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Identification of *MC1R* SNPs and their Association with Plumage Colors in Asian Duck

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The melanocortin 1 receptor (*MC1R*) gene is a candidate functional gene that controls the pigment production in melanocytes. The aim of this study was to identify polymorphisms and investigate the effect of the *MC1R* gene on plumage coloration in duck breeds, including Korean native ducks. Initially, 34 individuals from seven duck breeds were sequenced, obtaining 12 polymorphisms. Five single nucleotide polymorphisms (SNPs) in the coding region were non-synonymous, with mutations corresponding to amino acid changes. Among these, four SNPs were genotyped using polymerase chain reaction-restriction fragment length polymorphism method in 264 individuals from same seven duck breeds. Fisher's exact test was conducted to identify possible relationships between the *MC1R* gene polymorphisms and plumage color variations. Four non-synonymous SNPs, c.52A>G (p.Lys18Glu), and c.376 A>G (p.Ile126Val), c.409G>A (p.Ala137Thr) and c.649C>T (p.Arg217Cys), were associated with the two deduced genotypes (i.e., *E*/*E* and *e*+ /*e*+) based on plumage color phenotypes. In addition, we reconstructed *MC1R* gene haplotypes, where the haplotype AAGC showed its highest frequency in Nageswari duck breed, which presents an extended black phenotype. Our results indicate that the identified polymorphisms by this study can be used to explore associations with plumage color variations in Asian duck breeds.

Key words: extended black, Korean native duck, MC1R, plumage color, SNP

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Introduction

Melanin pigmentation, which in most vertebrates is regulated by the melanocyte-stimulating hormone (MSH) (Roulin and Ducrest, 2013), plays a vital role in plumage color variation in avian species (Mundy, 2005). The biological functions of MSH in mammals are regulated by five subtype of melanocortin receptors (Mountjoy *et al*., 1992; Gantz *et al*., 1993; Desarnaud *et al*., 1994, Chhajlani, 1996), including the melanocortin 1 receptor (*MC1R*).

The *MC1R* gene encodes a G protein-coupled receptor with seven transmembrane domains in the plasma membrane of melanocytes (Chhajlani and Wikberg, 1992; Mountjoy *et al*., 1992). The *MC1R* stimulates the adenylate cyclase via binding α-MSH, raising the intracellular cAMP formation, which increases the eumelanin biosynthesis through protein signaling pathways in the melanocytes. In contrast, the inhibition of cAMP production increases pheomelanin synthesis, due the presence of antagonist *Agouti* genes during hair development in mice (Hoekstra and Nachman, 2003). Extensive studies of melanism and *MC1R* variation have revealed mutations in cattle (Klungland *et al*., 1995), pigs (Kijas *et al*., 1998), chickens (Takeuchi *et al*., 1998); pocket mice (Nachman *et al*., 2003); and birds (Theron *et al*., 2001). The identification of genetic basis for color variation of *MC1R* gene has been performed in birds. On the other hand, this gene was also associated with growth and disease resistance (Ducrest *et al*., 2008; Gangoso *et al*., 2015). The production of melanin pigment is regulated by non-synonymous substitutions in the seven transmembrane domain regions from *MC1R*, with a major effect when substitutions occur in the first, second, and third domains (Theron *et al*., 2001; Mundy, 2005). For example, in bananaquits (p.Glu92Lys), lesser snow geese (p.Val185Met) and arctic skuas (p.Arg233His), a single non-synonymous

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mutation with different substitutions is associated with melanism (Mundy, 2005).

Over 40 loci are involved in controlling plumage color in chickens (Smyth, 1990), from which the *extended black* (*E*) locus is one of the most important ones. In mammals, *E* locus regulates the relative distribution eumelanin and pheomelanin (Takeuchi *et al*., 1996) which encoded by *MC1R* gene in human, mice and dog (Barsh, 2007; Robbins *et al*., 1993; Jackson, 1997). The ancestor of the domestic chickens, red jungle fowl (*Gallus gallus*) is homozygous for the e^{+} allele (Smyth, 1990). The recessive white loci have an epistatic effect on the *E* locus of *MC1R* gene and might also be located on other plumage color related genes. Therefore, the identification of the causative mutations of recessive white is difficult in white domestic duck breeds (Lancaster, 1990; Yu *et al*., 2012). Experimental studies on chickens described that *E* locus of the *MC1R* gene, located on chromosome 11, is controlling to the plumage color variants (Kerje *et al*., 2003). In addition, a substitution c.52 G>A of *MC1R* gene in domestic duck has been associated with *E* alleles (Yu *et al*., 2012).

