

Doctoral Thesis

Polymorphisms in Melanocortin 1 Receptor Gene (*MC1R*) and their Effects on Plumage Coloration in Native Japanese Chicken Breeds

(Summary)

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CHAPTER 1: General introduction

Native Japanese chicken breeds are unique for their various plumage coloration. Two most common melanins, eumelanin and pheomelanin, are responsible for plumage color variation in chickens. The melanocortin 1 receptor gene (*MC1R*) is the key gene to regulate melanin synthesis in the melanocyte. This study was aimed to investigate single nucleotide polymorphisms (SNPs) in the *MC1R* and their association with feather colors in native Japanese chicken breeds (Chapters 2 and 3). Approximately 45 chicken breeds have been created in Japan. Among them, Ô-Shamo and Chabo show many plumage color varieties and have been discussed in two different chapters (4 and 5).

CHAPTER 2: Comprehensive survey for the *MC1R* sequence and resultant haplotypes in native- and non-Japanese chicken breeds

The present study was performed to investigate and compare the *MC1R* sequence of 703 Japanese (29 breeds) and 155 non-Japanese (8 breeds) chicken breeds, which resulted in 13 SNPs. Ten were nonsynonymous (G178A, T212C, G274A, G376A, T398A/C, G409A, A427G, T637C, A644C, and C919G), which lead to amino acid substitutions (Ala60Thr, Met71Thr, Glu92Lys, Val126Ile, Leu133Gln/Pro, Ala137Thr, Thr143Ala, Cys213Arg, His215Pro, and Arg307Gly). Three were synonymous (C69T, G636A, and C834T). Based on the 13 SNPs, 15 haplotypes (H0–H14) were detected in Japanese chicken breeds. The Red Jungle Fowl showed two types of *MC1R* sequences (haplotypes H0 and H1, wild-type, e^+) that lead to cysteine or arginine at the 213 amino acid position. In native Japanese chicken breeds, almost all wild-type patterned chicken breeds had cysteine at the position (H0), but Ryujin-Jidori showed arginine (H1), which is a unique breed characteristic. The non-Japanese Brown Leghorn possessed H0 and H1 haplotypes evenly. Mie-Jidori and Nagoya, which show buff columbian plumage, had different haplotypes H5 (His215Pro) and H2 (Thr143Ala and Cys213Arg), respectively. Kumamoto and Tosakukin expressed buff and buff columbian plumage, respectively, and mostly possessed H2 haplotype (wheaten, e^{Wh} allele). Ingie and non-Japanese Rhode Island Red, which has mahogany plumage, were fixed with H2 haplotype. Summarizing these, the buff columbian, buff, and mahogany plumage chicken breeds were fixed with haplotype H2, with the unique H5 of Mie-Jidori. Among the Japanese breeds having black plumage, only Tômaru showed H4 haplotype (Leu133Gln and Cys213Arg, birchen, E^R allele) as like in non-Japanese black Minorca and Fayoumi, and had additional H11 haplotype (Glu92Lys, Val126Ile, and Cys213Arg). In Miyadidori, Kurokashiwa, Kinpa, Tômaru, Yakido, Kurekadori, black Ô-Shamo, and black Ukokkei that show black plumage, haplotypes H7 (Meth71Thr and Glu92Lys), H8 (Meth71Thr, Glu92Lys, and 213Arg), H9 (Meth71Thr, Glu92Lys, and 213Cys with G636A SNP), and H10 (Glu92Lys and Cys213Arg) were observed in a major percentage, where

substitution Glu92Lys was common for all haplotypes. Some Ô-Shamo (black) and Kinpa, with almost Yakido individuals possessed H9 haplotype, which was a new haplotype and can be a potential marker. White Ukokkei had novel haplotypes H13 (Ala60Thr, Glu92Lys, Cys213Arg, and Arg307Gly) and H14 (Glu92Lys, Ala137Thr, and Arg307Gly), with a common nonsynonymous substitution (Arg307Gly). Summarizing the above, three new haplotypes (H9, H13, and H14) were observed in the *MC1R* of Japanese chicken breeds. Non-Japanese breeds, on the other hands, showed no new haplotypes in the present study.

CHAPTER 3: Plumage colors expressed by the homozygous condition for haplotypes H5 and H14 of the *MC1R*

