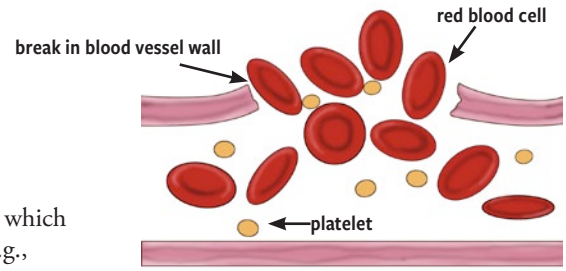
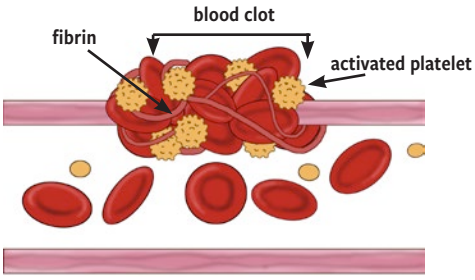
Inherited Feline Blood Clotting Disorders:

Genetic Testing and Breeder Practices

Lucy Drury





Most professional breeders of pedigree domestic cats screen their cats for inherited diseases and traits. Several veterinary genetic laboratories, both academic and commercial, offer multi-test profiles of a few dozen of the most popular genetic screens to more than 50. Several labs have added to their feline genetic screening profile two inherited blood clotting disorders: Factor IX (Christmas Factor) deficiency and Factor XII (Hageman) deficiency. Why are these important, and what should a breeder do if one of their cats is found to carry one of these traits? Before we answer those questions, a brief discussion of blood clotting mechanisms and current laboratory screening tests of the efficiency of those mechanisms is necessary.

The Miracle of Blood Clotting

We take it for granted. We are playing with Fluffy and his favorite toy, and our hand gets in the way of that paw batting at the toy. We yelp a little, look at the scratch oozing a tiny bit of blood, then wipe it off with the nearest available absorbent form of paper. Already stopped bleeding, we think to ourselves. We shake it off and think to ourselves that Fluffy is overdue for a nail trim.

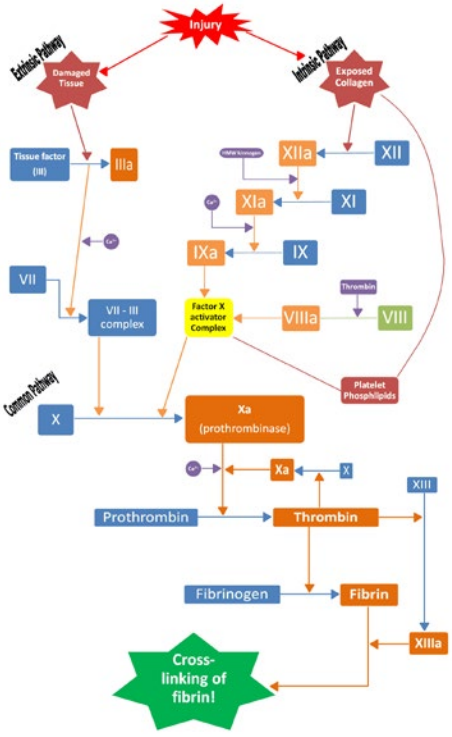
What we fail to appreciate is that not only does Fluffy need a nail trim, but those claws of his set off a cascade of biochemical reactions that has saved our life, time and again, and we don't even realize it. That process is more formally called hemostasis.[1] Hemostasis is essentially the same process in both humans and felines.

