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<sup>C70Y</sup>), including a total of 899 horses originating from eight different breeds (Achal Theke, Purebred Arabian, Purbred Arabian, Anglo-Arabian, Shagya Arab, Lipizzan and Noriker). Based on the data we collected we were able to demonstrate that, besides Quarter horses, the PAX3<sup>C70Y</sup> allele is also present in Noriker (seven out of 189) and Lipizzan (three out of 329) horses. The SBI 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 SBI- and PAX3<sup>C70Y</sup>-carrier horses for their characteristic white spotting patterns. None of the SBI/1 or SBI-1 carrier horses met the criteria defining the Sabino1 pattern according to current applied protocols. From 10 heterozygous PAX3<sup>C70Y</sup>-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; SBI, SNP KI16:1037A) is located in the KIT gene, and it is causative for the sabino1 (SBI) phenotype (Brooks & Bailey 2005), whereas SNP AX-103512392 (ECA6:11429753C>T; SNP PAX3<sup>C70Y</sup>) in the PAX3 gene is associated with the splashed white phenotype (Hauswirth et al. 2012, 2013).

The genotype distributions of SBI 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.
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 PAX3C70Y-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 PAX3C70Y-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 PAX3C70Y 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

| Breed           | n  | SB1/sb1 | PAX3C70Y/PAX3+ | Not typed SB1 | Not typed PAX3C70Y |
|-----------------|----|---------|----------------|---------------|-------------------|
| 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                |

**Table 1** Distribution of SB1- and PAX3C70Y-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.

**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.
phenotype was first detected in the Tennessee Walking horse (Brooks & Bailey 2005), and it was further documented in American Miniature, Paint Horse, Azteca, Missouri Fox Trotter, Shetland Pony and Spanish Mustang. According to this study, all heterozygous SB1/sb1-carrier horses showed the sabino phenotype or were multi-patterned. 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 PAX3C70Y allele (Table 1); homozygous animals for this locus were not observed. The PAX3C70Y allele occurred in only two breeds: seven Noriker horses (3.7%)
and three Lipizzan horses (0.9%), which were heterozygous at this locus. Among the 10 PAX3<sup>C70Y</sup> 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<sup>C70Y</sup> 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<sup>C70Y</sup> 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<sup>C70Y</sup> allele occurs exclusively in Quarter horses.

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).

| Breed   | Horse no. | Genotype       | Phenotype          | Extent of white markings | Base color |
|---------|-----------|----------------|--------------------|--------------------------|------------|
| Haflinger | 3        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 4 0 0                    | Chestnut   |
| Haflinger | 4        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 4 0 0                    | Chestnut   |
| Haflinger | 5        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 9 0 0                    | Chestnut   |
| Haflinger | 6        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 9 0 0                    | Chestnut   |
| Haflinger | 7        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 8 0 0                    | Chestnut   |
| Haflinger | 8        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 10 0 0                   | Chestnut   |
| Haflinger | 9        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 5 0 0                    | Chestnut   |
| Haflinger | 10       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | No phenotype       |                          |            |
| Haflinger | 11       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 8 4 0                    | Chestnut   |
| Haflinger | 12       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 3 0 0                    | Chestnut   |
| Haflinger | 13       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 2 0 0                    | Chestnut   |
| Haflinger | 14       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 8 0 0                    | Chestnut   |
| Haflinger | 15       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 8 0 0                    | Chestnut   |
| Haflinger | 16       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 5 0 0                    | Chestnut   |
| Lipizzan | 17       | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 2 0 0                    | Gray       |
| Noriker  | 1        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 1 5 0                    | Black      |
| Noriker  | 2        | SB1<sup>1</sup> sb1<sup>2</sup> +/+ | Criteria not met   | 6 0 0                    | Chestnut   |
| Noriker  | 26       | SB1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria not met   | 6 0 0                    | Chestnut   |
| Noriker  | 27       | SB1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | 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<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria not met   | 0 6 0                    | Black      |
| Noriker  | 22       | sb1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria not met   | 0 3 0                    | Black      |
| Noriker  | 18       | 0 C70Y/+         | Criteria met        | 11 0 0                   | Chestnut   |
| Lipizzan | 24       | sb1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria met        | 8 4 0                    | Gray       |
| Lipizzan | 23       | sb1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria not met   | 2 3 0                    | Gray       |
| Lipizzan | 25       | sb1<sup>1</sup> sb1<sup>2</sup> C70Y/+ | Criteria not met   | 2 0 0                    | Gray       |

