A KIT Variant Associated with Increased White Spotting Epistatic to MC1R Genotype in Horses (Equus caballus)

Laura Patterson Rosa 1,* , Katie Martin 1 , Micaela Vierra 1 , Erica Lundquist 1 , Gabriel Foster 1 , Samantha A. Brooks 2 and Christa Lafayette 1, *

1 Etalon, Inc., Menlo Park, CA 94025, USA; khoefs@etalondx.com (K.M.); mvierra@etalondx.com (M.V.); elundquist@etalondx.com (E.L.); gfoster@etalondx.com (G.F.)
2 Department of Animal Science, UF Genetics Institute, University of Florida, Gainesville, FL 32610, USA; samantha.brooks@ufl.edu
* Correspondence: laura.patterson@sulross.edu (L.P.R.); clafayette@etalondx.com (C.L.); Tel.: +1-650-380-2995 (C.L.)

Simple Summary: Although over 40 genetic variants are known to influence white spotting and markings on the horse, many still have unknown genetic causes. Furthermore, some seem to be influenced by pigmentation (base coat color) genes. We investigated two horses demonstrating a heritable white spotting pattern of no known genetic cause. Through sequencing of the coding region of candidate genes, we identified a mutation in the KIT proto-oncogene, receptor tyrosine kinase (KIT) gene changing the coded amino acid, predicted to be deleterious to protein function. We further evaluated this variant in a population of 147 horses, characterized using photographs scored by three independent observers using a standardized Average Grade of White score. The KIT mutation is significantly associated with a quantitative increase in white pattern ($p = 3.3 \times 10^{-12}$) and demonstrates an influence of the MC1R Extension locus. We also report a complete link between the previously reported KIT W19 allele and this mutation. We propose to name this mutation W34, following established nomenclature. Given the quantitative effect on white markings and MC1R influence, genetic testing for this allele can be of value for horse owners that desire to select for white patterns.

Abstract: Over 40 identified genetic variants contribute to white spotting in the horse. White markings and spotting are under selection for their impact on the economic value of an equine, yet many phenotypes have an unknown genetic basis. Previous studies also demonstrate an interaction between MC1R and ASIP pigmentation loci and white spotting associated with KIT and MITF. We investigated two stallions presenting with a white spotting phenotype of unknown cause. Exon sequencing of the KIT and MITF candidate genes identified a missense variant in KIT ($rs1140732842$, NC_009146.3:g.79566881T>C, p.T391A) predicted by SIFT and PROVEAN as not tolerated/deleterious. Three independent observers generated an Average Grade of White score for 147 individuals based on photographs. The KIT variant demonstrates a significant QTL association to AGW ($p = 3.3 \times 10^{-12}$). Association with the MC1R Extension locus demonstrated that, although not in LD, MC1R e/e (chestnut) individuals had higher AGW scores than MC1R E/- individuals ($p = 3.09 \times 10^{-17}$). We also report complete linkage of the previously reported KIT W19 allele to this missense variant. We propose to term this variant W34, following the standardized nomenclature for white spotting variants within the equine KIT gene, and report its epistatic interaction with MC1R.

Keywords: dominant white; white pattern; Arabian horse; American Quarter Horse; American Paint Horse; chestnut
1. Introduction

The easily observed expression of novel mutations causing white spotting phenotypes provides straightforward targets in studies of equine genetic variation. Forty-four genetic variants, most of which are located in the KIT proto-oncogene, receptor tyrosine kinase (KIT) and the melanocyt e inducing transcription factor (MITF) genes, are implicated in white spotting and depigmentation phenotypes in the horse (W1-W28, W30-W33 and SBI on KIT; SW1, SW3, SW5, SW6 and SW7 on MITF; SW2, SW4, LWO, TO and GR on other genomic locations) [1–16]. Phenotypes for white spotting alleles vary from white markings on the legs and extremities, as observed with KIT

$^{W20}$, to large white patches on the body, legs and head or completely white phenotypes in a few other mutant KIT, MITF or multiallelic individuals. Occasionally, carriers of well-documented white spotting alleles may present no to minimal white markings uncharacteristic of the spotting variant, a condition sometimes named “crypto” for its cryptic expression [17].

