Research Article

The Melanocortin 1 Receptor Gene Variations in Some Bali Cattle with Color Pattern Deviations

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Abstract | Coat color or pattern uniformity is one of the obvious outermost characteristics representing the quality of livestock breeds. Bali cattle have well known for their coat color or pattern uniformity standard and yet there are still found some irregularities in albino, *injin, gading*, *bang*, *poleng*, *mores*, *panjut*, and *cundang*. The MC1R (Melanocortin 1 Receptor) gene plays an important role in the pigmentation process and its variations are associated with differences in the skin and coat colors. The purpose of this study was to analyze the genetic variations of the MC1R gene in 10 Bali cattle reared in Bali Province which have aberrant color patterns. Blood samples were taken from the 10 Bali cattle consisting of three having normal coat color patterns, three albino, two *injin*, and two *poleng*. The blood samples were extracted using a commercial kit and the MC1R gene was then amplified by using a forward primer 5' AGT TGA GCA GGA CCC TGA GA 3', and a reverse primer 5' CCA GTC ACC ACA GAG CGT TA 3'. The PCR products were further sequenced and analyzed to see variations of the MC1R gene of all cattle. The results indicated that the sequence of MC1R gene in the 10 Bali cattle has a nucleotide variation of 0.31% generated from *Bos taurus* and 0.21% generated from *Bos indicus*. While the nucleotide variation of the MC1R gene from cattle with color pattern deviations to the Bali cattle, namely 1.36% albino, 0.21% *injin*, and 0.10% *poleng* Bali cattle.

Keywords | Albino, Bali cattle, Color pattern, *Injin*, MC1R gene, *Poleng*

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INTRODUCTION

These Bali cattle are one of the cattle that play an important role in livestock development in Indonesia. Currently, Bali cattle breeding programs are based on performance and pedigree information without knowing the genes that affect performance (Puja et al., 2018). No other cattle breeds are allowed reared in Bali Province. Furthermore, Bali cattle are regulated as national livestock germplasm, and its biodiversity in Bali Province needs to be preserved through sustainable pure breeding activities meeting the quality standards based on their genetic potential. Thus, Bali cattle as a preference of meat producers for national consumption must be maintained for their pureness.

The appearance of Bali cattle is easily distinguished from other local cattle breeds reared in Indonesia. Their color is also unique, both females and males from their birth up to their prior sexual maturity are in brick (terracotta) color.

While the females are remaining in brick color although they reach their sexual maturity, the sexual maturity males on the other hand turn to be black. When young males are castrated, their color remains brick, however, when sexually mature males are castrated their black coat color turn to be brick again (Oka, 1995).

In addition to these characteristics, Bali cattle also have other distinctive characteristics, namely the black line that extends like an eel from the back of the neck to the base of the tail. Their legs, starting from the top ruffled to the knees are white, have a white mirror on the rump and the tip of the tail is black. However, the Bali cattle breeding and development that occurred in Bali Province in many cases delivered calves with color deviations. Some of their color deviations namely albino (the whole body coat is white color), *injin* (the whole body coat is black color), the whole body coat is *gading* color, the whole body coat is *bang* color, *poleng* (the whole body coat is white and *gading*), *mores* (the whole body coat has white spots), *panjut* (the tip of the tail is white color where supposed to be black color), and *cundang* (white triangle shape on the forehead) (Hardjosubroto, 1994) (Figure 1).

Figure 1: Normal Bali cattle and some with color deviations.

