

# Mutations in the *melanocortin 1 receptor (MC1R)* gene are associated with coat colours in the domestic rabbit (*Oryctolagus cuniculus*)

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## Summary

We sequenced almost the complete coding region of the *MC1R* gene in several domestic rabbits (*Oryctolagus cuniculus*) and identified four alleles: two wild-type alleles differing by two synonymous single nucleotide polymorphisms (c.333A>G;c.555T>C), one allele with a 30-nucleotide in-frame deletion (c.304\_333del30) and one allele with a 6-nucleotide in-frame deletion (c.280\_285del6). A polymerase chain reaction-based protocol was used to distinguish the wild-type alleles from the other two alleles in 263 rabbits belonging to 37 breeds or strains. All red/fawn/yellow rabbits were homozygous for the c.304\_333del30 allele. This allele represents the recessive *e* allele at the *extension* locus identified through pioneering genetic studies in this species. All Californian, Checkered, Giant White and New Zealand White rabbits were homozygous for allele c.280\_285del6, which was also observed in the heterozygous condition in a few other breeds. Black coat colour is part of the standard colour in Californian and Checkered breeds, in contrast to the two albino breeds, Giant White and New Zealand White. Following the nomenclature established for the rabbit *extension* locus, the c.280\_285del6 allele, which is dominant over c.304\_333del30, may be allele  $E^D$  or allele  $E^S$ .

**Keywords** coat colour, domestic rabbit, *extension*, *MC1R*.

Pigmentation in mammals is mainly determined by the distribution of two chemically distinct types of melanin, pheomelanin and eumelanin, which produce red/yellow and dark phenotypes respectively. The relative amount of eumelanin and pheomelanin in the melanocytes is controlled primarily by two loci, *extension* and *agouti*, which show epistatic interactions (Searle 1968). The *extension* locus encodes the melanocyte-stimulating hormone receptor, also known as the melanocortin 1 receptor (MC1R), which is a seven-transmembrane G-protein-coupled receptor (Robbins *et al.* 1993). In mammals, mutations of the *MC1R* gene causing a constitutively active receptor are dominant and produce black coat colour, whereas inactivating mutations are recessive and result in red/yellow pigmentation (reviewed in Klungland & Våge 2003).

