White Coat in the Domestic Horse

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Traits such as shape, size, and color often influence the economic and sentimental value of a horse. Around the world, horses are bred and prized for the colors and markings that make their unique coat patterns stand out from the crowd. The underlying genetic mechanisms determining the color of a horse's coat can vary greatly in their complexity.

horse mutation coat color depigmentation white horse

1. Introduction

A streak of white, a patch of pink skin, a piercing blue eye. These traits add individuality, stunning beauty, and economic value to the domestic horse (*Equus caballus*). Specific color traits can help a horse qualify for color-specific registries. However, some of these prized alleles can cause detrimental phenotypes, such as an increased risk of deafness or blindness. Other combinations of these sought-after color traits fail to produce viable offspring. Understanding the etiology of white markings is crucial to ensure ethical care and breeding practices.

Recent advancements have decreased the cost of genome sequencing, enabling the elucidation of the characteristics of the genetic mutations causing many depigmentation phenotypes. *KIT*, *MITF*, *PAX3*, *HPS5*, *EDNRB*, *TRPM1*, and *RFWD3* represent the collection of genes associated with different white markings in the domestic horse (**Table 1**). While the genetic mechanisms underlying some of these color traits are not fully understood, the information collected on white spotting mutations can assist breeders in making optimal choices to breed unique and healthy herds.

Table 1. White coat color traits and their distinguishing characteristics along with the associated genes and encoded proteins.

Trait	Allele Symbol	Gene/Region	Encoded Protein (Complex)	Distinguishing Characteristics (Phenotype)
Dominant White	*W*	*KIT*	KIT	White spots or white coat, pink skin (sabino-like, all white)
Tobiano	*TO*	*KIT*	KIT	Large white spots covering the body and crossing the spine, white legs, pink skin (tobiano)

Trait	Allele Symbol	Gene/Region	Encoded Protein (Complex)	Distinguishing Characteristics (Phenotype)
Sabino	*SB1*	*KIT*	KIT	Jagged white markings or white coat, pink skin (sabino, all white)
Roan	*RN*	*KIT*	KIT	Interspersed white hairs distributed through the coat (roan)
Splashed White	*SW*	*MITF*, *PAX3*	MITF, PAX3	Deafness, blue eyes, smooth boarded white faces, abdomens, and legs (splashed white)
Eden White	*EDXW*	*HPS5*	HPS5 (BLOC-2)	White body spots, legs, and faces (sabino-like)
Lethal White Overo	*LWO*, *O*	*EDNRB*	EDNRB	White face, legs, and body spots that do not cross the spine (overo)
Leopard Spotting	*LP*	*TRPM1*	TRPM1	White hind quarter or white coat with colored spots, epistatic to *PATN1* (blanket or leopard)
Pattern 1	*PATN1*	*RFWD3*	RFWD3	Increases white spotting for individuals with a least one copy of *LP*

ends and e, whose phenotypes are often confused with *Grey* or *Cream*. Because horses homozygous for *Cream* have pink skin and hair diluted to a near-white color, it is difficult to differentiate between true white and homozygous *Cream* horses [1]. *Dominant White* horses are born with white markings and display pink skin below these areas, while individuals with *Grey* do not have pink skin and are not born with white hairs, but develop them with age [1]. The Romans knew of the phenotypic differences between gray and white, although it remains unknown if their terms for these colors correspond to modern designations [2]. Investigations into runs of homozygosity in 1476 horses of European descent revealed positive selection for base coat color on ECA3, but not in the region harboring *KIT* [3], while selection for white coat color patterns has been identified in ancient horse DNA [4].

The *Dominant White* locus is the equine locus with the largest number of known variants causing depigmentation (**Table 2**), and it is located on chromosome 3 within the *Proto-Oncogene*, *Receptor Tyrosine Kinase* (*KIT*) gene [2]. *Dominant White* uses the capital "*W*" followed by the integer in the series to indicate the specific dominant variant present in the genotype (e.g., *W35*). Originally, the *W* symbol was used for a small number of variants all following a true dominant pattern of inheritance and producing all white horses in the heterozygous state. The earlier dominant mutations were not observed in the homozygous state, leading to the adoption of an alternate term, *Lethal Dominant White*. Time has not honored this tradition as, to date, six *W* variants are known to be inherited in an incomplete dominant manner, with genotypes existing in the homozygous state in apparently healthy horses. Recent publications have started to refer to *Dominant White* as *White Spotting* to better account for the varied phenotypes at the *W* locus [5][6][7][8]. Phenotypes at the *Dominant White* locus are broadly characterized by horses displaying white areas with clear borders, or a completely white horse with pink skin underneath. Thirty-five *W* variants have been reported, and it seems likely that the number will continue to increase [2][9][10][11][12][13][14][15][16][17][18][19][20].