National Institute of Animal Science (NIAS) in Korea has been collected and reared Korean native ducks (KND) mainly on the basis of plumage color and other phenotypic appearance for conservation purposes. NIAS has expanded the breeding scheme of KND as an indigenous genetic resource and investigation has already been performed on their growth, carcass yield and meat quality traits (Kim *et al*., 2012; Heo *et al*., 2013). For example, flavor-related compounds of duck meat indicated that there were no significant results among breeds, while significant between sexes (Lee *et al*., 2015). A total of seven Asian duck populations (Korean native white and black duck populations, three Bangladeshi native duck varieties (Nageswari, Deshi white and common indigenous duck) and two Chinese breeds, Jinding and Peking duck, were used in this study. In this context, DNA markers for plumage color can be useful for breed differentiation. Previously, we identified single nucleotide polymorphisms (SNPs) in four candidate genes of plumage color (*ASIP, MITF, DCT*, and *MC1R*), and examined the genetic variations between Korean native black and commercial duck breeds (Sultana *et al*., 2015). The aim of this study was to identify polymorphisms in *MC1R* gene and to investigate their association with extended black (*E*) allele in Asian duck populations.

Materials and Methods

Animals and DNA Isolation

A total of 264 ducks were sampled, consisting 180 Korean ducks from three varieties, and 84 Bangladeshi ducks from four varieties (Table 1). Blood samples of Bangladesh ducks were preserved on Whatman FTA® cards (Whatman® Inc.), and genomic DNA was extracted using PrimePrep™ Genomic DNA Isolation Tissue Kit (GeNetBio, Korea), following the provided instructions. Moreover, Korean native duck (KND) blood samples were processed to isolate DNA by using PrimePrep™ Genomic DNA Isolation Blood Kit (GeNetBio, Korea). Concentration of isolated DNA was measured using NanoDrop2000 (Thermo Scientific, USA), and then each sample was diluted for adjustment at 25 ng/μl. Finally, isolated DNAs were stored at −20℃. Thirty-four samples (three Korean native black duck, three commercial white duck, eight Korean native white duck, and 20 samples of Bangladeshi duck, five from each variety) were used to verify plumage color polymorphisms.

PCR Reaction and DNA Purification

Three pairs of primers were used (Table 2), from which two (P1 and P3) were designed via Primer3 program 0.4.0 (http: //frodo. wi. mit. edu/primer3/) based on reference sequence data (GenBank Acc: HQ190952) from National Center for Biotechnology Information (NCBI). A total of 1,174 bp linear DNA sequences were used for designing the

Table 1. **Sample information for the seven Asian duck populations**

Duck breed/Variety	No. of sample	Sample code	Origin of sample	Location of sample collection site
White Korean native duck	92	WKND	South Korea	Yongin, Gyeonggi Province, and Chungnam National University farm, Korea
Black Korean native duck	68	BKND	South Korea	NIAS and Chungnam National University farm, Korea
Commercial (Peking) duck	20	CD	South Korea	Cherry Valley, Korea
Common Indigenous duck	13	BaL	Bangladesh	Sherpur and Mymensingh districts, Bangladesh
Deshi white	20	BaW	Bangladesh	BLRI, Dhaka, Bangladesh
Jinding	15	BaJ	Imported from China	CDBF, Narayanganj, Bangladesh
Nageswari (Deshi black)	36	BaB	Bangladesh	BLRI, Dhaka; CDBF, Narayanganj; Kishoreganj and Mymensingh districts, Bangladesh
Total	264			

Table 2. **Primers for PCR amplification and restriction enzyme information for genotyping of the *MC1R* gene in Asian ducks**

Primer no.	Primer sequences (5′ to 3′)	Primer size (bp)	Location	Annealing Temp (℃)	Use	Restriction enzyme
P1	F: CCATGTCCCCTTGACCTCG R: CCACTGCAAAGAGCCTTTATTCG	1221	−141 to +1080	63	Direct sequencing	
P2	F: GCTGAGGTCGGGGCCATGT R: CCGTGGCGTTGCTCTGGTTG	91	−15 to +76	63	c.52A＞G Genotyping	*Mnl*I
P3	F: GCTCTTCATGCTGCTGATGG R: GGCAGGTGACGATGAGGATG	515	+278 to +793	63	c.376A＞G, c.409A＞G, c.649C＞T, Genotyping	*Hpy*991 *Bcl*I *Bsp*MI

primer set (P1) used to scan SNPs, and one primer pair (P2) was used by following Yu *et al*. (2012) for genotyping. Polymerase chain reaction (PCR) was carried out in 20 μl volume containing approximately 50 ng genomic DNA, 10 μl of HS Premix 10× buffer (GeNet Bio, Korea), and 0.4 μl of 10 pmol of each primer. PCR reactions were performed in My-Genie 96 Thermal Block (Bioneer, Korea) with the following steps: pre-denaturation at 95℃ for 10 min, 35 cycles of 30 s at 95℃, 30 s at 63℃ annealing temperature, 30 s at 72℃, and a final extension step at 72℃ for 10 min. After finishing the PCR reaction, products were confirmed by electrophoresis on 2% agarose gels stained with ethidium bromide (GenetBio, Korea). Each PCR fragment was purified using an AccuPrep® PCR Purification Kit (Bioneer, Korea), following the manufacturer's instruction. Purified PCR products were also confirmed by electrophoresis with agarose gels and sequencing.

