With a Splash

The discovery of *Splashed White 5* made waves in 2019—here's what you need to know.

By JESSICA HEIN

Penny Kleinschmidt helped spark research into Splashed White 5. *She owns two* Splashed White 5 *horses, including TK Ollies After Shock.*

COURTESY PENNY KLEINSCHMIDT

When APHA member Penny Kleinschmidt of Indianapolis, Indiana, first looked over her horses' DNA results, she was puzzled. The test results indicated her Paints had none of the known alleles capable of producing white markings. Yet, her horses—TK Ollies After Shock and TK Scotchs Aftershock—weren't lacking chrome on their coats.

Meanwhile, across the Atlantic in France, another Paint Horse owner had the same question and the same perplexing DNA results. Angelique Levert's mare, Chelsea Dancin Wood, had plenty of chrome—a bold, mask-like apron face paired with blue eyes, tall stockings and a swath of white cradling her abdomen and barrel—and she was also deaf. Surely a genetic answer was at the core of these mysteries.

Both women—separately, but serendipitously—reached out to Christa Lafayette, founder and CEO of genetic testing company Etalon Diagnostics. Intrigued by the loudly marked horses and their corresponding lack of known white-producing alleles, Christa set the wheels in motion to discover a new spotting pattern that was lurking just out of reach of modern genetic testing. Soon, *Splashed White 5* was officially discovered.

On the Hunt

Shortly after the curious owners contacted her in early 2018, Christa jumped on the case from Etalon's base in Menlo Park, California—she, too, was interested in the potential genetic cause of these boldly marked, bald-faced, blue-eyed beauties.

"In the case of Ms. Levert's mare, we checked our most recent samples for related horses that appeared negative for known white-spotting genetics but displayed obvious white markings and, in this case, blue eyes as well," Christa explained. "We reached out to the owners of her mare's siblings, asking detailed questions and requesting more photos. Then, we began second-level testing where we looked in certain 'suspect' regions of the horses' genomes through DNA sequencing to see if we could find anything unusual. The results started to look promising."

Armed with her preliminary suspicions and a battery of sample cases, Christa reached out to genetics researcher Tosso Leeb, Ph.D., at the University of Bern in Switzerland. Intrigued, Tosso agreed to analyze the samples as part of a larger genome-sequencing project.

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"After about three decades of molecular genetic research, it has become quite clear that a small number of genes exist that might cause white-spotting phenotypes in mammals. The two most important of the genes are *KIT* and *MITF*. There are a few others, but the total number is less than 10," Tosso explained; "phenotype" refers to the horse's outward appearance.

KIT (Receptor Tyrosine Kinase) and *MITF* (Melanocyte Inducing Transcription Factor) are common culprits for white-spotting patterns. *KIT* is home to *Sabino 1* and the so-called *"White"* mutations (like *W5, W10* and *W20*),

Chelsea Dancin Wood was part of the original research into Splashed White 5*, along with several siblings and her sire. Researchers identified a deleted piece of DNA code on the* MITF *gene in some horses sharing bold white markings, blue eyes and, sometimes, deafness. This led to the discovery of the* Splashed White 5 *allele.*

COURTESY ANGELIQUE LEVERT

and is closely associated with *Tobiano*, as well. *MITF* is already home to *Splashed White 1* and *3*. Other genes of interest for Paint breeders include *PAX3* (Paired Box 3 Transcription Factor), home to *Splashed White 2* and *4*, and *EDNRB* (Endothelin Receptor Type B), which is home to *Frame Overo*.

"When we learn of a horse with an unexplained white-spotting phenotype, we determine its entire genome sequence, which consists of roughly 2.5 billion nucleotides. Subsequently, we focus on the known candidate genes for white-spotting phenotypes—which make up less than 1 million nucleotides—and analyze whether we find any DNA variation that is likely to impact the normal function of these genes," Tosso explained.

