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
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 appearance for conservation purposes. NIAS has expanded mainly on the basis of plumage color and other phenotypic appearance for conservation purposes. NIAS has expanded mainly on the basis of plumage color and other phenotypic appearance for conservation purposes. NIAS has expanded mainly on the basis of plumage color and other phenotypic appearance for conservation purposes. NIAS has expanded mainly on the basis of plumage color and other phenotypic appearance for conservation purposes.

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°C. 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

| 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
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 (GenetBio, 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 Haplovie 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 nonsynonymous, 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.

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**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: CCATGTCCCCCTGACCTCG R: CCATGTCCCCTGACCTCG | 1221 | -141 to +1080 | 63 | Direct sequencing | | |
| P2 F: GCTGAGGTCGGGCGCACTGT R: CCGTGGCCTGGCTCGTGGTG | 91 | -15 to +76 | 63 | c.52A>G Genotyping | Mull | |
| P3 F: GCTCTTCTGCTGCTGTAGG R: GGCGTGAAGCTGAGGATG | 515 | +278 to +793 | 63 | c.376A>G, c.409A>G, c.649C>T, Genotyping | Hpy99L BciI BspMI |
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. 1. Identification of MC1R gene polymorphisms and conformation of PCR-RFLP genotyping among four (A, B, C, and D) coding SNPs in Asian ducks.
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
Recently, the possibility of MC1R involvement in plumage color variations in Asian ducks (Xia 2008; Huang 2010; Yu et al., 2012), and p.Arg217His substitution, have been investigated in several duck breeds. The haplotype GGGT presented a high frequency in black Korean native ducks (5.1%), Deshi white (11%) and black Korean native ducks (5.1%). The haplotype AAGC was mainly observed in the extended black duck breeds (e.g., Black Orpington, Black East Indian) expresses the homozygous €e+ allele. Meanwhile, the appearance of mallard pattern or wild type duck color and pattern of plumage, and the $E$ locus (Lancaster, 1990). 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 $L$ 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) could increase the eumelanin synthesis.
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.376A>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 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
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 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        |         |
| **E/ E**         | 24       | 11      | 1         |         | 27        | 9       | 0         | <0.001  |
| **e+/e**         | 0        | 10      | 86        |         | 1         | 23      | 72        | <0.001  |
|                  |          |         |           |         | 0         | 3       | 33        | <0.001  |
|                  |          |         |           |         | 16        | 24      | 56        | <0.001  |
|                  |          |         |           |         | 30        | 5       | 1         | <0.001  |

*E/ E- homozygous allele group for extended black, e+/e- homozygous allele group for non-extended black.
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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### References

Barrett JC, Fry B, Maller J and Daly MJ. Haploview: analysis and visualization of LD and haplotype maps. Bioinformatics, 21: 263–265. 2005.

Barsh GS, How the dog got its spots. Nature Genetics, 39: 1304–1306. 2007.

Chhajlani V and Wikberg JE. Molecular cloning and expression of the human melanocyte stimulating hormone receptor cDNA. FEBS Letters, 309: 417–420. 1992.

Chhajlani V. Distribution of cDNA for melanocortin receptor subtypes in human tissues. Biochemistry and Molecular Biology International, 38: 73–80. 1996.

Choi Y, Sims GE, Murphy S, Miller JR and Chan AP. Predicting the functional effect of amino acid substitutions and indels. PLoS ONE, 7: e46688. 2012.

Desarroya F, Labbe O, Eggerickx D, Vassart G and Parmentier M. Molecular cloning, functional expression and pharmacological characterization of a mouse melanocortin receptor gene. Biochemical Journal, 299: 367–373. 1994.

Ducrest AL, Keller L and Roulin A. Pleiotropy in the melanocortin system, coloration and behavioural syndromes. Trends in Ecology & Evolution, 33: 502–510. 2008.

Gangoso L, Roulin A, Ducrest AL, Grande JM and Figuerola J. Morph-specific genetics and environmental variation in innate and acquired immune response in a color polymorphic raptor. Oecologia, 174: 1113–1123. 2015.