Sequencing and SNP Genotyping

Initially, 34 samples were selected from seven different duck varieties of Korean and Bangladeshi duck populations for DNA fragment amplification. To identify the polymorphisms, direct sequencing method was applied for sequencing the purified DNA fragments by Cosmogenetech company (www.cosmogenetech.com). PCR-restriction fragment length polymorphism (RFLP) for all of samples was applied for genotyping (Table 2), for which approximately 15 μL of PCR product was digested with two units of each restriction enzyme in 20 μL reaction volumes, based on the recommended protocol (New England Biolabs® Inc., UK). After digestion, RFLP fragments were confirmed by electrophoresis in 3% agarose gels stained with ethidium bromide to identify genotype variations, and DNA fragments were visualized under ultraviolet light (Fig. 1).

Determination of Proposed Genotypes Deduced from Plumage Colors

In this study, we found five pigmentation phenotypes among the seven Asian duck populations, and divided them into three groups on the basis of *E* locus (Yu *et al*., 2012). Nageswari (an indigenous black duck variety of Bangladesh) which is locally called "Nagi" because of snake deity head with black bill and bean (Fig. 2). The color of the plumage, shank and web of this duck is black or penciled black and white color extended from neck to the breast. Nageswari is predicted to be homozygous for *E* allele and compiled as the extended black (*E/E*) group (Lancaster, 1990). Common indigenous (Bangladeshi duck), Jinding, and Black Korean native ducks are assumed to be wild type and homozygote for e^+ allele. These latter three duck breeds are assembled as non-extended black (e^+/e^+) group (Table 4). Commercial (Peking), white Korean native, and Deshi white ducks are arranged as recessive white (E/e^+) group.

Statistical Analysis

Fisher's exact test was conducted to assess associations between the genotypes deduced from plumage colors and SNP genotypes of the *MC1R* gene, using SAS 9.3 (SAS Institute, NC), with *P*-values＜0.05 as statistically significant. The haplotype frequencies among four SNPs from the seven duck populations were explored using Haploview software (Barrett *et al*., 2005).

Results

Identification of Polymorphisms and Genotyping

In this study, we identified a total of 12 SNPs of MC1R gene consisting of single exon (945 bp) and flanking regions (5′ and 3′). These variations were detected from exon to downstream. Among the 12 SNPs, five were detected in the exon, while seven were detected in the 3′ -untranslated region of the gene (Table 3) from the samples of seven duck varieties. In addition, Table 3 showed the amino acid changes for the polymorphisms and their effects on protein types and functions. The amino acid substitution effects on protein function were investigated by PROVEN (http://provean.jcvi.org/seq_submit.php). According to the PROVEN score (Choi *et al*., 2012), two variants, K18E and I126V, may have neutral effects on protein function, whereas other three variants (A137T, R217C, R217H) have the deleterious effects. All substitutions in the exon region were found non-synonymous, and four A↔G transition substitutions were identified among five exonic SNPs (Fig. 1). Two adjacent SNPs at position c.649C＞T c.650G＞A were identified

