

Frameshift Variant in *MFSD12* Explains the Mushroom Coat

Color Dilution in Shetland Ponies

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Supplemental Tables and Figures

Table S1 Primer sequences for eight top prioritized GWAS SNPs. Genotyping was performed using Agena Bioscience MassArray technology.

MassArray Marker Assayed	Variant ID	Association*	Primer	Primer Sequence
7:718169	rs396258064	2.15 X 10 ⁻⁵	2 nd -PCR ^P ** 1 st -PCR ^P ** UEP_SEQ***	ACGTTGGATGCATCTGCCCTCGTTTCTATC ACGTTGGATGCGAGGTGCAAAATGACAAGG GCTGTTTCTGAGTTGGC
7:3290682	rs68661375	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGATGAGCCAGGTGGGTGTTTC ACGTTGGATGCTGCCTGACGTTCTAGAATG GCGCCGGCCCCGCAC
7:3293765	rs395756529	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGGACCTTGACTGAAACGCTTG ACGTTGGATGAAAGTCAAAGAGCTCCACCC AACGCTTGAGGCGAG
7:3305324	rs395273754	2.71 X 10 ⁻¹⁶	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGAAGAAAGCCGACTGTGGAAG ACGTTGGATGCTGTAAATGCCTCGCCGAAG ACTGTGGAAGACATTAATTACTC
7:3431918	rs1139100844	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGGCAATGCTCGGTCCCAAAC ACGTTGGATGTCCAAACGCGTTTCCAAAG CGCATCGTCTCCAAATT
7:3459389	rs68664417	5.20 X 10 ⁻¹⁵	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGACGCTTCTGTGCAACCATC ACGTTGGATGTTTGTCCGTGTCTTTGAGGG CGAACCATCTTAGCCC
7:3468232	rs68664452	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGCGACCCCACACGAAGCCAC ACGTTGGATGTGGTTCCATATGGTGATACG CCCCGAAGCCACCCCTGG
7:3482185	rs1148601322	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGGGTCCCTAAAGACTCATTGG ACGTTGGATGTGGAGCACTTTGCCCATCAC CCCCGGTTGCAGGAAACTCAC
7:3616751	rs394856954	9.84 X 10 ⁻¹⁷	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGGCAAGGAACAGCAATTCAGC ACGTTGGATGAAAGGGACAGTCAGAGTGAG TGTGTGTA AAAACGTGGACC
7:3749780	rs68666954	1.53 X 10 ⁻⁵	2 nd -PCR ^P 1 st -PCR ^P UEP_SEQ	ACGTTGGATGTTGCATACTGTGCCCAGATG ACGTTGGATGTTCCAGTTCATCCGCCGCTC CCCAGATGCATCTTGGGAA

* Association calculated under a recessive model. ** 2nd and 1st PCR^P denotes the forward and reverse primers. *** UEP_Seq denotes extension primer.

Table S2 Primer sequences for Sanger sequencing *PLIN3* and confirming *MFSD12* (c.600dupC)

Primer Name	Forward primer	Reverse primer
<i>MFSD12</i> Variant	GCTCACCCGGAACACCGGCA	ACCGCCAGTCTTGCGCCATC

Table S3 Ophthalmic Findings and Coat Color Genotypes for those Shetland Ponies Examined

Ophthalmic Findings										
Individual	ASIP ¹	MC1R ²	Mu ³	Cr ⁴	To ⁵	SW1 ⁶	Anterior ⁷	Posterior ⁸	Iris PPM ¹¹	Corpora Nigra Cyst OD ¹⁰ cysts
463	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/C</i>	<i>N/T</i>	<i>N/S</i>	OD hypo	hypo*		
466	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	hypo	pig**		
467	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/C</i>	<i>N/N</i>	<i>N/N</i>	hypo	pig	OU ⁹ : iris-iris PPMs	
477	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	hyper	pig		
478	<i>A/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	hyper	pig	OU: iris-iris PPMs	
479	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	hypo	pig	OU: iris-iris PPMs	
480	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo		
481	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	hypo	hypo	OU: iris-iris PPMs	
483	<i>a/a</i>	<i>e/e</i>	<i>M/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	hypo	hypo		
464	<i>A/a</i>	<i>e/e</i>	<i>N/M</i>	<i>N/C</i>	<i>T/T</i>	<i>N/S</i>	hypo	hypo		
465	<i>A/A</i>	<i>e/e</i>	<i>N/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	pig		
468	<i>a/a</i>	<i>e/e</i>	<i>N/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo		
469	<i>a/a</i>	<i>e/e</i>	<i>N/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo		
470	<i>a/a</i>	<i>E/e</i>	<i>N/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo		
471	<i>a/a</i>	<i>E/e</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	pig	pig	OU: iris-iris PPMs	OU cysts
472	<i>A/a</i>	<i>e/e</i>	<i>N/N</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo		
473	<i>A/a</i>	<i>E/e</i>	<i>N/N</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	pig	hypo	OU: iris-iris PPMs	
474	<i>a/a</i>	<i>e/e</i>	<i>N/M</i>	<i>N/N</i>	<i>N/T</i>	<i>N/N</i>	hypo	pig		
475	<i>A/a</i>	<i>e/e</i>	<i>N/M</i>	<i>N/C</i>	<i>N/T</i>	<i>N/N</i>	hypo	hypo	OU: iris-iris PPMs	
476	<i>A/A</i>	<i>E/e</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	<i>N/N</i>	pig	pig	OU: iris-iris PPMs	

¹ Agouti genotype. ² MC1R (red factor) genotype. ³ Mushroom genotype. ⁴ Cream genotype. ⁵ Tobiano genotype. ⁶ Splash white 1 genotype. ⁷ Anterior uveal pigmentation. ⁸ Posterior uveal pigmentation. ⁹ OU refers to both eyes.

