Short Communication

A novel *KIT* deletion variant in a German Riding Pony with white spotting coat colour phenotype

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Running title: *KIT* deletion in a German Riding Pony

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Summary

White spotting phenotypes in horses may be caused by developmental alterations impairing melanoblast differentiation, survival, migration and/or proliferation. Candidate genes for white spotting phenotypes in horses include *EDNRB*, *KIT*, *MITF*, *PAX3,* and *TRPM1*. We investigated a German Riding Pony with a sabino-like phenotype involving extensive white spots on the body together with large white markings on the head and almost completely white legs. We obtained whole genome sequence data from this horse. The analysis revealed a heterozygous 1273 bp deletion spanning parts of intron 2 and exon 3 of the equine *KIT* gene (Chr3:79,579,925_79,581,197). We confirmed the breakpoints of the deletion by PCR and Sanger sequencing. The knowledge on the functional impact of similar *KIT* variants in horses and other species suggests that this deletion represents a plausible candidate causative variant for the white spotting phenotype. We propose the designation *W28* for the mutant allele.

Keywords: *Equus caballus*; melanocyte; skin; pigmentation; coat colour; structural variant; whole genome sequencing

Melanoblast development, migration, survival and proliferation as well as the full differentiation into mature melanocytes are intricately regulated processes. White spotting phenotypes are the result of an altered embryonic development of the neural crest-derived melanocyte lineage (Thomas & Erickson, 2008). Candidate genes for such phenotypes in horses include *EDNRB*, *KIT*, *MITF*, *PAX3,* and *TRPM1* (OMIA 000629, 000209, 001688, 000214, 001341).

We investigated a one-year-old German Riding Pony with a sabino-like white spotting phenotype. The pony had extensive white body spots, and an almost completely white head and legs (Figure 1). Neither the father nor the mother showed a similar phenotype suggesting a potential *de novo* mutation event, which had given rise to a dominant white spotting allele. The pony was tested negative for 28 known white-spotting alleles (*SB-1*, *W1-W27*) in the *KIT* gene (Brooks & Bailey, 2005; Haase et al. 2007; Haase et al. 2009; Haase et al. 2010; Holl et al. 2010; Hauswirth et al. 2013; Haase et al. 2015; Dürig et al. 2017; Holl et al. 2017; Capomaccio et al. 2017; Hoban et al. 2018) as well as for the splashed white alleles (SW1-3) in the *MITF* and *PAX3* genes (Hauswirth et.al. 2012; Table S1).

We collected an EDTA blood sample from the pony and extracted genomic DNA using the Maxwell RSC Blood DNA Kit and a Maxwell RSC instrument. An Illumina TruSeq PCR-free DNA library with 350 bp insert size was prepared. We collected 240,866,812 million 2 x 150 bp read pairs on a NovaSeq 6000 instrument (29x coverage). Sequencing and read mapping to the EquCab 3 reference assembly was performed as previously described (Jagannathan et al. 2019). The sequence data were deposited under study accession PRJEB14779 and sample accession SAMEA5600769 at the European Nucleotide Archive. This analysis failed to reveal any single nucleotide or small indel variants in the functional candidate genes.

Using the Integrative Genomics Viewer (Robinson et al. 2011), we visually inspected the short read alignments for structural variants in the regions of functional candidate genes *EDNRB*, *KIT*, *MITF*, *PAX3,* and *TRPM1*. A 1273 bp deletion spanning parts of intron 2 and exon 3 of the *KIT* gene was detected, Chr3:79,579,925_79,581,197del (Figure 2).

A primer pair for the amplification of the mutant allele with the deletion was designed. Genomic DNA from the pony was amplified using forward primer ATCAGCGACGAACGTCAAGT, reverse primer GTGTCCTTTCCTTGGTGGGT and AmpliTaq Gold 360 Master Mix. The amplicon was treated with shrimp alkaline phosphatase and exonuclease I and sequenced on an ABI 3730 capillary sequencer. The Sanger sequencing data confirmed the breakpoints of the deletion (Figure 2). The deletion allele was not found in 88 genome sequences from genetically diverse horses (Table S2, Jagannathan et al. 2019).

Exon 3 of the equine *KIT* gene spans 282 bp, of which 75 bp are deleted in the mutant allele. As the deletion also removes the 3'-splice site of intron 2, splicing of the *KIT* mRNA most

likely is severely altered. Unfortunately, we had no suitable samples for RNA isolation, which would be required to investigate the functional consequences of the genomic deletion on the transcript level. A comparable deletion in the equine *KIT* gene spanning exons 10-13 gives rise to the *W22* allele that was identified in Thoroughbred Horses (Dürig et al. 2017). Heterozygous $KIT^{W22/+}$ horses have a similar white-spotting phenotype as the German Riding Pony described in this report.