Gantz I, Konda Y, Tashiro T, Shimoto Y, Miwa H, Munzert G, Watson SJ, DelValle J and Yamada T. Molecular cloning of a novel melanocortin receptor. Journal of Biological Chemistry, 268: 8246–8250. 1993.

Guo XL, Li XL, Li Y, Gu ZL, Zheng CS, Wei ZH, Wang JS, Zhou RR, Li LH and Zheng HQ. Genetic variation of chicken MC1R gene in different plumage colour populations. British Poultry Science, 5: 734–739. 2010.

Heo KN, Choo HJ, Kim CD, Kim SH, Kim HK, Lee MJ, Son BR, Choi HC and Hong EC. Changes of fatty acids and amino acids contents of Korean native commercial ducks meats with different raising periods. Korean Journal of Poultry Science, 40: 235–241. 2013.

Hoeska H and Nachman M. Different genes underlie adaptive melanism in different populations of rock pocket mice. Molecular Ecology, 12: 1185–1194. 2003.

Hoque MR, Jin S, Heo KN, Kang BS, Jo C and Lee JH. Investigation of MC1R single nucleotide polymorphisms and their relationships with plumage colors in Korean native chickens. Asian-Australasian Journal of Animal Science, 26: 625–629. 2013.

Huang H. Study on the molecular markers for plumage color trait in mule duck and their mother duck. Master Thesis, Fujian Agriculture and Forestry University, Fuzhou, China. 2010.

Jaap RG and Milby TT. Comparative genetics of blue plumage in poultry. Poultry Science, 23: 3–8. 1944.

Jackson DJ. Homologous pigmentation mutations in human, mouse, and other model organisms. Human Molecular Genetics, 6: 1613–1624. 1997.

Kerje S, Lind J, Schütz K, Jensen P and Andersson L. Melanocortin 1-receptor (MC1R) mutations are associated with plumage colour in chicken. Animal Genetics, 34: 241–248. 2003.

Kijas JM, Wales R, Torsten A, Chardon P, Moller M and Andersson L. Melanocortin receptor 1 (Mc1r) mutations and coat color in pigs. Genetics, 150: 1177–1185. 1998.

Kim HR, Kwon HJ, Oh ST, Yun JG, Choi YI, Choo YK, Kang BS,

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

| Haplotype | 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.
Kim HK, Hong EC, Kang CW and An BK. Effect of dietary metabolizable energy and crude protein concentrations on growth performance and carcass characteristics of Korean native ducks. Korean Journal of Poultry Science, 39: 167–175. 2012.

Klungland H, Vage DI, Gomez-Raya L, Adalsteinsson S and Lien S. The role of melanocyte-stimulating hormone (MSH) receptor in bovine coat color determination. Mammalian Genome, 6: 636–639. 1995.

Lancaster FM. Mutations and major variants in domestic ducks. In: Poultry Breeding and Genetics (Crawford RD ed), pp. 381–388. Elsevier. Amsterdam. 1990.

Lee HJ, Kim HJ, Yong HI, Khan MI, Heo KN and Jo C. Assessment of breed and sex-based variation in flavor-related compounds of duck meat in Korea. Korean Journal of Poultry Science, 42: 41–50. 2015.

Ling MK, Lagerstrom MC, Fredriksson R, Okimoto R, Mundy NI, Takeuchi S and Schioth HB. Association of feather colour with constitutively active melanocortin 1 receptors in chicken. European Journal of Biochemistry, 270: 1441–1449. 2003.

Nachman MW, Hoekstra HE and D’Agostino SL. The genetic basis of adaptive melanism in pocket mice. Proceeding of the National Academy of Sciences of the United States of America, 100: 5268–5273. 2003.

Nadeau JH, Minvielle F and Mundy NI. Association of a Glu92Lys substitution in MC1R with extended brown in Japanese quail (Coturnix japonica). Animal Genetics, 37: 287–289. 2006.

Oh D, Hyoeng K and Lee Y. Val126ile mutation within third transmembrane domain of the melanocortin 1 receptor (MC1R) is associated with a pigmentation of plumage color in chicken. Asian Journal of Animal and Veterinary Advances, 9: 321–322. 2014.

Phillips JC. Experimental studies of hybridization among pheasants and ducks. Journal of Experimental Zoology, 18: 69–144. 1915.