At its core, hemostasis is easy to understand, though it is a complex, multi-step biochemical process that has brought many medical, nursing, and allied health students to their knees while trying to learn all the steps in the process and how those processes can fail (the author was a member of that last group 45 years ago). Basically, hemostatis causes liquid blood to coagulate into a blood clot—thereby keeping blood inside the blood vessels where it belongs. Hemostasis can be broken down into three basic phases: vascular constriction, which is triggered when epithelial cells (e.g., skin) are damaged; primary hemostasis, where platelets—small cell fragments that circulate in our blood—attach to the wall of an injured blood vessel and form a temporary plug; and secondary hemostasis, where collagen released from the damaged cells triggers a cascade of thirteen coagulation factors that turn the soluble protein fibrinogen (found in blood) into insoluble fibrin. While detailed as separate processes, they are occurring simultaneously and working together to form a solid clot. The blood vessels constrict, narrowing the passage that needs to be plugged. The platelets plug the damaged blood vessel while fibrin, along with trapped red blood cells, create a seal for it: the clot. Over time, through a process called fibrinolysis, the clot is dissolved and replaced with the same kind of tissue as the damaged tissue.[4]

Multiple Steps, Multiple Opportunities for Failure

As shown in the image below, the coagulation cascade is a complex, multi-step process. Like any other process of this nature, there are multiple places where things can go horribly wrong. If one of the clotting factors is depleted or missing, a cat or a human can take a long time to stop bleeding and even bleed to death, depending on the severity of the deficiency. Often, bleeding problems are secondary problems caused by another disease. Many of the coagulation factors are manufactured in the liver, so liver disease can result in factor deficiency. Cancer and leukemia can cause bleeding problems because leukemia and cancer cells are replacing the cells in the bone marrow that produce the platelets needed for clotting.

However, bleeding disorders can have a genetic root. Hemophilia A (Factor VIII deficiency) and B (Factor IX, Christmas), Von Willebrand disease, Hageman Factor (Factor XII) deficiency, and inherited thrombocytopenia (low platelet count) are all inherited disorders.[2] One of the challenges with this group of disorders is that they often go undetected until the human or feline suffers an injury. Then, there may be bruising disproportionate to the severity of the trauma, or a cut that refuses to stop bleeding. Slower clotting times on minor cuts and scratches go unnoticed because if one of the three phases of hemostasis is impaired, the other two can compensate. But when there is a severe bleeding challenge to the body, such as a major injury or surgery, some unnoticed clotting deficiencies can be life-threatening.



Laboratory Testing for Coagulation Disorders

Though much more automated today, screening tests for clotting disorders are essentially unchanged from when they were first devised by researchers in the mid 20th century.[6] Two of these tests, the Prothrombin Time (PT) and the activated Partial Thromboplastin Time (aPTT) are frequently run (or should be) before any major surgery. They are highly recommended before any procedure that could trigger heavy bleeding or require anti-coagulation therapy following the procedure (e.g., implanting a pacemaker). Both tests follow the same basic procedure: the patient's blood is collected in a container that contains an anti-coagulant that inhibits specific parts of the coagulation pathway and keeps blood in its liquid state. The blood is centrifuged, separating out the cells from the plasma (liquid part of the blood). A small amount of the plasma is placed in a cup, then the substances that were inhibited by the anticoagulant are added back in. A timer is started and stopped when a clot begins to form. Patients with bleeding disorders or factor deficiencies may have prolonged clotting times when compared to patients with no deficiencies, whether inherited or acquired.

If one or both screening tests are abnormal, then more specific testing is ordered to locate the exact malfunction in the clotting process. Today, unlike in the mid-20th century, laboratory scientists can test for many of the specific clotting factors and protein substances in the coagulation cascade. Together, the PT & aPTT tests offer clues as to which additional tests should be ordered to identify the specific deficiency. [10]

Hemophilia, Genetic Screening, and Breeding Programs

A clotting disorder due to a deficiency in a clotting factor is called hemophilia. The most common one in humans and present in cats is Factor XII deficiency, or Hemophilia A. The gene causing the defect is located on the X chromosome, making the disease more prevalent in males than females. Females may be carriers of the defect but will generally not manifest symptoms. Males will develop the disease if they receive a carrier X chromosome from their mother. Fathers will always pass the defect on to their daughters but cannot pass it on to their sons. Daughters will not develop the disease unless they also receive a defective X chromosome from their mother.