Table 2. Genomic location, variant type, and phenotypes of *Dominant White KIT* variants.

Allele	Genomic Coordinate EquCab3.0	Type	Phenotype	Homozygotes	References
W1	chr3:79545942G>C	nonsense	All White	Not Observed	[2]
W2	chr3:79549540C>T	missense	All White	Not Observed	[2]
W3	chr3:79578535T>A	nonsense	All White	Not Observed	[2]
W4	chr3:79549780G>A	missense	All White	Not Observed	[2]
W5	chr3:79545900delC	small deletion	Sabino-like	Not Observed	[12]
W6	chr3:79573754C>T	missense	Sabino-like to All White	Not Observed	[12]
W7	chr3:79580000C>G	splice site	All White	Not Observed	[12]
W8	chr3:79545374C>T	splice site	Sabino-like	Not Observed	[12]
W9	chr3:79549797C>T	missense	All White	Not Observed	[12]
W10	chr3:79566925_79566928del	small deletion	Sabino-like to All White	Not Observed	[12]
W11	chr3:79540429C>A	splice site	All White	Not Observed	[12]
W12	chr3:79579755_79579779delAGACG	small deletion	Sabino-like	Not Observed	[17]
W13	chr3:79544066C>G	splice site	All White	Not Observed	[14]
W14	chr3:79544151_79544204del	gross deletion	All White	Not Observed	[14]
W15	chr3:79550351A>G	missense	Sabino-like to All White	Observed	[14][18]
W16	chr3:79540741T>A	missense	All White	Not Observed	[14]

Allele	Genomic Coordinate EquCab3.0	Type	Phenotype	Homozygotes	References
W17a	chr3:79548265T>A	missense	All White	Not Observed	[14]
W17b	chr3:79548244A>G	missense	All White	Not Observed	[14]
W18	chr3:79553751C>T	splice site	Sabino-like	Not Observed	[15]
W19	chr3:79553776T>C	missense	Sabino-like	Observed	[15]
W20	chr3:7948220T>C	missense	No markings to Sabino-like	Observed	[15]
W21	chr3:79544174delG	small deletion	Sabino-like	Not Observed	[13]
W22	chr3:79548925_79550822del1898	gross deletion	Sabino-like	Not Observed	[11]
W23	chr3:79578484C>G	splice site	All White	Not Observed	[18]
W24	chr3:79545245C>T	splice site	All White	Not Observed	[10]
W25	chr3:77769878T>C	missense	All White	Not Observed	[16]
W26	chr3:79544150del	small deletion	Sabino-like	Not Observed	[16]
W27	chr3:79552028A>C	missense	All White	Not Observed	[16]
W28	chr3:79579925_79581197del	gross deletion	Sabino-like	Not Observed	[19]
W30	chr3:79548244T>A	missense	All White	Not Observed	[20]
W31	chr3:79618532_79618533insT	fs nonsense	Sabino-like	Not Observed	[7]
W32	chr3:79538738C>T	missense	No markings to Sabino-like	Observed	[7]
W33	chr3:79545248T>A	missense	Sabino-like	Not Observed	[5]

Many *W* alleles have been traced to a founding individual and are limited to those descendants, yet others are observed in diverse breeds, including a few that have likely circulated among diverse breeds over centuries, transmitted in cross-breed matings and by shipment of horses around the globe. The introduction of white alleles to new breeds is also promoted by registries opening studbooks to foreign horses with hopes of reducing inbreeding. As an example, researchers identified *W13* in American Quarter Horses (AQH) in 2011 [14], but more recently

Allele	Genomic Coordinate EquCab3.0	Type	Phenotype [24]	Homozygotes	References
W34	chr3:79566881T>C	missense	No markings to Sabino-like	Observed	[8]
W35	chr3:79618649A>C	UTR variant	No markings to Sabino-like	Observed	[6]

t from the genotypes g variants of alleles ations will help prevent the introduction of white alleles into registries that select against white markings and mitigate the potential crossing of lethal pairs.

