

By a hair

By REBECCA OVERTON



	N	O
N	NN	NO
O	NO	OO

Consider a hair.

If it gets in your eye, you want it out. If it lands on your clothes, you want it off. Each year, balding men spend millions of dollars in the hope of replenishing the diminishing supply on their heads.

To a Paint Horse breeder, a hair can mean the difference between holding your breath for 11 months to see if a foal will be born with Overo Lethal White Syndrome (OLWS), or knowing you made a genetic cross that ensures you won't get a foal with the dreaded disease.

Using hair samples from a horse's mane or tail, geneticists at the University of California–Davis can determine if a horse is at risk for producing lethal white foals. The test reveals whether a horse carries the mutation associated with lethal white syndrome by looking at DNA extracted from a hair follicle.

Offered by the Veterinary Genetics Lab at the UC Davis School of Veterinary Medicine, the test costs \$50 and can be ordered by completing a form and returning it to the lab.

The test requires 25–30 samples of hair, which must be pulled, not cut, so that the roots are intact. A horse needs to be tested only once. Results are confidential and available within two to three weeks.

The test was developed by Dr. Danika Bannasch while Bannasch was conducting post-doctoral research on canine and equine genetics. The university began offering the test to the public in 1998.

During the last five years, the genetics lab has conducted 4,000 to 5,000 tests, a number that researchers there say is surprising.

“That is a relatively small number of horses tested when you compare it with the large number of horses registered,” said Dr. Cecilia Penedo, associate director of the Veterinary



A simple DNA test using mane or tail hair samples can help Paint Horse breeders avoid the heartbreak of producing lethal white foals.

Genetics Lab. Penedo has worked at UC Davis as a geneticist since 1982.

“The risk of producing a lethal white comes when people breed two horses that carry the overo lethal white gene,” she explained. “By using the test, horse owners can determine if white-spotted horses that are predominantly overo carry the mutation responsible for lethal white syndrome.”

It's a mystery

The curse of Paint Horse breeders, lethal white syndrome is one of the most heartbreaking conditions to afflict spotted horses. Also known as congenital intestinal aganglionosis, the disease occurs in Paint Horses that are all, or predominantly all, white.

Although the foal may at first appear to be normal, it usually begins exhibiting signs of colic within 12 hours of birth because of a non-functioning colon, similar to Hirschsprung disease in humans. Because surgery is not successful, OLWS is always fatal, so foals are often euthanized shortly after birth.

The affliction occurs more often in breeds such as Paint and Quarter Horses that have white in their coats. It rarely occurs in Thoroughbreds.

The disease is most often associated with horses that have an overo spotting pattern. Overo includes three primary patterns—frame overo, sabino and splashed white—that are genetically different.

Frame overo horses are characterized by white patches centered in the neck and body that are framed by colored areas. Research indicates that frame overo horses are most likely to produce lethal whites.

The mutation associated with OLWS also has been found in horses that have the sabino and splashed white patterns. The sabino pattern can range from being minimally expressed in a horse that has one or more white legs and a blaze to horses that are almost or completely white. Most sabinos that are largely white are quite roaned and speckled.

In the splashed white pattern, a horse's legs and the bottom parts of its body are usually white, which makes the horse look like it has been dipped in white paint. Its head is often white and its eyes are frequently blue.

However, seeing is not always believing when it comes to the complex world of genetics. Although it has been shown that frame overos can produce lethal white foals, the disease can also occur in tobianos and solid-colored Paint and Quarter Horses that carry the OLWS gene.

For decades, Paint Horse owners sought a reliable, scientific way to identify the deadly characteristic. When none could be found, many accepted the risk of getting a lethal white foal as the cost of breeding Paints.

Then, in 1997, three independent research groups made a significant discovery. Geneticists at the University of Minnesota, the University of California and at a hospital in Australia located the DNA mutation and gene associated with OLWS. The Minnesota research was funded by a grant from the American Paint Horse Association.

By analyzing the DNA sequence of the gene in affected foals and their parents, scientists determined that lethal white foals have two copies of the gene responsible for the disease. As in all genetics, the offspring gets one copy of a gene from each parent.

Once the gene was located, the mystery began to unravel.



APHA PHOTOS

The overo pattern includes three primary patterns that are genetically different. Frame overo horses (top) are the most likely to produce lethal white foals. Although the mutation linked with OLWS has been found in sabino (middle) and splashed white (bottom) horses, the risk of their producing lethal whites is less.

