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SHORT COMMUNICATION

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MC1R polymorphism associated with plumage color variations in *Coturnix chinensis*

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Summary

The *melanocortin 1 receptor* (*MC1R*) gene was investigated as a candidate for plumage variations in Chinese painted quail, *Coturnix chinensis*. Four silent and two missense nucleotide polymorphisms were identified. The correspondent amino acid changes, p.Glu92Lys and p.Pro292Leu, were found in Blue Face and Red Breasted animals respectively. Blue Face is a melanic phenotype similar to the co-dominant Extended Brown of Japanese quail, and both share the p.Glu92Lys mutation. The association of p.Pro292Leu with the recessive Red Breasted was confirmed in 23 animals from an experimental F2 cross.

Keywords coloration, feather, polymorphism, quail

Melanocyte production of black eumelanins and red phaeomelanins is regulated mainly by the activity of the melanocortin 1 receptor (MC1R) and has great influence in fur or plumage color. The *MC1R* gene has only one exon and has been reported to cause color variation in both mammals and birds (Andersson 2003). In chicken (Kerje *et al.* 2003), quail (Nadeau *et al.* 2006) and bananaquit (Theron *et al.* 2001), the same polymorphism, p.Glu92Lys, causes dominant black plumages likely through a MC1R constitutive activation (Robbins *et al.* 1993). In contrast, recessive pheomelanic colors have been linked to the inactivation of the receptor, at least in mammals (Kijas *et al.* 1998).

Chinese painted quail (*Coturnix chinensis*) is the smallest of the domesticated quail species and has an accentuated sexual dimorphism in plumage color. Wild females display a brown coloration similar to that of Japanese quail, whereas Wild males show a general blue pattern on its back and a red tail (Fig. 1). Because breeders value the colors of these animals, several plumage varieties have already been established. Some of these are compatible with variations in melanin content, such as the incomplete dominant Blue Face and maybe the recessive Red Breasted. Interestingly, although Blue Face males and females have darkened eumelanic plumages, there is a strong contrast between the Red Breasted sexes: females display lighter brown colors, whereas males display a completely blackened face and a ventral extended red patch (Fig. 1).

Thirteen quails of two different origins displaying Wild ($n = 5$), Blue Face ($n = 3$) and Red Breasted ($n = 5$) plumages were sampled to assess *MC1R* variability. A Wild male and a Red Breasted female were used to generate an experimental cross, and 15 Wild and eight Red Breasted F2 individuals were analyzed. In all cases, DNA was extracted from growing feathers as described by Vidal *et al.* (2010a).

Primers MC1R-F1 5′-ACGGCCCCAGCCAGGGGTCCT-3′ and MC1R-R1 5′-AGGCACACATCACTGCAAAG-3′ were designed from published sequences of *C. chinensis* (accession no. AB201632) and *Gallus domesticus* (accession no. D78272.1) to amplify 1200 bp including the full coding region of the gene. Two internal primers (MC1R-F2 5′-CCTCATCCTCATCGTCACCT-3′ and MC1R-R2 5′-TACCAGGAGCACAGCACCAC-3′) were designed to fully sequence the 942 bp of the coding region of the *MC1R* gene. PCR products were purified with the ExoSAP-IT PCR Product Cleanup (Thermo Fisher Scientific) and sequenced with the BigDye Terminator v3.1 Cycle Sequencing kit (Thermo Fisher Scientific). Sequences were aligned using MULTALIN software (Corpet 1988). The effects of new non-silent polymorphisms were assessed with PANTHER-PSEP (Tang & Thomas 2016).

The sequencing of 13 individuals allowed for the identification of six polymorphisms comprising five haplotypes (see Table 1). Two of the polymorphisms are missense and cause the amino acid changes p.Glu92Lys (haplotype H3) and p.Pro292Leu (haplotypes H4 and H5).

The p.Glu92Lys change has been demonstrated to cause dominant melanism in mice (Robbins *et al.* 1993), chicken (Takeuchi *et al.* 1996) and bananaquit (Theron *et al.* 2001). This melanism is related to a constitutive activation of the

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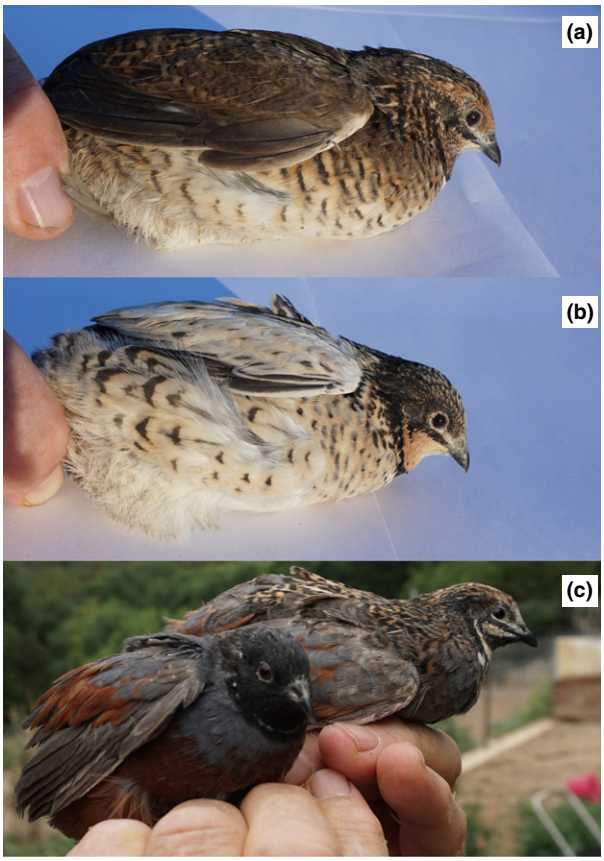