In conclusion, based on the extensive knowledge on the functional effect of *KIT* variants, it is highly plausible that the reported deletion spanning parts of intron 2 and exon 3 causes the white spotting phenotype in the investigated German Riding Pony. In line with previously named equine *KIT* alleles, we propose to designate the newly identified allele *W28*.

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Figure 1. White spotting phenotype in the German Riding Pony. The distribution of the unpigmented skin and hairs resembles the sabino spotting pattern.

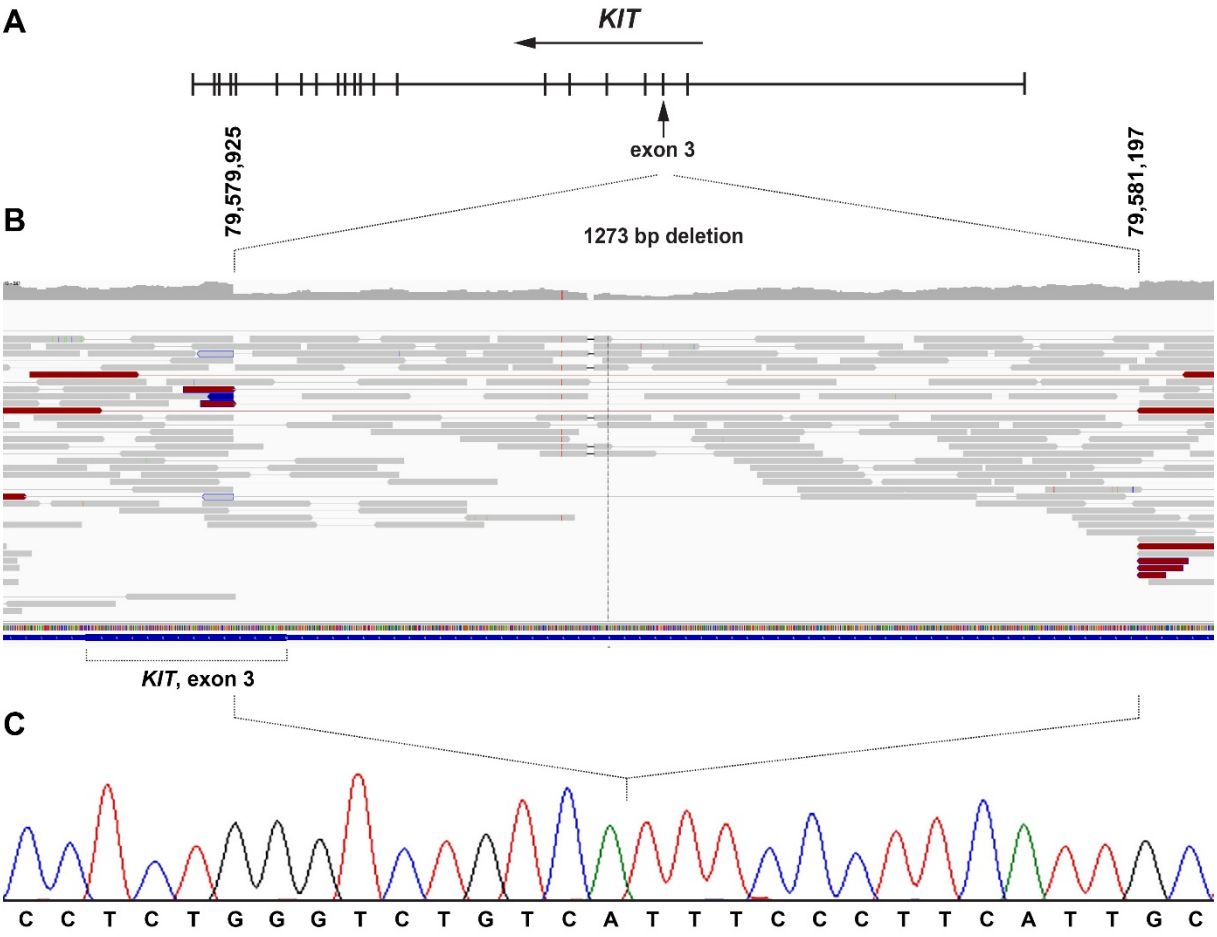

Figure 2. Details of the *KIT* deletion. **(A)** A schematic illustration of the *KIT* gene with its 21 exons. **(B)** IGV screenshot of the illumina short reads in the region of the heterozygous 1273 bp deletion spanning a part of intron 2 and exon 3 of the *KIT* gene. Note the drop in coverage and the truncated read-alignments at the deletion breakpoints. Reads coloured in red indicate read-pairs that map farther apart on the reference genome than the average insert size of the sequencing library. Such read-pairs are indicative for deletions in the sequenced sample. **(C)** Sanger sequence of a PCR amplicon obtained with primers flanking the deletion breakpoints. The deletion can be designated as Chr3:79,579,925_79,581,197del with regards to the EquCab 3 assembly.

Supplementary Material

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

Table S2. Accessions of 88 horse genomes and their genotypes at the Chr3:79,579,925_79,581,197del variant.