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# Incidence of Mutation for Silver Coat Color in Black Forest Horses

**Short Communication**

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## ABSTRACT

Black Forest horses are typically chestnut colored with flaxen mane and tail. However, as their coat color can get very dark, they are sometimes also indicated as silver, a color depending on a black base color. To analyse if the silver allele is present in the Black Forest horse population, we genotyped 250 horses of this breed for formerly reported coat color mutations within *MC1R* and *SILV*. As a result, all Black Forest horses of this study were chestnut colored due to *MC1R* genotyping. Surprisingly, the silver mutation (*SILV*-1852C>T) occurred with a prevalence of 0.8%. As chestnut coat color is predominant in this breed, the silver mutation is expected to have very few to no phenotypic appearance in Black Forest horses and presumably results from incrossing of another breed.

**KEY WORDS** Black Forest horses, *MC1R*, *SILV*, silver mutation.

## INTRODUCTION

Black Forest horses typically show reddish to very dark coat color with flaxen to white mane and tail. In the population of this breed, 97.8% of all mares are registered as chestnut colored (Sambraus, 1999), while other coat colors such as bay and gray are rare.

Chestnut coat color depends on a homozygous *MC1R*-248C > T transition, that causes an amino acid exchange from Serine to Phenylalanine (S83F) (Marklund *et al.* 1996). Some chestnuts carry an additional *MC1R*-250G > A mutation (Wagner and Reissmann, 2000). This latter mutation was only found compound with *MC1R*-248T.

Though it causes an amino acid exchange from Aspartic acid to Asparagine (D84N), an effect on the phenotype was not observed. The flaxen mutation has not yet been identified.

It is inherited recessively (Reissmann *et al.* 2007) and causes a dilution of the pheomelanin in chestnut longhair to a flaxen or almost white color.

However, on eumelanin pigment of black horses it does not have any effect. On the other hand, the dominant silver mutation at *SILV*-1852C > T (Brunberg *et al.* 2006; Reissmann *et al.* 2007), which changes the amino acid Arginine to Cysteine (R618C), dilutes eumelanin pigment of mane and tail in black or bay horses to white, but has no effect on pheomelanin.

However, there are still persistent suggestions that some of the Black Forest horses with a very dark coat color might be black ones carrying the silver mutation instead of being dark chestnuts with flaxen.

The objective of this study was to survey this hypothesis. For this purpose, we genotyped 250 Black Forest horses for their status at the *MC1R* mutations to assure their base color. Simultaneously, their status at *SILV*-1852C > T was determined. Genealogically, Black Forest horses belong to the Noriker horse group (Aberle *et al.* 2004) and selected Noriker stallions were approved for breeding with them. Therefore, 92 Noriker horses were genotyped for the three mutations as well.

## MATERIALS AND METHODS

We used samples of 250 Black Forest horses and 92 Noriker horses in this study. The coat colors of the Black Forest horses ranged from red to a very dark coat color, which appeared black. All these horses showed flaxen to white manes and tails. DNA was extracted from EDTA-blood samples using an in-house desalting method. Primers for amplification of the *MC1R*-248C > T, *MC1R*-250G > A and *SILV*-1852C > T mutations were designed using PRIMER3 software (http://frodo.wi.mit.edu/primer3). PCRs were carried out according to the standard protocol advised by the manufacturer of the *Taq* DNA polymerase (Qbiogene, Heidelberg, Germany). A 540 bp product within *MC1R* was amplified using the primers:

F: 'TCCTGCTTCCTAGAGGGACT'.
R: 'GGACACTAACCACCCAGATG'.

This product contained *MC1R*-248C > T as well as *MC1R*-250G > A and was enzymatically digested using *TaqI* and *Hpy188I* separately. The digestion scheme of both enzymes for each individual provides the genotype of both mutations. For genotyping of the silver locus (*SILV*-1852C>T) we used PCR primers:

F: 'TGAACCCTGTTTGTGAGGA'.
R: 'GTGGTACACCTCCCTCATTT'.

This resulting is in a 525 bp product. Enzymatic digestion was performed using the *HhaI* restriction enzyme. In horses positive for the silver mutation we sequenced a PCR product of the same primers for validation. Sequencing was performed using the ABI Big Dye Terminator v3.1 sequencing kit (Life Technologies, Darmstadt, Germany) and an automated ABI 3500 capillary sequencer (Life Technologies). Relationship analyses between individual horses were performed using the Opti-Mate software, version 3.88.

