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Novel insights into Sabino1 and splashed white coat color patterns in horses

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Summary

Within the framework of genome-wide analyses using the novel Axiom® genotyping array, we investigated the distribution of two previously described coat color patterns, namely sabino1 (SBI), associated with the *KIT* gene (KI16+1037A), and splashed white, associated with the *PAX3* gene (ECA6:g.11429753C>T; $PAX3^{C70Y}$), including a total of 899 horses originating from eight different breeds (Achal Theke, Purebred Arabian, Partbred Arabian, Anglo-Arabian, Shagya Arabian, Haflinger, Lipizzan and Noriker). Based on the data we collected we were able to demonstrate that, besides Quarter horses, the $PAX3^{C70Y}$ allele is also present in Noriker (seven out of 189) and Lipizzan (three out of 329) horses. The *SB1* allele was present in three breeds (Haflinger, 14 out of 98; Noriker, four out of 189; Lipizzan one out of 329). Furthermore, we examined the phenotypes of *SB1*- and $PAX3^{C70Y}$-carrier horses for their characteristic white spotting patterns. None of the *SB1*/*sb1*-carrier horses met the criteria defining the Sabino1 pattern according to current applied protocols. From 10 heterozygous $PAX3^{C70Y}$-carrier horses, two had nearly a splashed white phenotype. The results of this large-scale experiment on the genetic association of white spotting patterns in horses underline the influence of gene interactions and population differences on complex traits such as Sabino1 and splashed white.

Keywords association, sabino, single nucleotide polymorphism, white spotting patterns

The paint or pinto coat color pattern, a term that was proposed by Sponenberg (2009), summarizes a wide range of coat color phenotypes within horses that covers patterns of white spotting from extensive white markings, over symmetrical or non-symmetrical patches of white spread over the body, to completely white horses. To date, several genes and loci (*KIT*, *MITF*, *PAX3*, *Sabino1*, *EDNRB*) have been associated with this phenotypic variability, which has been structured into defined categories, such as tobiano, overo, sabino and splashed white (Metallinos *et al.* 1998; Santschi *et al.* 1998; Yang *et al.* 1998; Brooks & Bailey 2005; Hauswirth *et al.* 2012, 2013; Brooks *et al.* 2017). Within the framework of genome-wide SNP (single nucleotide polymorphism) analyses, we genotyped a total of 899 horses originating from eight different breeds using the novel Axiom® genotyping array. After genotyping we screened our population samples for two SNPs on equine chromosome (ECA) 3 and 6. SNP *AX-103727726* (ECA3:77735520; *SB1*, SNP KI16+1037A) is located in the *KIT* gene, and it is causative for the sabino1 (SB1) phenotype (Brooks & Bailey 2005), whereas SNP *AX-103512392* (ECA6:11429753C>T; SNP $PAX3^{C70Y}$) in the *PAX3* gene is associated with the splashed white phenotype (Hauswirth *et al.* 2012, 2013).

The genotype distributions of *SB1* and *PAX3* in the dataset of the sampled horses ($n = 899$) are summarized in Table 1. The entire dataset comprised 899 horses. To investigate the population structure between the horses' origination from eight different breeds, we conducted a principal components analysis (PCA). We applied common filter criteria, removing SNPs with a call rate less than 90% and those with very low minor allelic frequency less than 0.01. After quality control, we included a total of 533 459 autosomal SNPs for the population structure analysis.

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Table 1 Distribution of *SB1*- and *PAX3*^{C70Y}-carrying horses in the sample of 899 horses originating from eight different breeds including Achal Theke, Haflinger, Lipizzan, Noriker, Purebred Arabian, Partbred Arabian, Anglo-Arabian and Shagya Arabian.

Breed	*n*	*SB1/sb1*	*PAX3*^{C70Y}/ *PAX3*$^{+}$	Not typed *SB1*	Not typed *PAX3*^{C70Y}
Achal Theke	36	0	0	1	0
Haflinger	98	14	0	12	1
Lipizzan	329	1	3	13	3
Noriker	189	4	7	9	6
Arabian	186	0	0	0	0
Partbred Arabian	21	0	0	0	0
Anglo Arabian	8	0	0	0	0
Shagya Arabian	32	0	0	0	0
Sum	899	19	10	35	10

Visualization of the entire dataset on the first three principal components (PCs) shows that, with the exception of only the highly related Arabian populations (Anglo Arabian, Purebred Arabian, Partbred Arabian and Shagya Arabian), all horses form distinct breed groups according to their genetic origin (Fig. 1).