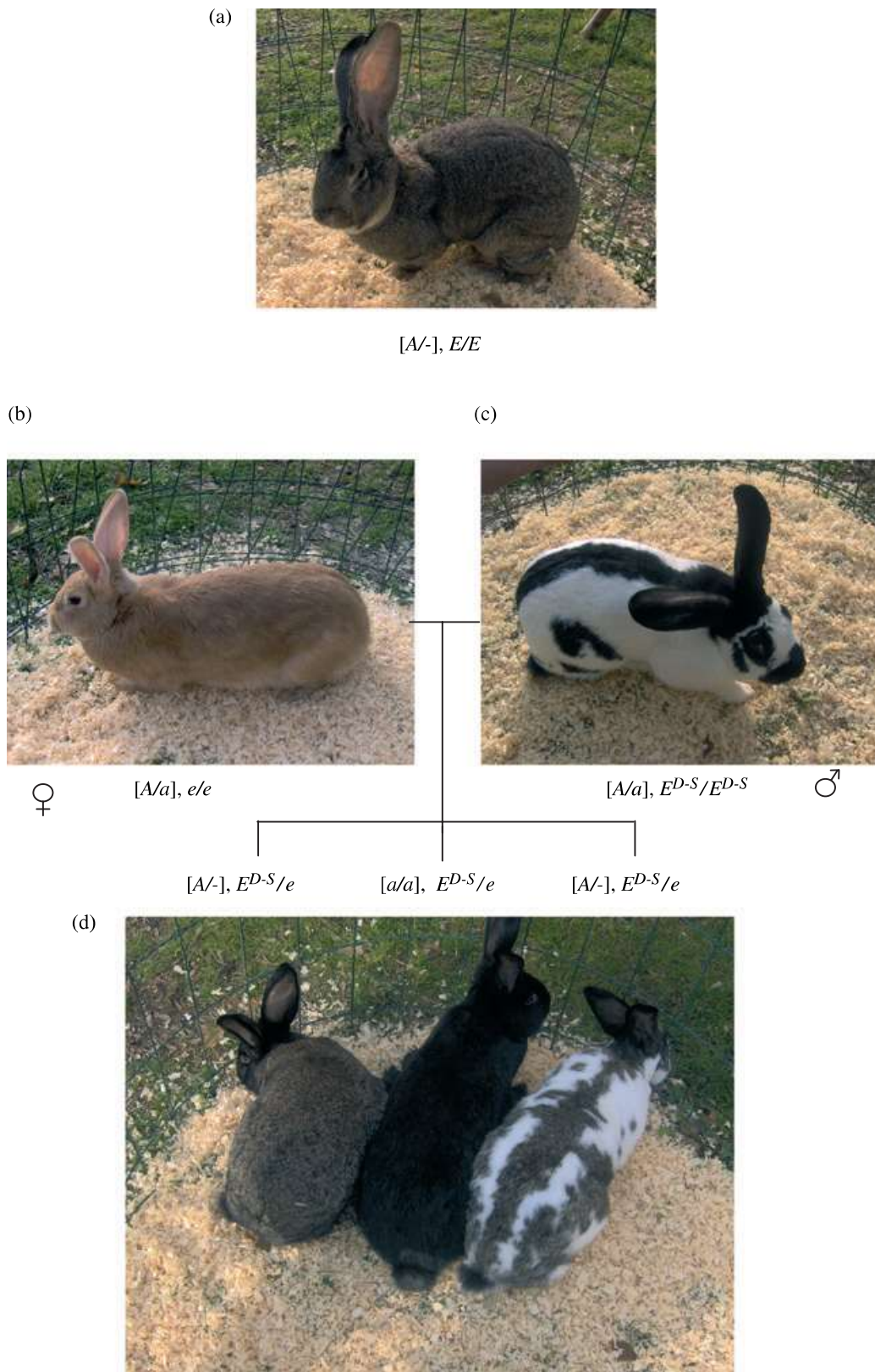
Up to now, the rabbit *extension* locus has been studied only by means of classical genetics, making it possible to assign it to linkage group VI (Fox 1994). Five alleles, listed according to their dominance level, have been reported:  $E^D$  (dominant black),  $E^S$  (steel, weaker version of  $E^D$ ),  $E$  (wild type),  $e^J$  (Japanese, black/yellow mosaic distribution) and  $e$  (non-extension of black, yellow/red) (Robinson 1958; Searle 1968; Fox 1994). Moreover, the most frequent genotype of some breeds at this locus has been deduced by pedigree studies. For example, all red or fawn breeds, like New Zealand Red, have been assigned the  $e/e$  genotype (Robinson 1958; Fox 1994). Here, we sequenced almost the complete coding sequence of the rabbit *MC1R* gene, report on the identification and analysis of mutations in different breeds and discuss the putative interactions between the *extension* and the *agouti* loci.

Sequencing of the *MC1R* gene from 16 rabbits across 12 breeds or strains showing diverse coat colours (Alaska,  $n = 1$ ; Belgian Hare,  $n = 2$ ; Blue Vienna,  $n = 1$ ; Burgundy Fawn,  $n = 2$ ; Californian,  $n = 1$ ; Checkered Giant with black markings,  $n = 2$ ; pale Siamese Coloured Dwarf,  $n = 1$ ; English Spot with Madagascar markings,  $n = 1$ ; Giant Grey,  $n = 2$ ; Russian,  $n = 1$ ; Silver,  $n = 1$ ; white commercial

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**Figure 1** (a) Giant Grey, (b) Burgundy Fawn, (c) Checkered Giant and (d) F<sub>1</sub> (obtained from a Burgundy Fawn × Checkered Giant cross) rabbits. The determined genotype at the *extension* locus, together with the assumed (a) or deduced (b, c and d) genotype at the (*agouti*) locus, is shown below each photograph. Only rabbits showing the three different F<sub>1</sub> coat colour types have been included in frame d.

