Short Communication

Whole genome sequencing reveals a large deletion in the *MITF* gene in horses with white spotted coat colour and increased risk of deafness

Jan Henkel[1,2], Christa Lafayette[3], Samantha A. Brooks[4], Katie Martin[3], Laura Patterson-Rosa[4], Deborah Cook[3], Vidhya Jagannathan[1,2], Tosso Leeb[1,2]

[1] Institute of Genetics, Vetsuisse Faculty, University of Bern, 3001 Bern, Switzerland

[2] DermFocus, University of Bern, 3001 Bern, Switzerland

[3] Etalon Inc., Menlo Park, CA 94025, USA

[4] Department of Animal Sciences, University of Florida, Gainesville, FL 32611-0910, USA

Running title: Equine *MITF* deletion

Address for correspondence

Tosso Leeb
Institute of Genetics
Vetsuisse Faculty
University of Bern
Bremgartenstrasse 109a
3001 Bern
Switzerland

Phone: +41-31-6312326
Fax: +41-31-6312640
E-mail: Tosso.Leeb@vetsuisse.unibe.ch

Summary

White spotting phenotypes in horses are highly valued in some breeds. They are quite variable and may range from the common white markings up to completely white horses. *EDNRB*, *KIT*, *MITF*, *PAX3,* and *TRPM1* represent known candidate genes for white spotting phenotypes in horses. For the present study, we investigated an American Paint Horse family segregating a phenotype involving white spotting and blue eyes. Six of eight horses with the white-spotting phenotype were deaf. We obtained whole genome sequence data from an affected horse and specifically searched for structural variants in the known candidate genes. This analysis revealed a heterozygous ~63 kb deletion spanning exons 6-9 of the *MITF* gene (chr16:21,503,211_21,566,617). We confirmed the breakpoints of the deletion by PCR and Sanger sequencing. PCR-based genotyping revealed that all 8 available affected horses from the family carried the deletion. The finding of an *MITF* variant fits well to the syndromic phenotype involving both depigmentation and an increased risk for deafness and corresponds to human Waardenburg syndrome type 2A (WS2A). Our findings will enable more precise genetic testing for depigmentation phenotypes in horses.

Keywords: Equus caballus; melanocyte; pigmentation; coat colour; splashed white; structural variant; heterogeneity

White spotting in horses and other mammals may result from an altered embryonic development of the neural crest melanocyte lineage and a lack of mature melanocytes in the unpigmented skin areas ("leucism"). Candidate genes for such phenotypes in the horse include *EDNRB*, *KIT*, *MITF, PAX3*, and *TRPM1* (Thomas & Erickson 2008; OMIA 000629, 000209, 001688, 000214, 001341).

The horse currently represents the species with the largest number of molecularly defined white spotting alleles among domesticated animals. A missense variant in the *EDNRB* gene causes the frame overo white spotting pattern or lethal white foal syndrome, if present in heterozygous or homozygous state, respectively (Santschi *et al.* 1998). Variants in *MITF* and *PAX3* cause the so-called splashed white phenotype, which closely resembles human Waardenburg syndrome (Pingault et al. 2010; Hauswirth *et al.* 2012; Hauswirth *et al.* 2013; Dürig et al. 2017). A variant in the equine *TRPM1* gene causes the so-called leopard complex spotting (Bellone *et al.* 2013). Furthermore, according to our knowledge, 29 different functional alleles at the equine *KIT* gene have been described so far. These include the alleles for sabino-1 and tobiano spotting, and an allelic series termed *W1* - *W27* (Brooks & Bailey 2005; Brooks *et al.* 2007; Haase *et al.* 2007; Haase *et al.* 2008; Haase *et al.* 2009; Holl *et al.* 2010; Haase *et al.* 2011; Hauswirth *et al.* 2013; Haase *et al.* 2016; Capomaccio et al. 2017; Holl et al. 2017; Hoban et al. 2018; Table S1).

We studied a family of American Paint horses segregating for a white spotting phenotype resembling the splashed white pattern that could not be explained by any of the previously published white spotting alleles (Figure 1). We had access to samples from one affected stallion and eight of his daughters (Figure S1). Seven of the daughters also had white spotting patterns that could not be explained by their genotypes at the known white spotting variants. One of the daughters had a regular frame overo phenotype, which was inherited from the dam. The coat colour phenotypes in this family were quite variable as all horses carried additional known white-spotting alleles. All affected horses had white faces. The amount of body pigmentation was variable and one of the studied horses was even completely white. At least six of the affected horses had blue eyes. The quality of our photos of the two remaining affected horses did not allow unambiguous determination of their eye colour. Six out of eight horses with unexplained white spotting phenotypes were deaf.