2.1. Phenotype

Phenotypes associated with the *W* locus are characterized by either white patterning or an entirely white coat with pink skin underneath. Mild white spotting phenotypes are described as sabino-like, with white legs, facial stripes, and a collection of other white facial markings (called stars or snips depending on the size, shape, and location), and, less commonly, patches of white hair across the abdomen [22]. Strongly deleterious mutations (frameshift, stop-gain, indel) typically result in completely white horses with pink skin (**Figure 1**). In contrast, *W20*, *W32*, *W34*, and *W35* horses can be solid (non-white) in color in the absence of other white alleles but may magnify white markings caused by other alleles. For example, when an individual carries one copy of *W22* and an out-of-phase copy of *W20*, the resulting phenotype is an all-white or almost all-white horse, despite each of these individual alleles typically producing less pronounced depigmentation phenotypes [11][23]. The amplification of the degree of white spotting is also observed with *W5/n* and *W20/n* compound heterozygotes, as these horses display an all-white phenotype [24]. Variants *W19*, *W21–W23*, *W28*, and *W31–W35* produce a sabino-like phenotype sometimes accompanied by depigmentation on the abdomen with jagged borders. *KIT* variants also sometimes cause a rare phenotype of blue eyes when the depigmentation covers the entire face, including the eyes.

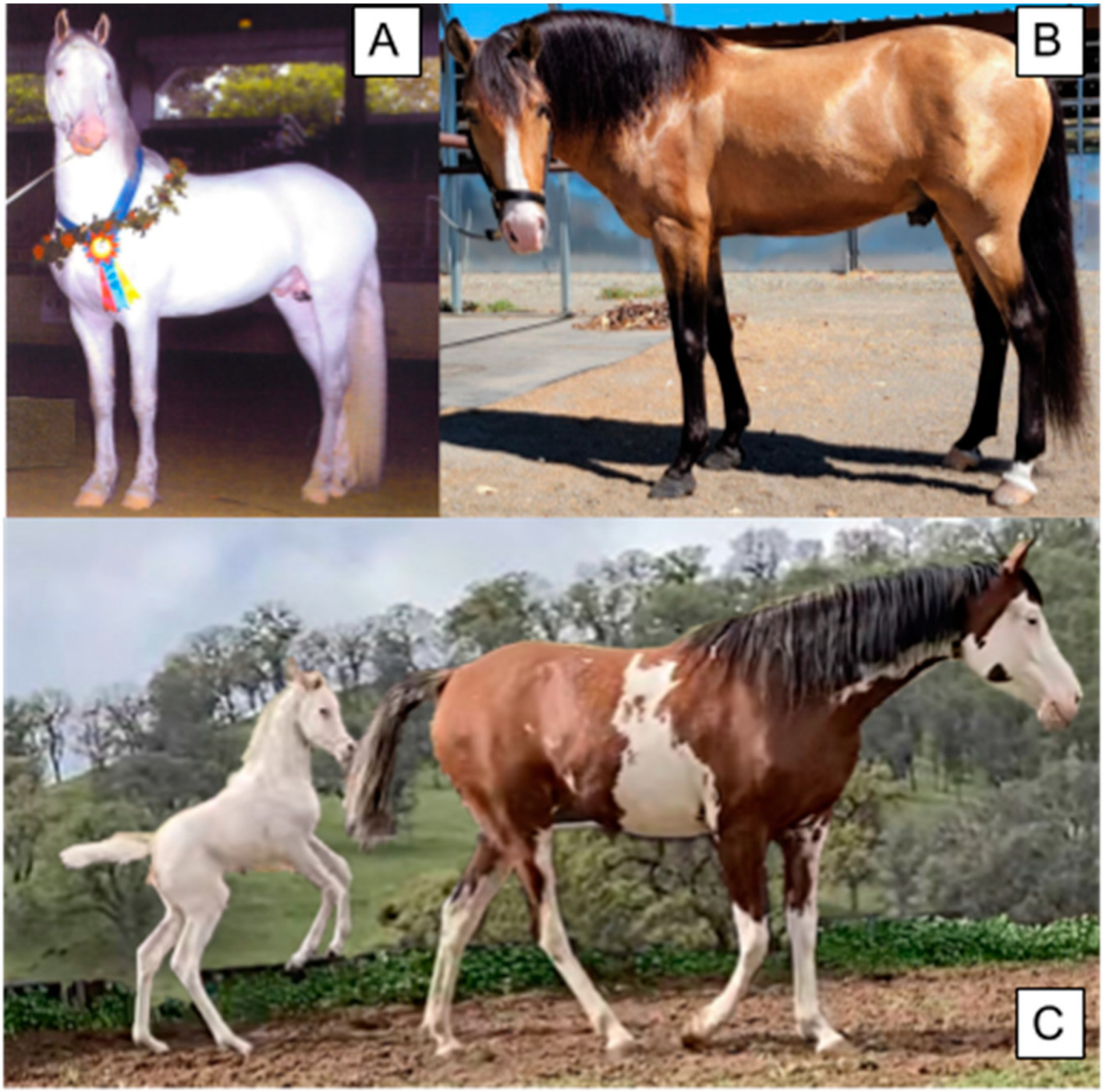

Figure 1. Various phenotypes caused by variants at the W locus, including full depigmentation, a sabino-like pattern, and minimal markings. Coat color genotypes and breeds are as follows: (**A**) *a/a E/e* (black) *W13/n*, Friesian-American White Horse cross. (**B**) *A/a E/e Cr/n* (Buckskin). (**C**) Foal—*A/A E/E* (bay) *W15/15*, Arabian, Dam —*A/A E/e* (bay) *W15/n*, Arabian.