Loci likely altering the eumelanin vs. pheomelanin pigment proportion are also associated with the extent of depigmentation observed in the horse. The “chestnut” coat color, caused by a Melanocortin 1 Receptor (MC1R), termed the “Extension” locus, symbol “E” or “e”) loss-of-function mutation (e/e) and resulting in predominantly pheomelanin pigmentation, displays greater KIT-associated white markings [18–20]. Comparatively, “black” and “bay” coat colors, possessing the dominant eumelanin-producing functional MC1R (E/E) [20], demonstrate greater MITF-associated white markings [18]. Interactions between KIT and MC1R could be due to linkage, as both genes are located on Equus caballus Autosome 3 (ECA3), although separated by ~42 Mbps and a centromere [21–23]. Alternatively, the e/e genotype may decrease melanocyte quantity or migration, intensifying the white spotting QTL effect of KIT mutations [18].

White markings and spotting phenotypes are varying selection in some horse breeds, depending on breeding goals and registry requirements. For the American Paint Horse, a white spotting phenotype enables registration in the “Regular” registry rather than the “Solid Paint-Bred” registry, significantly impacting the economic value of the horse [8,24]. The American Quarter Horse Association did not previously allow registration of horses with white spotting phenotypes (a rule that changed in 2004), yet statements noting white spotting as “undesirable” and “uncharacteristic” are maintained in the regulations (AQHA Official Handbook, 67th Edition, 2019). Therefore, white markings or spotting phenotypes with unknown/novel associated genetic loci are of commercial interest for the equine industry, encouraging further studies on these variants.

We describe the investigation of a white spotting phenotype (extended white markings on limbs and the ventral thoracic region), yet negative for all published white variants (W1-W28, W30-W33, SW1-SW7, LWO, SBI and TO) [1–16]. Using a quantitative white score phenotype, we tested the association of this allele along with the MC1R Extension and ASIP Agouti loci in 41 cases and 94 breed-matched controls lacking published white variants. We report the significant association of a missense KIT polymorphism with a quantitative increase in white spotting on the coat, as well as the epistatic interaction of this allele with the MC1R loss-of-function genotype (chestnut). We also report the complete linkage of the previously published KIT c.1322A>G; p.(Y441C) (W19) allele with this variant in 12 genotyped individuals.

2. Materials and Methods

2.1. Horses and Putative Candidate Variant Inspection

An Arabian and a Mangalarga stallion, submitted to Etalon Diagnostics (Menlo Park, CA, USA) for commercial genotyping services, demonstrated a notable white spotting phenotype but no spotting or depigmentation alleles at any of the 44 known loci (GR, W1-W33, SW1-SW7, LWO, SBI and TO) [1–16]. Both individuals exhibited white markings extending past the distal part of the carpal and tarsal joints, as well as facial white markings extending from the forehead to the upper and lower lips. Additionally, the Arabian showed a large white spot on the ventral region of the body. The Mangalarga stallion was also
reported to produce similar white phenotypes on its offspring (Figure 1). Given the likely heritable phenotype, we pursued further evaluation of coding regions of candidate genes KIT and MITF (known to cause phenotypically similar white spotting patterns in the horse), using previously described methods of targeted exon sequencing and alignment [13].

Figure 1. The subject stallions (a, b), demonstrating spotting phenotype extending past the distal part of the carpal and tarsal joints, as well as a facial white marking extending from the forehead to the upper and bottom lips along homozygote MC1R e/e phenotype, (a) also possess ventral white markings extending past the ribcage; (c–f) the respective offspring of the Mangalarga (b) stallion demonstrating the heritable phenotype.

Putative candidate polymorphisms were further evaluated by predicting functional impacts using the PROVEAN [25] and SIFT [26] webtools, using the NCBI Equus Caballus annotation release 103. To test for associations between novel variants and white spotting phenotypes, while controlling for confounding effects originating from other known white pattern variants, 41 unrelated individuals carrying only the candidate variant, as well as 94 negative control individuals that did not possess other known white spotting or depigmentation alleles (of 1431 previously genotyped by Etalon Diagnostics [27] representing the general horse population) having submitted photographs, were selected for further phenotyping (n = 135). Following genotypic selection for the novel candidate variants, we observed that all W19 individuals (n = 12, four times the number of individuals in the original W19 publication) in the Etalon Diagnostics genotyped population possessed at least one allele of the candidate KIT variant; thus, we performed a second analysis including this allele (n = 147).
2.2. Linkage Disequilibrium Analysis with Dominant White 19 and MC1R

Due to the co-localization of loci on the *Equus caballus* autosome 3 (ECA3), we calculated the linkage disequilibrium (LD) between candidate *KIT* loci, *W19* and the *MC1R* polymorphism using Haploview V4.1 (Broad Institute, MIT and Harvard), in the 147 phenotyped individuals.