## RESULTS AND DISCUSSION

All 250 Black Forest horses analysed showed the homozygous *MC1R*-248T genotype encoding for chestnut coat color. Of these horses, 34.3% were heterozygous at the second locus within *MC1R* (*MC1R*-250G > A) and 2.5% were homozygous for *MC1R*-250A. At the silver locus (*SILV*-1852C>T), no Black Forest horse was homozygous for *SILV*-1852T, but two (0.8%) were heterozygous for the mutation. These two horses showed a relationship of 12.0% with each other. They had 12 common ancestors, of which two were females and ten were males, including one Noriker, one Ardenner, and one Freiberger stallion. Except for the Freiberger stallion (A) and one of the mares (B), these ancestors prevail in pedigrees of most Black Forest horses.

The Freiberger stallion (A) occurs in the pedigrees of the two horses three and seven generations ago, respectively. The mare (B) is the maternal grandmother and the maternal great-grandmother, respectively. Regarding the pedigree of mare (B) herself, the maternal grandmother is unknown.

None of the Noriker horses analysed in this study carried the silver mutation. Of the Noriker horses, 29.3% showed the homozygous *MC1R*-248T genotype for chestnut color and 70.7% showed the genotype for black with 33.7% heterozygous and 37.0% homozygous for *MC1R*-248C. At *MC1R*-250G > A, all Noriker horses were homozygous for the wildtype (*MC1R*-250G).

All Black Forest horses showed the genotype for chestnut coat color (homozygous for *MC1R*-248T). This is in agreement with Sambraus (1999), who reported that 97.8% of all Black Forest mares were chestnut colored. Two horses were heterozygous at *SILV*-1852C > T and therefore carried a genotype for silver. However, the silver mutation has no phenotypic effect on chestnuts (Brunberg *et al.* 2006). Therefore, it was probably introduced coincidentally by incrossing of other breeds. There were twelve common ancestors in the two horses heterozygous for silver. Of these ancestors nine were frequently used in all of the 250 Black Forest horses genotyped within the last 50 years and the incidence of *SILV*-1852C > T in the population would be expected to be much higher, if it would have been of their origin. The Noriker stallion common in both pedigrees was one of those ancestors. Therefore, and also because *SILV*-1852C > T was not present in any of the 92 Norikers genotyped in this study, it is unlikely to be the origin of the silver allele. The Freiberger stallion (A) which was ancestor of both horses was less frequently used in the Black Forest population. In the whole population, the gene proportion of Freiberger horses was estimated at 0.86% (Biedermann and Schröter, 2003). However, Freiberger horses are bay, chestnut and gray (Mele *et al.* 2007) while silver color does not occur. Therefore, it is unlikely that the silver allele is of Freiberger origin. At the end of the 19$^{th}$ century, Ardenner horses were used for breeding with Black Forest horses. These horses have already been reported to segregate for silver (Brunberg *et al.* 2006). In the pedigrees of both Black Forest horses carrying the silver allele, a common Ardenner ancestor was found. Though the stallion was a popular breeder in the Black Forest horse population, it was used a century ago. As there is hardly selection pressure for silver in the mainly chestnut colored Black Forest horse population, the silver mutation might have largely been lost over the following generations. On the other hand, the mare (B) present in both pedigrees was born in the seventies of the last century.

**Table 1** Genotypes for *SILV1*852C > T, *MC1R*-248C > T and *MC1R*-250G > A in a number (n) of Black Forest horses and Noriker horses. Coat color alleles with Z (silver, *SILV1*852T), z (wildtype, *SILV1*852C), E (black, *MC1R*-248C), e (chestnut, *MC1R*-248T and *MC1R*-250G), and $e^a$ (chestnut, *MC1R*-248T and *MC1R*-250A) are given. The silver allele has no phenotypic effect on a chestnut based coat color. The color indicated as phenotype depends on the genotypes analysed. There may be further color variations not tested for in this study

| Horse breed | n | Genotype *SILV1*852C > T | Genotype *MC1R248*C > T | Genotype *MC1R*250G > A | Alleles | Phenotype |
|---|---|---|---|---|---|---|
| Black Forest | 161 | C / C | T / T | G / G | zz ee | Chestnut |
| | 81 | C / C | T / T | A / G | zz $ee^a$ | Chestnut |
| | 6 | C / C | T / T | A / A | zz $e^ae^a$ | Chestnut |
| | 2 | C / T | T / T | G / G | Zz ee | Chestnut |
| Noriker | 27 | C / C | T / T | G / G | zz ee | Chestnut |
| | 31 | C / C | C / T | G / G | zz Ee | Black |
| | 34 | C / C | C / C | G / G | zz EE | Black |

In the pedigree of mare (B), the maternal grandmother is unknown, so that horse might have carried the silver allele.

## CONCLUSION

In conclusion, the silver mutation (*SILV*-1852C>T) does occur in the Black Forest horse population with a prevalence of about 0.8% due to this study. The allele presumably originated from Ardenner incrossing or from an unknown ancestor of the maternal line. As chestnut coat color is predominant in this breed, the silver mutation is expected to have very few to no phenotypic appearance in Black Forest horses.

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