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SHORT COMMUNICATION

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Explicit evidence for a missense mutation in exon 4 of *SLC45A2* gene causing the pearl coat dilution in horses

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Summary

Four loci seem responsible for the dilution of the basic coat colours in horse: Dun (D), Silver Dapple (Z), Champagne (CH) and Cream (C). Apart from the current phenotypes ascribed to these loci, pearl has been described as yet another diluted coat colour in this species. To date, this coat colour seems to segregate only in the Iberian breeds Purebred Spanish horse and Lusitano and has also been described in breeds of Iberian origin, such as Quarter Horses and Paint Horse, where it is referred to as the 'Barlink Factor'. This phenotype segregates in an autosomal recessive manner and resembles some of the coat colours produced by the champagne CH^{CH} and cream C^{Cr} alleles, sometimes being difficult to distinguish among them. The interaction between compound heterozygous for the pearl C^{prl} and cream C^{Cr} alleles makes *SLC45A2* the most plausible candidate gene for the pearl phenotype in horses. Our results provide documented evidence for the missense variation in exon 4 [*SLC45A2*: c.985G>A; *SLC45A2*:p.(Ala329Thr)] as the causative mutation for the pearl coat colour. In addition, it is most likely involved as well in the cremello, perlino and smoky cream like phenotypes associated with the compound C^{Cr} and C^{prl} heterozygous genotypes (known as cream pearl in the Purebred Spanish horse breed). The characterization of the pearl mutation allows breeders to identify carriers of the C^{prl} allele and to select this specific coat colour according to personal preferences, market demands or studbook requirements as well as to verify segregation within particular pedigrees.

Keywords Barlink Factor, coat colour, cream pearl, cremello, *Equus caballus*, *MAPT*, perla, perlino, smoky cream

Human preferences for different colour phenotypes have played a major role in the development of the broad variety of coat colours exhibited by domestic species like horses, favouring rare alleles by selective breeding. Most coat and skin colours follow relatively simple models of Mendelian inheritance, with a few genes implicated in particular phenotypes acting either on pigment synthesis or on the function of melanocytes, the cells producing pigments (Sponenberg 2009). Four loci have been described in the horse as responsible for the dilution of the basic coat colours (see revision in Rieder 2009): Dun (D), Silver Dapple (Z), Champagne (CH) and Cream (C). Dun and Champagne loci can cause dilution phenotypes affecting pheomelanin and eumelanin, whereas the Silver Dapple locus acts mostly on eumelanin and has no or little effect on pheomelanin. The C^{Cr} allele of the Cream locus acts in a codominant manner, diluting pheomelanic and eumelanic bay coats in the heterozygous state but with limited phenotypic effect on the eumelanic black background. The homozygote C^{Cr} allele dilutes all basic colour phenotypes and is characterized by rosy-pink skin, cream-coloured coats that can appear almost white and pale blue eyes. Mariat *et al.* (2003) located a single base substitution in exon 2 of the *solute carrier family 45, member 2* (*SLC45A2*) gene, also known as *membrane-associated transporter protein* (*MATP*), fully associated with the phenotypes segregating with C^{Cr}.

Apart from these variations, another diluted coat colour has been described in horse: pearl (Fig. 1a,b). To date, this coat seems to segregate only in the Iberian breeds Purebred Spanish horse and Lusitano and has also been described in breeds of Iberian origin, such as Quarter Horses and Paint Horse, where it is referred to as the 'Barlink Factor'. This phenotype segregates in an autosomal recessive manner and resembles some of the coat colours produced by the champagne CH^{CH} and cream alleles C^{Cr}, sometimes being difficult to distinguish among them. Animals carrying two copies of the

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pearl allele exhibit a dilution of both eumelanin and pheomelanin similar to that produced by the champagne allele CH^{CH} and the heterozygous genotype of the cream allele C^{Cr}. The effect of the pearl allele in heterozygote state cannot be seen unless it is combined with the cream allele C^{Cr}, producing a pseudo-double-diluted phenotype difficult to distinguish from the homozygote $C^{Cr}C^{Cr}$. The interaction between pearl and cream alleles makes *SLC45A2* the most plausible candidate gene for the pearl phenotype.

A second mutation [p.(Ala329Thr)] in *SLC45A2* has been proposed to generate the causative allele of pearl (Genbank accession no. EU272924; Wutke *et al.* 2016), but so far this has not been documented. It has not been included in the survey by Rieder (2009) or the OMIA database.

For this study, genomic DNA was isolated from blood and all seven *SLC45A2* exons were PCR-amplified using primers designed based on the publicly available sequence with PRIMER3 (http://bioinfo.ut.ee/primer3-0.4.0/primer3/) (Table S1). The sample set included a total of 16 Purebred Spanish horse samples: four horses exhibiting a phenotype compatible with $C^{wt}C^{Cr}$ or $C^{prl}C^{prl}$ genotypes but not carrying the C^{Cr} allele, six samples from horses with a cremello or perlino phenotype but not carrying two copies of the C^{Cr} allele and six control horses not displaying a dilute phenotype (Table 1). *SLC45A2* sequences were analysed using the software SEQUENCING ANALYSIS 5.2 (Applied Biosystems) and aligned to the equine EquCab2 genome reference sequence.