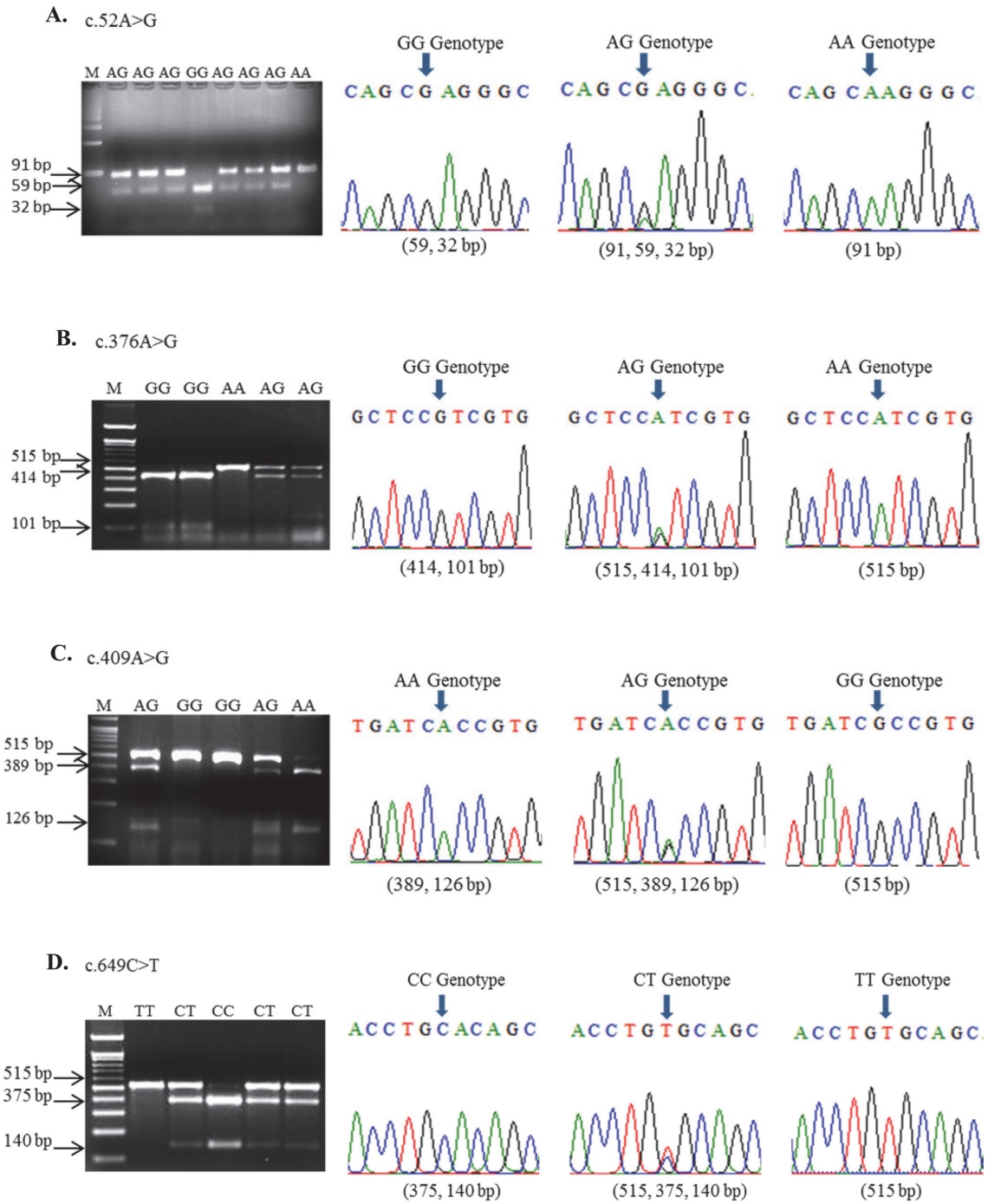

Fig. 1. **Identification of *MC1R* gene polymorphisms and conformation of PCR-RFLP genotyping among four (A, B, C, and D) coding SNPs in Asian ducks.**

from the sequenced samples of duck varieties where the SNP c.649C>T was only considered for genotyping of all samples. Finally, haplotype reconstruction was performed based on the identified four SNPs c.52A>G, c.376A>G c.409A>G and c.649C>T.

The identified genotype data of the SNPs in different Korean and Bangladeshi duck varieties are presented together with proposed genotypes based on phenotypic classification in Table 4. Birds having AA genotypes for c.52A>G SNP present higher frequencies in extended black