The process of coloring coat color (melanogenesis) is determined by the pigment melanin which is found in the melanosomes of melanocytes. Melanins can be produced in two chemically distinct types eumelanin and pheomelanin (Song et al., 2019). Eumelanin is responsible for the black-to-brown pigmentation of the skin and coat color, while pheomelanin is responsible for the red-to-yellow coloring in the coat color of mammals. Eumelanin production depends on the stimulation of a G-protein coupled receptor called the melanocyte-specific melanocortin receptor (MC1R) by agonists of α-melanocyte-stimulating hormone (α-MSH) (Herraiz et al., 2021). The pigmentation of fur in livestock is determined by the relative distribution of pheomelanin and eumelanin pigments. Pigmentation is influenced by genetic factors, environment, and endocrine modulating the amount, type, and distribution of melanin within the skin (Hearing and Tsukamoto, 1991; Slominski et al. 2018; Jablonski, 2021). Both of these pigments are expressed by melanocyte cells through the mechanism of melanogenesis (Jung et al., 2020). The expression of pigment produced by melanocytes is controlled by the melanocortin 1 receptor and alleles of the agouti locus (Jung et al., 2021). The synthetic shift of eumelanin to pheomelanin is linked to a mutation in the MC1R gene that results in a change in coat color.

Abnormal changes or mutations in the MC1R gene have been shown to affect coat color in a large number of mammals, including cattle (Klungland et al., 1995; Girardot et al., 2006). This gene is widely used to determine the variation in several different color patterns. Research on five breeds of cattle reared in Italy showed high variations in the MC1R gene (Crepaldi et al., 2005). Meanwhile, in some cattle reared in Indonesia, more variations were found in cross-bred cattle compared to the other Indonesian local cattle (Hartatik, 2016), including Bali cattle reared in Kupang in NTT Province (Tabun et al., 2013; Tabun et al., 2014). However, until recently, there has been no evidence of this gene variation/diversity in Bali cattle with aberrations in color patterns that exist in Bali Province.

Therefore, the objective of this study was to find out the sequence variation/diversity of the MC1R gene in Bali cattle with color pattern aberrations that exist in Bali Province. The information gained in this study is expected to be used as a marker in selecting bulls for semen resources for artificial insemination purposes to ensure that Bali cattle reared in Bali Province have more uniform coat colors thus minimizing the coat color aberrations occurred.

MATERIALS AND METHODS

Blood Sampling and DNA Isolation

Blood samples were collected from 10 Bali cattle reared in Bali Province. Those cattle consisted of 3 Bali cattle having normal color, 3 Bali cattle having albino, 2 Bali cattle having *poleng*, and 2 Bali cattle having *injin* colors. EDTA-containing vacutainers were used to collect their blood through jugular veins. Genomic DNA was extracted using readymade Qiagen DNeasy blood and tissue DNA extraction kit (Qiagen, Hilden, Germany) following the manufacturer's instructions. DNA concentration was determined by spectrophotometer (Nanophotometer, Implan, Germany). Then, the DNA was put into a 1.5 ml tube and stored at -20 0C for further processing. The study was approved by the Animal Ethics Committee, Faculty of Veterinary Medicine Udayana University.

Amplifying MC1R Gene and Sequencing

DNA amplification used a forward primer 5' AGT TGA

GCA GGA CCC TGA GA 3', and a reverse primer 5' CCA GTC ACC ACA GAG CGT TA 3'. Both primers were designed based on Design Program Primer 3 method by Triinu Koressaar and Maido Remm (2007). The DNA amplification process was carried out with a total volume of 20 µl consisting of 5 µl DNA, 10 µl HotStar Taq plus master mix, 1 µl forward primer, 1 µl reverse primer, and 3 µl RNase free water. Amplification of specific DNA fragments in the MC1R gene of 954 bp using a Thermal Cycler machine (Senquest – Labcycler-Germany) with pre-denaturation conditions of 95 0C for 5 minutes, denaturation of 940C for 30 seconds, annealing of 57 0C for 30 seconds and extension at 72 0C for 30 seconds, repeated for 30 seconds as many as 30 cycles, and the final extension at a temperature of 72 0C for 10 minutes. After that, the DNA was visualized through electrophoresis on 1.2% agarose gel in 1x TBE solution. The fragments were stained with ethidium bromide after being migrated for 40 minutes at a voltage of 85 V. Stained DNA fragments were then viewed under an ultraviolet illumination chamber (UVITEC-Cambridge). The MC1R gene PCR product was then sent to Macrogen Inc., Singapore for sequencing.