Two new single nucleotide polymorphisms (SNPs) were found in the *SLC45A2* gene: *SLC45A2*:c.828C>T in the third exon and *SLC45A2*:c.985G>A in the fourth exon (accession no. PRJEB27771). The SNP in exon 3 was synonymous, whereas the SNP in exon 4 causes a substitution of a hydrophobic alanine for a polar threonine amino acid at position 329 [*SLC45A2*:p.(Ala329Thr)] located in the transmembrane (TM) helix 7 (Fig. 1c). The mutated variant would most likely affect the substrate specificity and/or transport activity of the SLC45A2 protein (Ward & Milligan 2002; Haimeur *et al.* 2004). The replacement caused by the *SLC45A2* mutation in exon 2—*SLC45A2*:p.(Asp153Asn)—allele C^{Cr}, results in the substitution of a polar acidic aspartate with a polar neutral asparagine in TM helix 4. Taking into account the codominant vs. recessive mode of inheritance and the more extreme diluted

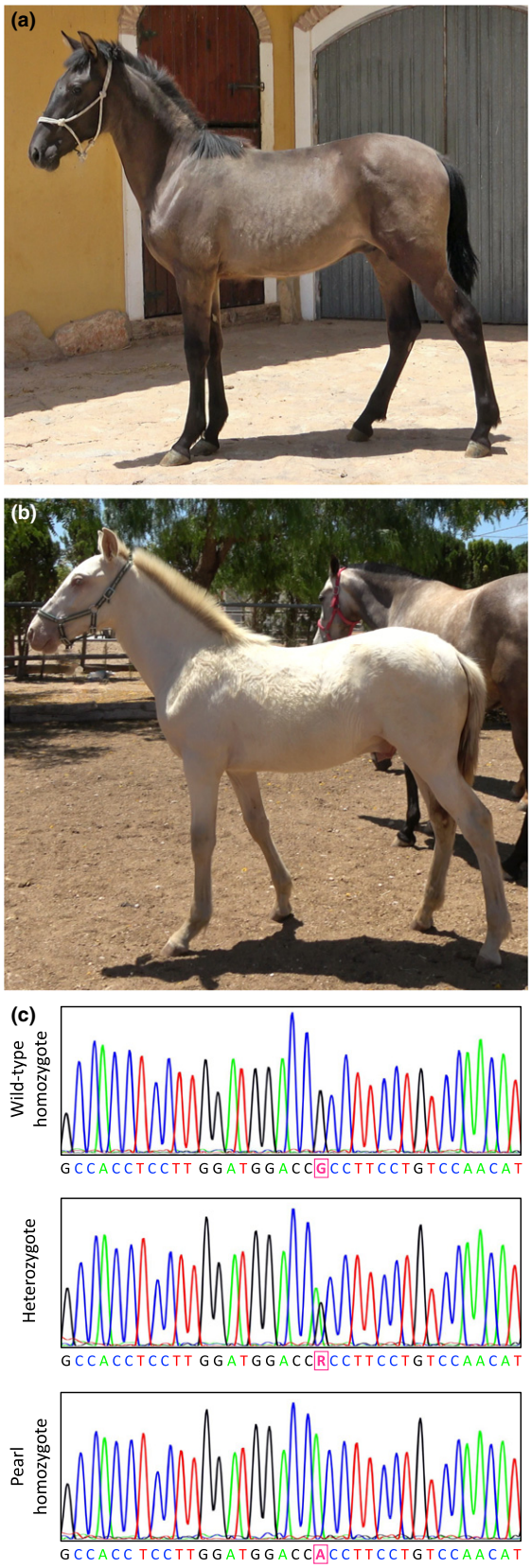

Figure 1 Pearl coat dilution in horses. (a) Example of the effect of homozygous pearl genotype action on a black horse ($E^{E}E^{e}$ $A^{a}A^{a}$ $G^{g}G^{g}$ $C^{prl}C^{prl}$). (b) Example of the effect of a compound *SLC45A2* heterozygote, pearl and cream, on a bay horse ($E^{E}E^{e}$ $A^{A}A^{a}$ $G^{g}G^{g}$ $C^{Cr}C^{prl}$). (c) Sanger sequencing showing the wild-type homozygous, heterozygous and pearl homozygous genotypes for the *SLC45A2*:p.(Ala329Thr) mutation. Photographs courtesy of Yeguada Carthago, Spain.

Table 1 Phenotypes and genotypes of the Purebred Spanish horse samples included in the study.