As mentioned in Chapter 2, Mie-Jidori was fixed with H5 haplotype (His215Pro) of the *MC1R*. However, direct effect of the H5 on plumage color was unclear, because the Mie-Jidori has columbian (*Co*) gene besides the H5. As also described in Chapter 2, white Ukokkei possessed a new *MC1R* haplotype H14. Like the case of the Mie-Jidori, the direct effect of the H14 haplotype on plumage color was unknown, because the white Ukokkei was fixed with the tyrosine gene (*c*) that is epistatic to the *MC1R*. To reveal the independent plumage colors expressed by the H5/H5 and H14/H14 conditions under the wild-type genetic background, mating experiments were performed between the wild-type Tosa-Jidori male and Mie-Jidori female and between the wild-type Tosa-Jidori male and white Ukokkei female. The F₂ individuals homozygous for the H5 haplotype from the Mie-Jidori showed brown-tinged cream color in neonatal plumage. In the adulthood, the female showed wheaten-like plumage. In addition to this, the Hinaidori female homozygous for the H5 also showed wheaten-like plumage, and the Ô-Shamo male homozygous for the H5 exhibited wild-type plumage. The F₂ birds homozygous for the H14 haplotype from white Ukokkei expressed stripes on the back in neonatal plumage. In adulthood, the male and female homozygous for the H14 haplotype showed wild-type and wild-type-like plumage, respectively. In the females, the breast feathers had black markings, differing from the salmon brown coloration of usual wild-type female breast feathers. The fact that individuals homozygous for the H14 haplotype exhibited wild-type or wild-type-like plumage suggests that G409A (Ala137Thr) and/or C919G (Arg307Gly) substitution(s) possessed by the H14 haplotype may downregulate the effect of G274A (Glu92Lys) mutation that usually expresses black plumage.

CHAPTER 4: *MC1R* polymorphisms in Japanese Large Game, Ô-Shamo

Ô-Shamo is popular for its cock fighting behavior. So, in breeding policy the target becomes body structure and aggressive behavior, instead of its plumage color. Plumage color variation in Ô-Shamo makes this breed as an ideal model to understand phenotypic effects from *MC1R* polymorphisms. In total, 104 blood samples were sequenced for the *MC1R* to identify the polymorphisms in Ô-Shamo. Eight SNPs were detected, and 6 were nonsynonymous, including a newly found SNP. In total, 9 haplotypes (H0–H8) were created from the 8 SNPs. The wild-type and wild-type-like plumage varieties showed H0 haplotype (wild type, e^+), besides the H4 (His215Pro, e^m allele), and H5 (Met71Thr, Glu92Lys, and His215Pro; e^{bc}). The wheaten and wheaten-like plumage birds also showed H0 and H4 haplotypes, besides the H3 haplotype (e^{wh} allele). Moreover, the above-mentioned chickens showed the new haplotype H2 in a low frequency. The Ô-Shamo that has brown plumage showed H0 and H5 in even frequency. The

buff columbian showed H4 instead of H5, comparing with haplotypes observed in brown plumage. The white and black plumage Ô-Shamo mostly showed H8 haplotype (Meth71Thr, Glu92Lys, and 213Cys with G636A SNP) which was related to extended black *E* allele. Summarizing all, Ô-Shamo showed huge *MC1R* polymorphisms and possessed one novel haplotype H2, including a new nonsynonymous substitution.

CHAPTER 5: The *MC1R* in the Japanese Bantam, Chabo: Association between genotypes and phenotypes

The small and extraordinary chicken breed Chabo is very popular for its various plumage coloration. The *MC1R* is the major gene to regulate plumage colors, but other color regulating genes, such as columbian (*Co*), silver (*S*), mottled (*mo*), recessive white (*c*), dominant white (*I*), blue (*Bl*), and barring (*B*) have effects to make this breed beautiful and attractive to fancy fowl keepers. In total, 108 Chabo samples were sequenced for *MC1R*, which led to 6 SNPs (C69T, T212C, G274A, A427G, G636A, and T637C) and resultant 5 haplotypes (H0–H4). Out of the 6 substitutions, 4 and 2 were nonsynonymous (T212C, Met71Thr; G274A, Glu92Lys; A427G, Thr143Ala; T637C, Cys213Arg) and synonymous (C69T and G636A), respectively. Although, the white columbian, buff columbian, brown mottled, white, and golden barred plumage varieties differ phenotypically, all were fixed with H1 (Thr143Ala and Cys213Arg). The tri-colored mottled, pyle, and black-breasted red plumage varieties of Chabo mostly showed H0 haplotype (wild type, e^+), along with H1. The mottled, black, barred, and blue plumage varieties mostly exhibited H3 haplotype, where G274A (Glu92Lys) for the expression of black pigmentation was commonly observed, in addition to Met71Thr, Glu92Lys, and Cys213Arg. Summarizing all, it was revealed that Chabo plumage varieties mostly possessed H0 (wild type, e^+), H1 (wheaten, e^{wh}), and H3 (extended black, *E*) haplotypes of *MC1R*, although they showed a large difference in plumage colors.

In conclusion, this study revealed that native Japanese chicken breeds show large diversity in the *MC1R*, including two new nonsynonymous nucleotide substitutions and four novel haplotypes (H9, H13 and H14 in Chapter 2 and H2 in Chapter 4). Moreover, the plumage colors expressed by the homozygotes for H5 and H14 haplotypes were identified under the wild-type genetic background (Chapter 3). The detailed and extensive information on *MC1R* from 1,054 chickens (899 and 155 chickens from 30 Japanese- and 8 non-Japanese breeds, respectively) will contribute to the development of chicken genetics. The information will also be helpful to produce new brand chickens and be important for fancy fowl keepers for their future breeding plans to avoid unexpected plumage colors from their stocks.