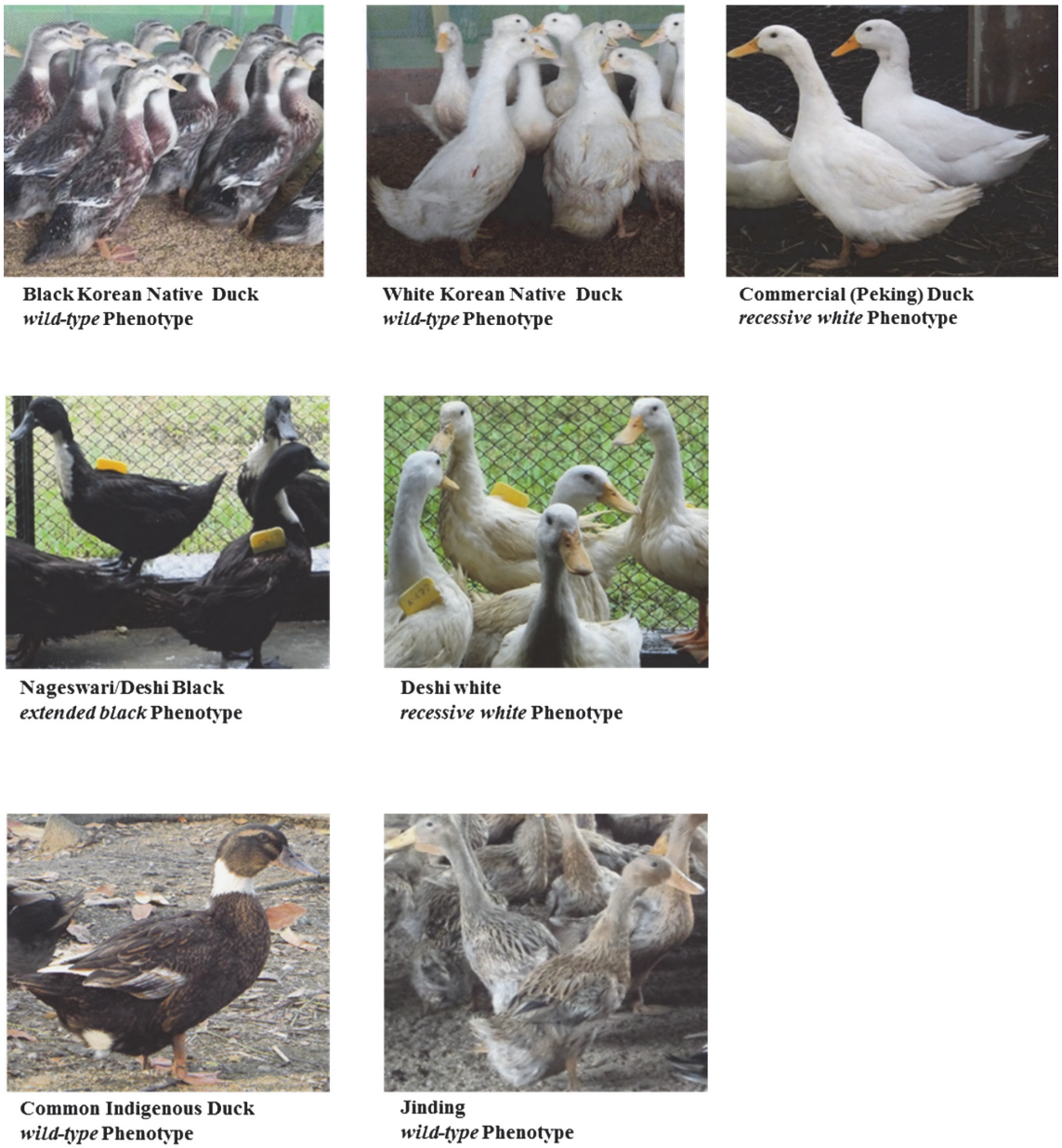

Fig. 2. **Pigmentation phenotypes for the Asian duck populations.**

group phenotype, while ducks having GG genotypes present the highest frequency within the e^+/e^+ proposed genotype group. Similar genotypic patterns for E/E and e^+/e^+ groups were detected in c.376A>G and c.52A>G SNP. On the other hand, the highest GG genotype-frequency among extended black duck phenotype is described in c.409 G>A SNP, and the highest frequency of CC genotype is present in the extended black duck phenotype (in c.649 C>T SNP) (Table 4). All associations between the missense substitutions and the proposed genotype groups were found significant (Fisher's exact test, $P<0.001$; Table 5).

The position of the substitution p.Lys18Glu is in the N-terminus extracellular membrane of the *MC1R* protein of duck is shown in Fig. 3. No substitutions were found in the second transmembrane domain, and two substitutions (p.Ile126Val and p.Ala137Thr) took place in the third one. The 4th and 5th missense mutations occurred at the same site with different substitutions (p.Arg217Cys and p.Arg217His, respectively) in the third intracellular loop.

Haplotype Frequency

The haplotypes and their frequencies of the four missense SNPs from seven Asian duck varieties are described in Table 6, with the detection of 13 haplotypes. Haplotype AAGC showed the highest frequency (75.5%) in Nageswari, and

Table 3. **Identified polymorphisms in *MC1R* gene from seven duck populations**

Nucleotide position	Amino acid change	Effect on protein due to amino acid change	Effect on protein function due to substitution*
c.52A>G	Missense, p.Lys18Glu	Basic to acidic	0.753, Neutral
c.376A>G	Missense, p.Ile126Val	Non-polar to Non-polar	−0.074, Neutral
c.409G>A	Missense, p. Ala 137Thr	Non-polar to Polar (uncharged)	−3.935, Deleterious
c.649C>T	Missense, p.Arg217Cys	Polar (positively charged) to Polar (uncharged)	−5.893, Deleterious
c.650G>A	Missense, p.Arg217His	Polar (positively charged) to Polar (positively charged)	−3.047, Deleterious
g.1019C>T	Not applicable		
g.1020A>G	Not applicable		
g.1023C>T	Not applicable		
g.1024A>G	Not applicable		
g.1028A>G	Not applicable		
g.1033A>G	Not applicable		
g.1038A>G	Not applicable		

* Variants with a score above −2.5 are considered "neutral" and variants with a score equal to or below −2.5 are considered "deleterious" (Choi *et al*., 2012)

lowest frequencies in commercial (Peking) (27.5%), white Korean native (12%), Deshi white (11%) and black Korean native ducks (5.1%). The haplotype AAGC was absent in Jinding and common indigenous ducks (Bangladesh). Haplotype GGGT presented a high frequency in black Korean native (56.4%), Deshi white (48.4%), commercial (Peking) (46.1%), and in white Korean native ducks (45.5%), and low frequencies in common indigenous ducks (Bangladesh) (5.3 %), Jinding (3.3%), and Nageswari (2.3%). The GGGT and GGGC were the only two haplotypes found in all seven duck breeds. The common indigenous duck (Bangladesh) presented the highest number of haplotypes, and the highest frequency of haplotype GGAC was identified in Jinding ducks (86.5%).