hybrid,  $n = 1$ ; Table S1) was carried out. The assembled sequence of 910 bp obtained from the Giant Grey rabbits (AM180878), considered as carriers of the wild type *E* allele (Robinson 1958; Searle 1968; Fig. 1a), contained 14 bp of the 5'-untranslated region and 896 bp of the predicted 951 bp complete open reading frame. The deduced 299-amino acid sequence showed 80% identity with the corresponding human MC1R protein. The nucleotide sequence obtained from the Giant Grey rabbits was the same as obtained from the Russian rabbit but differed by two synonymous single nucleotide polymorphisms (SNPs; c.333A>G;c.555T>C) from the sequence obtained from Belgian Hare, Alaska, Blue Vienna and Silver rabbits (AM180879). The sequenced commercial hybrid was heterozygous at the two polymorphic sites.

The sequences obtained from the fawn/red/yellow animals (Burgundy Fawn, English Spot with Madagascar markings and Siamese rabbits; AM180881) showed an in-frame 30-bp deletion (c.304\_333del30) causing the loss of 10 amino acids (from positions 102 to 111) that are part of the first extracellular loop. This deletion may disrupt the functionality of the MC1R protein resulting in the yellow/red phenotype that is a characteristic of the sequenced rabbits. The 30-bp deletion eliminates the first SNP position of the wild-type alleles. However, at the second SNP position, all c.304\_333del30 sequences were T, as in the Giant Grey rabbits.

The sequences produced from Californian and Checkered Giant rabbits showed another in-frame deletion of 6 bp (c.280\_285del6; AM180880), resulting in the elimination of two amino acids at positions 94 and 95, close to the extracellular end of the second transmembrane domain in which gain-of-function missense mutations have been reported in several species (i.e. Robbins *et al.* 1993; Klungland *et al.* 1995). At the two SNP positions, all c.280\_285del6 sequences were like the Giant Grey sequence, with A and T respectively.

To analyse the two deletions in a larger sample of rabbits belonging to different breeds (Table 1) a polymerase chain reaction-based method called amplified product length polymorphism (APLP) was used (Table S1). All rabbits of breeds with typical fawn/red/yellow coat colour (Burgundy Fawn, English Lop, Gold Saxony, New Zealand Red and Thuringian) and other animals showing the pheomelanin phenotype (Madagascar, shaded yellow/brown and Siamese colour) were homozygous for allele c.304\_333del30 (Table 1). Moreover, black Dutch rabbits that were heterozygous *E/c.304\_333del30* indicated that allele c.304\_333del30 is recessive to allele *E*. An *E/c.304\_333del30* genotype was observed also in an albino commercial rabbit, but in this case the mutated *C* locus masked all other loci (Searle 1968).

Allele c.280\_285del6 was homozygous in all genotyped Californian, Checkered, Giant White and New Zealand White rabbits, and it was identified in three out of eight

Dutch animals and in the heterozygous state (*E/c.280\_285del6*) in only one Champagne d'Argent rabbit (Table 1). Black or dark blue is part of the coat colour of the listed breeds except for the two albino breeds (Giant White and New Zealand White) that cannot express any colour, as indicated above.

All other rabbits belonging to breeds with a wide variety of coat colours did not show the reported deletions and were considered having genotype *E/E* (Table 1). For these animals that showed self-black coat colour or black markings (or dark colours), the *a/a* genotype at the *agouti* locus has been suggested to determine the eumelanin phenotype (Robinson 1958; Searle 1968; Fox 1994).