One reason Hemophilia A it so common is that is does not usually result in spontaneous abortion of the fetus or neonate death due to the cutting of the umbilical cord. It is treatable and easily identified by laboratory testing. Currently, there are no genetic screens readily available for Hemophilia A and it is not part of any of the feline genetic screening kits offered to the public, however, when hemophilia A is identified in a breeding cat, all females should be removed from the breeding program whether the identified cat is the sire or dam. All males should be removed from the breeding program if the dam has the disease or suspect of being a carrier through pedigree analysis. Males may safely remain in the program if their sire has the disease, as there is no chance of father passing it on to son as the sire only passes a Y chromosome to their male offspring. [2,4]

Factor IX deficiency, also known as Christmas disease or Hemophilia B, is far rarer than Hemophilia A. It has primarily been reported in British Shorthairs, Himalayans, and some Siamese crosses. Kittens with severe Factor IX deficiency (less than 1% of normal activity) will likely not survive long after birth. Kittens and cats with 5 – 10% of the normal factor levels may develop blood clots, joint bleeding, oozing of blood into body cavities or organ bleeding. Some animals may have no symptoms until they suffer an injury or have surgery.[3] Hemophilia B, like Hemophilia A, is also an X-chromosome linked trait; however, it is included now in several feline genetic screen panels. Because suspected carriers can be screened, there is no need to remove a female from a breeding program if she screens negative for this deficiency.

There is one other clotting factor deficiency that has recently become part of several feline genetic screen panels: factor XII or Hageman factor deficiency. This factor is the most common inherited factor deficiency in cats. Unlike Factor XIII and Factor IX deficiencies, Factor XII deficiency typically does not cause any detectable bleeding disorders. Why, then, is it part of a routine panel screen?

While Factor XII deficiency itself may not cause coagulation challenges in the body, it does prevent blood from clotting outside an animal's body. If blood is drawn into a clean glass test tube with no anti-coagulants present, it will typically form a large blood clot in the tube in less than 20 min. However, if a person or animal is Factor XII deficient, their blood will take much longer than 20 minutes to clot in the glass tube. Though the whole blood clotting test is no longer routinely done except on victims of a venomous snake bite, the aPTT test previously mentioned is also affected and its clotting time increased, as it is with Hemophilia A and B. In the case of Factor XII deficiency, the patient is in no danger of severe or prolonged bleeding and no treatment is required. If the patient is asymptomatic for any kind of bleeding disorder but has a prolonged aPTT and is not receiving any medications that might prolong the aPTT, Factor XII deficiency is suspected, and a vet or physician will order a test that specifically measures the amount of Factor XII circulating in the blood to confirm the diagnosis. If Factor XII deficiency is confirmed, no treatment is required.[11]

It also should be noted that in a 2014 study of a cat colony specifically bred to study the Factor XII mutations, there were a significantly more stillborn kittens in first-time mothers who were homozygous for the mutation when compared to those who were heterozygous or clear for the mutation. Factor XII deficient human females have also demonstrated higher spontaneous abortion rates in the first trimester. However, the authors were quick to point out that pregnant mice had no such difficulties when factor XII deficient. They noted other factors in the population, such as a higher coefficient of inbreeding due to the need for homozygous test subject could have been responsible for the kitten mortality.[1]

Back to our opening question in this section. If a Factor XII deficiency is not harmful to a cat, then why is part of routine genetic screening now? Why bother if it requires no treatment?

Ignoring for a moment the stillborn kitten issue, that answer lies in the other reasons why an aPTT clotting time could be prolonged. One of those is Disseminated Intravascular Coagulation (DIC), which can loosely be described as a complete collapse of the clotting system.[3] Many conditions can trigger DIC: serious infection, trauma or even COVID-19 in humans and FIP in cats. For example, a cat or a person may suffer a trauma that does not result in a life-threatening injury, such as a broken neck or ruptured spleen, but they have cuts and bruises all over their body along with a few broken bones. Their body goes to work forming clots to stop the internal and external bleeding, but it can go a little crazy and overdo the clot formation in small