In a second step, we compared the genotypes of identified *SB1*- and *PAX3*^{C70Y}-carrier animals with their phenotypes following the system of Brooks & Bailey (2005) (Fig. 2) and Hauswirth *et al.* (2012) (Fig. 3). Additionally we scored the white markings of *SB1*- and/or *PAX3*^{C70Y}-carrier horses according to the protocol of Rieder *et al.* (2008) to describe the extent of the depigmentation areas. The coat color phenotype was derived from the identification protocols and additionally from photographs. According to Brooks & Bailey (2005), Sabino spotted horses must fulfill three out of four characteristics: (i) two or more white feet or legs, (ii) a blaze, (iii) jagged margins around white areas and (iv) white spots or roaning in the midsection of the body. To describe splashed white phenotypes associated with the *PAX3*^{C70Y} allele according to Hauswirth *et al.* (2012), we used the system they proposed, estimating the percentage of white face area and white body area by visual examination. Hauswirth *et al.* (2012) classified horses with 3% or less of white face area and 0% white body area as solid colored and horses with 20% or more white face area and 10% or less white body area as splashed white. Horses with white face area ranging from 3 to 20% were classified as unknown phenotype, and horses with 20% or more of white face area and less than 10% white body area were considered to have another white spotting phenotype.

Within the total dataset of 899 genotyped horses, 19 animals (2.11%) were heterozygous for the *SB1* locus and none of the horses was homozygous (Table 1). Regarding the breed-associated frequency of animals carrying the variant, no *SB1* allele could be identified in Purebred Arabians, Partbred Arabians, Anglo-Arabians, Shagya Arabians and Achal Theke samples, altogether comprising 283 animals. One copy of the *SB1* allele was present in 14 Haflinger horses (14.3%), one Lipizzan (0.3%) and four Noriker horses (2.1%). Among these 19 *SB1*-carrier horses (14 Haflinger, 4 Noriker, 1 Lipizzan), the color phenotype for one Haflinger was not available. The remaining 18 horses could not be phenotypically classified as sabino according to the criteria of Brooks & Bailey (2005) (Table 2). Sixteen horses had no or only head markings with scores ranging from 0 to 12, according to Rieder *et al.* (2008). The *SB1* allele associated with the sabino