Discussion

MC1R gene is associated with extended black feather color (Yu *et al*., 2012). We observed four non-synonymous substitutions (p.Lys18Glu, p.Ile126Val, p.Thr137Ala, and p.Arg217Cys) in the *MC1R* gene in Asian ducks (Xia 2008; Huang 2010; Yu *et al*., 2012), and p.Arg217His substitution was observed exclusively in Bangladeshi duck breeds. Recently, Yu *et al*. (2012) reported that one non-synonymous substitution (p.Glu18Lys) was highly associated with the extended black variant for plumage color variations in domestic ducks. In Korean native chickens, the c.376G>A SNP causes p.Val126Ile substitution and a high frequency of GG genotype observed in Korean native black chicken, and AA genotype frequency high in White Leghorn (Hoque *et al*., 2013). In this study, we observed the same substitution, with a high frequency of the AA genotype in Nageswari, extended black duck, and a high frequency of the GG genotype in wild type and recessive white duck breeds (Table 4). The most relevant finding presented in Table 4 is that two non-synonymous SNPs, c.52A>G and c.376 A>G (p. Lys18Glu and p. Ile126Val, respectively), seem to have a major responsibility on the extended black type mutation.

The wild type plumage pattern has minor variations, which are responsible for the distinct features and appearance in some duck breeds (Lancaster, 1990). In this study, the four pigmentation phenotypes of our duck breeds were deduced by classical genetics from *MC1R* genotyping according to the color and pattern of plumage, and the *E* locus (Lancaster, 1990). The appearance of mallard pattern or wild type duck fully expresses the homozygous e^{+} allele, meanwhile the extended black duck breeds (e.g., Black Orpington, Black East Indian) expresses the homozygous *E* allele (Phillips, 1915; Jaap and Milby, 1944). The presence of extended black (*E)* locus, which is autosomal and dominant to other non-extended black (e^{+}), affects all areas to express solid black color except white spotting. Therefore, the *E* locus has the complete epistatic effect to the genes for white spotting at the *Li* and *M* loci (Lancaster, 1990). Our results from Table 5 between the two genotypes (i.e., *E*/*E* and *e*+ /*e*+) were provided the significant association of extended black (*E*) locus and *MC1R* in plumage color. Here, we found that the AAGC haplotype was mainly observed in the extended black duck group. On the other hand, haplotypes GGGT and GGGC showed higher frequencies in non-extended black and recessive white ducks (Table 6). The constructed haplotypes of this study could be applied in selection of pure stock from their base population for the development of particular breed/varieties with unique phenotypic features.

Robust evidence from domestic animals and birds suggests that most substitutions occur in the second transmembrane domain and nearby the intracellular and extracellular membrane of the *MC1R* (Robbins *et al*., 1993; Ling *et al*., 2003; Majerus and Mundy, 2003). In this study, one substitution (p. Glu18Lys), located in the extracellular N-terminus of *MC1R* could increase the eumelanin synthesis via stimulating *MC1R* activity (Yu *et al*., 2012), while in rock pocket mice it was demonstrated that a different substitution (p.Arg18Cys)

Table 4. **Genotype distribution of the SNPs in *MC1R* gene in different colored duck breeds**

