Running title: MC1R in guinea pig

Deleterious mutations of *MC1R* in Guinea Pig

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Source/description

In guinea pigs (*Cavia porcellus*) the *Extension* locus has three alleles $E > e^{p} > e$ related to black eumelanic, bicolor and brown phaeomelanic phenotypes (Figure 1), respectively [1]. This locus has been molecularly characterized as the melanocortin receptor 1 (*MC1R*) gene in other animals [2,3], and it codes for a G-coupled receptor that regulates the synthesis of eumelanins and phaeomelanins. Genetic changes causing the inactivation of the receptor lead to the exclusive production of yellow-brown phaeomelanins, while the constitutive activation is linked to the synthesis of black eumelanins [2]. In this context, the objective of this study is to molecularly characterize *MC1R* and its variations in guinea pig, and to assess their associations with the coat colors of the *Extension* locus.

Animals/Methods

Ear clips of 5 individuals (1 brown, 2 black and 2 bicolor, all spotted in white, Figure 1) and two F2 experimental crosses generated from unrelated brown males including 11 (5 brown, 6 bicolor) and 7 (3 brown, 4 bicolor) individuals were used to obtain using the Real Pure Genomic DNA Extraction Kit (Durviz).

The region containing *MC1R* in the *C. porcellus* genome was identified using the rat sequence AB306978 as a template for the BLAST tool in ENSEMBL (http://www.ensembl.org/Tools/Blast). Primers on this region were designed with Primer3 software (http://bioinfo.ut.ee/primer3-0.4.0/) (Table S1). Reactions were carried out in a volume of 30 µl with a final concentration of 1.5 mM MgCl2, 200 µM dNTPs, 0.2 µM of each primer, 25 ng of genomic DNA and 0.3 U DNA polymerase (Biogen). The thermal profile was 94ºC 3 min followed by 35 cycles of 94ºC 30 s, 60ºC 1 min and 30 s and 72ºC 1 min and 30 s, followed by a final extension at 72ºC 5 min. Fragments were purified with ExoSAP-IT (Thermo Fisher Scientific) and subsequently sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems) using both PCR primers. Sequences were aligned with Multalin software (http://multalin.toulouse.inra.fr/multalin/). The effects of new non-silent polymorphisms were assessed with Panther-Psep [4] and PolyPhen-2 [5].

Polymorphisms

Three polymorphisms causing two amino acid changes were identified in the coding region (PCR1, Table S1). The distribution of these haplotypes and their dominance patterns point out to a correspondence to the *E*, e^{p} and *e* alleles (Table 1).

The p.Leu250Gln is found only in brown animals and it is a strong candidate to have deleterious effects both in Panther-Psep and Polyphen predictions (p = 0.94). Thus, the *MC1R*2* allele might correspond with the *e* allele of the *Extension* locus, as the inactivation of the receptor is most likely to cause the production of brown phaeomelanin [2]. An experimental

cross confirmed this association: in the F2, brown animals were *MC1R*2* homozygotes while of the bicolor animals, 1 was homozygote *MC1R*3* and 5 were heterozygote.

The p.Ala291Val mutation is predicted to be probably benign by Panther-Psep and it has a relatively low Polyphen probability (p = 0.617) to be damaging. Therefore, this polymorphism doesn't seem capable to alter the receptor's functionality. Additionally, the two different alleles *MC1R*2* (*e,* brown) and *MC1R*3* (e^p, bicolor) share the Valine in position 291, reinforcing the idea that it is not a good candidate to be the cause of phenotypic changes.

It was impossible to amplify *MC1R* in the brown guinea pigs of the second experimental cross. This suggested a deletion in this genomic region. This location was investigated by blindly amplifying up to 8 kb upstream and downstream, until a fragment within the expected size range was produced (PCR2, Table S1). After sequencing, a deletion of 2760 pb including all the *MC1R* coding region was found. All the brown animals of this cross were genotyped (PCR3, Table S1) and were found to be homozygote for this deletion, confirming its association with the brown phenotype and the existence of a second *e* allele (Table 1). With these results, it becomes clearer that the brown phenotype in *C.porcellus* is caused by a loss of function of the receptor MC1R. Thus, the predicted deleterious effects of p.Leu250Gln are further supported.

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References

1. Ibsen (1919) *Genetics* **4**. 595-606.
2. Robbins *et al.* (1993) *Cell* **72**. 827-34.
3. Vidal *et al.* (2010) *Poult Sci* **89**. 1599-1602.
4. Tang *et al.* (2016) *Bioinformatics* **32**. 2230-32.
5. Adzhubei *et al.* (2010) *Nat Methods* **7**. 248-9.

Table 1. Polymorphic positions and resulting haplotypes.

Haplotype	Genbank accession number	*Extension* locus alelle	Position		
			DNA		
			(Amino acid)		
			654	749	872
				(250)	(291)
*MC1R*1*	MH444202	*E*	T	T	C
				(Leu)	(Ala)
*MC1R*2*	MH444203	*e*	C	A	T
				(Gln)	(Val)
*MC1R*3*	MH444204	e^p	-	-	T
					(Val)
*MC1R*4*	MH450218, MH450219	*e*	Deletion		

Figure 1. Fur colors in cavies: brown (left), bicolor (middle) and black (right), all spotted on white.