Data Analysis

The results of the sequences then were analyzed using Mega version X software (Kumar et al., 2018), so that variations or polymorphisms of the MC1R gene in all samples are known.

RESULTS AND DISCUSSIONS

The amplification results of the MC1R gene of all samples showed that the primer pair amplified the DNA fragment by 954 base pairs (bp). The DNA fragment recognized by the primer flank the nucleotide sequence at 233 to 1187 bp from GenBank with accession number NM_174108.2. Multiple alignments (Multi Alignment ClustalW) of bovine MC1R gene sequences was carried out, then it was analyzed for its nucleotide diversity after that amino acid composition was compared to sequences of other from GenBank, namely *Bos taurus* (NM_174108.2), and *Bos indicus* (MG373644.1). Results of the present study revealed several polymorphisms in Bali cattle with *Bos taurus* and *Bos indicus* showed in Figure 1.

Identification of polymorphism/variation from all samples (Bali cattle, albino, *injin*, and *poleng*) and MC1R gene sequences found 3 SNPs with nucleotide changes compared to *Bos taurus* MC1R gene from GenBank, whereas Bali cattle when compared with *Bos indicus* MC1R gene sequences found 2 SNPs. The nucleotides that changed were p201 C>T, p296 C>T, p876 T>C. Variations or nucleotide diversity of all samples to other cattle from GenBank were presented in Table 1.

The alignment results of all sequences showed that there were 13 polymorphisms found in albino Bali cattle, 2 polymorphisms in *injin* Bali cattle, and 1 polymorphism in *poleng* Bali cattle (Table 1).

Table 1: Nucleotide similarity of MC1R gene of Bali cattle and other breeds.

	Length (bp)	Total Nucleotide diversity	Nucleotide Similarity
Bali cattle vs *Bos taurus*	954	3 (0.31%)	951 (99.69%)
Bali cattle vs *Bos indicus*	954	2 (0.21%)	952 (99.79%)
Albino vs brick Bali cattle	954	13 (1.36%)	941 (98.54%)
Injin vs brick Bali cattle	954	2 (0.21%)	952 (99.79%)
Poleng vs brick Bali cattle	954	1 (0.10%)	953 (99.90%)

The nucleotide changes that occurred in albino Bali cattle of a total of 13 nucleotides, 8 of them caused changes in amino acids. In *injin* Bali cattle, on the other hand, both two nucleotide changes caused amino acid changes. Differing from the *Poleng* Bali cattle, the nucleotide changes did not cause amino acid changes. The results of multiple alignments of MC1R gene sequences (954 bp) for Bali cattle, albino Bali cattle, *injin* Bali cattle and Bali *poleng* cattle with standard sequences from the NCBI GenBank can be seen in Figure 2.

The synthesis of melanin is determined by the complex regulation of various aspects, one of which was the melanocortin 1 receptor (MC1R) gene. The normal pigmentation process run as it should, of course, if the MC1R gene has the right sequence. Changes in the nucleotide base content of a gene cause variations in the expression of the gene concerned (Huang et al., 2002), where one of the causes of this change could be due to mutation (Agisimanto and Supriyanto, 2007).

The results of the present study indicated that Bali cattle, both those that have normal color patterns and those that deviate by looking at their MC1R gene sequence were still in the same group with *Bos taurus* and *Bos indicus* breeds with 99.69% and 99.79% nucleotide similarities, respectively (Table 2). This finding was also similar to the study found by Tabun et al. (2014), which used Bali cattle with brick color, *injin*, and albino in Kupang Province. This showed that although Bali cattle were generated from *Bos javanicus* (banteng) from the view of the color pattern construction, they also had high similarity with *Bos taurus* and *Bos indicus*.