ID	Phenotype	*MC1R* c.901C>T (chestnut/sorrel)	*ASIP* c.191_201 (black)	*STX17* duplication intron 6 (greying)	*SLC45A2* p.(Asp153Asn) (cream) p.(Ala329Thr) (pearl)
1	Pearl	$E^{E}E^{E}$	$A^{A}A^{A}$	$G^{g}G^{g}$	$C^{prl}C^{prl}$
2	Pearl[1]	$E^{E}E^{E}$	$A^{A}A^{a}$	$G^{g}G^{G}$	$C^{prl}C^{prl}$
3	Pearl	$E^{E}E^{e}$	$A^{a}A^{a}$	$G^{g}G^{g}$	$C^{prl}C^{prl}$
4	Pearl	$E^{E}E^{E}$	$A^{A}A^{a}$	$G^{g}G^{g}$	$C^{prl}C^{prl}$
5	Cream pearl (cremello like)	$E^{e}E^{e}$	$A^{A}A^{A}$	$G^{g}G^{g}$	$C^{Cr}C^{prl}$
6	Cream pearl (perlino like)[1]	$E^{E}E^{E}$	$A^{A}A^{a}$	$G^{G}G^{G}$	$C^{Cr}C^{prl}$
7	Cream pearl (perlino like)	$E^{E}E^{E}$	$A^{A}A^{A}$	$G^{g}G^{g}$	$C^{Cr}C^{prl}$
8	Cream pearl (perlino like)[1]	$E^{E}E^{E}$	$A^{A}A^{A}$	$G^{g}G^{G}$	$C^{Cr}C^{prl}$
9	Cream pearl (perlino like)	$E^{E}E^{E}$	$A^{A}A^{A}$	$G^{g}G^{g}$	$C^{Cr}C^{prl}$
10	Cream pearl (perlino like)[1]	$E^{E}E^{E}$	$A^{A}A^{a}$	$G^{g}G^{G}$	$C^{Cr}C^{prl}$
11	Black	$E^{E}E^{E}$	$A^{a}A^{a}$	$G^{g}G^{g}$	$C^{wt}C^{prl}$
12	Chestnut/sorrel	$E^{e}E^{e}$	$A^{A}A^{a}$	$G^{g}G^{g}$	$C^{wt}C^{prl}$
13	Chestnut/sorrel[1]	$E^{e}E^{e}$	$A^{A}A^{a}$	$G^{g}G^{G}$	$C^{wt}C^{wt}$
14	Bay[1]	$E^{E}E^{E}$	$A^{A}A^{A}$	$G^{G}G^{G}$	$C^{wt}C^{wt}$
15	Bay	$E^{E}E^{E}$	$A^{A}A^{a}$	$G^{g}G^{g}$	$C^{wt}C^{wt}$
16	Chestnut/sorrel	$E^{e}E^{e}$	$A^{A}A^{a}$	$G^{g}G^{g}$	$C^{wt}C^{wt}$

[1]Progressive greying over time.

phenotypes caused by the C^{Cr} allele compared with C^{prl}, the first mutation seems to have a more severe effect on the functionality of this transport protein implicated in melanin synthesis. The combination of alleles C^{Cr} and C^{prl} may also cause a severe disruption of the gene product.

Although other missense variations have been described in exons 1 and 3 of the *SLC45A2* gene, none of them segregated in our sample set, so they were discarded as causative mutations of the pearl phenotype. The exonic SNP *SLC45A2*:c.985G>A in exon 4 of the *SLC45A2* gene identified in this study fits the distribution of the pearl/cream phenotype (Table 1). Among the analysed samples, one horse exhibiting a chestnut/sorrel coat colour and another with black coat were found to be carriers for the C^{prl} allele. This new allele may also explain the very light palomino coat colour of one horse carrying only one C^{Cr} copy described by Mariat *et al.* (2003), with it probably being a *SLC45A2* compound heterozygote.

In conclusion, we provide evidence for the *SLC45A2:* p.(Ala329Thr) variation as the causative mutation for the pearl coat colour and the cremello, perlino and smoky cream like phenotypes associated with the compound C^{Cr} and C^{prl} heterozygous genotype (known as cream pearl in the Purebred Spanish horse breed). Similar evidence has been provided by Holl *et al.* (2019). This coat colour can be difficult to distinguish from the effects of the cream C^{Cr} and champagne CH^{CH} alleles and can also be masked by the grey locus (G), whose G^{G} dominant allele produces progressive greying with age, independent of the background colour (Pielberg *et al.* 2008). Thus, the characterization of the pearl mutation allows breeders to identify carriers of the C^{prl} allele and to select this specific colour phenotype according to personal preferences, market demand or studbook requirements as well as to verify segregation within particular pedigrees.

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Conflict of interest

Our laboratory carries out commercial tests on the pearl mutation.

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Supporting information

Additional supporting information may be found online in the Supporting Information section at the end of the article.
Table S1. Primer pairs used to amplify *SLC45A2* exons in horse.