Breed/Varity	No. of bird	Proposed genotypes*	Phenotype	Genotype (Number of bird)		
				c.52 A>G		
				AA	AG	GG
Black Korean native duck (BKND)	68	e^+/e^+	Wild- type	0	7	61
Commercial(Peking) duck (CD)	20	E/e^+	Recessive white	3	5	12
White Korean native duck (WKND)	92	E/e^+	Recessive white	1	20	71
Common Indigenous duck (BaL)	13	e^+/e^+	Wild- type	0	1	12
Jinding (BaJ)	15	e^+/e^+	Wild- type	0	2	13
Deshi white (BaW)	20	E/e^+	Recessive white	0	8	12
Nageswari (BaB)	36	*E/ E*	Extended black	24	11	1
				c.376 A>G		
				AA	AG	GG
Black Korean native duck (BKND)	68	e^+/e^+	Wild- type	0	17	51
Commercial(Peking) duck (CD)	20	E/e^+	Recessive white	4	6	10
White Korean native duck (WKND)	92	E/e^+	Recessive white	3	31	58
Common Indigenous duck (BaL)	13	e^+/e^+	Wild- type	1	6	6
Jinding (BaJ)	15	e^+/e^+	Wild- type	0	0	15
Deshi white (BaW)	20	E/e^+	Recessive white	3	9	8
Nageswari (BaB)	36	*E/ E*	Extended black	27	9	0
				c.409 G>A		
				AA	AG	GG
Black Korean native duck (BKND)	68	e^+/e^+	Wild- type	3	15	50
Commercial(Peking) duck (CD)	20	E/e^+	Recessive white	0	1	19
White Korean native duck (WKND)	92	E/e^+	Recessive white	2	20	70
Common Indigenous duck (BaL)	13	e^+/e^+	Wild- type	1	6	6
Jinding (BaJ)	15	e^+/e^+	Wild- type	12	3	0
Deshi white (BaW)	20	E/e^+	Recessive white	0	2	18
Nageswari (BaB)	36	*E/ E*	Extended black	0	3	33
				c.649 C>T		
				CC	CT	TT
Black Korean native duck (BKND)	68	e^+/e^+	Wild- type	14	30	24
Commercial(Peking) duck (CD)	20	E/e^+	Recessive white	5	10	5
White Korean native duck (WKND)	92	E/e^+	Recessive white	24	51	17
Common Indigenous duck (BaL)	13	e^+/e^+	Wild- type	5	6	2
Jinding (BaJ)	15	e^+/e^+	Wild- type	14	1	0
Deshi white (BaW)	20	E/e^+	Recessive white	4	10	6
Nageswari (BaB)	36	*E/ E*	Extended black	30	5	1

* *E/ E*- homozygous allele for extended black, e^+/e^+- homozygous allele for non-extended black

at the same site is associated with reduction of eumelanin (Nachman *et al*., 2003). Yu *et al*. (2012) reported that p.Glu18Lys substitution might have a salt bridge connection with the N-terminus extracellular membrane and the *MC1R* protein function, stimulating the eumelanin production. A mutation c.96G>A generated a premature stop codon (p. W32X) occurs in the extracellular N-terminus in turkeys (Vidal *et al*., 2010), which is near to the p.Lys18Glu substitution observed in ducks. In domestic chickens, mice, Japanese quails, and bananaquits, the p.Glu92Lys substitution is associated in the second transmembrane domain by the vital activation of *MC1R* gene (Robbins *et al*., 1993; Ling *et al*., 2003; Mundy, 2005; Nadeau *et al*., 2006). In the same site, another single non-synonymous substitution was found in lesser snow geese (p.Val185Met) and in cow and pig (p.Leu99Pro) (Kijas *et al*., 1998; Klungland *et al*., 1995; Majerus and Mundy, 2003). Therefore, it is suggested that, at least in ducks, the two missense SNPs, c.52A>G and c.376 A>G (p.Lys18Glu and p.Ile126Val respectively), may have a major effect on the extended black phenotypes. Additionally, the p.Val126Ile substitution in the third transmembrane domain of *MC1R* is associated with plumage pigmentation in chicken and duck (Kerje *et al*., 2003; Guo *et al*., 2010; Yu *et al*., 2012; Oh *et al*., 2014).

In this study, we identified SNPs in the *MC1R* gene, and their association with extended black genotypes deduced

Table 5. **Association analysis of the SNPs in *MC1R* gene between genotype groups**

Groups*	c.52 A>G			*P*-value	c.376 A>G			*P*-value	c. 409 G>A			*P*-value	c. 649 C>T			*P*-value
	AA	AG	GG		AA	AG	GG		AA	AG	GG		CC	CT	TT	
E/ E	24	11	1	<0.001	27	9	0	<0.001	0	3	33	<0.001	30	5	1	<0.001
e^+/e^+	0	10	86		1	23	72		16	24	56		33	37	26	

* *E/ E*- homozygous allele group for extended black, e^+/e^+- homozygous allele group for non-extended black