To evaluate the putative dominant behaviour of allele c.280\_285del6 and the recessive mode of inheritance of allele c.304\_333del30, two crosses between Checkered Giant (with black markings and genotype c.280\_285del6/c.280\_285del6) and Burgundy Fawn (with genotype c.304\_333del30/c.304\_333del30) rabbits were carried out (Fig. 1). All eight weaned  $F_1$  animals resulted in agouti-black (with black extremities) or self-black; thus the fawn phenotype was always masked (Fig. 1). This result confirmed the recessive behaviour of allele c.304\_333del30 and indicated the dominance of allele c.280\_285del6. Thus the latter allele may mimic allele  $E^D$  or  $E^S$  (Robinson 1958; Searle 1968; Fox 1994), but because we could not distinguish which of the two alleles it corresponds to, we indicated it as  $E^{D-S}$ . Furthermore, a partial epistatic interaction between the *MC1R* alleles and alleles at the *agouti* locus may describe the presence of agouti-black and self-black rabbits that, in our crosses, resulted in the exact 3:1 ratio. Following this reasoning and inferring the parental genotype at the *agouti* locus (*A/a* for both parents; Fig. 1), the proposed model suggests that genotype *a/a* may strengthen black colour even when the rabbits are heterozygous  $E^{D-S}/e$ , while carriers of the *A* allele in such heterozygous animals may result in agouti-black production. In animals with  $E^{D-S}/E^{D-S}$  or  $e/e$  genotype, no visible effect may be obtained if the *A* allele is present at the *agouti* locus. Moreover, one-half of the  $F_1$  rabbits showed the Checkered markings, confirming that the Checkered Giant animals were heterozygous *En/en* at the *English* locus, where *En* is the mutated (semi) dominant allele that, only in the heterozygous state with the normal *en* allele (non-English spotting), confers the typical markings of the breed (Robinson 1958; Searle 1968).

In the present work, we identified two new natural mutations of the *MC1R* gene, adding further information for the disclosure of the role of amino acid residues in the functionality of this receptor. Pharmacological investigations of the c.304\_333del30 and c.280\_285del6 alleles will be important to confirm their roles in the shown phenotypes. Moreover, other studies are needed to characterize the *agouti* locus (as well as other coat colour loci) in domestic rabbits and to clarify the interaction with the *extension* locus and the inferred phenotypic effects in several breeds.