Figure 1 Plumages of Wild and Red Breasted quail. (a) Wild female, (b) Red Breasted female, (c) (front) Red Breasted and (back) Wild males. A complete color chart can be found at http://www.featheredobsessions.com/Button_Quail_Colors.php.

MC1R receptor, which increases the concentration of cAMP inside the melanocytes, and eumelanins are synthesized almost exclusively (Robbins *et al.* 1993; Ling *et al.* 2003). Interestingly, in Japanese quail, this same polymorphism has been associated with the darker Extended Brown plumage (Nadeau *et al.* 2006), an autosomal incomplete dominant mutation. The constitutive activation of MC1R has been suggested as the molecular mechanism for this phenotype as well (Nadeau *et al.* 2006). We found that all Blue Face animals carried one copy of haplotype H3, whereas none of the others animals did. Because the pattern of inheritance of this melanic plumage mimics the Extended Brown of Japanese quail, and although the number of available samples is low, we suggest that an activation of MC1R caused by p.Glu92Lys is a likely mechanism explaining this phenotype in Chinese painted quail.

The sequencing of five Red Breasted animals showed that three of them were homozygous for the H4 haplotype and two were homozygous for H5. These haplotypes differ in one silent position, g.601C>T, and both carry the g.872C>T missense polymorphism causing a p.Pro292Leu amino acid change (see Table 1). An experimental F2 cross was generated to confirm its association with the Red Breasted plumage, and 15 Wild and eight Red Breasted F2 animals were genotyped. As expected all the Red Breasted animals were homozygous for the H4 haplotype, whereas of the Wild animals, 10 were heterozygous H1H4 and five were homozygous H1.

The analysis of the p.Pro292Leu polymorphism using PANTHER-PSEP (Tang & Thomas 2016) suggests a high probability of deleterious effects. So far, deleterious mutations of this gene in mammals have been related to an increase in phaeomelanin production caused by the receptor's inactivation. This mechanism has been described in yellow mice (Robbins *et al.* 1993), and it likely explains red coloration in other mammals, such as pig (Kijas *et al.* 1998). In humans, MC1R mutations causing red hair are considered to act in this same way, through deleterious mutations. Interestingly, a polymorphism with very intense effects, p.Asp294His, has been located in the transmembrane domain 7 (Lightner 2008), which is therefore suspected to be functionally relevant. Both p.Asp294His and p.Pro292Leu are thus highly likely to be deleterious by altering this transmembrane domain.

In this sense, a hypothetical loss of function caused by p.Pro292Leu would be compatible with Red Breasted being recessive; however, the colors found in Red Breasted (especially males) do not match other typical *MC1R* phenotypes. In fact, clear *MC1R* pheomelanic phenotypes have not been found in birds yet (Roulin & Ducrest 2013), and brown and red plumages have been associated with *SOX10* in chickens (Gunnarsson *et al.* 2011) and pigeons (Domyan *et al.* 2014).

Table 1 MC1R variation in Chinese painted quail.

			DNA (amino acid)[1]					
Haplotype	Genbank accession no.	Allele	117	271 (92)	601	633	714	872 (292)
H1	MG520490	*Wild*	C	G (Glu)	C	G	G	C (Pro)
H2	MG520492	*Wild*	T			A		
H3	MG520491	*Blue Face*		A (Lys)			A	
H4	MG520493	*Red Breasted*						T (Leu)
H5	MG520494	*Red Breasted*			T			T (Leu)

[1]Position in Japanese quail protein sequence accession no. BAD91489.1.

Interestingly, *MC1R* polymorphisms that could be inactivating the receptor have been linked to recessive dark phenotypes in guinea fowl (Vidal *et al.* 2010b) and turkey (Vidal *et al.* 2010a). These phenotypes do not display the typical white dots found in Wild guinea fowl nor the white bars of Wild plumage turkeys, thus resulting in a darker general appearance. It could be possible then that these colorations are not related to neat increases in eumelanin synthesis.

In this context, the association of the putatively deleterious p.Pro292Leu mutation with the Red Breasted quail could be in consonance with the inactivation of the receptor in birds not directly affecting eumelanin/pheomelanin balance but, instead, plumage pattern and/or color distribution. This could imply a significant difference in the function of MC1R of mammals and birds that should be further studied.

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