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CLUSTAL O(1.2.4) multiple sequence alignment

NM_174108.2     ATGCCTGCACTTGGCTCCCAGAGGCGGCTGCTGGGTTCCCTTAACTGCACGCCCCCAGCC  60
MG373644.1      ATGCCTGCACTTGGCTCCCAGAGGCGGCTGCTGGGTTCCCTTAACTGCACGCCCCCAGCC  60
Bali_70         ............................................................  60
BALI_ALBINO     .....................A...T..........C...................G.....  60
BALI_INJIN3     ....................................T.................G.....  60
Bali_poleng     ............................................................  60
                ********************.*** ********** * ********** *****

NM_174108.2     ACCCTCCCCTTCACCCTGGCCCCCAACCGGACGGGGCCCCAGTGCCTGGAGGTGTCCATC  120
MG373644.1      ACCCTCCCCTTCACCCTGGCCCCCAACCGGACGGGGCCCCAGTGCCTGGAGGTGTCCATC  120
Bali_70         ............................................................  120
BALI_ALBINO     ..........................................G.G..............T..  120
BALI_INJIN3     ............................................................  120
Bali_poleng     ............................................................  120
                ****************************************.* **************:**

NM_174108.2     CCTGACGGGCTCTTTCTCAGCCTGGGGCTGGTGAGTCTCGTGGAGAACGTGCTGGTAGTG  180
MG373644.1      CCTGACGGGCTCTTTCTCAGCCTGGGGCTGGTGAGTCTCGTGGAGAACGTGCTGGTAGTG  180
Bali_70         ............................................................  180
BALI_ALBINO     ....................C.......................................  180
BALI_INJIN3     ............................................................  180
Bali_poleng     ............................................................  180
                ****************** ****************************************

NM_174108.2     GCTGCCATTGCCAAGAACCGCAACCTGCACTCCCCCATGTACTACTTTATCTGCTGCCTG  240
MG373644.1      GCTGCCATTGCCAAGAACCGCAACCTGCACTCCCCCATGTACTACTTTATCTGCTGCCTG  240
Bali_70         ......................T.....................................  240
BALI_ALBINO     A.....................T.....................................  240
BALI_INJIN3     ......................T.....................................  240
Bali_poleng     ......................T.....................................  240
                .****************** ****************************************

NM_174108.2     GCTGTGTCTGACTTGCTGGTGAGCGTCAGCAACGTGCTGGAGACGGCAGTCATGCCGCTG  300
MG373644.1      GCTGTGTCTGACTTGCTGGTGAGCGTCAGCAACGTGCTGGAGACGGCAGTCATGCTGCTG  300
Bali_70         ..........................................................T....  300
BALI_ALBINO     ..........................................T.........T....  300
BALI_INJIN3     ..........................................................T....  300
Bali_poleng     ..........................................................T....  300
                ********************************************* ********.****

NM_174108.2     CTGGAGGCCGGTGTCCTGGCCACCCAGGCGGCCGTGGTGCAGCAGCTGGACAATGTCATC  360
MG373644.1      CTGGAGGCCGGTGTCCTGGCCACCCAGGCGGCCGTGGTGCAGCAGCTGGACAATGTCATC  360
Bali_70         ............................................................  360
BALI_ALBINO     ............................................................  360
BALI_INJIN3     ............................................................  360
Bali_poleng     ............................................................  360
                ************************************************************


NM_174108.2     GACGTGCTCATCTGCGGATCCATGGTGTCCAGCCTCTGCTTCCTGGGTGCCATTGCTGTG  420
MG373644.1      GACGTGCTCATCTGCGGATCCATGGTGTCCAGCCTCTGCTTCCTGGGTGCCATTGCTGTG  420
Bali_70         ............................................................  420
BALI_ALBINO     .....................A............A.........................  420
BALI_INJIN3     ............................................................  420
Bali_poleng     ................................A...........................  420
                ********************.***********.****************************