We prepared an Illumina TruSeq PCR free genomic DNA library and re-sequenced the genome of one affected horse at 19x coverage using 2 x 150 bp reads on an Illumina NovaSeq 6000 instrument. Sequencing and read mapping to the EquCab 3 reference assembly was performed as previously described (Jagannathan et al. 2018). Data were deposited at the European Nucleotide Archive (study accession PRJEB14779, sample SAMEA4822838). This analysis failed to reveal any single nucleotide or small indel variants in the candidate genes *EDNRB*, *KIT*, *MITF, PAX3*, and *TRPM1*. In order to search for large structural variants we

visually inspected the read alignments for these genes in the Integrative Genomics Viewer (IGV; Robinson *et al.* 2011).

The analysis revealed a large heterozygous deletion spanning the last four exons of the *MITF* gene corresponding to exons 6 – 9 of the *MITF-M* transcript isoform (Figure 2). Specifically, 63,407 bp were missing (chr16:21,503,211_21,566,617). There is currently no RefSeq entry for the equine melanocyte-specific MITF-M transcript isoform available. We therefore used the accessions JN896378.1 (mRNA) and AFH66983.1 (protein) to analyse the putative effects of the deletion on the transcript and protein. Assuming regular splicing and polyadenylation of the remaining exons, the deletion removes 706 nucleotides (56%) from the open reading frame (JN896378.1:c.555_1260del). This includes the codons required to encode the functionally important bHLH-Zip domain required for DNA binding. We did not find any other obvious structural variants in the *EDNRB*, *KIT, PAX3*, and *TRPM1* genes.

We designed primers flanking the deletion (TTAGCAATAAGCCACTGGTC, TCATTGTGTCCAGGCTGCTG) and confirmed the breakpoints of the deletion by PCR and Sanger sequencing (Figure 2). A PCR with these primers and ATG360 polymerase (ThermoFisher) was used as diagnostic assay to genotype additional horses for the deletion. All eight affected horses from this family, from which genomic DNA was available, carried the deletion. The phenotype resembling the splashed white pattern with an extremely large blaze on the head and frequently associated with blue eyes is similar to the phenotypes of horses with other mutant *MITF* alleles. Most of the horses carrying this deletion were deaf. This confirms earlier observations that horses with a lack of functional *MITF* have an increased risk for deafness (Hauswirth et al. 2012). All deaf horses carried additional white spotting alleles, which may have exacerbated the functional impact of the *MITF* deletion.

In conclusion, our study revealed a large structural variant at the equine *MITF* gene, which most likely causes a splashed white depigmentation phenotype and predisposes to deafness.

Acknowledgements

The authors would like to thank all involved horse owners for donating samples and pictures and for sharing pedigree information of their horses. The authors also wish to thank Nathalie Besuchet, Muriel Fragnière, and Sabrina Schenk for expert technical assistance. The Next Generation Sequencing Platform of the University of Bern is acknowledged for performing the whole genome re-sequencing experiments and the Interfaculty Bioinformatics Unit of the University of Bern for providing high performance computing infrastructure. This study was supported by grant 31003A_172964 from the Swiss National Science Foundation. Conflicts of interest: Christa Lafayette, Katie Martin, and Deborah Cook are affiliated with a genetic testing laboratory offering tests for white spotting in horses.

References

Bellone RR, Holl H, Setaluri V, Devi S, Maddodi N, Archer S, Sandmeyer L, Ludwig A, Foerster D, Pruvost M, Reissmann M, Bortfeldt R, Adelson DL, Lim SL, Nelson J, Haase B, Engensteiner M, Leeb T, Forsyth G, Mienaltowski MJ, Mahadevan P, Hofreiter M, Paijmans JL, Gonzalez-Fortes G, Grahn B, Brooks SA (2013) Evidence for a retroviral insertion in *TRPM1* as the cause of congenital stationary night blindness and leopard complex spotting in the horse. *PLoS One* **8**, e78280.

Brooks S. A. & 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 & Genome Research* **119**, 225-30.