**Table 1** Breeds and animals genotyped with the amplified product length polymorphism (APLP) method.

Breeds (no. of animals)	Coat colour [proposed <i>agouti</i> and <i>extension</i> genotype] <sup>1</sup>	APLP genotype <sup>2,3</sup>				
		<i>del30/del30</i>	<i>E/del6</i>	<i>del6/del6</i>	<i>E/del30</i>	<i>E/E</i>
Alaska (7)	Self black [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	7
Belgian Hare (6)	Reddish laced with black [ <i>A/-</i> , <i>E/-</i> ]	–	–	–	–	6
Blanc de Hotot (4)	White with black markings [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	4
Blue Vienna (20)	Dark blue [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	20
Burgundy Fawn (27)	Fawn [ <i>A/-</i> , <i>e/e</i> ]	27	–	–	–	–
Californian (28)	White with black markings [ <i>a/a</i> , ?]	–	–	28	–	–
Champagne d'Argent (7)	Silver as surface colour and dark blue as under-colour [ <i>a/a</i> , <i>E/-</i> ]	–	1	–	–	6
Checkered Giant (10)	White with black (9) or blue (1) markings [ <i>a/a</i> , <i>E<sup>D</sup>/-</i> ]	–	–	10	–	–
Checkered Small (4)	White with black markings [ <i>a/a</i> , <i>E<sup>D</sup>/-</i> ]	–	–	4	–	–
Coloured Dwarf (6)	Blanc de Hotot (1) [ <i>a/a</i> , <i>E/-</i> ]; blue (1) [ <i>a/a</i> , <i>E/-</i> ]; bristle white (1) [?, ?]; hare-grey (1) [ <i>A/-</i> , <i>E/-</i> ]; Havana (1) [ <i>a/a</i> , <i>E/-</i> ]; pale Siamese (1)* [?, <i>e/e</i> ]	1*	–	–	–	5
Dutch (8)	With black markings [ <i>a/a</i> , <i>E/-</i> or <i>E<sup>D</sup>/-</i> or <i>E<sup>S</sup>/-</i> ]; with Madagascar markings (1)* [?, <i>e/e</i> ]	1*	–	3	3	1
English Lop (1)	Shaded yellow/brown [ <i>a/a</i> , <i>e/e</i> ]	1	–	–	–	–
English Spot (6)	White with black markings (5) [ <i>a/a</i> , <i>E/-</i> ]; Madagascar markings (1)* [?, <i>e/e</i> ]	1*	–	–	–	5
Ermine (4)	White albino (2) [?, ?]; white with blue eyes (2) [?, <i>E/-</i> ]	–	–	–	–	4
Fairy Marburg (3)	Grey-light blue [?, <i>E/-</i> ]	–	–	–	–	3
Fairy Pearly (7)	Pearling grey [?, <i>E/-</i> ]	–	–	–	–	7
Fox (1)	Dark blue [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	1
Giant Chinchilla (6)	Chinchilla [ <i>A/-</i> , <i>E/E</i> ]	–	–	–	–	6
Giant Grey (6)	Wild-grey [ <i>A/-</i> , <i>E/E</i> ]	–	–	–	–	6
Giant White (3)	White albino [?, ?]	–	–	3	–	–
Gold Saxony (2)	Red [?, <i>e/e</i> ]	2	–	–	–	–
Havana (3)	Dark brown [ <i>a/a</i> , <i>E/E</i> or <i>E<sup>D</sup>/E</i> ]	–	–	–	–	3
Lop (15)	Wild-grey [ <i>A/-</i> , <i>E/-</i> ]; with Madagascar markings (4)* [?, <i>e/e</i> ]	4*	–	–	–	11
Lop Dwarf (7)	White (1) [?, ?]; pearling grey (1) [?, <i>E/-</i> ]; white and Madagascar (3)* [?, <i>e/e</i> ]; Madagascar (1)* [?, <i>e/e</i> ]; shaded yellow/brown (1)* [ <i>a/a</i> , <i>e/e</i> ]	5*	–	–	–	2
Lynx (2)	Silver blue [?, ?]	–	–	–	–	2
Marten (1)	Brown [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	1
Mini Lop (2)	Wild-grey [ <i>A/-</i> , <i>E/-</i> ]	–	–	–	–	2
Mini Silver (4)	Black with silvering [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	4
New Zealand Red (7)	Red [ <i>A/-</i> , <i>e/e</i> ]	7	–	–	–	–
New Zealand White (23)	White-albino [?, ?]	–	–	23	–	–
Rex (4)	Black dalmatian (3) [ <i>a/a</i> , <i>E/-</i> ]; black (1) [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	4
Rhineland (7)	White with black and yellow markings [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	7
Russian (2)	White with black markings [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	2
Silver (5)	Black with silvering [ <i>a/a</i> , <i>E/-</i> ]	–	–	–	–	5
Tan (6)	Black fire (5) [ <i>a<sup>t</sup>/a<sup>t</sup></i> , <i>E/-</i> ]; Havana (1) [ <i>a<sup>t</sup>/a<sup>t</sup></i> , <i>E/-</i> ]	–	–	–	–	6
Thuringian (3)	Shaded yellow/brown [ <i>a/a</i> , <i>e/e</i> ]	3	–	–	–	–
White Vienna (4)	White-blue eyes [?, <i>E/-</i> ]	–	–	–	–	4
Commercial hybrids (2)	White-albino [?, ?]	–	–	–	1	1
Total (263)		52	1	71	4	135

<sup>1</sup>In breeds for which animals of different coat colour have been sampled, the number of the rabbits showing the distinctive phenotypes is indicated between parentheses. Brackets contain the proposed genotype at *agouti* (for which three alleles have been reported in rabbit: *A*, *a* and *a<sup>t</sup>*, the latter producing the tan phenotype) and *extension*, when reported or deduced from the literature (Robinson 1958; Fox 1994).

<sup>2</sup>Alleles *c.304\_333del30* and *c.280\_285del6* have been indicated as *del30* and *del6* respectively. The number of animals showing the different genotypes is reported. An asterisk (\*) has been included to link the genotyped animals with their coat colour description when more phenotypes were sampled for a breed.

<sup>3</sup>A first evaluation of the distribution of the *c.333A>G* polymorphic site (present only in the *E* and *del6* alleles) was obtained by analysing 61 rabbits of different breeds by polymerase chain reaction-restriction fragment length polymorphism (Table S1).

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## Supplementary Material

The following supplementary material is available for this article online from: <http://www.blackwell-synergy.com/doi/abs/10.1111/j.1365-2052.2006.01494.x> (This link will take you to the article abstract).

**Table S1** PCR analyses.

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