blood vessels, depleting the clotting factors, which in turn severely limits the person's ability to form clots in the areas where they are really needed. The outcome of this is the victim could potentially bleed to death by depleting their clotting factors. This is a life-threatening situation, and immediate medical or veterinary intervention is needed. DIC does not usually happen immediately, but once the coagulation cascade starts, it can end badly very quickly. The veterinarian or physician can order blood tests on patients to detect the early stages of DIC if they are suffering from a condition that could trigger a DIC event and start therapy to combat the condition. One of those tests is the aPTT; a prolonged result can suggest an imminent DIC, or it could be nothing more than a benign Factor XII deficiency. The aPTT can be modified to differentiate between a truly prolonged clotting time inside the body ("in vivo") or a Factor XII deficiency where the blood won't clot outside the body ("in vitro") unless a substance simulating clotting factors found in tissue is added to the sample.[3,10]

If a cat is in a situation where DIC becomes a possibility or any other acute acquired bleeding problem, it is helpful to know if they have an inherited Factor XII deficiency. If a cat is rushed to an ER and the aPTT is prolonged, most vets won't think about a Factor XII artifact. They will be thinking about all the in vivo conditions that can cause a prolonged aPTT. The treatment for DIC and other clotting/bleeding disorders is often fresh, frozen plasma (FFP) and other blood products along with anti-coagulants (blood thinners). None of these are a good idea for a feline or human patient if they don't need them.

With Factor XII deficiency now detectable by a genetic screen and included on several commercial panels, knowing whether your cat is Factor XII deficient is now cheap and easy. If a cat is a carrier (only one copy of the mutation) it will have no abnormalities when coagulation tests are run. If a cat has two copies of the mutation, their blood will not clot normally outside the body.

Unlike Factor VIII and Factor IX, there is probably no need to remove a quality, healthy cat from a breeding program for a Factor XII deficiency alone. If a cat's screening pre-operative tests are abnormal, the aPTT test can be modified to confirm that the Factor XII deficiency is the only cause for the abnormal test result. If only one of a breeding pair tests positive, there is no need to screen any of the kittens if they are not to be used for breeding, at most they would only have one copy of the defect. If both parents carry the defect, then the breeder should consider screening all the kittens in case any of them are homozygous (two copies) for the defect. That information should be passed on as part of the cat's medical records in case they need to have pre-operative or emergency coagulation testing done.

To Breed or Not to Breed

One might ask why a breeder should leave a cat in a breeding program with a known defect, particularly if there's a yet unproven link to increased stillborn kittens? Consider this: there are between 2.5 and 3.0 million base pairs (building blocks of DNA) in a cat, on about 20,000 genes.[7] Mutations occur frequently in an animal's DNA daily. This may sound like a high number but is insignificant when one considers the even higher number of base pairs in a cat or any other mammal. In a population with a low COI or randomly bred, the odds of a random mutation matching up to produce a homozygous defect is very low.

However, pedigreed cats are not a randomly bred population. They have been specifically bred to express a particular group of phenotypical traits and in most cases are a closed population, with no or limited outcrosses allowed. When cats and their lines are removed from a population because of genetic defects, the homozygosity of the gene pool increases, creating the opportunity for those spontaneous mutations to pair up. While the entire genetic genome for a cat has been sequenced, only a small percentage of the genome has been mapped to a certain activity or trait at present, though the number of mapped conditions and traits increases yearly—as does the number of screening tests available.

In the future, as Leslie A. Lyons, PhD. has observed, researchers will map the entire feline genome, and every potential defect will be identified, giving veterinarians and breeders a powerful diagnostic tool.[9] What will breeders do then? Already, there are healthy debates regarding line breeding and inbreeding to set desired phenotypic traits. Ethical breeders remove from their breeding lines carriers of inherited diseases such as polycystic kidney disease (PKD) and hypertrophic cardiomyopathy (HCM). But is it necessary to remove cats that carry mild defects such as Factor XII deficiency?

This question will surface for breeders more and more frequently as the mapping of the feline genome continues to advance. Each breeder will need to decide for themselves if the elimination of a particular defect from their lines is of more benefit to the genetic health of their breed versus the narrowing of the available gene pool and loss of heterozygosity in their lines.

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