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Sequence Variation of Melanocortin 1 Receptor (*MC1R*) Gene and Association with Plumage Color in Domestic Geese

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In contrast to other domestic and wild animals, the genetic variation of *MC1R* gene and association with plumage color has not been investigated in domestic goose. In the present study, we sequenced 714 bp fragment of the *MC1R* for 176 individuals from two goose breeds with three plumage color pattern (Landes goose, gray plumage, $n=58$; Landes goose, spotted plumage, near black, $n=36$; Zhedong White Goose, white plumage, $n=82$). A total of five single nucleotide polymorphisms (SNPs) were detected, including c.210C>T, c.321C>T, c.411C>T, c.525C>T, and c.756G>A, which subsequently determined seven haplotypes (H1−H7). Among them, H2 and H4 were the predominant haplotypes. Association analysis revealed that haplotypes H2 and H5 were significantly associated with white plumage of Zhedong white goose, whereas H3 was significantly associated with gray plumage in Landes goose ($p<0.01$). Diplotypes H2H2 and H3H4 were associated with white plumage in Zhedong white goose and gray plumage in Landes goose ($p<0.01$), respectively. The results suggested that the genetic variation of *MC1R* is also significantly associated with the plumage color in domestic goose.

Key words: Landes goose, *MC1R*, plumage color, SNPs, Zhedong white goose

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Introduction

The dazzling plumage is always the favorable appearance for all kinds of birds, which is determined by the relative content of two main melanin pigment of eumelanin (black-brown pigment) and phaeomelanin (yellow-red pigment) in melanocyte. Genetically, only a few loci are involved in this process, such as agouti signaling protein (*ASIP*), tyrosinase (*TYR*), and tyrosinase-related protein 1 (*TYRP1*) (Chang *et al*., 2006; Hiragaki *et al*., 2008). Previous studies in mammals had suggested that the *Extension* (E) locus, which encodes the melanocortin 1 receptor (MC1R), plays a critical role for regulating biosynthesis and distribution of eumelanin and phaeomelanin (Robbins *et al*., 1993; Jackson *et al*., 1994).

MC1R is a G protein-coupled seven-pass transmembrane domain receptor and contains ~310 amino acids (Cone *et al*., 1996; Ollmann *et al*., 1998). After linking to ligands of α-melanocyte stimulating hormone (α-MSH) and adrenocorticotrophic hormone (ATCH), MC1R causes a change of the G-protein coupling the receptor from inactive guanosine diphosphate (GDP) to active form guanosine triphosphate (GTP), which subsequently triggers Adenylate Cyclase system in membrane and cAMP synthesis. This cAMP finally leads to activation and production of huge amount of the rate-limiting melanogenic enzyme tyrosinase and increased production of dark (eumelanin). In contrast, the biogenesis of tyrosinase would be reduced when the above pathway was disturbed, which will result in a wide expression of phaeomelanin in melanocytes (Robbins *et al*., 1993; Ballotti, 2000; Slominski *et al*., 2004).

Therefore, *MC1R* gene mutations have been proposed to be associated with different coat color phenotypes in a number of mammals species, such as mouse (Robbins *et al*., 1993), cattle (Klungland *et al*., 1995), horse (Marklund *et al*., 1996), sheep (Vage *et al*., 1999), fox (Vage *et al*., 1997), goat (Fontanesi *et al*., 2009), dog (Newton *et al*., 2000), rabbit (Fontanesi *et al*., 2006), but not in yak (Chen *et al*., 2009). In avian, until now, limited information was available for the genes controlling the plumage and skin pigmentation. Takeuchi *et al*. (1996a) first cloned and sequenced the *MC1R* gene in chicken. Subsequent studies showed that *MC1R* gene variants were also associated with plumage color vari-

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ation in chicken (Takeuchi *et al*., 1996b), bananaquits (Theron *et al*., 2001), and Japanese quail (Nadeau *et al*., 2006).

China has a rich genetic resource of domestic goose, which can be divided into Chinese goose and Yili goose (Qiu *et al*., 1988). The ancestor of Chinese goose is *Anser cygnoides* and the Yili goose is domesticated from *A. anser*. Zhedong white goose (*A. cygnoides*) is a local breed distributed in the Eastern Zhejiang province of China, which is known as the fast growth rate and good meat quality. The Landes goose (*A. anser*) is initially produced in Landes of South France, which is a special variety of world famous goose foie gras. Landes goose has dust-color plumage, near black neck-dorsum color, silver gray breast, and white undersides. However, there is no report for the *MC1R* gene variants and association with plumage color yet. The aim of the present study is to explore the possible association between SNPs of the *MC1R* gene and goose feather color phenotype.