NM_174108.2     GACCGCTACATCTCCATCTTCTACGCCCTGCGGTACCACAGTGTTGTGACACTGCCCCGA  480
MG373644.1      GACCGCTACATCTCCATCTTCTACGCCCTGCGGTACCACAGTGTTGTGACACTGCCCCGA  480
Bali_70         ............................................................  480
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NM_174108.2     GCGTGGAGGATCATTGCGGCCATCTGGGTGGCCAGCATCCTCACCAGCCTGCTCTTCATC  540
MG373644.1      GCGTGGAGGATCATTGCGGCCATCTGGGTGGCCAGCATCCTCACCAGCCTGCTCTTCATC  540
Bali_70         ............................................................  540
BALI_ALBINO     ............................................................  540
BALI_INJIN3     ............................................................  540
Bali_poleng     ............................................................  540
                ************************************************************

NM_174108.2     ACCTACTACAACCACAAGGTCATCCTGCTGTGCCTCGTTGGCCTCTTCATAGCTATGCTG  600
MG373644.1      ACCTACTACAACCACAAGGTCATCCTGCTGTGCCTCGTTGGCTTCTTCATAGCTATGCTG  600
Bali_70         ............................................................  600
BALI_ALBINO     ............................................................  600
BALI_INJIN3     ............................................................  600
Bali_poleng     ............................................................  600
                ************************************************************

NM_174108.2     GCCCTGATGGCCGTCCTCTACGTCCACATGCTGGCCCGGGCCTGCCAGCATGCCCGGGGC  660
MG373644.1      GCCCTGATGGCCGTCCTCTACGTCCACATGCTGGCCCGGGCCTGCCAGCATGCCCGGGGC  660
Bali_70         ............................................................  660
BALI_ALBINO     ............................................................  660
BALI_INJIN3     ............................................................  660
Bali_poleng     ............................................................  660
                ************************************************************

NM_174108.2     ATTGCCCGGCTCCAGAAGAGGCAGCGCCCCATTCATCAGGGCTTTGGCCTCAAGGGCGCT  720
MG373644.1      ATCGCCCGGCTCCAGAAGAGGCAGCGCCCCATTCATCAGGGCTTTGGCCTCAAGGGCGCT  720
Bali_70         ............................................................  720
BALI_ALBINO     ............................................................  720
BALI_INJIN3     ............................................................  720
Bali_poleng     ............................................................  720
                ************************************************************

NM_174108.2     GCCAACCTCACCATCCTGCTGGGCGTCTTCTTCCTCTGCTGGGGCCCCTTCTTCCTGCAC  780
MG373644.1      GCCACCCTCACCATCCTGCTGGGCGTCTTCTTCCTCTGCTGGGGCCCCTTCTTCCTGCAC  780
Bali_70         ............................................................  780
BALI_ALBINO     ............................................................  780
BALI_INJIN3     ............................................................  780
Bali_poleng     ............................................................  780
                ************************************************************

NM_174108.2     CTCTCGCTCATCGTCCTCTGCCCCCAGCACCCCACCTGTGGCTGCATCTTCAAGAACTTC  840
MG373644.1      CTCTCGCTCATCGTCCTCTGCCCCCAGCACCCCACCTGTGGCTGCATCTTCAAGAACTTC  840
Bali_70         ............................................................  840
BALI_ALBINO     ............................................................  840
BALI_INJIN3     ............................................................  840
Bali_poleng     ............................................................  840
                ************************************************************

NM_174108.2     AACCTCTTCCTGGCCCTCATCATTTGCAACGCCATTGTGGACCCCCTCATCTATGCCTTC  900
MG373644.1      AACCTCTTCCTGGCCCTCATCATTTGCAACGCCATTGTGGACCCCCTCATCTATGCCTTC  900
Bali_70         ..................................C.........................  900
BALI_ALBINO     ..................................C.........................  900
BALI_INJIN3     ..................................C.........................  900
Bali_poleng     ..................................C.........................  900
                ********************************** ************************

NM_174108.2     CGCAGCCAGGAGCTCCGGAAGACGCTCCAAGAGGTGCTGCAGTGCTCCTGGTGA        954
MG373644.1      CGCAGCCAGGAGCTCCGGAAGACGCTCCAAGAGGTGCTGCAGTGCTCCTGGTGA        954
Bali_70         ......................................................        954
BALI_ALBINO     ......................................................        954
BALI_INJIN3     ......................................................        954
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Figure 2: The results of multiple alignments of the MC1R gene sequence (954 bp) in Bali cattle, albino bali cattle, injin bali cattle and poleng bali cattle with sequences standards from GenBank NCBI