2.3. Phenotyping and Statistical Analysis

Three blinded observers (LPR, KM and EM) scored the white phenotypes based on anonymized photographs of individual horses, following a previously published procedure for white pattern scoring [19]. In short, photos submitted by the owners were reviewed by KM and EM, selecting for 1 or 2 photographs that best represented the individual, where all 4 legs, the front of the head and any ventral/lateral white were visible for scoring purposes. Aside from the 12 compound *KIT* *W19* individuals, only two horses (EdX1476 and EdX4739) showed uneven body markings; these were scored based on the side with the highest amount of white. We then scored the amounts of white on the head, legs and body for each individual horse, which were then combined in a total score for each observer, summed, then averaged by the number of observers (*n* = 3), generating the “Average Grade of White” (AGW) value [19]. We modified the original score, awarding one point for white within a square comprising the ventral thorax and ribcage, and one point if white patterns were observed outside of the delimited region, to better quantify body markings (Figure 2). The modified scheme graded white from a minimum score of 0 to a maximum of 38, with a minimal effect on the original score maximum value of 36 [19]. Correlations between observer scores were evaluated using Pearson’s pairwise multivariate correlation on SAS JMP Pro V15 (SAS Institute, Cary, NC, USA).

Figure 2. Average Grade of White phenotyping system as modified from that published by Rieder et al. [19]. Dashed lines demonstrate anatomical locations and limits for each score. Leg scores range from 0 (no white) to 5 (white above the respective dotted line).
A multiple linear regression modeling the effects of candidate loci, coat color (MC1R and ASIP genotype) and KIT W19 on AGW was done using SAS JMP Pro V15 (SAS Institute, Cary, NC, USA). We independently evaluated the genotype impact of MC1R, ASIP and the candidate mutation with (n = 147 horses) and without (n = 135 horses) the presence of W19 on the AGW (Table 1 and Table S2). Genotypes for all variants were obtained through the Etalon Diagnostics (Menlo Park, CA, USA) commercial testing.

**Table 1.** Effect sizes of MC1R Extension, ASIP Agouti and KIT rs1140732842 loci on AGW (n = 135 horses) using binomial regression.

| Description                                           | AICc     | MC1R Extension | ASIP Agouti | KIT rs1140732842 |
|-------------------------------------------------------|----------|----------------|-------------|-----------------|
| AGW                                                   | 918.99   |                |             |                 |
| AGW + MC1R Extension                                  | 903.95   | 17.99          |             |                 |
| AGW + ASIP Agouti                                     | 915.54   | 5.57           |             |                 |
| AGW + KIT rs1140732842                                | 869.13   |                |             | 32.51           |
| AGW + MC1R Extension + KIT rs1140732842               | 843.23   | 30.26          |             | 40.51           |
| AGW + MC1R Extension + ASIP Agouti                    | 904.51   | 13.51          | 1.54        |                 |
| AGW + MC1R Extension + ASIP Agouti + KIT rs1140732842 | 845.29   | 28.14          | 0.13        | 39.09           |
| p-value (full model)                                  | 4.71 × 10^{-7} | 0.7199       | 5.09 × 10^{-14} |                 |

*p*-values are given for the full model incorporating AGW and genetic effects.

3. Results

3.1. Variant Analysis Suggests a Candidate in KIT Influenced by MC1R

Exon sequencing of both stallions identified homozygosity for two non-synonymous variants, NC_009159.3:g.21551234C>G in MITF and NC_009146.3:g.79566881T>C in KIT, respectively recorded as rs1148371483 and rs1140732842 on the Ensembl Variation Annotation release 104 [28]. We did not observe an association between the MITF variant and the AGW phenotype in the 135 horses (p = 0.4256; W19 individuals excluded). Functional predictions also support that the MITF variant, a glycine to alanine change, is not a likely candidate, as SIFT and PROVEAN predicted its effect as neutral (score = 0.23, SIFT; score = −0.356, PROVEAN) [29].