Capomaccio S., Milanesi M., Nocelli C., Giontella A., Verini-Supplizi A., Branca M., Silvestrelli M. & Cappelli K. (2017) Splicing site disruption in the *KIT* gene as strong candidate for white dominant phenotype in an Italian Trotter. *Animal Genetics* **48**, 727-8.

Dürig N., Jude R., Jagannathan V. & Leeb T. (2017) A novel *MITF* variant in a white American Standardbred foal. *Animal Genetics* **48**, 123-4.

Haase B., Brooks S.A., Schlumbaum A., Azor P.J., Bailey E., Alaeddine F., Mevissen M., Burger D., Poncet P.-A., Rieder S. & Leeb T. (2007) Allelic heterogeneity at the equine *KIT* locus in dominant white (*W*) horses. *PLoS Genet* **3**, e195.

Haase B., Jude R., Brooks S.A. & Leeb T. (2008) An equine chromosome 3 inversion is associated with the tobiano spotting pattern in German horse breeds. *Animal Genetics* **39**, 306-9.

Haase B., Brooks S.A., Tozaki T., Burger D., Poncet P.-A., Rieder S., Hasegawa T., Penedo C. & Leeb T. (2009) Seven novel *KIT* mutations in horses with white coat colour phenotypes. *Animal Genetics* **40**, 623-9.

Haase B., Rieder S., Tozaki T., Hasegawa T., Penedo M.C., Jude R. & Leeb T. (2011) Five novel *KIT* mutations in horses with white coat colour phenotypes. *Animal Genetics* **42**, 337-9.

Hauswirth R., Haase B., Blatter M., Brooks S.A., Burger D., Drögemüller C., Gerber V., Henke D., Janda J., Jude R., Magdesian K.G., Matthews J.M., Poncet P.-A., Svansson V., Tozaki T., Wilkinson-White L., Penedo M.C., Rieder S. & Leeb T. (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., Bellone R.R., Archer S., Holl H., Brooks S.A., Tozaki T., Penedo M.C.T., Rieder S. & Leeb T. (2013) Novel variants in the *KIT* and *PAX3* genes in horses with white-spotted coat colour phenotypes. *Animal Genetics* **44**, 763-5.

Hoban R., Castle K., Hamilton N. & Haase B. (2018) Novel *KIT* variants for dominant white in the Australian horse population. *Animal Genetics* **49**, 99-100.

Holl H., Brooks S. & Bailey E. (2010) *De novo* mutation of *KIT* discovered as a result of a non-hereditary white coat colour pattern *Animal Genetics* **41 Suppl 2**, 196-198.

Jagannathan V., Gerber V., Rieder S., Tetens J., Thaller G., Drögemüller C. & Leeb T. (2018) Comprehensive characterization of horse genome variation by whole genome sequencing of 88 horses. *Animal Genetics*, manuscript submitted for publication.

Pingault V., Ente D., Dastot-Le Moal F., Goossens M., Marlin S. & Bondurand N. (2010) Review and update of mutations causing Waardenburg syndrome. *Human Mutation* **31**, 391-406.

Robinson J.T., Thorvaldsdóttir H., Winckler W., Guttman M., Lander E.S., Getz G. & Mesirov J.P. (2011) Integrative genomics viewer. *Nature Biotechnology* **29**, 24-6.

Santschi E.M., Purdy A.K., Valberg 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.

Thomas A.J. & Erickson C.A. (2008) The making of a melanocyte: the specification of melanoblasts from the neural crest. *Pigment Cell & Melanoma Research* **21**, 598-610.