The diversity of color pattern aberrations to Bali cattle was 1.36%, 0.21%, and 0.10% for the albino, *injin*, and *poleng* Bali cattle, respectively. There was no other similar study was carried out to this study in Indonesia. Tabun et al.. (2014), however, conducted a slightly different study in Kupang Province, in that study albino and *injin* cattle sequences were compared to the sequence of the breed from the gene bank instead of Bali cattle with brick color. In addition, they also observed a shorter sequence (296bp) than to the present study 954 bp.

The number of nucleotide variations of the MC1R gene

Table 2: Nucleotide Variations and Amino Acid Changes by MC1R Gene of Bali Cattle with Color Deviations

Cattle	Nucleotide variations	Amino acid variations
Albino	p21G>A p25C>T p36 T>C p55C>G p101A>G p103T>Gp118A>T p140G>Cp181G>A p287C>Tp386C>A p392G>Ap479G>A	Valin7Valin Alanin9Treonin Prolin12Prolin Glisin19Arginin Valin34Alanin Treonin35Prolin Lisin40Lisin Serin47Triptofan Arginin61Tirosin Arginin96Histidin Isoleusin127Isoleusin Serin131Serin Alanin160Valin
Injin	p38C>Tp55C>G	Arginin13Lisin Glisin19Arginin
Poleng	p392G>A	Serin13Serin

sequence in the three Bali cattle that had color pattern deviations in the present study was 13, 2, and 1 for albino Bali cattle, *injin* Bali cattle, and *poleng* Bali cattle, respectively, from the brick color pattern of Bali cattle (Table 2). Mutations in the MC1R gene caused the function of the MC1R gene and it is associated with an increase or lower in the two types of melanin (Eumelanin and pheomelanin) productions which resulted in color changes (Wolf Horrell et al., 2016). Of all the mutations that occurred in the samples, however, there was not a single specific mutation that could be associated with the color of a particular sample. In albino Bali cattle, changes occurred in the four nucleotides, namely A, T, G, and C. In Bali *Injin* cattle, however, two nucleotides changed from C to T, and G, and in *Poleng* cattle, the nucleotides G became A. No previous studies have found the same variation as this study. According to Garcia-Borron et al. (2005), nucleotide C was important in the formation of black pigment so changes in the nucleotide C caused lighter colors. In line with Klungland et al. (1995) who also reported that nucleotide changes T296C were responsible for the black color of Angus cattle. In the recent study, only the sequence of *Bos taurus* from the Gen Bank (code NM_174108.2) has nucleotide C, the rest sequences *Bos indicus* (MG373644.1), Bali cattle, albino, *injin*, and *poleng* was nucleotide T instead (Figure 3). In contrast to Klungland et al. (1995), Garcia-Borron et al. (2005), and Tabun *et al.*(2014), this study found changes of nucleotide C to T in *injin* Bali cattle do not make the color lighter, it was black from birth. This was probably due to nucleotide C changing to G as well and this situation kept the formation of black color. Differ from albino and *injin* cattle, *poleng* cattle have only 1 nucleotide change was G to A, and this does not give rise to amino acid change (Serine to Serine), it can be said that these cattle have similar protein as normal Bali cattle. Further study on the nutrition and health status of these cattle was necessary to get a better understanding of this color emergence. Meanwhile, the albino Bali cattle had 13 nucleotides which were differ from the normal Bali cattle have the four nucleotides changed, from the results of this study it could not be said which of the nucleotides changes caused a change in the formation of a dark color (black) to a light color or albino. It was probably due to the small sample used in this study and in addition, there was no association found between certain mutations to specific color deviation occurred.