Materials and Methods

Animals and Phenotypes

We totally collected 176 blood samples, including 58 gray plumage Landes geese (completely gray at dorsum and breast), 36 spotted feather Landes geese (near black at dorsum and breast), 82 white plumage Zhedong white geese (pure white) (Figure 1). All samples used in this study were collected from the Research Poultry Farm Veterinary Research Institute of Shanghai Academy of Agricultural Sciences and were raised with the same levels of nutrition and management in poultry house. This study was approved by the Institutional Animal Care and Use Committee of Sichuan Agriculture University.

DNA Extraction, Amplification and Sequencing of the MC1R Gene

The genomic DNA was extracted from whole blood by the standard phenol/chloroform method. Part of coding region of the *MC1R* gene (754 bp) was amplified using primers: M-F: 5′-GCCCAATGAACTCTTCCTCAC-3′ and M-R: 5′-CACCGAGTTGCAGATGATGAG-3′, which were designed based on the *MC1R* gene encoding sequence (GenBank Accession Number FJ170062). PCR reaction was carried out in a total volume of 50 μL (including 100 ng genomic DNA, 1.5 μL of each primers, 25 μL 2xTaq Master Mix [TaKaRa Biotech Co. Ltd., Dalian, China] and 18 μL ddH_2O) under the following thermal cycle conditions: a denaturation cycle at 94℃ for 5 min, followed by 35 cycles at 94℃ for 30 s, 53.5℃ for 45 s, 72℃ for 50 s, and ended with an extension cycle at 72℃ for 10 min. PCR products were purified with centrifugal columnar agarose gel DNA extraction kit (Tian Gen Biotech Co. Ltd., Beijing, China) and were sequenced directly on an ABI Prism 3700 DNA sequencer.

Data Analysis

Sequences were edited and aligned by DNAstar package (DNASTAR, Madison, WI, USA). We used MEGA 4.0 to export sequence variations. Haplotypes were deduced by using the PHASE 2.0 program (Stephens *et al*., 2001). The Hardy-Weinberg equilibrium (HWE) was examined when more than one genotype were observed in a certain breed. In order to evaluate the potential association between the *MC1R* alleles and plumage colors, chi-square test for independence was performed using SAS V8.1 (SAS Institute Inc. Cary, NC, USA).

Results

We obtained 714 bp coding fragment of the *MC1R* gene, which was deduced to encode 238 amino acids. A total of five SNPs (c.210C>T, c.321C>T, c.411C>T, c.525C>T, and c.756G>A) were detected in 176 samples. Among them, SNPs c.210C>T, c.321C>T, and c.756G>A were only found in Zhedong White goose, while c.411C>T and c.525C>T were exclusively found in Landes goose. All these SNPs were synonymous and did not cause amino acid change. The allele and genotype frequencies of these SNPs were displayed in Table 1.

A total of seven haplotypes (H1-H7) were determined (Table 2). Among them, haplotypes H1, H2, H5, H6, and H7 were only found in Zhedong white goose, whereas H3 and H4 were exclusively found in Landes. Haplotype H2 was a dominant haplotype of Zhedong white goose and H4 had the highest frequency in Landes. Haplotypes H2 and H5 were

Fig. 1. **The plumage color of (A) gray plumage Landes goose, (B) spotted feather Landes goose, and (C) Zhedong white goose.**

Table 1. **Genetic variants of the *MC1R* gene in goose populations**

SNP	Breed	Mutant allele frequency	Genotype frequency[a]			HW[b]
			RR	RV	VV	
c.210C>T	Gray feather Landes	0.00	0.33 (58)	0.00 (0)	0.00 (0)	—
	Spotted feather Landes	0.00	0.20 (36)	0.00 (0)	0.00 (0)	—
	Zhedong White goose	0.11	0.36 (64)	0.10 (18)	0.00 (0)	0.26
c.321C>T	Gray feather Landes	0.00	0.33 (58)	0.00 (0)	0.00 (0)	—
	Spotted feather Landes	0.00	0.20 (36)	0.00 (0)	0.00 (0)	—
	Zhedong White goose	0.16	0.33 (58)	0.13 (22)	0.01 (2)	0.96
c.411C>T	Gray feather Landes	0.55	0.00 (0)	0.15 (26)	0.18 (32)	0.03
	Spotted feather Landes	0.41	0.00 (0)	0.02 (4)	0.18 (32)	0.72
	Zhedong White goose	0.00	0.47 (82)	0.00 (0)	0.00 (0)	—
c.525C>T	Gray feather Landes	0.16	0.18 (32)	0.15 (26)	0.00 (0)	0.03
	Spotted feather Landes	0.05	0.18 (32)	0.02 (4)	0.00 (0)	0.72
	Zhedong White goose	0.00	0.47 (82)	0.00 (0)	0.00 (0)	—
c.756G>A	Gray feather Landes	0.00	0.33 (58)	0.00 (0)	0.00 (0)	—
	Spotted feather Landes	0.00	0.20 (36)	0.00 (0)	0.00 (0)	—
	Zhedong White goose	0.09	0.40 (70)	0.06 (10)	0.01 (2)	0.05

[a] R=reference nucleotide; V=mutant nucleotide. The number of geese harbouring certain genotype was included in parentheses.
[b] *P* value of the Hardy-Weinberg equilibrium test only in these breeds with observable genetic variation.

