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Known loci in the *KIT* and *TYR* genes do not explain the depigmented white coat colour of Austro-Hungarian Baroque donkey

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ABSTRACT

The Italian Asinara donkey and the White Austro-Hungarian Baroque donkey share an identical coat colour phenotype which is characterised by unpigmented skin, white hair, white hooves and blue eyes. Whereas for the Asinara donkey the white coat colour phenotype was assigned to a recessive inherited missense mutation in the Tyrosinase gene (*TYR*), the underlying genetic background in the White Austro-Hungarian Baroque donkey has not been studied yet. Historical documents derived by the presence of Austro-Hungarian prisoners in the Asinara Island during the First World War might suggest a possible common origin of the same coat colour phenotype in the two breeds. Genotyping of this mutation in the *TYR* gene and the loci in the *KIT* gene associated with Dominant White and White Spotting phenotype, revealed, that none of the mutated alleles segregated in the White Austro-Hungarian Baroque Donkey breed. Also sequencing analysis of the *TYR* gene did not result in the detection of further candidate variants. Therefore, the *TYR* gene can be excluded as a possible candidate gene for this specific coat colour in the White Austro-Hungarian Baroque donkey. This result excludes a common genetic origin of the white coat colour of the Asinara and White Austro-Hungarian Baroque donkeys that historical documents could have suggested.

HIGHLIGHTS

- Historical information indicated that the white (albino) Asinara donkey breed and the White Austro-Hungarian Baroque donkey breed could be genetically related.
- The *TYR* mutation identified in Asinara donkeys and known polymorphic sites in the *KIT* gene associated with depigmented white coat colour in donkeys do not segregate in the White Austro-Hungarian Baroque Donkey.
- Due to different underlying genetic background of one identical phenotype in two populations, no common ancestors can be assumed between Asinara donkey and White Austro-Hungarian Baroque donkey throughout the last century.

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Introduction

With 44 million of animals worldwide, donkeys represent the biggest population of working animals (Starkey and Starkey 2000). Despite of this high economic relevance, the scientific interest in donkeys has been comparable low. Especially in the field of coat colour genetics, where in horses' huge progress has been achieved in the last decades (Rieder 2009), the investigation of the genetic background of colour variation in donkeys is in its beginnings. Although

donkeys exhibit comparable high phenotype variation of coat colours as horses, up to date only a few determining loci are known. Abitbol et al. (2014) identified a recessive inherited mutation (c.629T > C) in the melanocortin-1-receptor gene (*MC1R*) gene, responsible for chestnut coat colour in donkeys. The same group of researchers (Abitbol et al. 2015) identified a recessive genetic variant (c.349T > C) in the agouti-signaling protein (*ASIP*) gene, associated with the absence of light points around eyes, muzzles and belly. Furthermore, three mutations were detected to be

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involved in white phenotypes in donkeys. Haase et al. (2015) identified two variants in the *KIT* gene, encoding the KIT proto-oncogene, receptor tyrosine kinase, as candidate mutations for Dominant White and white spotting phenotypes in donkeys. One missense mutation (c.662A > C) in exon 4 of the *KIT* gene was found in a Hungarian donkey with pink skin, white hair and dark eyes (Haase et al. 2015) and the white spotted phenotype was assigned to a variant affecting the splice donor site (c.1978+2T > A) in the *KIT* gene. Utzeri et al. (2016) identified a recessive inherited mutation (c.604C > G) in the Tyrosinase (*TYR*) gene in the Italian Asinara donkey. This semiferal managed breed mainly lives in the Asinara Island, a small island geographically positioned in the North-West of Sardinia (Italy). The animals of this breed are characterised by unpigmented skin and hair, white hooves and blue eyes. The White Austro-Hungarian donkey breed exhibits an identical coat colour phenotype (unpigmented skin and hair, white hooves, blue eyes) as the Asinara donkey. A few historical reports suggested a possible link between these two breeds. Austro-Hungarian soldiers were imprisoned in the Asinara island during the First World War and this event that is testified by the presence of an Austro-Hungarian ossuary in this island (Gorgolini 2011; Ughi and Rubino 2015). It could be possible that this historical event might have created the possibility of exchange of donkeys in the two directions.