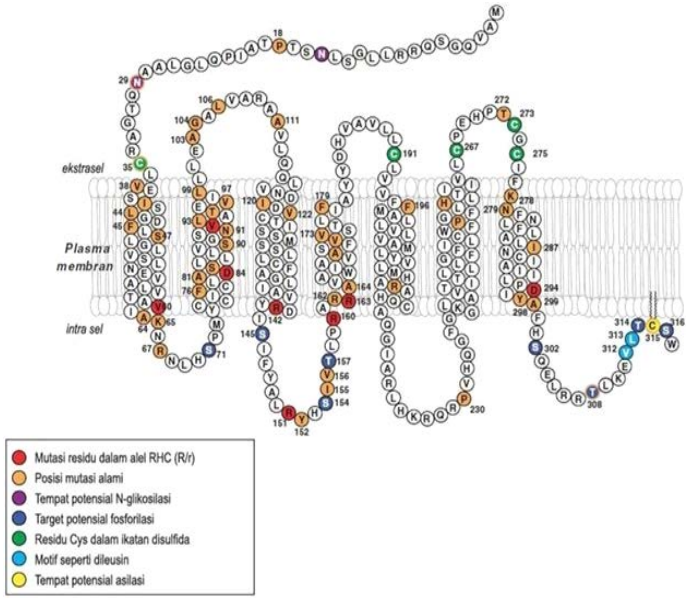

Figure 3: Protein structure of melanocortin1 receptor was made up by the 317 amino acid residues with their specific characteristics of GPCR i.e., N-extra cell terminals, three eclipses extra cells, seven trans membrane fragments, three eclipses intra cells, and C- intra cell terminals. The position of trans membrane helix fits to 2D model picture by Ringholm et al., (2004).

Other than nucleotide changes, color changes occurred also dictated by the location of the amino acid changes on the MC1R gene protein structures. These receptors were categorized into G- Protein Coupled Receptor (GPCR) group. MC1R was part of the integral membrane made up of the 317 amino acid residues (Figure 3) with the specific structure of GPCR such as N-extra cell terminals, three extracellular loops (els), seven trans-membrane (TM) fragments, three intracellular loops and C-intra cell terminals (Ringholm et al., 2004).

The changes in amino acids in the 10 Bali cattle in the present study occurred on N-terminal, transmembrane 1, 2, dan 3 (albino), N-terminal (*injin*). N-terminal caught peptide signals from free protein. The only effect was the amino acid residues after the number 27. Amino acid

changes from Ser to Ala in that region were reported to cause significant decreases in the affinity of the receptor (Chhajlani et al., 1996). On the transmembrane fragment, 11 natural mutations cluster in TM2 of the MC1R (Figure 3) and several of them had important functional consequences. Changes in amino acids on the 10 Bali cattle in the present study were not found in the critical position mentioned in the previous structures i.e., N-terminal and transmembrane fragments. Further study with a large sample will allow determining which SNPs affected the certain phenotype. That would make it possible to identify the phenotypic variations, to help in selecting the most important for association study.

CONCLUSION

Results of the present study showed that MC1R gene of albino, *injin* and *poleng* Bali cattle had variant sequences or different nucleotide orders from Bali cattle reared in Bali Province. The proportion of the variant sequences of MC1R gene of albino, *injin*, and *poleng* Bali cattle was 1.36%, 0.21%, and 0.10%, respectively. No specific nucleotide changes were found to be associated with any color abnormality in Bali cattle.

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CONFLICT OF INTEREST

We certify that there is no conflict of interest with any financial, personal, or other relationships with other people or organizations related to the material discussed in the manuscript.

NOVELTY STATEMENT

This research demonstrated that there was a difference in the MC1R gene sequence in Bali cattle with color deviation.

AUTHORS CONTRIBUTION

Ni Putu Sarini, I Wayan Suarna, I Gusti Agung Arta Putra, I Ketut Puja, Lindawati Doloksaribu designed the experiments, executed them, and wrote the manuscript. I Gde Suranjaya, I Ketut Gde Natakusuma, I Gusti Ngurah Bagus Rai Mulyawan assisted in collecting data and analysis. All authors read and approved the final version of the manuscript.

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