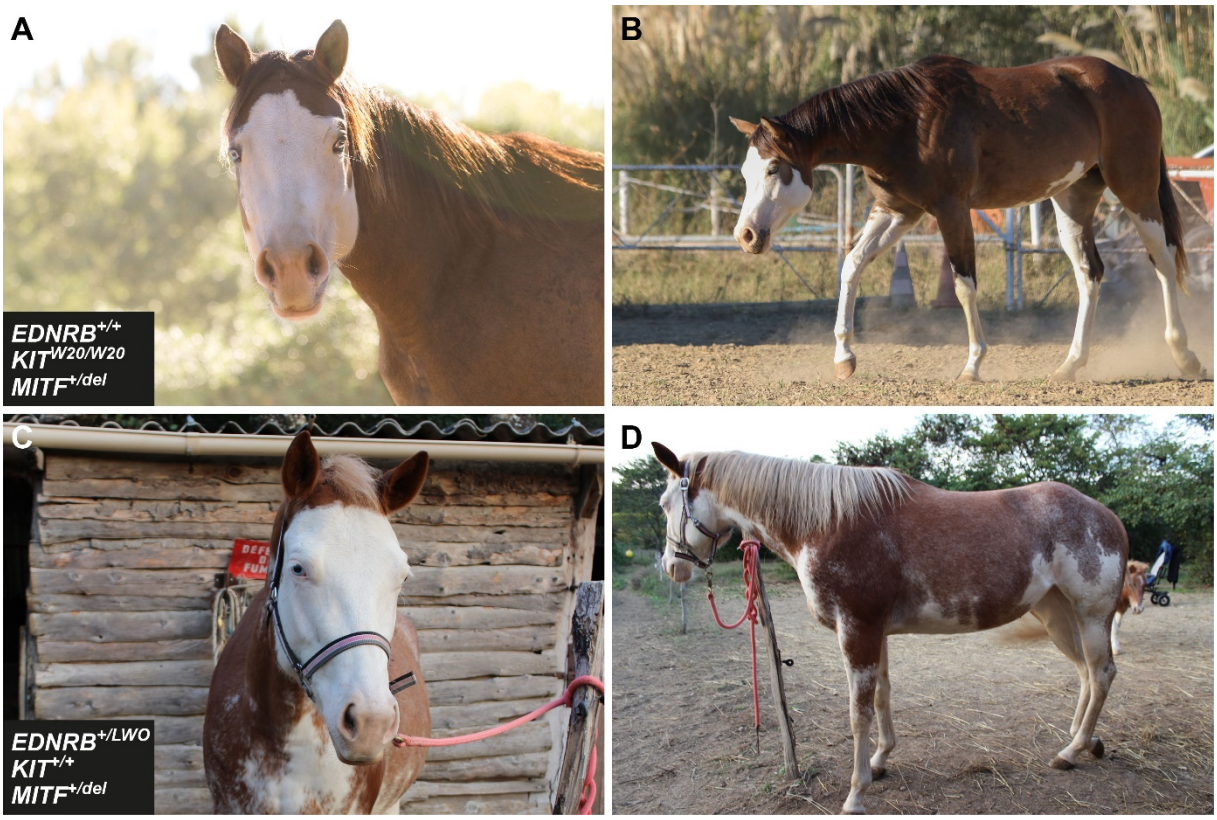

Figure 1 White spotting phenotype resembling the splashed white pattern. **(A, B)** This horse had light blue eyes, a white face, white legs and a small white belly spot. The horse was deaf. **(C, D)** This horse had a more pronounced white spotting phenotype compared to its full sibling shown in (A) and (B). It also had light blue eyes and was deaf. Genotypes at important white spotting loci are indicated. *MITF*^{del} designates the new *MITF* allele identified in this study.

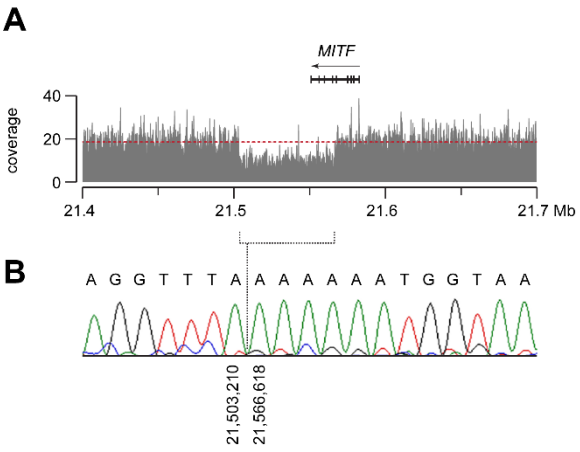

Figure 2 Details of the *MITF* deletion. **(A)** A coverage plot of the whole genome sequence data indicates a heterozygous ~63 kb deletion comprising exons 6-9 of the *MITF* gene. **(B)** Sanger sequencing of a PCR product from the deletion allele precisely defined the breakpoints of the deletion (Chr16:21,503,211_21,566,617del, EquCab 3 assembly).

Supplementary Material:

Figure S1 Pedigree of the studied horses.

Table S1 Compilation of variants in candidate genes for white spotting.