How it works

Inherited diseases, such as lethal white syndrome, are caused by mutations or changes in DNA. In lethal whites, a receptor gene contains a change in the order of the basic DNA building blocks, which results in a mutation that affects the normal development of the foal's intestines.

Animals receive one copy of each gene, which may be dominant or recessive, from their parents. A horse that has two copies of the lethal white gene will be a lethal white.

By knowing a horse's genetic makeup and avoiding the crossing of a stallion and mare that both carry the lethal white gene, it is possible to produce foals that are overo, but not lethal white.

With a Punnett Square, scientists can predict the possible gene combinations when crossing two horses. If one uses the letter "O" to symbolize the overo lethal white gene and "N" for horses that do not have that gene, lethal white foals can be symbolized as "OO" because they have two copies of the lethal white gene.

The overo parents of the aforementioned foal would be represented genetically as "NO." White-spotted or solid horses that do not have the mutation would be "NN."

When two overos carrying one copy of the OLWS gene, or (NO), are bred, the resulting ratio of offspring as solids, overos and lethal whites is 1:2:1. This means that each time two overos who carry the lethal white gene are crossed, there is a 25 percent chance of producing a lethal white foal.

This percentage may not be readily apparent in real life because not all lethal white foals survive birth. Some are resorbed by the mare during gestation or they are aborted.

When an overo that carries the lethal white gene (NO) is bred to a solid-colored horse that does not have the OLWS gene (NN), there is a 50 percent chance of producing an overo and a 50 percent chance of getting a solid. There is no chance of producing a lethal white foal.

Many people believe they increase their chance of getting an overo foal

	N	O
N	25% NN Solid	25% NO Overo
O	25% NO Overo	25% OO Lethal

	N	O
N	25% NN Solid	25% NO Overo
N	25% NN Solid	25% NO Overo

Many people believe they increase their chances of getting an overo foal if they breed two overos. However, as the Punnett Square on the left shows, these breeders have a 25 percent chance of getting a lethal white. If an overo is bred to a solid-colored horse that does not carry the OLWS gene (square at right) they have a 50 percent chance of getting an overo—the same as when breeding two overos—but without the risk of producing a lethal white.

if they breed two overos, said Penedo. But research reveals this is not the case.

"If they breed an overo to a solid-colored horse that does not carry the OLWS gene, their chance of getting overo offspring is 50 percent, the same as if they bred two overos," she explained. "But they eliminate the 25 percent chance of getting a lethal white."

Equine genetics can be very tricky. You can't always tell by looking at a horse if it carries an overo gene. Tobianos, toveros and solid-colored horses can all produce lethal white foals if they have the OLWS gene in their pedigrees.

That's where the test comes in.

Using a process called allele specific polymerase chain reaction (ASPCR), the test makes it possible for scientists to look at the region of the gene responsible for the white pattern.

"Almost everyone working with DNA uses this process because it allows us to look at a very specific, very short piece of DNA that contains the mutation," Penedo said.

Although no other mutations associated with OLWS have been discovered, there is the rare possibility that two non-overo horses (NN) could have a lethal white foal if there is a mutation at a site different from the one the test detects.

The test is also useful to breeders who want to identify pedigree sources of the overo pattern that may be valuable to their breeding programs.

Looks can be deceiving

Just as you cannot tell whether a horse is at risk for producing lethal white foals by looking at its coat, a completely white horse is not always a lethal white.

Interestingly enough, the white gene (W) is dominant over all other coat colors, including gray. This means that a foal that receives only one dominant white gene from either parent will be white. This horse has white hair, pink skin and dark eyes.

But even though the white gene is dominant, scientists know that two dark-colored horses have produced a white foal. One explanation is that the white gene may have a high rate of spontaneous mutation because foals produced by such crosses have gone on to breed as if they had a dominant white gene.



Dr. Cecilia Penedo is associate director of the Veterinary Genetics Lab at UC Davis.

UC DAVIS PHOTO

Whites aren't always lethal

Peg Fultz couldn't believe her eyes as her Paint Horse mare, Ambers Romance, began to foal during the morning of May 4, 2001.

Peg and her husband, Daniel, had bred the bay tobiano mare to their sorrel overo stallion, Sonnys Zippin It.