Deleterious *Dominant White* alleles result in more extensive white markings and are likely lethal in the homozygous state [25]. *W1–W14*, *W16–W18*, *W21–28*, *W30*, *W31*, and *W33* have not been observed in the homozygous state, and are predicted to be homozygous lethal due to their similarities to mutations observed in other species [2][25]. Progeny ratios for white alleles causing fully white phenotypes also stray from Mendelian expectations. When

heterozygous white horses were crossed, the resulting offspring possessed a 2:1 ratio of white foals to solid foals, supporting the hypothesis that *W/W* is lethal during early gestation [25]. These two observations suggest homozygous embryos are not viable for certain alleles, but too few births have occurred to conclusively determine the lethality of each variant. *W15*, originally thought to be embryonic lethal, was later reported in two homozygous individuals [18]. A horse homozygous for *W19* was also recently identified, which also boasted two copies of *W34* and *W35* each, for a total of six white spotting variants [26]. Cases such as this support the hypothesis that other white variants could be viable in the homozygous state but have not yet been observed. Conclusions regarding the lethality of homozygous *Dominant White* variants will only be elucidated through continued monitoring and expanded genetic testing for these variants.

Many *KIT* variant haplotypes are reported in horses, and the resulting phenotypes are extremely varied and not fully documented. Phenotypes of horses with multiple white alleles depend on the specific white allele combination but generally result in increased depigmentation when compared to individuals with only one variant. Despite the impressive number of publications on the *Dominant White* locus, there are few studies focusing on phenotypes of horses with multiple white alleles. There are even fewer studies focusing on the health effects, and specifically, the reproductive effects, of horses with *KIT* variants, despite reports of *KIT* variants being associated with health defects in other species [2].

2.2. Mechanisms and Genetics

KIT transmits transmembrane signals critical for survival and plays an important role in melanogenesis [27][28][29]. During development, melanoblasts begin to migrate from the neural crest to populate the rest of the body and eventually develop into melanocytes (pigment-producing cells). Melanocyte development is in part controlled by interactions between KIT, KIT Ligand (KITL), and Melanocyte Inducing Transcription Factor (MITF) [28][29][30][31][32][33][34][35]. After binding with KITL in the extracellular domain, KIT self-dimerizes and phosphorylates MITF, activating the transcription factor and upregulating target genes involved in pigmentation [28][29]. Mutations affecting the function or binding sites of KIT protein disrupt this pathway, resulting in downregulated pigment genes and melanocytes failing to develop in some or all of the tissues.

There are a variety of mutations at the *Dominant White* locus including deletions, insertions, missense, nonsense, and splice site variants. More impactful mutations alter the protein conformation and function to a greater degree, and cause greater disruptions to KIT pathways, resulting in fewer melanoblasts properly migrating and a more depigmented individual. More tolerated *KIT* variants such as *W20*, *W32*, and *W35* have subtle effects on the protein or protein expression and result in milder phenotypes. However, because the failure of a melanoblast to migrate is a chance event, mild variants on their own may still cause extensive depigmentation. The stochastic nature of white spotting events can cause individuals with the same genotype to display very different phenotypes. Commercial genetic testing for all *W* alleles exists, but assays for *W10*, *W13*, *W19*, *W20*, and *W22* are among the more readily available tests since these alleles are more common.

Up to three *KIT* variants have recently been found linked together, resulting in complex haplotypes. To date, the *W22* allele has only been observed in combination with the *W20* allele [11][23]. *W19* has been observed by itself and in linkage with *W34* and *W35*. The *W19W34W35* haplotype likely occurred by a crossover event because it was only identified in an inbred family, while the *W19* allele has been found out of phase of *W34* and *W35* in multiple families [26]. Sixteen haplotypes, including combinations of *W20*, *W32*, *W34*, and/or *W35* with other variants, have been identified, but the genesis of these complex haplotypes is not completely understood. Founder horses have not been reanalyzed for recently discovered alleles to reveal if novel variants occurred on the background of other alleles or if the haplotype occurred via a crossover event. While phenotypic records of all known multilocus genotypes are incomplete, it is likely that more white variants increase the amount of white patterning on a horse.

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