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Original article

A mutation in the *MATP* gene causes the cream coat colour in the horse

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Abstract – In horses, basic colours such as bay or chestnut may be partially diluted to buckskin and palomino, or extremely diluted to cream, a nearly white colour with pink skin and blue eyes. This dilution is expected to be controlled by one gene and we used both candidate gene and positional cloning strategies to identify the "cream mutation". A horse panel including reference colours was established and typed for different markers within or in the neighbourhood of two candidate genes. Our data suggest that the causal mutation, a G to A transition, is localised in exon 2 of the *MATP* gene leading to an aspartic acid to asparagine substitution in the encoded protein. This conserved mutation was also described in mice and humans, but not in medaka.

horse / coat colour / underwhite / cream / *MATP*

1. INTRODUCTION

In mammals, coat colour is defined by two pigments in the skin and the hair, the black eumelanin and red pheomelanin. The specific colour of an animal thus depends on the pattern, the geographical distribution of the two pigments on the body, and on the relative quantity of both pigments. If we do not consider the pattern of white, the basic colours of horses are bay, black, brown and chestnut [32]. Many, but not all, colours derived from the four basic ones are diluted colours. A first locus dilutes the bay to wild bay, black to smoky and chestnut to wild chestnut. A second independent locus dilutes bay to dun or buckskin (pale body with dark mane, tail and points), depending if it acts on a wild bay or a bay, chestnut to palomino (a yellow horse with nonblack points, pale mane, tail and body) but has no or little effect on the black colour. This locus corresponds to the cream gene conferring, in its homozygote state,

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the most pale (cream) colour to horses with pink skin and blue eyes. Some of these horses can be nearly white. The cream colour associated with blue eyes (BEC) is considered as a defect preventing in certain breeds the registration to the Stud-book.

Several genes may be involved in hypopigmentation. Because of its major role in the melanin pathway [14] and its involvement in type I Oculocutaneous albinism [24], tyrosinase is a major candidate [2] well described in humans [7] and in mice [13]. The pink-eyed dilution locus (*p*) encodes a melanosomal membrane protein [29] also involved in the melanin-synthesis pathway by interference with tyrosine supply [25,31]. Mutations in the *p* locus result in hypopigmentation in the eye and coat [3], and are responsible for type II Oculocutaneous albinism [28]. In the mouse, one of the diluted coat colour loci was identified as encoding myosin VA [20], which acts as an organelle motor for melanosomes within dendritic extensions [35]. As a member of the myosin family, *MYO10* is also an actin-based molecular motor [1]. In spite of the biochemical differences [12], *MYO10* could act in a similar way as *MYO5A*, and was therefore considered as a candidate gene. In addition, *MYO10* has already been proposed as a candidate gene for the mouse *uw* locus because of its localisation on MMU15 [11]. The underwhite mutation (*uw*) was first reported in the nineteen-sixties [4], when light coloured mice lacking the black eye pigment were described, as well as the involvement of a unique autosomal recessive gene. Further studies confirmed the role of the *uw* locus in melanogenesis, in the reduction of pigmentation in the eye and coat and its preferential action in the melanosome structure [15,33]. Recently the mouse *uw* locus was identified as the *AIM1*, also called *MATP* gene [5,23] which encodes a transporter protein also involved in hypopigmentation of gold-fish medaka [6] and in human type IV Oculocutaneous albinism.

In the horse, the latest data published by Locke *et al.* [17] described the localisation of the cream dilution locus on chromosome 21. Therefore, some of our candidate genes were rejected, such as *TYR* which is on ECA7 [16], *MYO5A* on ECA1 [18], and *p* expected to be on ECA1 since it is localised on HSA15 partly homologous to ECA1 according to Zoo-FISH [26].

Therefore our study was focused on *MYO10* and *MATP* as candidate genes, since both of them are expected to be localised on ECA21.

BAC clones containing genes were sequentially isolated from our Inra library when exonic sequences were available in order to detect internal polymorphism. This polymorphism was used to detect a linkage and association between markers and coat colour in a panel of unrelated and family animals.

Here, we provide molecular evidence for a mutation in *MATP*, responsible for the cream coat colour in the horse.

Table I. Distribution of colour phenotypes in our family and an unrelated horse panel.

