitely be a differential, although in some cases, mild hemophiliacs may have a normal aPTT.
Fortunately, some additional screening can be performed to help identify hemophilia and whether it is hemophilia A or hemophilia B. The distinction is very important, as we will discuss.

One of the most important coagulation tests you can perform for suspected hemophilia is measurement of the patient’s factor activity levels, especially factor VIII and factor IX activity levels.

Very briefly, factor VIII and factor IX are proteins that float around in blood, created by the factor VIII or factor IX genes. Although important, we’re not going to review the coagulation cascade here because it is hideously complex.

All you need to know is that these factors are proteins in the blood that help blood clot to stop an injury, and the less functional factor you have, the higher your risk of bleeding to death. And, if you have significantly less of it than what has been observed as normal in humans, that indicates a problem in the gene that produces it. If the lab work comes back indicating a deficiency of either factor VIII or IX, or less than 40% of what is considered normal for a human, that is the point at which you can begin to seriously consider genetic testing for your patient.

Just a brief aside here, should you have a patient with an apparent deficiency in factor VIII, you will also want to perform vonWillebrand factor antigen and activity
levels to rule-out von Willebrand disease, another hereditary bleeding disorder that can mimic mild hemophilia A.
But since we are focusing on hemophilia, you must know 3 important things about genetic testing for male patients.

If your patient does have a deficiency in either of these factors, you pretty much have enough to clinically diagnose him with hemophilia A if he’s got less than 40% factor VIII activity OR with hemophilia B if he’s got less than 40% factor IX activity.
However, although hemophilia can be diagnosed based on low factor levels, genetic testing can still be very helpful for confirming the disease and for assessing prognosis and the risk for the development of inhibitors, which are a very grave complication of this disorder.

Additionally, genetic testing can be very helpful for members of his family for all of these reasons listed here:

Genetic testing can be important because not every hemophiliac has the same mutation in the F8 or F9 gene.

And, knowing the specific mutation can make testing easier, less expensive, and more informative for family members.

Also, the identification of the familial mutation is required prior to any prenatal testing on a male fetus at risk for hemophilia.
Just to illustrate, this is just 1 exon in the F9 gene. Each of those red squares represents a specific mutation, either a single nucleotide substitution, or tiny deletions or duplications, etc. As you can see there many, and keep in mind this is only 1 exon out of 8.

So, yes, it would be wonderful if each hemophiliac had the exact same genetic mutation because that would make testing easy, but sadly it is not the case.
The third thing to note about this situation is that before the patient is tested, he should undergo genetic counseling so that he understands the risk, benefits, and limitations of the testing. I hope it goes without saying that no one should ever be forced to or unknowingly undergo genetic testing.
So, that was the first situation in which you should consider genetic testing, and this is the second: When you have a female patient with a confirmed or reported family history of hemophilia, especially if this male is a first-degree relative.

For example, if a woman’s or girl’s father was a confirmed hemophiliac, it is a virtually certainty that she and all of her sisters who share the same father are carriers. Because of that, these women are called obligate carriers.

In fact, if you are able to test this obligate carrier instead of her affected male relative that may in some cases be preferable because you probably want to minimize the number of times you stick your male hemophiliac patient.

If a woman has maternal male relative (like a brother or nephew) who is affected with hemophilia and then has a son with hemophilia, she too is an obligate carrier. Hemophilia is rare enough we wouldn’t consider that coincidental.

However, a woman with no prior family history of hemophilia who has a son with hemophilia may or may not be a carrier. Overall, there is a 2% to 20% chance of her not being a carrier, depending on the type of mutation in the son. This happens sometimes because the hemophilia mutation can arise very early on in the development of the little male embryo, in which case, he’d have the mutation but his mother would not. It’s rare, but it happens.
Other women at risk for carrying hemophilia include those with an affected second- or third-degree male relative, when her mother has not been excluded as a carrier. Although in these cases it is best to test her mom first, because if mom is not a carrier, none of her children will have the mutation either.
Why is it so important to consider which side of the family – Mom’s side or Dad’s side – has a bleeding issue when we are thinking about hemophilia?

If you are ordering genetic testing, I hope you are familiar with genetic inheritance patterns. In the case of hemophilia, the disease is X-linked, meaning the gene that is mutated in the disease is on the X-chromosome, which men can only inherit from their mother.
Our lab has received orders for hemophilia genetic testing from clinicians who want to evaluate their female patients for hemophilia based on heavy periods and easy bruising, but there is no family history of affected males.

I would like to reassure you that is **very unlikely** these patients have hemophilia or are carriers of hemophilia. Unless, of course, she happens to have significantly reduced factor VIII or factor IX activity levels.
Abnormally heavy periods can be caused by hormone imbalance, fibroids, and medications.

Only 15%-20% of women with heavy periods have some type of genetics-related bleeding diathesis.

- 80%-85% do not.