The presence of the alternate allele at rs1140732842 is associated with a substantial effect on the AGW quantitative phenotype (F (2, 132) = 32.51, (ANOVA) p = 3.3 × 10^{-12}) (Table 1). The KIT variant rs1140732842 is predicted by the SIFT method as not tolerated (score = 0.03), and as deleterious by PROVEAN (score = −3.363). This variant substitutes an uncharged polar threonine to a nonpolar alanine in the KIT protein structure (p.T391A). The MC1R genotype alone also has a small yet significant effect on AGW (F (1, 133) = 17.99, (ANOVA) p = 4.12 × 10^{-5}), with e/e (chestnut) individuals demonstrating higher AGW scores on average (mean score of 7.63) than MC1R E/- (eumelanin dominant) individuals (mean score of 2.54) (Figure 3). The ASIP Agouti locus has no significant effect on the AGW phenotype (Table 1). The KIT rs1140732842 variant appears to have an incomplete penetrance autosomal dominant, or additive mode of inheritance for AGW, that could be cryptic in MC1R E/- individuals. When evaluating the effect of W19 in AGW (Table S2, N = 147), the best-fitting model included all four loci (AICc = 919.73). Notably, the AGW scores from the three independent observers were highly correlated (r(147) > 0.9846, p < 4.38 × 10^{-87}), demonstrating the repeatability of the AGW scoring methodology in this study.
3.2. Linkage Disequilibrium between KIT and MC1R

We did not observe linkage disequilibrium between MC1R alleles and the KIT rs1140732842 variant in the 147 horses \( (r^2 = 0.0001, \ D' = 0.065, \ LOD = 0.06) \). Similarly, Brooks et al. [24] demonstrated that the KIT W20 allele (exon 14) was not in linkage disequilibrium with the MC1R Extension locus in a cohort of 364 American Paint Horses [25]. However, the KIT W19 mutant allele (exon 8, 13.1 Kb apart) is in perfect linkage with the rs1140732842 (exon 7) C variant \( (r^2 = 0.17, \ D' = 1, \ LOD = 6.49) \). Three horses demonstrated compound genotypes (EdX4400 and EdX1926: KIT rs1140732842 C/C W19/KIT+ and EdX2927: KIT rs1140732842 C/C W19/W19), indicating that the KIT W19 may have appeared after the rs1140732842 mutant variant, as we observed heterozygosity of the W19 allele in the presence of homozygosity of the KIT rs1140732842 C allele, yet not the opposite (Figure 4, File S2).
Figure 4. Phenotypic examples of the rs1140732842 (W34) and compound W19 allele action in different MC1R Extension and ASIP Agouti loci, as well as respective Average Grade of White (AGW) scores.

4. Discussion

Based on VGNC:19433 and NP_001157338.1 annotations as wild-type models, the computational analysis of the protein change in folding free energy upon mutation predicts that the p.T391A variant is destabilizing (Supplementary File S1) [30]. The amino acid threonine is highly conserved (Genomic Evolutionary Rate Profiling (GERP) score = 3.33) in this position in 91 eutherian mammals, including humans and mice [28], which could explain the observed low (1.51%) minor allele frequency of the rs1140732842 variant in 1431 genotyped horses.

The effects of black or chestnut base coat colors on white spotting patterns were previously observed in the Arabian [31,32], the Franches-Montagnes horse [18,19] and the American Paint Horse [24]. While there is no linkage between the MC1R Extension locus and the candidate KIT variant in our cohort, the epistatic effect might be explained by other biological mechanisms. It is possible that the MC1R c/c genotype negatively affects the proliferation and differentiation of melanocytes, as observed in the murine recessive yellow (McIr°) model [33]. Lower activity of the MC1R receptor, as is likely to result from the loss-of-function variant, promotes phaeomelanin production [34]. As KIT is also involved in melanocyte pigmentation and development, the combined effect of deleterious alleles at both loci likely promotes a higher likelihood of failed melanocyte migration or maturation, resulting in unpigmented skin devoid of melanocytes [35]. Furthermore, exon screening cannot rule out the possibility that rs1140732842 is tagging a haplotype bearing a non-coding regulatory change in the KIT gene.