Family	Mares	Foals	Total	Cream	Buckskin	Palomino	Bay	Chesnut	Grey	Black	Roan
A	7	11	19	3	2	0	6	2	13	1	0
B	11	17	29	3	7	4	8	2	4	0	2
C	1	2	4	2	0	0	0	0	1	1	0
E	2	8	11	3	3	0	1	0	3	1	0
F	3	5	9	3	3	0	2	0	2	0	0
G	2	4	7	0	4	0	1	0	2	1	0
H	2	3	6	1	0	1	0	0	5	0	0
I	4	7	12	2	6	1	0	0	3	2	0
J	1	1	3	1	1	0	0	0	1	0	0
K	1	1	3	1	0	0	0	0	2	0	0
L	1	1	3	0	0	0	2	0	2	0	0
Total	35	60	106	19	26	6	20	4	38	6	2
				18%	24%	5%	19%	4%	35%	5%	2%
Unrelated horses											
Connemara			21	5	3	1	3	0	7	1	0
Welsh			11	7	1	3	0	0	0	0	0
Welsh-Cob			2	0	1	0	0	0	0	1	0
Barbe			1	0	1	0	0	0	0	0	0
Total			35	12	6	4	3	0	7	2	0

2. MATERIALS AND METHODS

2.1. Resource individuals

Our collaboration with many breeders, mostly from the "Association française du Poney de Connemara", allowed us to collect 141 DNA samples from related and unrelated horses, of different coat colors (Tab. I). Eleven paternal half-sib families in which diluted colours segregated, were collected for linkage analysis. The resource panel also included unrelated individuals from Connemara, Welsh, Welsh-Cob and Barbe breeds. The colour phenotypes were taken from the official registration papers of the horses or directly collected from the owners.

2.2. Primer design and BAC clone isolation

The available exonic sequences from candidate genes were aligned using the BLAST programme at NCBI (www.ncbi.nlm.nih.gov/BLAST/). The primers

Table II. Genes included in this study. The consensus primers were used to screen the Inra BAC library and to sequence the corresponding fragments amplified from each specific BAC. BLAST sequence identities between the horse and other species are indicated.

Gene symbol	Gene name	Consensus primers	Identity horse / other
MATP (AIM-1)	Membrane-associated transporter protein (underwhite)	CACAGGTTTTGGAGGTGCCC GAACATGACCTGGAATTCTG	0.95 / mouse
MYO10	Myosin X	GCCATCAAGATATTCAATTC GTCAGGATCTGCCAGCTGTA	0.92 / human

were designed in the regions of homologies to provide intra-exonic consensus primers which were used to screen the Inra BAC library (Tab. II) as described in [18]. Recent data identified the *MATP* gene as the *uw* locus in mice, humans and fish [5, 6, 23], and allowed us to design such primers. The library consists of 108 288 clones distributed in 47 super-pools as described in [22]. It was screened by PCR under conventional conditions [9]. The identity of the BAC clones was systematically confirmed by sequencing of the PCR product obtained with consensus primers and homology scoring with the BLAST programme.

2.3. Microsatellite and deletion characterisation and typing

The BAC clones were subcloned in a pGEM4Z plasmid vector, after digestion with Sau*3A*. The subsequent clones were screened with $(TG)_{12}$ and $(TC)_{12}$ oligonucleotides, using the DIG luminescent detection kit of Boehringer Mannheim, as already described in [8].

The polymorphism of the isolated microsatellites was observed on a panel of ten DNA from 2 Black horses (Frison), 2 Bay (Connemara), 2 Buckskin (Connemara), 2 Palominos (Connemara) and 2 Cream (Connemara). The PCR products were detected on an ABI PRISM 373 Sequencer (PE Applied Biosystems) as described in [18].

A deletion was detected by sequencing the 5′ region of the *MATP* gene. Specific primers were designed to amplify this region and were used to type the deletion by conventional agarose gel electrophoresis.

2.4. Amplification of *MATP* introns

Several anchor primers were designed in each of the seven exons of the *MATP* by comparison of the human and murine sequences (Tab. III) for exon amplification. BACs were subcloned in pGEM4Z after digestion with Bam*HI*

Table III. Single anchor primers used to amplify and sequence part of the *MATP* gene. PCR amplification was performed with a second universal or reverse plasmid primer on the BAC subclone fragments. The extension column refers to gene orientation.