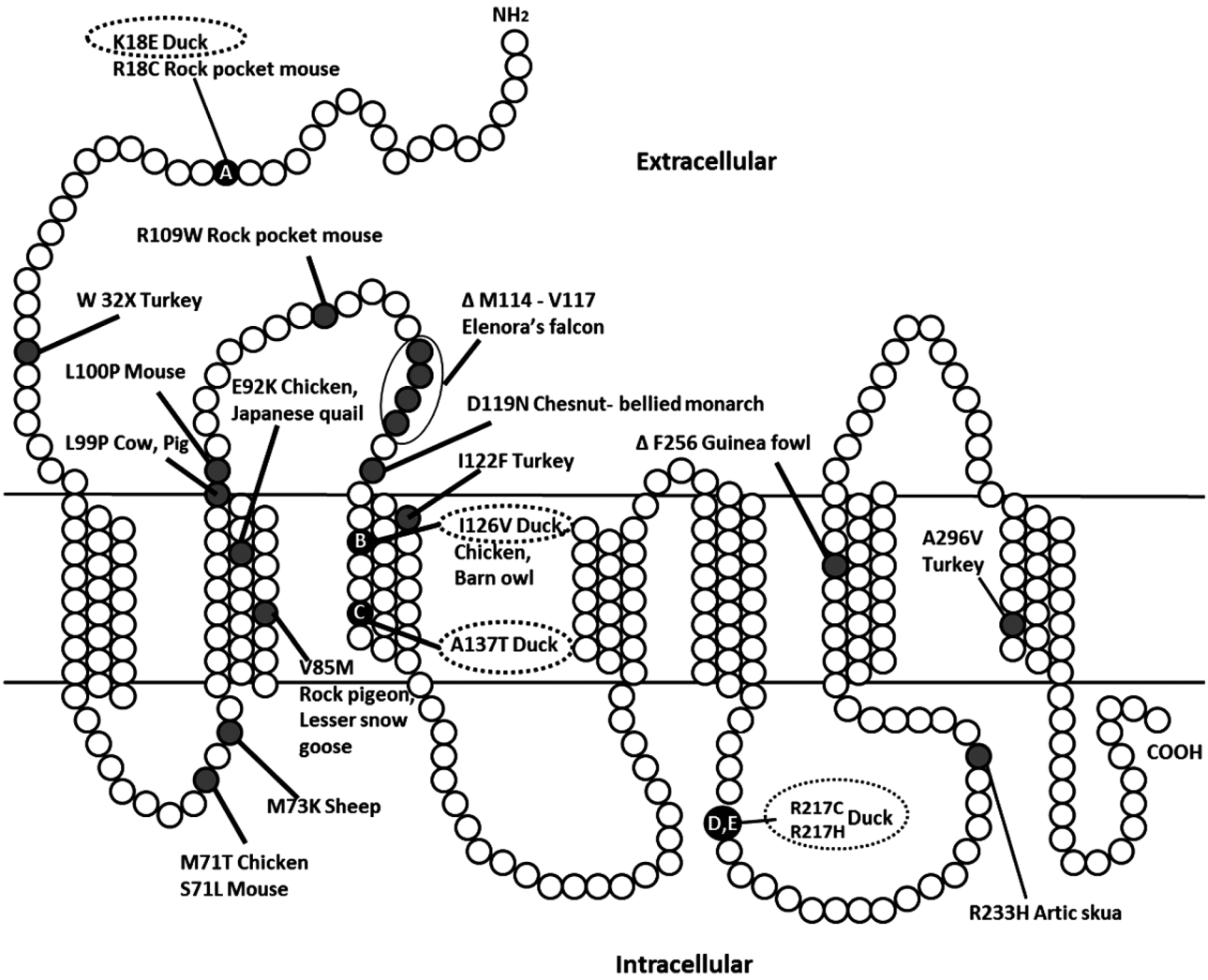

Fig. 3. **Hypothetical structure of the *MC1R* amino acid variants in birds and domestic animals.** Substitutions are shown using single letter amino acid code and solid colors show substitutions linked with melanism. We assessed the five substitutions (A, B, C, D, and E) of *MC1R* for duck. This figure is based on the references of *MC1R* diversity in birds (Theron *et al*., 2001; Kerje *et al*., 2003; Mundy *et al*., 2005; Nadeau *et al*., 2006; Yu *et al*., 2012; San-José *et al*., 2015) and in mouse (Majerus & Mundy, 2003).

Table 6. **Reconstructed haplotypes and their frequencies for the *MC1R* gene in seven duck breeds***

Haplotype	Haplotype frequencies (%)						
	BKND	WKND	CD	BaB	BaW	BaJ	BaL
GGGT	56.4	45.5	46.1	2.3	48.4	3.3	5.3
GGGC	15.7	21.3	16.4	6.1	5.1	3.5	39.3
GGAC	15.4	12.4	2.5	4.2	—	86.5	10.3
GAGC	6.4	8.2	3.6	4.6	24.0	—	5.9
AAGC	5.1	12.0	27.5	75.5	11.0	—	—
AAGT	—	—	—	6.5	—	—	—
GAGT	—	—	3.9	—	2.6	—	18.8
AGAC	—	—	—	—	5.0	3.5	—
AGGT	—	—	—	—	4.0	—	—
AGGC	—	—	—	—	—	3.2	—
GGAT	—	—	—	—	—	—	14.4
AAAC	—	—	—	—	—	—	3.8
GAAC	—	—	—	—	—	—	2.3

* WKND -White Korean native duck, BKND- Black Korean native duck, CD- Commercial (Peking) duck, BaB- Nageswari (Deshi black), BaW- Deshi white, BaJ- Jinding, BaL- Common Indigenous duck.

from plumage color phenotypes. Among these, the two alleles, c.52A and c.376A that substitute p.Lys18 and p.Ile126, respectively, may have a relevant effect on enhancing *MC1R* activity and thus the eumelanin deposition in duck plumage. Variations in the plumage color genes were investigated for possible use in breed identification of different colored Korean native ducks.

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