¹⁰OD refers to the right eye. ¹¹PPMs are persistent pupillary membranes. *hypopigmentation. **pigmented (normal).

*** This pony had one blue eye likely due to splash white 1.

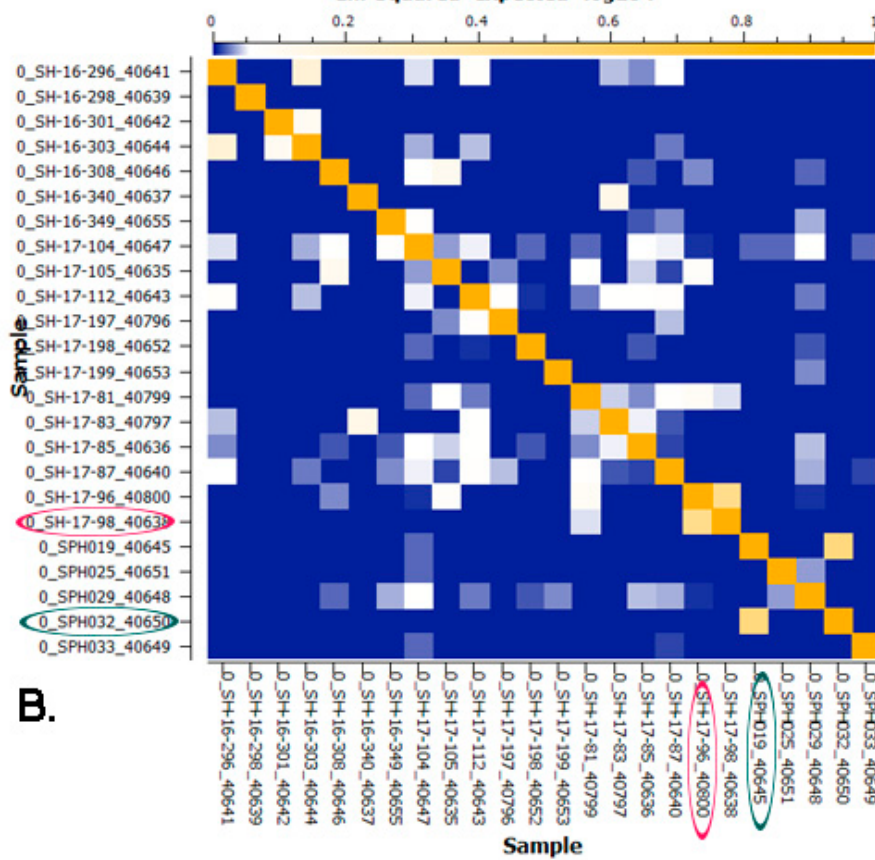
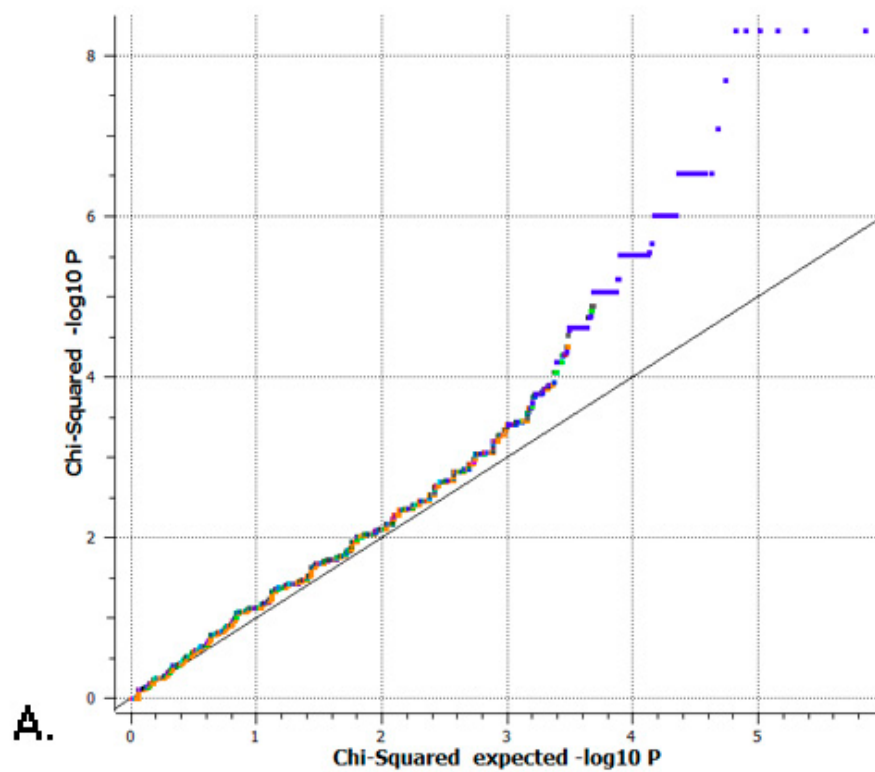