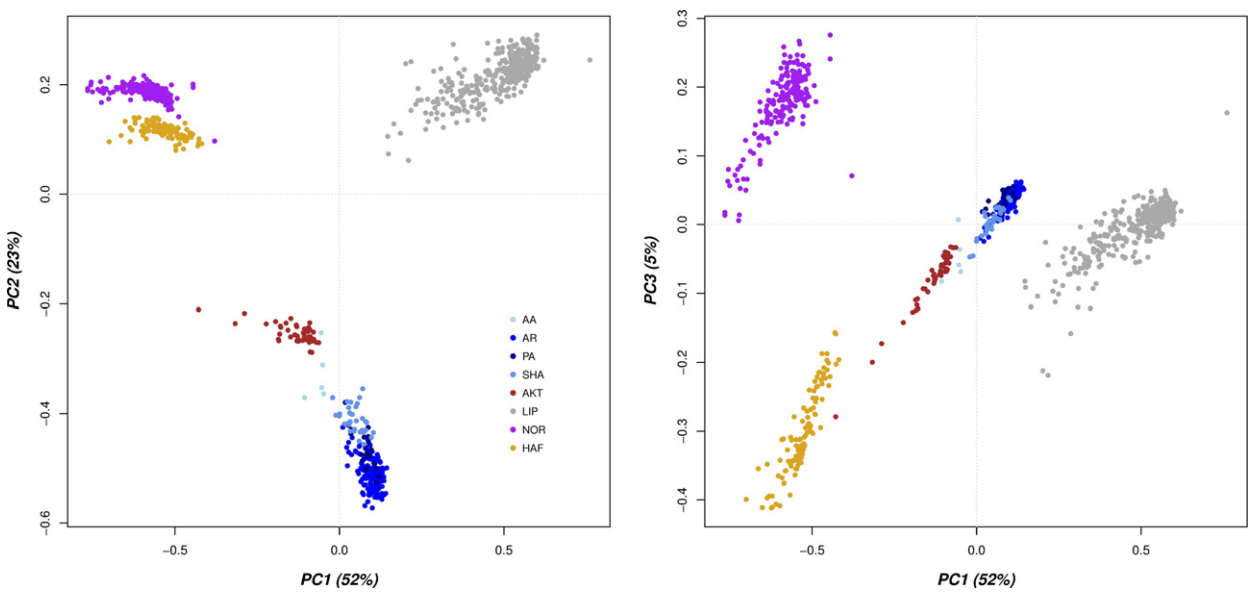

Figure 1 Population structure of the sampled horses on the first three principal components (PCs). AA, Anglo Arabian; AR, Purebred Arabian; PA, Partbred Arabian; SHA, Shagya Arabian; AKT, Achal Theke; LIP, Lipizzan; NOR, Noriker; HAF, Haflinger.

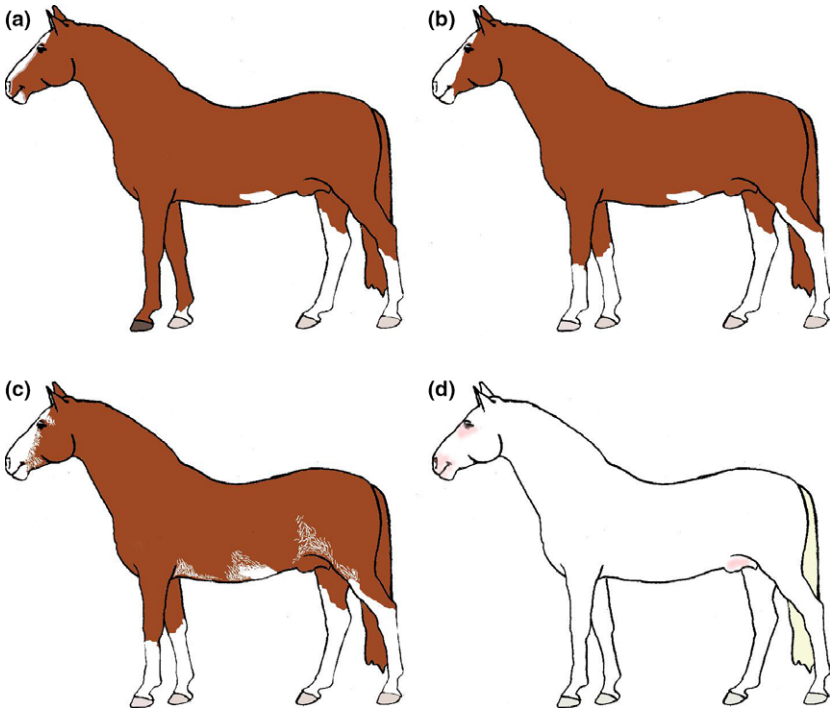

Figure 2 Examples of different sabino1 (SB1) phenotypes; according to Brooks & Bailey (2005) three criteria need to be fulfilled: (i) a blaze, (ii) two or more white feet and legs, (iii) jagged margins around white areas, and (iv) white spots or roaning in the midsection of the body. (a) SB1 horse having two white legs, one stocking, a broad blaze and a white spot on the belly. (b) SB1 horse with four white legs, broad and extended blaze and white spot on the belly. (c) The same as image (b) but also including roaning in the midsection and on the borders of white area. (d) Homozygous SB1 horse is nearly white (illustrations by T. Druml).