Location	Exonic anchor primers	Extension
MATP exon 1	ACATGGCCATGCTGTGCATG	Reverse
	CATGCACAGCATGGCCATGT	Forward
	CCATTGAGGTACAGAGCCAT	Reverse
	CAGGAATCCCAGGATGGGGC	Reverse
	GCCCCATCCTGGGATTCCTG	Forward
	ATGGCTCTGTACCTCAATGG	Forward
MATP exon 2	GGAGAAGGGCCTCCACTACC	Forward
	CATCAAAGCCTACTTATTTG	Forward
	GGTAGTGGAGGCCCTTCTCC	Reverse
	CAAATAAGTAGGCTTTGATG	Reverse
MATP exon 3	GAACATGACCTGGAATTCTG	Reverse
	CAGAATTCCAGGTCATGTTC	Forward
MATP exon 4	CAGCCACCTCATTGGATGGAC	Forward
	GTCCATCCAATGAGGTGGCTG	Reverse
MATP exon 5	GATGCACAAGCCCCAACATC	Reverse
	GATGTTGGGGCTTGTGCATC	Forward
MATP exon 6	TGCTGGACATTACACCAAAC	Reverse
	GAGGTTAAAGGGCACAGTGT	Reverse
	GTTTGGTGTAATGTCCAGCA	Forward
	ACACTGTGCCCTTTAACCTC	Forward
MATP exon 7	GGTGATCACAGCGTCTGCGG	Forward
	CCGCAGACGCTGTGATCACC	Reverse

or Sau*3A*, and PCR amplification of the specific regions was performed by the use of one exonic anchor primer and one plasmid primer (universal or reverse). For each exon, all primer combinations were tested, and we could amplify the regions corresponding to introns 1, 2 and 4.

2.5. Linkage analysis

Linkage between some markers and the colour phenotype was analysed using the two-point option of the CRIMAP programme version 2–4 [10]. Linkage disequilibrium between the cream locus and polymorphic markers, was analysed using the maximum likelihood method of [34] with the DISLAMB programme obtained from the Rockefeller Institute (http://linkage.rockefeller.edu).

2.6. cDNA synthesis

One hundred mg of skin from a bay horse and a cream horse, and 100 mg of testicular tissue from a bay horse, were separately homogenised in the RNA NOW reagent (Ozyme), and total RNA was extracted as described by the manufacturer. The yield was 1.5 μg of RNA per mg of tissue. Reverse transcription was performed using the Superscript II RNase H-Reverse Transcriptase from GIBCO BRL, as described by the manufacturer.

The PCR reaction was performed using conventional conditions by using intra-exonic consensus primers designed from human and mouse *MATP* sequences (Tab. III).

2.7. PCR amplification of exons

Most PCR reactions were performed in 25 μL, with 20 pmoles of each primer, 2 mM MgCl$_2$, 0.25 mM of dNTP and 1 unit of Taq polymerase (Promega). The PCR conditions were as follows: 5 min at 94 °C followed by 35 cycles of 30 s at 94 °C, 30 s at 55 °C, and 30 s at 72 °C except amplification of *MATP* exon 2 for which the cycles were set at 20 s.

For the amplification of exon 2 of *MATP*, the horse primers used are

$$5'\text{-GGTAGTGGAGGCCCTTCTCC-}3'$$

and

$$5'\text{-TGCTGACCGAAGGAAGAAG-}3'.$$

3. RESULTS

3.1. Resource individuals and families

Our horse panel was designed to represent all the genotypes expected at the cream locus. The cream colour (C^{cr}C^{cr}) is well represented in the family
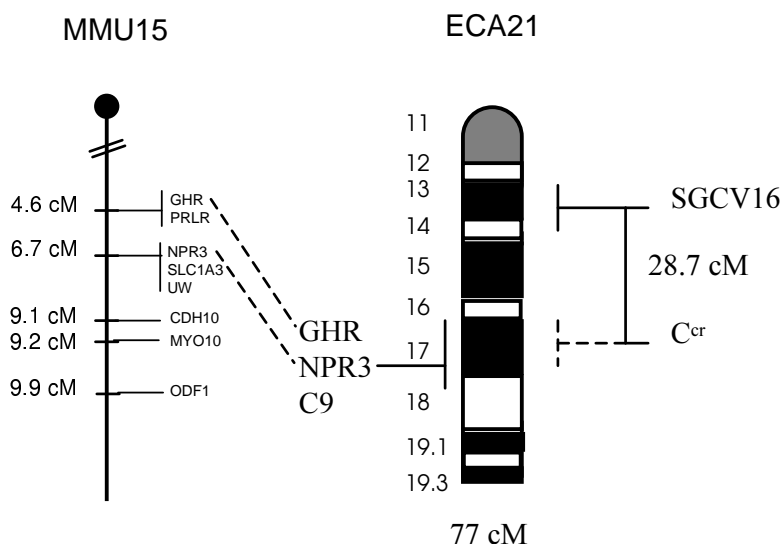
Figure 1. Distribution of markers around the underwhite locus on horse chromosome 21 and mouse chromosome 15.

panel (18%), as well as buckskin (CC^{cr}, 26%), bay (CC, 20%), while palomino (CC^{cr}) and chestnut (CC) represent only 6% and 4% respectively (Tab. I). The table also shows that the grey colour often occurs in our Connemara panel since it represents 35% of all horse colours.