Individuals possessing at least one rs1140732842 alternate allele © included the Arabian and its crosses, as well as Warmblood, Rocky Mountain Horse, American Quarter Horse, American Paint Horse, Appaloosa, Mustang, Mangalarga, Mangalarga Marchador and Morgan breeds (Table S1). Notably, the three original individuals in the KIT W19 publication are also recorded as compound rs1140732842 heterozygotes [5]. The W19 allele seems to further increase the AGW ($p = 1.88 \times 10^{-20}$) and, given the AICc results, has some effect on the ASIP locus that we could not properly access in our study due to the
small sample size and confounding effect of the rs1140732842 variant. Due to the observed linkage, further evaluation of the W19 allele’s phenotypic effects alone is suggested, along with a possible effect of base coat color, including respective genotypes at ASIP and MC1R as suggested by the model.

5. Conclusions
We report a white spotting QTL associated with the KIT variant rs1140732842 and modified by the presence of the MC1R loss-of-function pheomelanin genotype in the horse, as well as the observed linkage of KIT W19 to this variant in our population and in the original publication. We propose to designate this polymorphism as W34, following the standardized nomenclature for white spotting variants within the KIT gene. Given the KIT rs1140732842 alternate allele’s significant association and QTL effect on white markings and its MC1R epistatic influence, genetic testing for this variant can be of value for horse owners that desire to select for quantitative white phenotypes.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/ani12151958/s1, Table S1: Genotypes and AGW phenotypic information for the 147 individuals; Table S2: Effect sizes of MC1R Extension, ASIP Agouti, KIT W19 and KIT rs1140732842 loci on AGW (n = 147 horses) using binomial regression; File S1: DUET—Protein Stability Change Upon Mutation prediction results for the p.T391A variant impact on protein stability; File S2: Graphical representation of the Linkage Disequilibrium heatmap between MC1R, KIT W19 and rs1140732842 (W34), as well as respective allele frequency and haplotype demonstration of the W19 C genotype ($r^2 = 0.17$, $D' = 1$, LOD = 6.49) to W34 C genotype in 147 horses.

Author Contributions: Conceptualization, L.P.R., K.M., S.A.B. and C.L.; methodology, L.P.R. and S.A.B.; software, L.P.R. and G.F.; validation, L.P.R., K.M., G.F. and S.A.B.; formal analysis, L.P.R.; investigation, L.P.R. and K.M.; resources, E.L. and C.L.; data curation, L.P.R., K.M., M.V. and G.F.; writing—original draft preparation, L.P.R.; writing—review and editing, L.P.R., K.M., M.V., E.L., G.F., S.A.B. and C.L.; visualization, L.P.R.; supervision, K.M., E.L., S.A.B. and C.L.; project administration, K.M., E.L. and C.L.; funding acquisition, E.L., K.M. and C.L. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Informed consent was obtained from all owners of the animal subjects involved in the study.

Data Availability Statement: Not applicable.

Acknowledgments: The authors would like to thank Deedra Holloway, José Roberto Petrucci, Emelia Wolf, Nancy Groenenboom, Natalie DiBerardinis, Roberta Nugent, Tammie Patrick, Sherry Bumbarger, Laetitia Lhéritier, Stephanie Wind, Steve Hugus, Janet Macdonald, Honey Belshe, Brandi Fulgo and João Antônio Rodrigues Caldas, as well as all the owners of subject horses, who provided samples, photos and background information for the W34 study, and Erik Martin (EM), for his collaboration in this study.

Conflicts of Interest: All authors are affiliated with Etalon Diagnostics, which offers testing for white pattern variants.