The Fultzes, who own Prism Paints & Quarter Horses in Bagley, Minnesota, have bred and raised horses for 15 years. Originally, the couple wanted to breed "Sonny" to another mare who was a frame overo, but they decided against it after having both horses tested for Overo Lethal White Syndrome (OLWS).

"We knew about lethal whites after being on horse farms and learning about it from the *Paint Horse Journal*," Peg noted. "After the test revealed both horses had one copy of the frame overo gene, we decided not to breed them.

"We felt it was a matter of responsibility because we knew there was a

25 percent chance of their producing a lethal white foal and we didn't want to create that situation."

Instead, they bred Sonny to Ambers Romance because she is a tobiano, which doesn't carry the lethal white gene unless the horse has overo in its background.

So, when Peg saw a white coat emerge as "Amber" began to foal, she felt uneasy.

"I looked at the baby as it was being born and saw more and more white coming out," she said.

After the foal was finally born, Peg was devastated. It was a solid white colt with pink skin and blue eyes.

"He didn't have a dark spot anywhere," said Peg. "Believe me, I looked.

"I almost died because I thought it was a lethal white. It was heartbreaking.

"Daniel wouldn't even look at the foal. He just knew we were going to have to put him down."

PPQ Spirit Of Romance (left) is an example of a viable white horse. His dam, Ambers Romance (right), is a bay tobiano. His sire, Sonnys Zippin It (below), is a sorrel overo.



The couple knew veterinarians advised euthanizing lethal white babies so that they wouldn't suffer.

Peg immediately logged on to www.horsesmidwest.com, an equine chat line created by horse owner Pat Bores, for advice and comfort from on-line friends.

"I wanted to talk with them about what I thought was a horrible situation," she recalled.

"Some said to wait and see if the foal exhibited lethal white symptoms. They advised me to wait before doing anything."

Lethal white foals have non-functioning colons, so they are unable to pass waste. Because of this, they begin to show signs of colic within the first 12 hours after they are born.

When Peg finally saw the colt pass meconium, a newborn's first excrement, she was elated.

But her joy quickly turned to despair.

"I thought we were out of the woods," she said, "but then I found out we needed for the milk feces to go through. I didn't see it from Friday morning, when the foal was born, until Sunday night.

"Finally, after the third day, it came. Boy, was I happy!"

The foal's registered name is PPQ Spirit Of Romance, but the Fultzes call him "Houston." He remained healthy, and is a good example of a viable white.

Viable whites are usually a combination of equine white spotting patterns. Scientists believe such horses are white because they have two white spotting patterns that overlap.

One of the genes may carry the overo lethal white characteristic, but the other gene carries another white pattern.

After further research into the pedigree of Houston's sire and dam, Peg believes he carries the genes for four patterns—tobiano, frame overo, sabino and splashed white. His base color is red, or sorrel, according to the results of a red-factor test conducted by the Veterinary Genetics Lab at the University of California—Davis.

The Fultzes also had Houston tested for the lethal white gene, which revealed he has one copy. So they bred him to PPQ Zena Skips, a smoky black Breeding Stock who does not carry the OLWS gene.

They can hardly wait for Houston's first baby to be born this spring. The foal could be sabino, splashed white, frame overo, tovero or Breeding Stock, says Peg.

"Horses like Houston are sometimes referred to as 'extreme overos,' which have more than an 80 percent chance of throwing color," she said.

"But most important, he's got great bloodlines and conformation. His sire is a full brother to APHA Champion Sonny Dee Zippin. He can do everything from hunter under saddle to halter to Western pleasure."

Peg credits her on-line friends for convincing her to wait to see if Houston was healthy.

"If it weren't for my horse forum friends, I don't know if he would be here today," she said.

"Later, I had some very heart-felt, tearful conversations with people who said they had put down some all-white foals who hadn't shown any symptoms because their vets told them they would probably die anyway."

The Fultzes are big supporters of OLWS testing because it helps breeders avoid crossing horses that can produce a lethal white foal.

"Financially, the test saves you a whole year of breeding," Peg said.

"I don't think a mare owner should breed to any stallion, regardless of what he looks like, unless he has been tested for OLWS. He could still have the lethal white gene. You can't assume Quarter Horse mares don't have it, either."

The test doesn't discourage people from breeding for the popular frame overo pattern.