3.2. The study of an ECA21 region

3.2.1. Comparative mapping

The localisation of the cream locus on horse chromosome 21 reported by Locke *et al.* [17] was confirmed in our horse families. Genetic linkage was found between the C^{cr} locus and the LEXO60 and CORO73 microsatellites ($\theta = 0.05$, Z = 2.49 and $\theta = 0.05$, Z = 2.47) respectively.

Therefore, taking into account genetic and cytogenetic data (Fig. 1), it appeared that C^{cr} should be localised in the ECA21p17 region, since C^{cr} is in a distal position at 30 cM from SGCV16, and that the estimated length of ECA21 is 77 cM. Three genes have been localised on the horse genome in the ECA21p17 region, *i.e. GHR*, *NPR3* and *C9*.

When looking at the mouse linkage map (www.informatics.jax.org) we found that *GHR* is localised on chromosome 15 at 4.6 cM from the centromere, and *NPR3* at 6.7 cM (Fig. 1). Interestingly, *uw* is reported at 6.7 cM and *MYO10* at 9.2 cM on MMU15, among other genes.

3.2.2. Identification of BAC clones containing candidate genes

The Inra BAC library was screened for two candidate genes, namely underwhite (*MATP*), and myosin10 (*MYO10*). We thus identified three BAC clones containing *MATP* (356-E-3, 778-F-4, and 992-G-4), and two containing *MYO10* (160-C-9 and 174-A-3).

The presence of the expected gene was confirmed by sequencing the DNA fragment amplified with specific primers on the BAC clones and by sequence alignment analysis (Tab. II). Sequence homologies ranged from 92 to 95% depending on the species.

3.2.3. Microsatellite markers and linkage analysis

In a first step, we searched for microsatellites in BAC clones containing *MATP*, *MYO10* genes. We found a large microsatellite (HMS77) with a $(TG)_{20}N(GA)_{17}GC(GA)_5$ repeat in the BAC clone containing *MYO10* (Tab. IV). On the contrary, no microsatellite was found in the BAC clones containing *MATP*.

When amplified on a DNA panel of ten unrelated individuals from two different breeds (Frison and Connemara) and five different coat colors (black, bay, buckskin, palomino and cream), HMS77 exhibited seven alleles.

A linkage study on our horse families suggests that HMS77 (*MYO10*) and the C^{cr} locus were not linked nor in linkage disequilibrium. This result indicates that the C^{cr} locus is distinct from *MYO10* and that this gene is not responsible for the cream coat colour.

3.2.4. SNP markers, association and linkage analysis around the MATP gene

SNP were searched in the mean time in introns and exons of the *MATP* gene by sequencing regions flanking the seven exons. Three SNP in the first intron (SNP2, 4 and 5), one SNP in the second intron (SNP1), and one SNP in the fourth intron (SNP3), as well as a 75 bp deletion upstream of the first exon (Fig. 2, Tab. V) were identified.
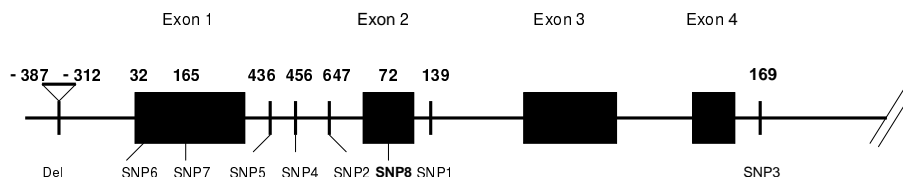

Figure 2. Polymorphism identified in the horse *MATP* gene. Exons are represented by black boxes and introns and UTR by lines. The relative positions of SNP are indicated. Exon and intron nucleotides are numbered from the first nucleotide of each exon or intron. Figure not drawn to scale.

Table IV. Characterisation of the microsatellite identified in the BAC containing the *MYO10* gene.

Microsatellite	Primers	Repeat	Polymorphism	Size
HMS77 *(MYO10)*	TCATTGTAATGGGCTACACC CATGGTGTTGTGACATGGTC	$(TG)_{20}N(GA)_{17}GC(GA)_5$	7 alleles	196–220 bp

Table V. Characterisation of the SNP and deletion polymorphisms reported in this study. Primers were used to detect and identify the two types of markers. The positions are described in the legend of Figure 2. The allelic frequencies of the variants were estimated from the restricted horse panel.