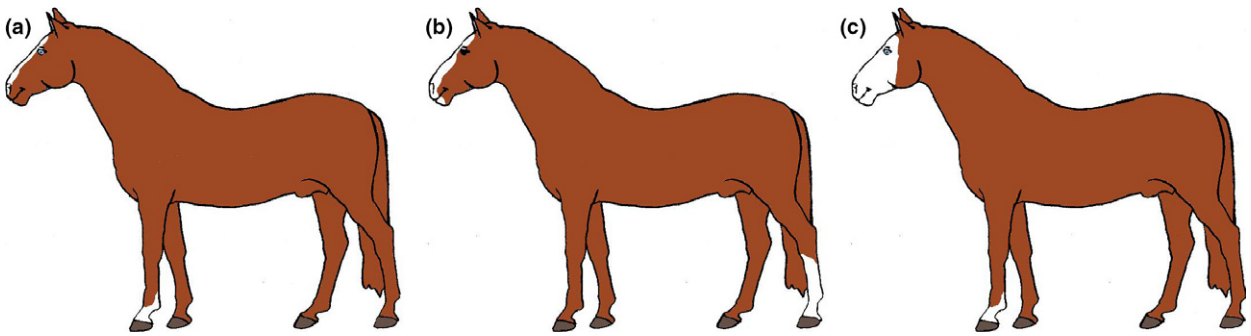

Figure 3 Examples of different Splashed White phenotypes as reported by Hauswirth *et al.* (2012). Typical is a 20% or more white face area, often in combination with blue eyes or iris heterochromia, and white leg markings. (a) Splashed white horse with a relatively small blaze (around 20% white face area), blue eyes and a stocking. (b) Splashed white horse with a broader blaze (more than 20%) and a white hind leg. (c) Splashed white horse with an extended white face area, blue eyes and a stocking (illustrations by T. Druml).

phenotype was first detected in the Tennessee Walking horse (Brooks & Bailey 2005), and it was further documented in American Miniature, Paint Horse, Azteca, Missouri Foxtrotter, Shetland Pony and Spanish Mustang. According to this study, all heterozygous *SB1*/*sb1*-carrier horses showed the sabino phenotype or were multipatterned. Homozygous *SB1*/*SB1* horses were nearly white. Furthermore it was demonstrated that, within breeds representing the classical Sabino phenotype such as Shire Horse or Clydesdale, the *SB1* allele did not segregate, a result that was also confirmed by Reissmann *et al.* (2016). Among the 18 *SB1*/*sb1* animals in our sample, four Norikers and one Lipizzan showed a mean total score of 4.2 (on a scale of 0–6) regarding the amount of white markings, whereas among the 13 Haflingers the variation ranged from a score of 2–12 (mean 6.7). The frequency of *SB1*/*sb1* horses (14.3%) was highest in the Haflinger sample. According to Brooks & Bailey (2005), we would expect the existence of a small percentage of homozygous *SB1*/*SB1* horses in this breed, which should be phenotypically white. As far as we can determine from the breeding records of 18 521 Haflinger horses from the Austrian Haflinger breeding association, no white horse has been reported within the last four decades (Druml *et al.* 2016).

From the 899 genotyped horses, 10 animals (1.11%) carried one $PAX3^{C70Y}$ allele (Table 1); homozygous animals for this locus were not observed. The $PAX3^{C70Y}$ allele occurred in only two breeds: seven Noriker horses (3.7%)

Table 2 Distribution of *SB1* and *PAX3* genotypes in three different breeds and white spotting pattern classification for sabino1 (SB1) and splashed white according to Brooks & Bailey (2005) and Hauswirth *et al.* (2012). The extent of white markings on head, legs and body was scored according to the protocol of Rieder *et al.* (2008).

		Genotype		Phenotype		Extent of			
Breed	Horse no.	*KIT*[1]	*PAX3*	SB1[2]	Splashed white[3]	White head	White legs	White body	Base color
Haflinger	3	*SB1/sb1*	+/+	Criteria not met		4	0	0	Chestnut
Haflinger	4	*SB1/sb1*	+/+	Criteria not met		4	0	0	Chestnut
Haflinger	5	*SB1/sb1*	+/+	Criteria not met		9	0	0	Chestnut
Haflinger	6	*SB1/sb1*	+/+	Criteria not met		9	0	0	Chestnut
Haflinger	7	*SB1/sb1*	+/+	Criteria not met		8	0	0	Chestnut
Haflinger	8	*SB1/sb1*	+/+	Criteria not met		10	0	0	Chestnut
Haflinger	9	*SB1/sb1*	+/+	Criteria not met		5	0	0	Chestnut
Haflinger	10	*SB1/sb1*	+/+	No phenotype					
Haflinger	11	*SB1/sb1*	+/+	Criteria not met		8	4	0	Chestnut
Haflinger	12	*SB1/sb1*	+/+	Criteria not met		3	0	0	Chestnut
Haflinger	13	*SB1/sb1*	+/+	Criteria not met		2	0	0	Chestnut
Haflinger	14	*SB1/sb1*	+/+	Criteria not met		8	0	0	Chestnut
Haflinger	15	*SB1/sb1*	+/+	Criteria not met		8	0	0	Chestnut
Haflinger	16	*SB1/sb1*	+/+	Criteria not met		5	0	0	Chestnut
Lipizzan	17	*SB1/sb1*	+/+	Criteria not met		2	0	0	Gray
Noriker	1	*SB1/sb1*	+/+	Criteria not met		1	5	0	Black
Noriker	2	*SB1/sb1*	+/+	Criteria not met		6	0	0	Chestnut
Noriker	26	*SB1/sb1*	*C70Y*/+	Criteria not met	Criteria not met	6	0	0	Chestnut
Noriker	27	*SB1/sb1*	*C70Y*/+	Criteria not met	Criteria not met	0	0	0	Black
Noriker	19	0	*C70Y*/+		Criteria not met	0	0	0	Black roan
Noriker	20	0	*C70Y*/+		Criteria not met	5	0	0	Chestnut
Noriker	21	*sb1/sb1*	*C70Y*/+		Criteria not met	0	6	0	Black
Noriker	22	*sb1/sb1*	*C70Y*/+		Criteria not met	0	3	0	Black
Noriker	18	0	*C70Y*/+		Criteria met	11	0	0	Chestnut
Lipizzan	24	*sb1/sb1*	*C70Y*/+		Criteria met	8	4	0	Gray
Lipizzan	23	*sb1/sb1*	*C70Y*/+		Criteria not met	2	3	0	Gray
Lipizzan	25	*sb1/sb1*	*C70Y*/+		Criteria not met	2	0	0	Gray