References
1. Haase, B.; Brooks, S.A.; Schlumbaum, A.; Azor, P.J.; Bailey, E.; Alaeddine, F.; Mevissen, M.; Burger, D.; Poncet, P.-A.; Rieder, S. Allelic heterogeneity at the equine KIT locus in dominant white (W) horses. PLoS Genet. 2007, 3, e195. [CrossRef] [PubMed]
2. Haase, B.; Brooks, S.A.; Tozaki, T.; Burger, D.; Poncet, P.A.; Rieder, S.; Hasegawa, T.; Penedo, C.; Leeb, T. Seven novel KIT mutations in horses with white coat colour phenotypes. Anim. Genet. 2009, 40, 623–629. [CrossRef] [PubMed]
3. Haase, B.; Rieder, S.; Tozaki, T.; Hasegawa, T.; Penedo, M.C.T.; Jude, R.; Leeb, T. Five novel KIT mutations in horses with white coat colour phenotypes. Anim. Genet. 2011, 42, 337–339. [CrossRef]
4. Hauswirth, R.; Haase, B.; Blatter, M.; Brooks, S.A.; Burger, D.; Drögemüller, C.; Gerber, V.; Henke, D.; Janda, J.; Jude, R. Mutations in MITF and PAX3 cause “splashed white” and other white spotting phenotypes in horses. *PLoS Genet.* 2012, 8, e1002653. [CrossRef]  
5. Hauswirth, R.; Jude, R.; Haase, B.; Bellone, R.R.; Archer, S.; Holl, H.; Brooks, S.A.; Tozaki, T.; Penedo, M.C.T.; Rieder, S. Novel variants in the KIT and PAX 3 genes in horses with white-spotted coat colour phenotypes. *Anim. Genet.* 2013, 44, 763–765. [CrossRef]  
6. Haase, B.; Jagannathan, V.; Rieder, S.; Leeb, T. A novel KIT variant in an Icelandic horse with white-spotted coat colour. *Anim. Genet.* 2015, 46, 466. [CrossRef] [PubMed]  
7. Brooks, S.A.; Bailey, E. Exon skipping in the KIT gene causes a Sabino spotting pattern in horses. *Mamm. Genome* 2005, 16, 893–902. [CrossRef] [PubMed]  
8. Brooks, S.A.; Lear, T.L.; Adelson, D.L.; Bailey, E. A chromosome inversion near the KIT gene and the Tobiano spotting pattern in horses. *Cytogenet. Genome Res.* 2008, 119, 225–230. [CrossRef]  
9. Holl, H.; Brooks, S.; Bailey, E. De novo mutation of KIT discovered as a result of a non-hereditary white coat colour pattern. *Anim. Genet.* 2010, 41, 196–198. [CrossRef]  
10. Dürig, N.; Jude, R.; Holl, H.; Brooks, S.A.; Lafayette, C.; Jagannathan, V.; Leeb, T. Whole genome sequencing reveals a novel deletion variant in the KIT gene in horses with white spotted coat colour phenotypes. *Anim. Genet.* 2017, 48, 483–485. [CrossRef]  
11. Holl, H.M.; Brooks, S.A.; Carpenter, M.L.; Bustamante, C.D.; Lafayette, C. A novel splice mutation within equine KIT and the W15 allele in the homozygous state lead to all white coat color phenotypes. *Anim. Genet.* 2017, 48, 497–498. [CrossRef] [PubMed]  
12. Martin, K.; Vierra, M.; Foster, G.; Brooks, S.A.; Lafayette, C. De novo mutation of KIT causes extensive coat white patterning in a family of Berber horses. *Anim. Genet.* 2020, 52, 135–137. [CrossRef] [PubMed]  
13. Patterson Rosa, L.; Martin, K.; Vierra, M.; Foster, G.; Lundquist, E.; Brooks, S.A.; Lafayette, C. Two variants of KIT causing white patterning in Stock-type horses. *J. Hered.* 2021, 112, 447–451. [CrossRef] [PubMed]  
14. Metallinos, D.L.; Bowling, A.T.; Rine, J. A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: An equine version of Hirschsprung disease. *Mamm. Genome* 1998, 9, 426–431. [CrossRef] [PubMed]  
15. Esaïe, E.; Till, B.; Kallenberg, A.; Fremeux, M.; Bickel, L.; Bellone, R.R. A de novo missense mutation in KIT is responsible for dominant white spotting phenotype in a Standardbred horse. *Anim. Genet.* 2022, 53, 534–537. [CrossRef] [PubMed]  
16. Patterson Rosa, L.; Martin, K.; Vierra, M.; Foster, G.; Brooks, S.A.; Lafayette, C. Non-frameshift deletion on MITF is associated with a novel splashed white spotting pattern in horses (*Equus caballus*). *Anim. Genet.* 2022, 53, 538–540. [CrossRef]  