The coat colour characteristics are the same as in the White Austro-Hungarian donkey breed, which was revitalised in the 1980ies. From historical records it is known that white donkeys have been bred from the 17th to the 19th century in private stud farms, mostly owned by noble families, throughout the area of the Austro-Hungarian monarchy. During the 20th century, this breed has been threatened by extinction. The revitalisation started in the 1980ies with a nucleus herd of the zoo Herberstein comprising 12 breeding animals and a couple of white Hungarian donkeys. A breeding pair of this core population was transferred in 1987 to the zoo of Erfurt in Germany. Later on, the animals and their offspring were transferred to the zoo of Stralsund, where the population was expanded and still is being bred. In Austria, two additional breeding herds were established in the 1990ies in the National Park Neusiedler See/Ilmitz and in Schloß Hof (Altmann 2002). Nowadays there are three nucleus populations in Austria, and additionally about 25 private small scale breeders. In total 257 animals are registered in the stud book of the breeder association of

the White Austro-Hungarian Baroque Donkey (Verein zur Erhaltung der Weißen Barockesel 2010).

The aim of the study was to investigate the genetic background of the specific coat colour in the White Austro-Hungarian Baroque donkey breed and to test already known loci associated with depigmented white colour phenotypes in donkeys for segregation in the respective breed.

Material and methods

Hair root samples and DNA from paternity control were available from 28 White Austro-Hungarian donkeys, which were all descending from white coloured parents. Additionally, samples from two white Hungarian donkeys of unknown origin, two white Asinara donkey, and three solid coloured Hungarian donkeys, which had depigmented offspring, were included in this study. As a control group, hair samples of nine donkeys of various coat colours (two dun, one bay, two black, two grey and two white spotted) were collected. Furthermore, 17 Asinara completed the data set for this study, where *TYR* (c.604C > G) genotypes were already available.

DNA from hair samples was extracted using a standard phenol–chloroform–isoamyl alcohol protocol (Sambrook et al. 1989).

In a first step, a cohort comprising eleven White Austro-Hungarian donkeys, two Asinara, three Hungarian donkeys (two dun, one black), two white spotted and one grey donkey, were tested for the *KIT* loci Dominant White (c.662A > C) and White Spotting (c.1978*2T > A) according to Haase et al. (2015). In the second step, 58 donkeys (28 Austrian white Baroque donkeys, five Hungarian (two white and three solid coloured) donkeys, 18 Asinara (17 samples were already genotyped from previous work), and seven non-white donkeys without known pedigree were genotyped for the recessive mutation (c.604C > G) in the *TYR* gene, which was associated with depigmented coat colour phenotypes in Asinara donkey according to Utzeri et al. (2016). We screened for further candidate variants associated with depigmented coat colour phenotypes by Sanger sequencing of the whole *TYR* gene (coding sequence, parts of the 5'-UTR and 3'-UTR and parts of intronic regions) in three white donkeys (two white Austro-Hungarian donkeys and one white Hungarian donkey). PCR primers and amplification conditions and details on Sanger sequencing are reported in [Supplementary Table 1](#).

Results and discussion

Due to the availability of well documented pedigree records in horses, the genetics of horse coat colour has been intensively studied, resulting in the detection of numerous causative mutations for a wide range of coat colours, shades and patterns (Rieder 2009). A systematic classification of donkey coat colour phenotypes was done by Sponenberg (2009), who used horse nomenclature as a reference and differentiated white depigmented coat colour in donkeys into grey, ivory/cameo and white born phenotypes. As in ivory/cameo the striping still is visible, Sponenberg (2009) proposed a dilution effect similar to cream/champagne and perlino in horses. Within white born donkeys, Sponenberg and Bellone (2017) distinguished between two phenotypes: white born donkeys with dark eyes and white-born donkeys with blue eyes. The first mentioned category was assigned to the *KIT* gene by Haase et al. (2015), who identified a mutation for Dominant White (c.662A > C) in one single white Hungarian donkey. This mutation was not found in the White Austro-Hungarian Donkey sample and the white Hungarian donkeys with unknown pedigrees. One Asinara donkey was heterozygous (Table 1) for this mutation in the *KIT* gene. The allele associated with White Spotting (c.1978*2T > A) was present in heterozygous state in one of the two phenotypically spotted donkeys, all other animals of the entire sample did not carry this *KIT* mutation (Table 1, Figure 1).