[1]The zero value accounts for non-successfully typed SNP.
[2]Brooks & Bailey 2005.
[3]Hauswirth *et al.* 2012.

and three Lipizzan horses (0.9%), which were heterozygous at this locus. Among the 10 $PAX3^{C70Y}$ allele carriers, eight did not fulfill the criteria of Hauswirth *et al.* (2012), as two horses did not show any white markings and three horses had only small head markings (total scores ranging from 2 to 6), two horses had only small leg markings (scores ranging from 3 to 6) and one horse had a small head marking and a small leg marking (scores of 3 and 6 respectively). Among the 10 $PAX3^{C70Y}$ allele carriers, one horse without any markings and the second horse with small head marking also had one copy of the *SB1* allele (Fig. 4). The $PAX3^{C70Y}$ mutation was discovered by Hauswirth *et al.* (2012) based on a Quarter horse sample. The authors traced this variant back to a Quarter horse mare born in 1987 and simultaneously demonstrated that this variation is absent in 12 additional analyzed breeds, a fact that led the authors to conclude that the $PAX3^{C70Y}$ allele occurs exclusively in Quarter horses.

Figure 4 Examples of heterozygous carrier horses for the *SB1* and/or $PAX3^{C70Y}$ alleles.

Negro *et al.* (2017) did not detect the $PAX3^{C70Y}$ allele in the Spanish breeds Pura Raza Espagnola and Menorca purebred. Based upon our data, we were able to show that this variant segregates at low frequencies in the Noriker (3.7% heterozygotes) and Lipizzan (0.9% heterozygotes). Eight of the 10 genotyped $PAX3^{C70Y}$-carrier horses did not show a typical splashed white phenotype with extended head markings, combined with blue eyes and leg markings. Only one chestnut Noriker stallion and one gray Lipizzan stallion had a white blaze covering about 20% of the head area, according to Hauswirth *et al.* (2012).

In this study, we demonstrated that the $PAX3^{C70Y}$ allele segregates at low frequencies in the Noriker (seven out of 189) and Lippizan (one out of 329) breeds. According to the association between genotype and phenotype following Hauswirth *et al.* (2012), who outlined a white face area of 20% or more as the primary phenotypic criterion, eight out of 10 horses did not exhibit a splashed white phenotype. For the *SB1* allele, which was present in three breeds and occurred in 14.3% of Haflinger horses, we could not report a corresponding sabino phenotype in our sample collection. In the Noriker, Haflinger and Lipizzan breeds, coat color represents a specific breeding objective, and selection favors horses with minimal amounts of white markings in the long term. Due to this selection pressure, phenotypes characterized by a larger extent of depigmentation shift to a low frequency in these populations, whereas in Quarter horses and/or Paint horses comparable phenotypes may segregate randomly. The expression of white markings/extent of depigmentation commonly are considered a complex trait influenced by multiple gene interactions and environmental factors (Hauswirth *et al.*2013).

Based upon our results, we conclude that the consistency between white spotting/depigmentation phenotypes and their reported causal variants might be affected by factors such as directional selection, population structure and gene interactions. Furthermore, we could also demonstrate that the analysis of large datasets can reveal new insights into the genetic association of white spotting patterns.

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