Table 2. **Distribution of the *MC1R* gene haplotypes in three goose populations**

Haplotype	No	Frequency	Number of Zhedong white goose	Number of Gray feather Landes	Number of Spotted feather Landes
H1 (CCCCA)	10	0.0283	10	0	0
H2 (CCCCG)	110	0.3125	110	0	0
H3 (CCCTG)	30	0.0852	0	26	4
H4 (CCTCG)	158	0.4489	0	90	68
H5 (CTCCG)	26	0.0739	26	0	0
H6 (TCCCA)	4	0.0114	4	0	0
H7 (TCCCG)	14	0.0398	14	0	0
Total	352	1.0000	—	—	—

significantly associated with white plumage trait of Zhedong white goose, and H3 was significantly associated with gray plumage trait ($p<0.01$). Ten diplotypes were estimated on the basis of these seven haplotypes (Table 3). In particular, H3H4 and H4H4 were only distributed in Landes (with an average distribution frequency in goose with gray and spotted-plumages), whereas the other diplotypes were restricted to Zhedong white goose. Diplotype H2H2 had the highest frequency and was associated with white plumage trait in Zhedong white goose, while H3H4 was associated with gray plumage trait of Landes ($p<0.01$).

Discussion

The plumage color of poultry is highly variable and has been widely used as a morphological maker for genetic selection. In addition to the species characteristics, the plumage color is an economic trait of living poultry that would be preferred by different customers. The *MC1R* gene polymorphisms were proposed to be responsible for the plumage colors and skin traits in domestic animals (Theron *et al*., 2001; Kerje *et al*., 2003; Mundy *et al*., 2004). In human, different alleles of the *MC1R* were associated with red hair color and fair skin, as well as, skin cancer risk (Beaumont *et al*., 2005). In our previous study, we showed that there was significant association between the *MC1R* genetic variation and chicken plumage and skin colors (Yang *et al*., 2008).

In the present study, we screened the *MC1R* gene variants in two goose breeds and discerned its potential association with plumage color. We identified five synonymous mutations in 176 individuals from three breeds. Although these SNPs did not cause amino acid change, association analysis also showed that they were significantly associated with goose plumage colors ($p<0.01$). In particular, SNPs c.411C>T and c.525C>T might have a positive effect on relative amount of eumelanin. The amino acids encoded by SNPs c.411C>T and c.525C>T were located in the third and fourth transmembrane domain of the MC1R protein, respectively. Previous studies have shown that different synonymous

Table 3. **Distribution of diplotypes in different goose populations**

Diplotype	No.	Frequency	Number of Zhedong white goose	Number of Gray feather Landes	Number of Spotted feather Landes
H1H2	4	0.02273	4	0	0
H1H5	4	0.02273	4	0	0
H1H6	2	0.01136	2	0	0
H2H2	38	0.21591	38	0	0
H2H5	16	0.09091	16	0	0
H2H7	14	0.07955	14	0	0
H3H4	32	0.18182	0	28	4
H4H4	62	0.35227	0	30	32
H5H5	2	0.01136	2	0	0
H5H6	2	0.01136	2	0	0
Total	176	1.0000	82	58	36

degenerate codon on these sites would affect protein translation efficiency and structural conformation, which finally lead to phenotypic changes (Kurland, 1991; Kimchi-Sarfaty *et al*., 2007; Komar, 2007). We cautiously speculated that these two synonymous variants may impact function of the MC1R transmembrane domain conformation and activity. In addition, there would be another possibility that these significant mutations are in strong linkage with the potential causing mutation within/outside *MC1R* gene.

Haplotype analysis is useful for detecting the association between candidate gene and phenotypes by taking into consideration of accumulated allele effect on different loci and interactions (Bader, 2001). We found that diplotypes were associated with plumage colors. In particular, H2H2 was significantly associated with white trait of Zhedong white goose and H3H4 was significantly associated with gray plumage trait of Landes goose. Intriguingly, diplotypes H3H4 and H4H4 were restricted to Landes goose, whereas the other diplotypes were only presented in Zhedong white goose. These results demonstrated that *MC1R* gene diplotypes bear the characteristic of regional distribution and were associated with plumage color in goose, despite a fact that plumage color control is a very complex trait.

HWE has been widely employed to examine the assortative mating, population stratification, and even genotyping errors involved in association analysis (Wigginton *et al*., 2005). Among them, the potential population stratification would be the most potential problem in farm animals partially resulted from the non-random mating. Therefore, it is reasonable to cast more cautions to these association analysis conclusions when they are not combined with the necessary functional investigation.

Conclusion

Based on the results obtained in this study, we concluded that there are significant associations between plumage colors and genetic variants of the *MC1R* gene in geese. However, further studies are essential to confirm this conclusion.

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