The genetic background for the second phenotype category, characterised by white hair, unpigmented skin and blue eyes, was clarified for Asinara donkeys by Utzeri et al. (2016), who identified a recessive inherited missense mutation (c.604C > G) in the *TYR* gene. Although the coat colour phenotype of the Austro-

Hungarian Baroque donkey is identical, the point mutation in the *TYR* gene was not present in the Austrian samples. Genotyping results of the mutation in the *TYR* gene revealed homozygosity for the wild-type allele C in all White Austro-Hungarian donkeys. The mutated G-allele was also not present in the non-white donkey sample, whereas all 18 tested Asinara donkeys were homozygous G/G for the mutated allele (Table 2).

Sanger sequencing did not result in the identification of further variants in the *TYR* gene associated with the classical depigmented coat colour phenotype in two White Austro-Hungarian donkeys and one white Hungarian donkey (Supplementary Table 1 and 2). Therefore, the *TYR* gene can be excluded as a possible candidate for this specific coat colour of the White Austro-Hungarian Baroque Donkey. We propose that further research should be conducted including genes like *OCA2* and *SLC45A2*, which are responsible for similar coat colour phenotypes in other species (Winkler et al. 2014; Caduff et al. 2017; Holl et al. 2019).

Conclusions

The results from this coat colour study are of relevance for the breeding history of the Asinara and the Austro-Hungarian Baroque Donkey. A direct exchange of breeding animals between both populations during the First World War, a theory which was based upon historical reports, seems not plausible taking our results into account. The absence of the causative *TYR* mutation in the Austro-Hungarian Baroque Donkey breed thus excludes a common genetic origin of this white coat colour phenotype. As a consequence, as

Table 1. Genotypes for the White Spotting locus (c.1978*2T > A) and the Dominant White locus (c.662A > C) in the *KIT* gene.

Breed	Coat colour	Genotype White Spotted (c.662A > C)	Genotype Dominant White (c.1978*2T > A)
Unknown origin	Grey	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
White Austro-Hungarian Baroque Donkey	White	T/T	A/A
Hungarian Donkey	Dun ^a	T/T	A/A
Hungarian Donkey	Dun ^a	T/T	A/A
Hungarian Donkey	Black ^a	T/T	A/A
Asinara Donkey	White	T/T	A/A
Asinara Donkey	White	T/T	A/C
Unknown origin	White Spotted	T/T	A/A
Unknown origin	White Spotted	T/A	A/A

^aWith known white offspring.



Figure 1. Different white coat colour phenotypes in donkeys: (a) grey coat colour (b) two white Austro-Hungarian White Baroque donkeys (c) white spotted donkey (d) white Asinara donkey.

Table 2. Genotypes for the *TYR* locus (c.604C > G) (Utzeri et al. 2016).

Breed	Coat colour	<i>n</i>	<i>TYR</i> c.604C > G
White Austro-Hungarian Baroque Donkey	White	28	C/C
Hungarian Donkey	White	2	C/C
Hungarian Donkey	Solid coloured	3	C/C
Asinara Donkey	White	18	G/G
Unknown origin	Solid coloured	7	C/C

both populations represent endangered breeds, an admixture in purpose of conservation genetics cannot be recommended.

Ethical statement

Hair samples were taken in the context of routine procedures according to GSP guidelines and national legislation.

Disclosure statement

No potential conflict of interest was reported by the author(s).

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