17. Stachurska, A.; Jansen, P. Crypto-tobiano horses in Hucul breed. *Czech J. Anim. Sci.* 2022, 707–715. [CrossRef] [PubMed]  
18. Haase, B.; Signer-Hasler, H.; Binns, M.M.; Obexer-Ruff, G.; Hauswirth, R.; Bellone, R.R.; Burger, D.; Rieder, S.; Wade, C.M.; Leeb, T. Accumulating mutations in series of haplotypes at the KIT and MITF loci are major determinants of white markings in Franches-Montagnes horses. *PLoS ONE* 2013, 8, e75071. [CrossRef]  
19. Rieder, S.; Hagger, C.; Obexer-Ruff, G.; Leeb, T.; Poncet, P.A. Genetic Analysis of White Facial and Leg Markings in the Swiss Franches-Montagnes Horse Breed. *J. Hered.* 2008, 99, 130–136. [CrossRef]  
20. Rieder, S.; Taourit, S.; Mariat, D.; Langlois, B.; Guérin, G. Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (*Equus caballus*). *Mamm. Genome* 2001, 12, 450–455. [CrossRef] [PubMed]  
21. Kalbfleisch, T.S.; Rice, E.S.; DePriest, M.S.; Walenz, B.P.; Hestand, M.S.; Vermeesch, J.R.; O’Connell, B.L.; Fiddes, I.T.; Vershinina, A.O.; Petersen, J.L.; et al. EquCab3, an Updated Reference Genome for the Domestic Horse. *bioRxiv* 2018, 306928. [CrossRef]  
22. Beeson, S.K.; Mickelson, J.R.; McCue, M.E. Exploration of fine-scale recombination rate variation in the domestic horse. *Genome Res.* 2019, 29, 1744–1752. [CrossRef] [PubMed]  
23. Beeson, S.K.; Mickelson, J.R.; McCue, M.E. Equine recombination map updated to EquCab3.0. *Anim. Genet.* 2020, 51, 341–342. [CrossRef] [PubMed]  
24. Brooks, S.A.; Palermo, K.M.; Kahn, A.; Hein, J. Impact of white-spotting alleles, including W20, on phenotype in the American Paint Horse. *Anim. Genet.* 2020, 51, 707–715. [CrossRef] [PubMed]  
25. Choi, Y.; Sims, G.E.; Murphy, S.; Miller, J.R.; Chan, A.P. Predicting the functional effect of amino acid substitutions and indels. *PLoS ONE* 2012, 7, e46688. [CrossRef] [PubMed]  
26. Ng, P.C.; Henikoff, S. SIFT: Predicting amino acid changes that affect protein function. *Nucleic Acids Res.* 2003, 31, 3812–3814. [CrossRef]  
27. Diagnostics, E. Etalon DNA Test Panel Comparison Chart. Available online: https://www.etalondx.com/compare-tests (accessed on 31 January 2022).  
28. Howe, K.L.; Achuthan, P.; Allen, J.; Allen, J.; Alvarez-Jarreta, J.; Amode, M.R.; Armean, I.M.; Azov, A.G.; Bennett, R.; Bhai, J.; et al. Ensembl 2021. *Nucleic Acids Res.* 2021, 49, D884–D891. [CrossRef]  
29. Choi, Y.; Chan, A.P. PROVEAN web server: A tool to predict the functional effect of amino acid substitutions and indels. *Bioinformatics* 2015, 31, 2745–2747. [CrossRef] [PubMed]  
30. Pires, D.E.V.; Ascher, D.B.; Blundell, T.L. DUET: A server for predicting effects of mutations on protein stability using an integrated computational approach. *Nucleic Acids Res.* 2014, 42, W314–W319. [CrossRef]  
31. Woolf, C.M. Common white facial markings in bay and chestnut Arabian horses and their hybrids. *J. Hered.* 1991, 82, 167–169. [CrossRef]
32. Woolf, C.M. Common white facial markings in Arabian horses that are homozygous and heterozygous for alleles at the A and E loci. *J. Hered.* 1992, 83, 73–77. [CrossRef] [PubMed]

33. Hirobe, T.; Abe, H.; Wakamatsu, K.; Ito, S.; Kawa, Y.; Soma, Y.; Mizoguchi, M. Excess tyrosine rescues the reduced activity of proliferation and differentiation of cultured recessive yellow melanocytes derived from neonatal mouse epidermis. *Eur. J. Cell Biol.* 2007, 86, 315–330. [CrossRef] [PubMed]

34. Ito, S.; Wakamatsu, K. Human hair melanins: What we have learned and have not learned from mouse coat color pigmentation. *Pigment Cell Melanoma Res.* 2011, 24, 63–74. [CrossRef] [PubMed]

35. D’Mello, S.A.N.; Finlay, G.J.; Baguley, B.C.; Askarian-Amiri, M.E. Signaling Pathways in Melanogenesis. *Int. J. Mol. Sci.* 2016, 17, 1144. [CrossRef]