Locus id.	Location	Forward primer	Reverse primer	Position	Variant type	Allelic frequency
SNP1	*MATP* Intron 2	CGGAAAGTCTCTCCTTTAGC	GGGGCCACCGAAGCAACACG	104	A/T	T: 0.55
SNP2	*MATP* Intron 1	AAACCTATCACCAGAATTAAC	GGCTCTGTACCTCAACGGGG	147	G/C	C: 0.55
SNP3	*MATP* Intron 4	TCACAGATTTCATGGGCCAG	CCATGTCACCCCTTTCATTC	169	C/T	C: 0.83
SNP4	*MATP* Intron 1	AAACCTATCACCAGAATTAAC	GGCTCTGTACCTCAACGGGG	333	A/G	A: 0.61
SNP5	*MATP* Intron 1	id.	id.	353	C/T	C: 0.55
SNP6	*MATP* Exon 1	CAACTCATTCATGTTTTAAC	AAACCTATCACCAGAATTAAC	32	A/C	A: 0.68
SNP7	*MATP* Exon 1	id.	id.	165	C/T	C: 0.87
SNP8	*MATP* Exon 2	GGTAGTGGAGGCCCTTCTCC	TGCTGACCGAAGGAAGAAG	72	A/G	G: 0.52
Del	Upstream *MATP* Exon 1	CCAGGTTGCCCACTGTTGCC	CAACTCATTCATGTTTTAAC	−330	deletion	d: 0.63

All five SNP markers were typed on the ten horse DNA panel. Three of them nearly fitted to the distribution of the cream phenotype. These preliminary data were confirmed by linkage analysis on our horse families between the loci at the deletion, SNP1 and the C^{cr} locus. A complete linkage between C^{cr} and both markers (Del-C^{cr} ($\theta = 0$, $Z = 3.31$), SNP1-C^{cr} ($\theta = 0$, $Z = 4.82$)) was found, suggesting that *MATP* may be responsible for the phenotypes under study.

Two SNP were found in the first exon MATP sequence: the first at position 32 (SNP6) where a C<u>A</u>C codon (His) is substituted for a C<u>C</u>C (Pro), and the second at position 165 (SNP7) where a TA<u>C</u> (Tyr) codon is substituted for a TA<u>T</u> (Tyr) (Fig. 3). In spite of the amino-acid modification induced by the first SNP, sequence comparisons between bay and cream horses did not reveal complete association between this SNP and the cream coat colour.

3.3. A mutation in exon 2 of the *MATP* gene

Considering the above genetic data, our main objective became to investigate *MATP* exons. Therefore we compared the partial cDNA sequences obtained from skin samples from bay and cream horses, and from testicular tissues from a bay horse as a control. Consensus exonic primers from exon 1 and exon 3 allowed us to amplify the whole exon 2 cDNA fragment. Comparison of the sequences of this fragment in bay and cream individuals revealed an SNP at position 72 (SNP8), where a <u>G</u>AT codon (Asp) in a bay horse is replaced by an <u>A</u>AT (Asn) in a cream horse (Fig. 3).

Although we failed to amplify the whole *MATP* cDNA, the exon 3 to exon 7 sequence was partially reconstituted from several overlapping sequences and compared between cDNAs from bay and cream individuals. No polymorphism was detected in this region.

We also performed a linkage analysis between SNP8, SNP1, the deletion and the C^{cr} locus. Recombination fractions and lod scores revealed tight linkages as follows: SNP8-C^{cr} ($\theta = 0$, $Z = 9.03$), Del-C^{cr} ($\theta = 0$, $Z = 3.31$), SNP1-C^{cr} ($\theta = 0$, $Z = 4.82$). Highly significant linkage disequilibrium was found between the cream locus and SNP8 (LRT = 88.5, $P < 10^{-15}$), SNP1 (LRT = 41.6, $P < 10^{-10}$) and Del (LRT = 32.5, $P < 10^{-8}$). On this family panel, all bay and cream horses were homozygous for alternative alleles for SNP8 while all diluted non-cream were heterozygous, as expected for a complete association. On the contrary, discrepancies occurred for SNP1 and Del, where heterozygous bay individuals were encountered as well as homozygous diluted non-cream individuals.

In addition, the SNP8 mutation was typed by sequencing the *MATP* exon 2 of the 141 individuals of our resource panel showing a complete association between SNP8 and the coat colour: all cream horses are homozygous A/A, all palominos and buckskin horses are heterozygous A/G, and all bay horses are homozygous G/G.