Table notes:
1 The zero value accounts for non-successfully typed SNP.
2 Brooks & Bailey 2005.
3 Hauswirth et al. 2012.

Figure 4 Examples of heterozygous carrier horses for the SB1 and/or PAX3<sup>C70Y</sup> alleles.
Negro et al. (2017) did not detect the PAX3<sup>3C70Y</sup> allele in the Spanish breeds Pura Raza Española and Menorca purebreds. 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<sup>3C70Y</sup>-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<sup>3C70Y</sup> allele segregates at low frequencies in the Noriker (seven out of 189) and Lipizzan (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.

References

Brooks A.S. & Bailey E. (2005) Exon skipping in the KIT gene causes a sabino spotting pattern in horses. Mammalian Genome 16, 893–902.

Brooks S.A., Lear T.L., Adelson D.L. & Bailey E. (2007) A chromosome inversion near the KIT gene and the tobiano spotting pattern in horses. Cytogenetic and Genome Research 119, 225–30.

Druml T., Sauer K., Elsbacher J., Grilz-Seger G. & Brem G. (2016) Analyse des Genpools, der genetischen Diversität und der Inzuchtverhältnisse der österreichischen Haflingerpopulation. Züchtungskunde 88, 379–94.

Hauswirth R., Haase B., Blatter M. et al. (2012) Mutations in MITF and PAX3 cause ‘splashed white’ and other white spotting phenotypes in horses. PLoS Genetics 8, e1002653.

Hauswirth R., Jude R., Haase B. et al. (2013) Novel variants in the KIT and PAX3 genes in horses with white-spotted coat colour phenotypes. Animal Genetics, 44, 763–5.

Metallinos D.L., Bowling A.T. & Rine J. (1998) A missense mutation in the endothelin-B receptor gene is associated with Lethal White Foal Syndrome: an equine version of Hirschsprung disease. Mammalian Genome 9, 426–31.

Negro S., Imsland F., Valera M., Molina A., Sole M. & Andersson L. (2017) Association analysis of KIT, MITF, and PAX3 variants with white markings in Spanish horses. Animal Genetics 48, 349–52.

Reissmann M., Musa L., Zkizadech S. & Ludwig A. (2016) Distribution of coat-color-associated alleles in the domestic horse population and Przewalski’s horse. Journal of Applied Genetics 57, 519–25.

Rieder S., Hagger C., Obexer-Ruff G., Leeb T. & Poncet P.A. (2008) Genetic analysis of white facial and leg markings in the Swiss Franches-Montagnes Horse Breed. Journal of Heredity 99, 130–6.

Santschi E.M., Purdy A.K., Valser S.J., Vrotsos P.D., Kaese H. & Mickelson J.R. (1998) Endothelin receptor B polymorphism associated with lethal white foal syndrome in horses. Mammalian Genome 9, 306–9.

Sponenberg P. (2009) Equine Color Genetics, 3rd edn. Wiley-Blackwell, Ames, IA.

Yang G.C., Croaker D., Zhang A.L., Manglick P., Cartmill T. & Cass D. (1998) A dinucleotide mutation in the endothelin-B receptor gene is associated with lethal white foal syndrome (LWFS) – a horse variant of Hirschsprung-disease (HSCR). Human Molecular Genetics 7, 1047–52.