More general bleeding and bruising problems can also be caused by things such as vitamin K deficiency, liver dysfunction, anemia, etc., which are more common than hemophilia.

Hemophilia may be the best known cause of abnormal bleeding, but it is far from the most common.

Why is this?

Abnormally heavy periods can be caused by such a long litany of things, such as hormone imbalance, fibroids, and medications, that a rare inherited bleeding disorder is pretty far down on the list of differentials. And more general bleeding and bruising problems can also be caused by many, many different things such as vitamin K deficiency, liver dysfunction, anemia, etc, that are more common than hemophilia.
Other Bleeding Diathesis Differentials

- von Willebrand disease (VWD):
  - Estimated to affect 1/100 people worldwide
  - Between 5% and 24% of women with menorrhagia had previously undiagnosed VWD (BJOG, 2004 Jul,111(7):734-740)
- Platelet function disorders (ie, Bernard-Soulier syndrome; Glanzmann thrombasthenia)
- Factor XI deficiency (ie, hemophilia C or Rosenthal syndrome) (AR)
  - Frequent nosebleeds or soft tissue bleeds, hemorrhaging after surgery, menorrhagia, and postpartum bleeding
- Prothrombin (factor II), factor V, factor X, and factor VII deficiencies
  - All are rare
  - Specific coagulation factor assays establish diagnosis
- Platelet-type VWD: similar phenotype to Type 2B VWD; caused by a rare gain-of-function mutation in platelet GPIb; a modified RIPA and genetic testing can help distinguish.
- Fibrinogen disorders

Even among the inherited causes of bleeding problems, von Willebrand disease, another clotting factor disorder, is more common than either hemophilia A or B, with an estimated prevalence of about 1/100.

And here is a partial list of even more inherited bleeding disorders for your reference. If you want to address your patient’s concern about a possible bleeding problem in the absence of a family history of hemophilia, there are very informative laboratory screening tests you can use to assess if there is evidence of such a problem, such as looking at prothrombin time or PT, activated partial thromboplastin time (aPTT), platelet count, and factor levels. Any genetic testing ordered prior to performing these screens and the consideration of other, more common, causes of bleeding is premature.
Just one more thing before we move on to the critical pieces of information you need to correctly order genetic testing for hemophilia.

Say you’ve got a patient who reports a family history of hemophilia and you run factor activity level labs and find she is not deficient in either factor VIII or factor IX levels? Doesn’t that mean I’ve excluded her as a carrier?”

• NO!

Situations 2—When Should You Consider Genetic Testing For Hemophilia?

“But what if I have a female patient who reports a family history of hemophilia A or hemophilia B but does not have below normal factor VIII or factor IX levels? Doesn’t that mean I’ve excluded her as a carrier?”

• NO!
If you see reduced factor levels, then you can be suspicious for the type of hemophilia associated with that factor. But if you don’t, you cannot rule it out because factor level screening is an unreliable way to detect carriers due to the fact that lots of things influence factor clotting activity in women such as pregnancy, oral contraceptive use, aerobic exercise, and chronic inflammation.

- Type O blood: 25% lower factor VIII levels
- The majority of known carriers, even carriers of severe hemophilia mutations, have normal factor activity.

Situation 2—When Should You Consider Genetic Testing For Hemophilia?

- Factor level screening tests in female carriers are unreliable for hemophilia carrier detection
- Clotting activity is increased with pregnancy, oral contraceptive use, aerobic exercise, and chronic inflammation
- Type O blood: 25% lower factor VIII levels
- The majority of known carriers, even carriers of severe hemophilia mutations, have normal factor activity.

If you see reduced factor levels, then you can be suspicious for the type of hemophilia associated with that factor. But if you don’t, you cannot rule it out because factor level screening is an unreliable way to detect carriers due to the fact that lots of things influence factor clotting activity in women such as pregnancy, oral contraceptive use, chronic inflammation, blood type in the case of factor VIII, and so on.

This is why genetic testing is important for women at risk for being carriers of a hemophilia mutation, and why such testing is made much easier by first identifying an affected male relative.

That said, the fact that activity levels are unreliable indicators of hemophilia in women should not be seen as a reason to go straight to genetic testing for hemophilia in women with bleeding issues, primarily because hemophilia is a relatively rare cause of abnormal bleeding. In the absence of any family history, these more likely causes should be considered and excluded first before thinking about genetics testing for hemophilia.
So, to review so far, there are basically 2 situations in which you might want to consider genetic testing for hemophilia.

If you have a patient in mind, and they don’t fit in either of these situations, you may want to consider doing something more useful than performing genetic testing for hemophilia for your patient because it is likely not the problem. If you have a patient who does fit into one of these situations, then please watch for Part 2 of this presentation in early 2017, when we will go over the 2 things you absolutely must know before you order genetic testing for hemophilia.
This concludes Part 1. I appreciate your attention.