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HORSE MATP^Cr  ATGGGTGGCAACAGTGGGCAGCCTGGCGTTCCCACCTATAAGTCCCTAGCTGAGGATGGCCCCTTTGGCTCCGTGGAGCT 80
                M  G  G  N  S  G  Q  P  G  V  P  T  Y  K  S  L  A  E  D  G  P  F  G  S  V  E  L
HORSE MATP     ATGGGTGGCAACAGTGGGCAGCCTGGCGTTCCCACCTATAAGTCCCTAGCTGAGGATGGCCCCTTTGGCTCCGTGGAGCT 80
                M  G  G  N  S  G  Q  P  G  V  P  T  Y  K  S  L  A  E  D  G  P  F  G  S  V  E  L
MOUSE MATP     ATGAGTGGAAGCAATGGGCCGACTGACACCCATACCTATCAATCCTTAGCCGAGGATTGCCCCTTTGGCTCTGTGGAGCA 80
                M  S  G  S  N  G  P  T  D  T  H  T  Y  Q  S  L  A  E  D  C  P  F  G  S  V  E  Q
HUMAN MATP     ATGGGTAGCAACAGTGGGCAGGCTGGCCGCCACATCTATAAATCCCTAGCTGATGATGGCCCCTTTGACTCTGTGGAGCC 80
                M  G  S  N  S  G  Q  A  G  R  H  I  Y  K  S  L  A  D  D  G  P  F  D  S  V  E  P

HORSE MATP^Cr  GCCCAAAAGATCCACGGGCAGGCTCGTCATGCACAGCATGGCCATGTTCGGCCGGGAATTTTGCTACGCGGTGGAGGCGG 160
                P  K  R  S  T  G  R  L  V  M  H  S  M  A  M  F  G  R  E  F  C  Y  A  V  E  A
HORSE MATP     GCCCAAAAGATCCACGGGCAGGCTCGTCATGCACAGCATGGCCATGTTCGGCCGGGAATTTTGCTACGCGGTGGAGGCGG 160
                P  K  R  S  T  G  R  L  V  M  H  S  M  A  M  F  G  R  E  F  C  Y  A  V  E  A
MOUSE MATP     ACCCAAGAGATCCACAGGGAGACTTGTCATGCACAGCATGGCCATGTTTGGCCGAGAGTTTTGCTATGCGGTGGAGGCAG 160
                P  K  R  S  T  G  R  L  V  M  H  S  M  A  M  F  G  R  E  F  C  Y  A  V  E  A
HUMAN MATP     GCCTAAAAGACCCACCAGACATCATCATGCACAGCATGGCCATGTTCGGAAGAGAGTTCTGCTACGCGGTGGAGGCAG 160
                P  K  R  P  T  S  R  L  I  M  H  S  M  A  M  F  G  R  E  F  C  Y  A  V  E  A

HORSE MATP^Cr  CCTACGTGACCCCAGTCCTGCTCAGCGTGGGCCTGCCCAAGCGTCTGTACAGCGTGGTGTGGCTGCTCAGTCCCGTCCTG 240
                A  Y  V  T  P  V  L  L  S  V  G  L  P  K  R  L  Y  S  V  V  W  L  L  S  P  V  L
HORSE MATP     CCTACGTGACCCCAGTCCTGCTCAGCGTGGGCCTGCCCAAGCGTCTGTACAGCGTGGTGTGGCTGCTCAGTCCCGTCCTG 240
                A  Y  V  T  P  V  L  L  S  V  G  L  P  K  R  L  Y  S  V  V  W  L  L  S  P  V  L
MOUSE MATP     CTTATGTGACTCCAGTTCTGCTCAGCGTGGGCCTGCCTAAGAGCCTGTACAGCATGGTGTGGCTCCTAAGCCCCATCTTG 240
                A  Y  V  T  P  V  L  L  S  V  G  L  P  K  S  L  Y  S  M  V  W  L  L  S  P  I  L
HUMAN MATP     CGTATGTGACCCCAGTCCTGCTCAGCGTAGGTCTGCCCAGCAGCCTGTACAGCATTGTGTGGTTCCTCAGCCCCATCTG 240
                A  Y  V  T  P  V  L  L  S  V  G  L  P  S  S  L  Y  S  I  V  W  F  L  S  P  I  L

