Genetic Variation of Muscovy Ducks MC1R Gene in a Different Feather Colors Population

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ABSTRACT

This research was aimed at gene sequence variations in the melanocortin 1 receptor (MC1R) and genotype relationships with different groups of feather colors in Muscovy ducks. Two hundred Muscovy days old ducks consisted of white feather male and female duck, black-white combination color male and female ducks. Primary design used Custal X, based on database from GeneBank Cairina moschata GH gene, partial cds (KX013541.1), primary Forward sequence: 5'-GGACCGCTACATCACCATCT-'3 and Reverse Primer: 5'-TGTAGAGCACCAGCATGAGG-'3. The results of the identification of feather color of Muscovy ducks shown there are variation on white color, white-black combination with white dominance and white-black color combination with black dominance. Variations of the feather color on the head, wings, breast, tail and plumage. The sequencing of PCR products obtained nucleotide polymorphism. GG genotype was observed in 293 nt only on white-black, CC in white-black and white feather color of male and female Muscovy ducks. Conclusively, Muscovy ducks had variation feather color, it was white and white-black combination. MC1R gene polymorphism was observed in Muscovy duck.

Keywords: Muscovy duck, Feather color, MC1R gene polymorphism

INTRODUCTION

Muscovy is a water poultry with potential development, particularly meat production. Muscovy spread throughout Indonesia because it has long been domesticated. The color of Muscovy in Indonesia is white, black and a combination of black and white. Genetic diversity of Muscovy in Indonesia is observable from the feather color on the wings, head, back, tail and abdomen. Most Muscovy is white and only a few has black color. Feather color gene, W gene, is the gene that controls feather color and has dominant characteristics to the mate (w gene). w gene is recessive to W gene and causes diverse feather colors (Ismoyowati, 2014). Feathers that belong to Muscovy diversity are black, white and blue (Su et al, 2006).

Different color of skin/feather in animals is caused by the pigment affected by Melanocortin 1 Receptor gene (MC1R) expressed on the surface of melanocyte. Melanocortin 1 Receptor gene (MC1R) is 7-transmembrane receptor expressed on melanocyte. The receptor affects the induction of tyrosinase enzyme that is responsible to the synthesis of eumelanin and pheomelanin. Generally, gene mutation in the MC1R coding occurs in mammals and birds that carry the dominant allele resulted from the extended locus in the active receptor that correlates with recessive allele and red-yellow color (Davila *et al*., 2014). Feather color in duck is the outcome response to melanocyte-stimulating hormone or

MSH (Melanocyt Stimulating Hormone) excreted by hypophysis (Price and Bontrager, 2001). Identifying Muscovy's feather color is important because it greatly defines the physical quality of carcass and consumers preferability. The present research was aimed to identify the variance of MC1R gene sequence in population of Muscovy with varied colors.

MATERIALS AND METHODS

Two hundreds day-old Muscovy consisted of white-plumed male and female, black and white-plumed male and female with dominant black. Blood sample, 3ml was taken from vena axillaries, put in a tube filled with anticoagulant (ETDA) and stored in the fridge. Deoxyribo Nucleic Acid (DNA) total genome was extracted from blood samples and isolated with DNA Isolation Kit (Geneaid). DNA isolation result was examined using 1% agarose gel electrophoresis. Primer design used Clustal X program with Cairina moschata melanocortin 1 receptor MC1R (MC1R) gene partial cds (KX013541.1) database from GeneBank. Primer base sequence of MC1R gene was forward: 5'-GCTCTTCATGCTGCTGATGG-'3 and reverse: 5'-GATGAAGACGGTGCTGGAGA-'3. Polymerase Chain Reaction (PCR) comprised several steps, namely DNA pre-denaturation at 95^{o}C for 5 min, DNA denaturation at 94^{o}C for 30s, annealing at 55.2^{o}C for 45s and elongation at 72^{o}C for 1 min. A final extension was performed at 72^{o}C for 10 min. PCR conducted 35 cycles. PCR products were subject to electrophoresis test with 1.5% agarose gel. The PCR products were visualized by using UV light. Sequencing PCR product of MC1R gene was done by PT Genetika Science Indonesia. Sequence product was read using software Sequence Scanner v1.0, in form of electrophoregram consisted of nucleotide sequence from MC1R gene samples of Muscovy ducks.

SNP genotyping was determined through BioEdit v7.2.0 program, by aligning sequence products according to the sequence in GeneBank KX013541.1database from ClustalW menu (an accesory application). Alignment result was seen in electrophoregram, so as to obtain SNP in particular position to use for genotyping. The base sequence of MC1R gene in Muscovy ducks (Chairina moschata) was on 726-bp. The SNP was confirmed based on the electrophoregram results and used for genotyping.