Upon analysis of DNA samples from Chelsea Dancin Wood, her sire Razor N Jills Paco, and seven additional offspring of that sire, Tosso's team identified a "deletion" of genetic code—a section of DNA where some information is missing, when compared to the horse reference genome used by researchers—in the *MITF* gene. This missing section was present in each sample horse that displayed bold white markings, blue eyes and, in some cases, deafness. Other sampled horses, who were related by pedigree but did not display similar coat color markings or deafness, were not missing the implicated portion of the *MITF* gene. *MITF* produces a protein that has a key role in the development and function of melanocytes, which are pigment-producing cells. As such, it can affect skin, hair and eye color, and contributes to the survival of pigment cells in the inner ear, thereby influencing hearing function.

"The genome of these horses was missing a piece of information found on the standard or 'wild type' equine genome," Christa explained, adding that the effect of a genetic deletion can be minor or major, depending on the extent of missing information. "We found this same deletion in all of the client horses displaying the characteristic bald face, blue eyes, other white body markings—usually, on the legs—and high incidence of deafness. Siblings of the loudly marked horses who did not display white markings, blue eyes or deafness did not have the deletion we now call *Splashed White 5*."

After discovery of the *Splashed White 5* deletion, Christa re-evaluated her other suspect horses' samples and expanded the testing pool.

Splashed White 5 *has been found in Paints of varied lineage and geographical regions. Testing is now available from APHA's partner labs.*

COURTESY AMY CHANDLER

In the Know: *Splashed White 5*

Discovery: 2019, University of Bern (Switzerland)

Location: *MITF* gene on chromosome 16
Note: This is the same gene affected by *Splashed White 1* and *Splashed White 3*

Cause: A deleted section of the *MITF* gene, which is required for the survival of pigment-producing cells

Distribution: Confirmed in a number of different pedigrees and geographic locations; suspected to go back to at least the 1970s, per Etalon researchers, though more samples will help narrow down a suspected founding horse.

Characteristics: Bald face, blue eyes, and white leg/abdomen markings are common; associated deafness in some horses

Testing: Available as a special request through Etalon Diagnostics; available at the University of California–Davis as part of the *Splashed White* test and APHA Color/Pattern panel (beginning December 2019)

The Science: Search "white spotted horse" on *ncbi.nlm.nih.gov/pubmed* to read the research abstract

Sure enough, they also had the *MITF* deletion; Penny's horses, TK Ollies After Shock and TK Scotchs Aftershock, as well as ALC Hope Always Wins, a 2010 sorrel overo mare owned by Amy Chandler of Morriston, Florida, joined the list of confirmed *Splashed White 5* horses.

"What's truly amazing about this kind of collaboration is that the horse owners get their answers; new genetic tools are discovered and made available to others; the academic scientists achieve a publication in record time; and APHA gets a new pattern. Everybody wins," Christa said. "Sharing observations and capabilities is a tremendous way to accelerate science. The horse owners' input is really key here; without their involvement, photos and observations, we would not have had any clues as to what was lying undiscovered in the DNA samples."

Puzzle Pieces

Though research into *Splashed White 5* is new, the following characteristics seem to be associated with this pattern:

- Bald face
- Blue eye(s)
- White lower legs, with markings sometimes extending on the barrel/abdomen (giving a "dipped in paint" look)
- Associated deafness in some, but not all, horses

"The increased risk for deafness is directly caused by the *MITF* variant," Tosso explained. "The inner ear needs some pigment cells to maintain its function. If the inner ear is completely devoid of pigment cells, it will degenerate and become deaf. Therefore, any genetic variants that lead to white heads are likely to increase the risk for deafness. *MITF* variants are known to also cause deafness in humans.

"Based on knowledge from other species, *SW5* is likely to act as a semi-dominant allele," Tosso continued; this means inheriting one copy of *Splashed White 5* has a different effect on the horse than when two copies are inherited. "One copy of *SW5* produces a white-spotting phenotype and an increased risk for deafness. Two copies of *SW5* in a homozygous horse will completely abolish *MITF* function and likely result in a very severe phenotype, causing blindness, deafness, completely white body color, potentially reduced lifespan or perinatal lethality.