Figure S1: Investigating Genomic Inflation in the GWAS Sample Set. Identity by descent and Population structure

A) Q-Q plot of chi-squared $-\log_{10} P$ vs chi-squared expected $-\log_{10} P$ calculated from a χ^2 basic allelic association test. Markers on ECA7 are represented in indigo. B) Heat map displaying identity by descent (IBD) in the sample set. Two pairs of samples (circled in pink and teal) shared up to 52% of their genome. Intensity of yellow shading on the heat map denotes level of genome sharing.

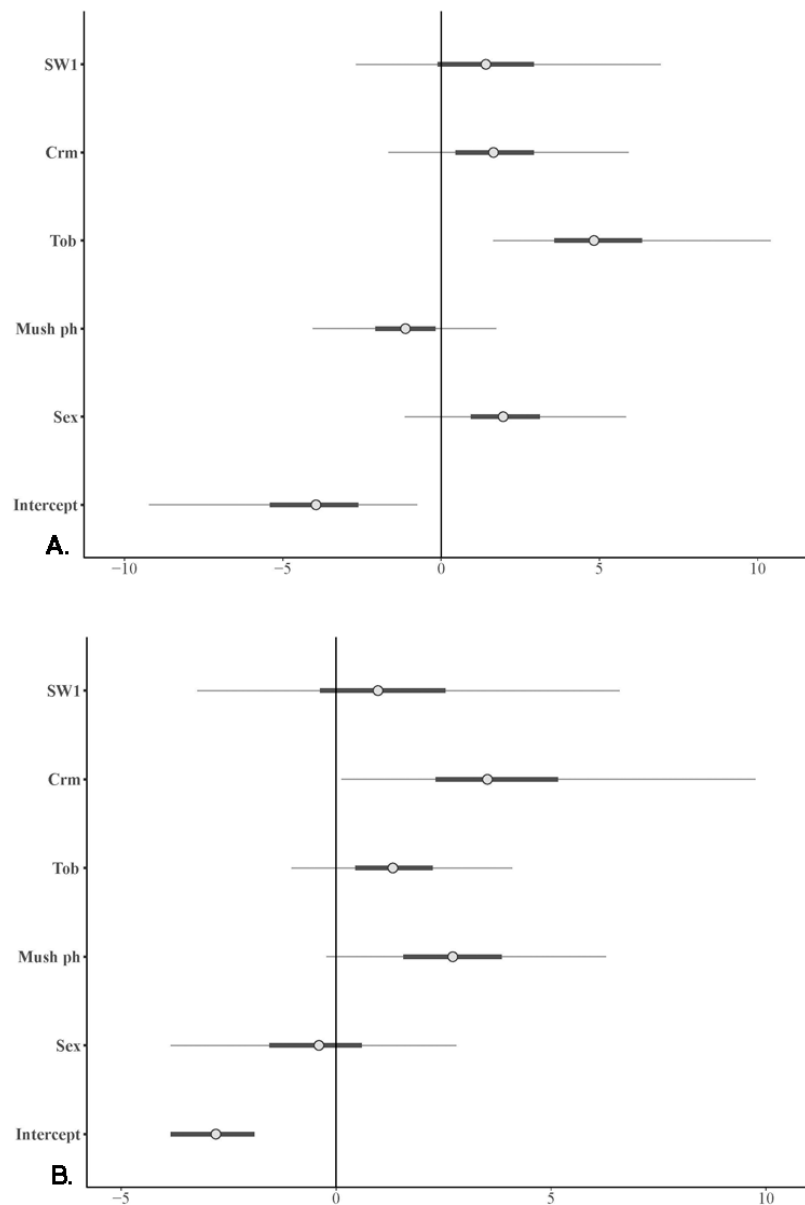


Figure S2: Regression Coefficient Plots for Potential Variables Explaining Posterior and Anterior Uveal Pigmentation Variation.

Both plots show the regression coefficients considering sex, mushroom phenotype (mush_ph), tobiano phenotype (tob), cream phenotype (crm), and splash white 1 phenotype (SW1) for posterior (A) and anterior (B) uveal pigmentation. The circles are an estimate of the coefficient, the thick line represents a 50% confidence interval and the thin line represents a 95% confidence interval. A) Regression coefficient plot for posterior uveal pigmentation. Tobiano looks to be the only variable tested that could explain hypopigmentation of the posterior uvea. B) Regression coefficient plot for anterior uveal pigmentation. The cream allele is the only variable tested explaining hypopigmentation of the anterior uvea.