Gene frequency was based on formula by Pirchner (1981) as follows:

$$FAn = \frac{\Sigma MC1RAgene}{\Sigma MC1RAgene + \Sigma MC1Rngene}$$

Keterangan :
Note:
FAn = gene A frequency at n-locus

Polymorphism was determined using Heterozygosity formula from Nei (1987) as follows:

$$h = 1 - \sum_{i=1}^{m} x^2$$

RESULTS AND DISCUSSION

Variation of Muscovy feather color

Muscovy was grouped according to the feather color white and combination black-white. The color pattern is located all over the body. Combined color variation is found on
the head, wings, chest, tails and plume as presented in Figure 1.

Figure 1. Feather color pattern in combined black-white Muscovy

Black color theoretically presents because of gene E factors, while the allele, e^b forms brown color. E is dominant over e^b. The source of color is caused by gene C factor, while the allele, that is c forms white color (recessive). White color is formed due to the absence of gene O (gene factor that supports gene C so that the color is formed during oxidation). Without gene O, gene C is in fact unable to show color, so the outcome is white (Mackay, 1990). This research showed that the dominant color of Muscovy is white. Percentage on white feather was 40% of the head, 63.3% on the plume, 96.7% on breast, 73.3% on wings and 46.7% on tail. It was contributed to the diversity controlled by gene W. Feather color is controlled by feather color gene or gene W (white) which is dominant to the allele (gene w). Gene w is recessive to gene W, resulting in the colored feather (Ismoyowati, 2014).

SNP MC1R gene in Muscovy

The PCR result showed that FSH gene is located in 203bp (Figure 2). PCR products were sequenced.

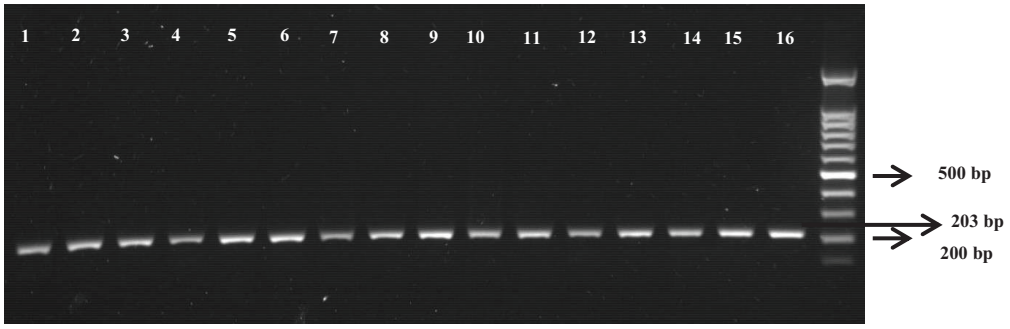

Figure 2. Electrophoresis product of PCR MC1R gene Muscovy 203 bp

Sequencing product of MC1R gene 203 bp was followed by the alignment sequence between *GenBank Acc. No.* KX013541 and data of sequence MC1R gene 203 bp. Sequencing of PCR products of MC1R 203 bp found sequence in 293 bp, located between 290 to 300 bp *GenBank Acc. No.* KX01354 (Figure 3).

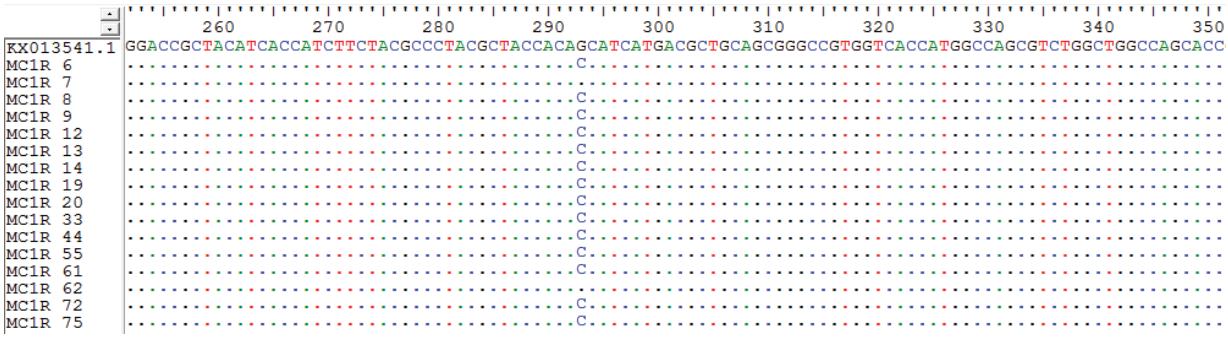

Figure 3. Result of alignment between *Genbank Acc.* No. KX01354 and sequencing Muscovy MC1R gene

Single Nucleotide Polymorphism (SNP) is 293 bp. Two SNP in 293 bp resulted in 2 genotypes, GG and CC. Sequencing result from 16 Muscovy ducks that was used as the sample showed GG and CC genotypes but not heterozygote genotype. In the group of 8 Muscovy with combined black-white color, 2 had GG genotype consisted of 1 male and 1 female, while 6 had CC genotype consisted of 3 male and 3 female. In the group of 8 white Muscovy with CC genotype, 4 are male and 4 are female.