"There are many different white-spotting phenotypes in horses. Often the exact genotype cannot be solely determined by looking at the phenotype of a horse and/or the genotypes of the parents, so breeders need to know whether a horse carries *SW5* or not."

Both APHA partner laboratories—Etalon Diagnostics and University of California–Davis—offer testing for *Splashed White 5*. At Etalon, it's an a la carte option available for $40 and it's not included on the All-Inclusive Mini-Panel for APHA at this time. Through UC–Davis, the test is included on the APHA Color & Pattern Panel and also offered as part of the individual *Splashed White* test, as of December 2019.

Ultimately, the official identification of *Splashed White 5* has several direct implications for Paint Horse breeders.

First, discovery of *Splashed White 5* provides Paint owners with greater insight—a genetic "answer" as to the cause of some horses' distinctive markings and, in some, potential for hearing impairment. Next, it offers breeders a new tool—either in color production potential or as a means to avoid the associated complications. Third, *Splashed White 5's* discovery on *MITF*, a gene that's well-established for its white-spotting mutations, reinforces the fact that almost all white-spotting alleles are concentrated in a few specific gene locations in

Vocab Lesson

Allele—variants of a given gene

Genotype—an organism's genetic makeup

Phenotype—an organism's outward appearance

Genome—an organism's complete genetic makeup

Deletion—a section of genetic code that's missing from the area where it's typically located in a species

Mutation—a deviation from the standard genetic makeup of a species

MITF—Melanocyte Inducing Transcription Factor; this gene produces a protein that affects the development and function of pigment-producing melanocytes

Increase your Paint genetics know-how by reading these past *Paint Horse Journal* articles:

- "The Color Inside—Parts 1 & 2:" January 2016 and February 2016 Read this article free online at *apha.com/breed/geneticeq*
- "In the Cards:" February 2017
- "Reading Between the Lines:" September/October 2018

Splashed White 5*'s discovery on* MITF *reinforces the fact that almost all white-spotting alleles are concentrated in a few specific gene locations.*

horses—so while Mother Nature might shake things up once in a while with a new mutation, the genes at the root of most white markings are well defined.

The Road Ahead

For Penny, discovery of *Splashed White 5* doesn't change the plans she has for her Paints, but it's gratifying to now have an answer to what once was a genetic mystery.

"I am the proud breeder and owner of TK Ollies After Shock—a special connection started between us as I watched him being born," Penny said about her 2017 sorrel overo stallion; "Chuck" made his showing debut in 2018 with Penny in in-hand events, and they progressed to riding events in 2019. "I intend to continue working on building his skills in different disciplines to eventually make him an awesome all-around horse. Chuck has the biggest heart and always works hard to please, no matter what's asked of him. It's also thrilling and inspiring to be part of a new color gene's discovery."

Jessica Hein is editor of the Paint Horse Journal.
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Confirmed *Splashed White 5* Cases

COURTESY AMY CHANDLER

ALC Hope Always Wins

2010 sorrel overo mare

Intensely Handsome x Sonnys Lucky Image
Owned by Amy Chandler, Morriston, Florida
SW5/n, W20/n

COURTESY PENNY KLEINSCHMIDT

TK Ollies After Shock

2017 sorrel overo stallion

Sonnys After Shock x Ollies New Design
Owned by Penny Kleinschmidt, Indianapolis, Indiana
SW5/n

COURTESY ANGELIQUE LEVERT

Chelsea Dancin Wood

2016 bay overo mare

Razor N Jills Paco x Southern Dottie
Formerly owned by Angelique Levert, Villeneuve Du Latou, France; now owned by Sarah Grand
SW5/n, W20/W20

EYE OF THE HORSE PHOTOGRAPHY

TK Scotchs Aftershock

2017 sorrel overo gelding

Sonnys After Shock x Annies Classy Scotch (QH)
Owned by Penny Kleinschmidt, Indianapolis, Indiana
SW5/n