"So many people love that pattern," said Peg. "It's beautiful."

"You can have frame overos, but still prevent lethal whites from occurring. People just have to be informed."



DARRELL DODDS

Solid white Paint Horses and Thoroughbreds are rare. Arctic White is both. Bred and raised by Dalene Knight, Arctic White is the only living white Thoroughbred stallion registered with the Jockey Club.

If two white horses are bred, 50 percent of their foals will be white and 25 percent will be dark. The other 25 percent will be lethal, but these foals, which are homozygous white (WW), are usually resorbed before birth.

These two lethal conditions, homozygous white (WW) and overo lethal white (OO), are produced by two different genes.

APHA lists white as a color on its registration certificate. The Appaloosa Horse Club and Jockey Club also recognize white as a color that may be registered, but the American Quarter Horse Association does not.

Solid white horses are rare in Paint Horses, Appaloosas and Thoroughbreds. In fact, the only living white

Thoroughbred stallion registered with the Jockey Club is registered as a Paint Horse, as well.

The horse, Arctic White, was sired by Airdrie Apache, who is also a double-registered Paint/Thoroughbred stallion. Arctic White's dam is Out of Tropicana Anna (TB).

Arctic White was bred and raised by Dalene Knight of Painted Desert Farm in Redmond, Oregon. In 2003, he was sold to Shadow Mountain Stables in Norco, California, which plans to use him to breed white Thoroughbred racehorses.

Horses of other colors, such as perlino and cremello, can look white, but they are also genetically different from white overos. Perlinos and cremellos



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*Perlino*s may look white, but they are actually bay horses whose color has been lightened twice by a cream gene. RFF Starbuck, shown here with owner Milynda Milam, is APHA's first registered perlino.

are double-diluted, which means they carry two copies of a color-dilution gene called the cream gene.


The cream gene in double dose lightens, or dilutes, black and red, the two basic pigments of equine coat color, to a very pale cream that sometimes may appear to be white. It has nothing to do with the lethal white gene. For example, a perlino is a bay horse whose color has been lightened by two doses of the cream gene. A cremello is a red horse whose color has been lightened by two doses of the cream gene.

Then there are horses called viable whites that are often a combination of two white patterns.

APHA registers viable whites as Breeding Stocks because the animals have no other color with which to contrast the white. This means they don't have a qualifying spot that enables them to be eligible for the Regular Registry, explained APHA Registration Manager Cindy Grier.

But some lethal whites have pigment somewhere on their bodies, she noted.

"They may have a small dark spot on their nose or under their tails," she said. "That pigmented area does not ensure that a horse is not a lethal white foal.

"However, it's equally important to note that just because a horse is white or 99 percent white doesn't mean it's lethal. Only time or the test will tell." 

Want to know more?

If you would like to order the test for Overo Lethal White Syndrome, or for more information about it, visit the University of California–Davis Web site at www.vgl.ucdavis.edu, or call (530) 752-2211.

The following pamphlets, books and Web sites are also helpful:

- American Paint Horse Association's *Guide to Coat Color Genetics*. To order, call (817) 834-2742, extension 271. APHA's Web site also has information about coat color genetics at www.apha.com/breed/geneticeq.html.
 - *Equine Color Genetics* by Dr. D. Phillip Sponenberg. Published by Iowa State University Press. To order, call (800) 862-6657.
 - *Horse Genetics* by Dr. Ann T. Bowling. Published by Oxford University Press. To order, call (800) 445-9714.
 - *Horse Color Explained* by Jeanette Gower. Published by Trafalgar Square Publishing. To order, call (800) 423-4525.
 - The Cremello and Perlino Educational Association Web site at www.doubledilute.com.
- The following scientific papers on lethal white syndrome are available at many public libraries:
- Endothelin receptor B polymorphism associated with Lethal White Foal Syndrome in horses. By E. M. Santschi, A. K. Purdy, S. J. Valberg et al. *Mammalian Genome* 9:306-309 (1998).
 - A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschsprung disease. By D. L. Metallinos, A. T. Bowling and J. Rine. *Mammalian Genome* 9: 436-431 (1998).
 - A dinucleotide mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome (LWFS); a horse variant of Hirschsprung disease (HSCR). By G. C. Yang, D. Croaker, A. L. Zhang et al. *Human Molecular Genetics* 7(6):1047-1052 (1998).