Single Nucleotide Polymorphism in 293 bp MC1R gene indicated polymorphism in Muscovy. Polymorphism in MC1R gene had been reported in several mammals such as bull, (Chen *et al.,* 2009), goat (Fontanesi *et al.,* 2009) and poultry like Magelang duck (Purwantini *et al.,* 2013), chicken and quail (Gunnarsson *et al*., 2006). Polymorphism occurs because of a mutation that results in black/dark color, while the absence of mutation function causes red/yellow or white color (Fontanesi *et al.,* 2009. Observation of electropherogram figure (Figure 4) reveals the base conversion from Guanine to Cytosine. Base conversion of SNP c.293G>C showed mutation transition. Mutation transition occurs because of the substitution between one purine base (adenine and guanine) and the other purine base or between one pyrimidine (thymine and cytosine) and the other pyrimidine base (Windelspecht, 2007).

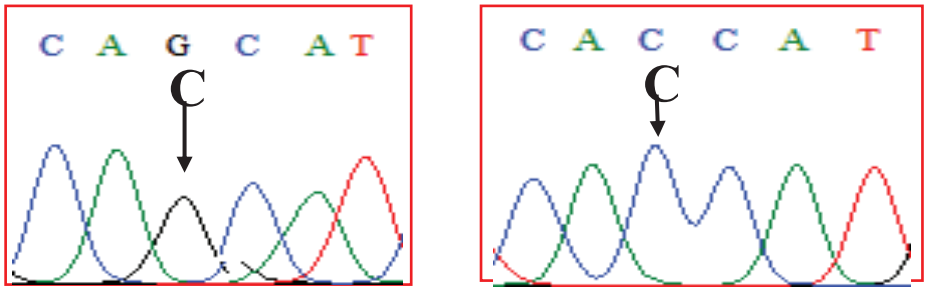

Figure 4. Electhroporegram Polimorfisme of Muscovy MC1R gene

Melanocortin 1 Receptor (MC1R) belongs to the small subfamily *G Protein-Coupled Receptor* (GPCR). *Melanocortin Receptor* (MCR) 1 to 5. MCR is activated by a group of peptide hormones that contribute to the regulation of various physiological process such as production of glucocorticoid hormone in adrenal gland (MC2R), food intake and energy homeostatis (mainly MC4R), sebaceous gland activity (MC5R) and others (Yang, 2011).

Melanocortin is the bioactive peptide produced at the cleft of precursor prohormone *Protein Pro-Opiomelanocortin* (POMC) in several locations by two endoprotease (Garcia-Borron *et al.,* 2014).

Pigment color is theoretically produced as a response to *Melanocyt Stimulating Hormone* (MSH) secreted by hypophysis (Price and Bontrager, 2001). MC1R activity occurs when MCS is bound so tyrosinase activity improved and inhibited melanin synthesis. Eumelanin will be produced when tyrosinase enzyme reaches the highest level in melanocyte, thus the black or brown color occurs. Otherwise, the absence of MSH stimulation from MC1R causes the level of tyrosinase low, so pheomelanin that forms dull red and yellow color is produced. When the concentration of *cAMP* increased in the cell, it would activate nucleotide C. Moreover, tyrosinase synthesis would also improve and caused an increase in eumelanine synthesis and reduce pheomelanin synthesis. It caused the formation of black or brown skin (Garcia-Borron *et al.,* 2014).

One of the factors that control several programs related to neurogenesis and neural crest is *Micropthalmia-associated transcription factor* (MITF). After birth, MITF controls 3 enzymes that are potential to control melanin production. One of the enzymes, tyrosinase, produces eumelanin an dpheomelanin. A mutation in MITF will inhibit melanin production, thus eumelanin and pheomelanin are not produced and the outcome color is white (Hallsson *et al.,* 2007).

Muscovy with GG genotype are 12.5% or 0.125 genotype frequency from the total of 16 sequenced PCR products, while genotype CC are 87.5% or 0.875 genotype frequency. Noor (2000) stated that polymorphism rate in a population can be measured with Heterozygosity parameter. Heterozygosity is scored or parameter to measure the rate of polymorphism in a population (Nei, 1987). The heterozygosity score in this research is 0,069. Criteria of heterozygosity ranges from 0 (zero) to 1 (one). If heterozygosity approaches 0 (zero), then polymorphism is low, and if heterozygosity approaches 1 (one) then polymorphism is high (Mulliadi, 2010). The heterozygosity score in this research is 0,069 or closer to 0 (zero), therefore Muscovy polymorphism according to feather color is low.

CONCLUSIONS

Research result concluded that polymorphism MC1R gene in Muscovy DNA (*Cairina moschata*) was based on feather color of white and combined black-white. The Polymorphism rate of Muscovy based of white feather and combined black-white feather was low, as shown from the heterozygosity score 0,069.

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