HORSE MATP^Cr  GGATTCCTGCTGCAACCTGGTGGTGGGATCGGCCAGCGACCACTGCCGGGCCAGGTGGGGCCGGCGGGAGACCCTACATTCT 320
                G  F  L  L  Q  P  V  V  G  S  A  S  D  H  C  R  A  R  W  G  R  R  R  P  Y  I  L
HORSE MATP     GGATTCCTGCTGCAACCTGGTGGTGGGATCGGCCAGCGACCACTGCCGGGCCAGGTGGGGCCGGCGGGAGACCCTACATTCT 320
                G  F  L  L  Q  P  V  V  G  S  A  S  D  H  C  R  A  R  W  G  R  R  R  P  Y  I  L
MOUSE MATP     GGATTCCTGCTCCAGCCTGTGGTGGGATCAGCCAGTGATCACTGCAGGGCCCGTTGGGGTCGCCGGGAGACCATACATCCT 320
                G  F  L  L  Q  P  V  V  G  S  A  S  D  H  C  R  A  R  W  G  R  R  R  P  Y  I  L
HUMAN MATP     GGATTCCTGCTGCAGCCCGTGGTCGGATCGGCCAGCGACCACTGCCGGTCCAGGTGGGGCCGCCGGAGACCCTACATCCT 320
                G  F  L  L  Q  P  V  V  G  S  A  S  D  H  C  R  S  R  W  G  R  R  R  P  Y  I  L

HORSE MATP^Cr  GGCCCTGAGCGTCATAATGCTCCTGGGTATGGCTCTGTACCTCAACGGGGACGCGTGTCATATCAGCTTTGATTGCTGACC 400
                A  L  S  V  I  M  L  L  G  M  A  L  Y  L  N  G  D  A  V  I  S  A  L  I  A  D
HORSE MATP     GGCCCTGAGCGTCATAATGCTCCTGGGTATGGCTCTGTACCTCAACGGGGACGCGTGTCATATCAGCTTTGATTGCTGACC 400
                A  L  S  V  I  M  L  L  G  M  A  L  Y  L  N  G  D  A  V  I  S  A  L  I  A  D
MOUSE MATP     GACTCTGGCCATTATGATGCTCTTGGGAATGGCTCTGTACCTCAATGGAGATGCGGTGTCGTATCAGCTTTGGTTGCTAACC 400
                T  L  A  I  M  M  L  L  G  M  A  L  Y  L  N  G  D  A  V  V  S  A  L  V  A  N
HUMAN MATP     CACCCTGGGAGTCATGATGCTCGTGGGCATGGCTCTGTACCTCAATGGGCTACTGTTGTAGCAGCTTTGATTGCTAACC 400
                T  L  G  V  M  M  L  V  G  M  A  L  Y  L  N  G  A  T  V  V  A  A  L  I  A  N

HORSE MATP^Cr  GAAGGAAGAAGCTGACCTGGGCCATAACCATCACCATGATAGGTGTGGTTCTCTTT[A]ATTTTGCTGCTGACTTCATTGAT 480
                R  R  K  K  L  T  W  A  I  T  I  T  M  I  G  V  V  L  F  [N]  F  A  A  D  F  I  D
HORSE MATP     GAAGGAAGAAGCTGACCTGGGCCATAACCATCACCATGATAGGTGTGGTTCTCTTT[G]ATTTTGCTGCTGACTTCATTGAT 480
                R  R  K  K  L  T  W  A  I  T  I  T  M  I  G  V  V  L  F  [D]  F  A  A  D  F  I  D
MOUSE MATP     CAAGGCAGAAGCTGATCTGGGCCATAAGCATCACCATGGTAGGTGTGGTTCTCTTCTTTCTGCTGACTTCATTGAC 480
                P  R  Q  K  L  I  W  A  I  S  I  T  M  V  G  V  V  L  F  D  F  A  A  D  F  I  D
HUMAN MATP     CAAGGAGGAAGCTGGTTTGGGCCATAAGTGTCACCATGATAGGTGTCGTTCTCTTTGATTTTGCTGCCGACTTCATTGAT 480
                P  R  R  K  L  V  W  A  I  S  V  T  M  I  G  V  V  L  F  D  F  A  A  D  F  I  D

HORSE MATP^Cr  GGGCCCATCAAAGCCTACTTATTTGATGTCTGCTCCCATCAGGACAAGGAGAGGGGCCTCCACCACCAGCTCTCTTCAC 560
                G  P  I  K  A  Y  L  F  D  V  C  S  H  Q  D  K  E  R  G  L  H  H  H  A  L  F  T
HORSE MATP     GGGCCCATCAAAGCCTACTTATTTGATGTCTGCTCCCATCAGGACAAGGAGAGGGGCCTCCACCACCAGCTCTCTTCAC 560
                G  P  I  K  A  Y  L  F  D  V  C  S  H  Q  D  K  E  R  G  L  H  H  H  A  L  F  T
MOUSE MATP     GGGCCCATCAAAGCCTACTTATTTGATGTCTGCTCCCATCAGGACAAGGAGAAGGGGCCTCCACTACCGCCTCTTCAC 560
                G  P  I  K  A  Y  L  F  D  V  C  S  H  Q  D  K  E  K  G  L  H  Y  H  A  L  F  T
HUMAN MATP     GGGCCCATCAAAGCCTACTTATTTGATGTCTGCTCCCATCAGGACAAGGAGAAGGGGCCTCCACTACCGCCTCTTCAC 560
                G  P  I  K  A  Y  L  F  D  V  C  S  H  Q  D  K  E  K  G  L  H  Y  H  A  L  F  T

HORSE MATP^Cr  AG 562
HORSE MATP     AG 562
MOUSE MATP     AG 562
HUMAN MATP     AG 562
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Figure 3. Conservation of nucleotide and amino-acid wild-type sequence of the horse, mouse and human exon 1 and exon 2 of the *MATP* gene. SNP6 and SNP7 positions are represented by dots (position 32 and 165 respectively in the nucleotide sequence). SNP8 position is represented by frames (position 457 in the nucleotide sequence, and position 153 in the amino-acid sequence). Identical residues are shaded. The first exon is 385 nucleotides long. (Accession numbers AY187092, AY187093).

These results show that SNP8 is completely associated with the cream coat colour in different horse breeds and suggest that it can be considered as the causal mutation.

4. DISCUSSION

Comparative mapping predicts the localisation of the *uw* locus on ECA21 (Fig. 1), which is consistent with the results of Locke *et al.* (2001), making *MATP* a good positional candidate gene, in addition to its biological functions.

Moreover, HMS77, isolated from a MYO10 BAC in this study, was not significantly linked to the cream locus which rejects *MYO10* as the gene responsible for horse coat colour dilution.

Thus, in this report we show that the candidate gene strategy, comparative mapping, and genetic linkage support the fact that the *MATP* gene is responsible for the coat colour dilution. We describe genetic markers closely associated to *MATP*, which are strongly linked to the C^{cr} locus, and a mutation in the exon 2 of *MATP* completely linked and associated to the cream locus. The same mutation, a G to A transition in codon 153 resulting in an aspartic-acid to asparagine substitution (N153D) has been described in humans [23] and mice [5] as being responsible for hypopigmentation in the eye and fur. This mutation must be involved in a transmembrane domain of the transport protein and its substitution is likely to disrupt the secondary local structure. The gene encodes a transport protein that can be partially or totally disrupted, a situation consistent with the semi-dominant status of the observed horse phenotypes. Taking these arguments into account, we propose that the N153D (SNP8) mutation can be considered as the causative mutation for the diluted colours including buckskin, palomino and blue eye cream in the horse.

In the Connemara breed, grey colour is common and represents more than one third of our family panel. At the grey locus G, the grey (G^{G}) is a dominant allele which interacts with basic colour genes at separate loci. It can be expressed at birth or becomes expressed with age so that horses can turn grey when aging whatever the basic coat colour. In such cases, the identification of the phenotype can be puzzling and the resort to molecular tests, such as *MATP* mutation typing, can help breeders identify carriers of the C^{cr} allele otherwise obscured by the grey phenotype.

In a similar way, black horses can be heterozygous carriers for the C^{cr} allele with no or very little coat dilution, while black horses homozygous for the C^{cr} mutation have a smoky cream coat [32]. Some horses of our panel, first identified as dark bay, were found to be carriers for the C^{cr} allele. But a DNA test for the mutation at the agouti locus [27] revealed that the horses were in fact black, with a cryptic C^{cr} allele. Their coat colour was thus initially wrongly identified.

As another peculiarity, we found a horse first identified as a bay, but a carrier of the C^{cr} allele, who turned out to be buckskin, with the presence of yellow hair and spots in his coat, only noticed after *MATP* genotyping. Similarly, a cream horse who carried only one C^{cr} allele was in fact a very light palomino as revealed by his verified coat colour at birth and by his brown eyes.

Finally, the identification of coat colours is still subjected to uncertainties due to the involvement of a number of modifying genes causing shades or to alleles, such as black or grey, masking the effect of other alleles such as the cream allele. These cryptic alleles nevertheless segregate and hamper the action of horse owners to plan breeding to obtain their favourite colours.

The *MATP* mutation is the fourth causal mutation for coat colour described in horses, after the mutation responsible for chestnut [19], lethal white [21, 30] and black [27]. Therefore, these data increase the panel of molecular tools available to horse breeders for improving horse identification and enabling genetic counseling for a better efficiency in breed management.

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