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# Allelic and Genotypic Distribution in Single Nucleotide Polymorphism (SNP) G.676A > G of Melanocortin-1 Receptor (*MC1R*) Gene in Indonesian Goat Breeds

Research Article

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## ABSTRACT

The melanocortin-1 receptor (*MC1R*) gene has been investigated by many studies regarding the pigmentation variation in various species. In order to determine its allelic and genotypic distribution, we sequenced the goat *MC1R* gene from 78 individuals in ten populations (Gembrong, Senduro, Ettawa Grade, Boerawa, Boerka, Kosta, Samosir, Muara, Boer and Kacang). Direct sequencing method was performed to detect the single nucleotide polymorphisms (SNPs). Three SNPs (g.676A>G, g.748G>T and g.801C>G) were identified in the gene target. The SNP g.676 A > G was used to genotype the investigated animals by using polymerase chain reaction restriction fragment length polymorphism (PCR-RFLP) method with *EarI* restriction enzyme. In all studied populations, the A allele had the highest frequency (83%) and the G allele had the lowest frequency (17%). The frequency of homozygous AA genotype was the highest (90%) in Kacang goats, while that of heterozygous AG genotype was fixed in Senduro and Boer goats. Analysis of observed heterozygosity, expected heterozygosity, Nei's (1973) expected heterozygosity, and Shannon index were found to be 0.3425, 0.2858, 0.2838 and 0.4578, respectively. The distribution of *MC1R* gene in overall breeds confirmed with Hardy-Weinberg equilibrium (P>0.05).

**KEY WORDS** allele distribution, local goat, *MC1R* gene.

## INTRODUCTION

Goat is one of potential livestock species alongside cattle because of its ability to produce triple purposes: meat, milk and fur. The meat type of goat has an important leading market because the national consumption of goat meat ranks second after non-poultry meat such as beef. National meat demand amounted to 2,880,340 tons supported by approximately 23 percent from non-poultry meat consumption followed by 3 percent of goats, 2 percent of sheep and 1% of buffalo (Ditjenpkh, 2017). Indonesia has various local goat breeds including Gembrong, Senduro, Ettawa Grade, Boerawa, Boerka, Kosta, Samosir, Muara, Boer and Kacang. The coat color of the goats is varying from white to black and brown, or mix. The coat color is often an important preferable market point to select the animals. The farmers prefer to keep the certain goat color for increasing their income. To select the animal with preferable coat color, a molecular genetics approach can be performed through detection of the gene encoding the trait. Melanocortin-1-receptor (MC1R) is a potential candidate gene regulating the confirmation of animal coat color.

The gene encodes a protein called the MC1R which plays an important role in normal pigmentation. The melanocytes produce melanin which is the substance that gives skin, hair and eyes their color.

There are two forms of melanin namely eumelanin and pheomelanin. The *MC1R* gene controls the melanin which is produced by melanocyte. Whenever the receptor of the gene is activated, the production of eumelanin was stimulated, but if the receptor is blocked, melanocyte produces pheomelanin instead of eumelanin. Individual having mostly eumelanin, tends to have brown or black hair and dark skin color. In contrast, individual producing more pheomelanin tends to have red or blond hair, freckles and light-colored skin.

The *MC1R* gene has been widely detected in cattle, pig, yak, sheep and goat in order to identify the relationship with coat color (Rouzaud *et al.* 2000; Kijas *et al.* 1998; Vage *et al.* 1999; Fontanesi *et al.* 2009). In Murciano-Granadina Black goats, the p267W missence mutation of *MC1R* gene had been identified but no polymorphism had been detected in the same goat with brown coat color (Fontanasi *et al.* 2009). The mutation at position 676 bp of *MC1R* gene was found in Boer goat with red head and neck color (Wu *et al.* 2006).

The recent study reported that SNP g.676A > G of *MC1R* gene performed equal distribution in three different groups of color in Ettawa Grade goat (Maharani, *et al.* 2016). In order to have more information of the gene, the objective of this study was to identify the allele and genotype distribution of *MC1R* gene in ten Indonesian local goats. The results of study may give intention of learning of genetic basis on coat color that lead to give evidence for preferable market based on coat color.

## MATERIALS AND METHODS

Seventy-three blood samples from ten local breeds of Indonesian goat: Gembrong (n=5), Senduro (n=3), Ettawa Grade (n=3), Boerawa (n=7), Boerka (n=25), Kosta (n=4), Samosir (n=8), Muara (n=1), Boer (n=7) and Kacang (n=10) were used for DNA collection samples. The profile of the investigated Indonesian goat breeds are presented in Figure 1. All studied animals were reared under same feeding and management in Goat Research Center (GOATRES) in Sei Putih, North Sumatera, Indonesia. The blood samples were collected for genomic DNA isolation using gSYNC$^{TM}$DNA Extraction Kit (Geneaid, New Taipei City, Taiwan).

The used primers were according to Wu *et al.* (2006). The information of primers used, PCR product size and restriction enzyme for PCR-RFLP is shown in Table 1. Amplification was performed at 10 min at 94 ˚C, 35 cycles of 30 s at 94 ˚C, 30 s at 64 ˚C and 30 s at 72 ˚C, followed by final extension of 10 min at 72 ˚C. The PCR products were visualized on a 1.5% standard agarose gel stained with ethidium bromide.

In order to confirm the gene target and SNP position, the PCR products were sequenced using the same primers by 1$^{st}$ Base DNA sequencing service. The DNA sequences were analyzed with BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA) and SNPs detection was confirmed based on the electrophoregram results. The SNP g.676A > G is located in chromosome 18 (14208837..14212670 based on NC_022310.1) and used for genotyping of all investigated animals using PCR-restriction fragment length polymorphism (PCR-RFLP) method.

The restriction enzyme digestion was performed in 20 µL reaction volume with approximately 15 µL of PCR products and 2 units of each restriction enzyme. The digested products were run on a 3% agarose gel. Analysis for amino acid changes was performed by using expasy translate tool (https://web.expasy.org/translate).

The allelic and genotypic frequencies, as well as genetic diversity indices including $\chi^2$ test for Hardy-Weinberg equilibrium, observed heterozygosity, expected heterozygosity, Nei's (1973) expected heterozygosity, and Shannon index were determined using the POPGENE version 1.32 software (Yeh and Yang, 1999).

## RESULTS AND DISCUSSION

### Polymorphism of *MC1R* gene

Three SNPs were detected in the MC1R target gene located in exonic region of chromosome 18. They were SNP g.676A > G, SNP g.748G > T and SNP g.801C > G (Figure 2). Two SNPs were detected that lead to an amino acid change. The amino acid variants of K-E (lysine to glutamic) and F-V (phenylalanine to valine) were detected in SNP g.676A > G and SNP g.748G > T, respectively. However, no amino acid variant was detected in SNP g.801C > T. Wu *et al.* (2006) identified an amino acid variant K-E in SNP g.676A > G in Boer goat and reported 5 SNPs. Among the 5 SNPs, two variants locating at the sixth transmembrane regions and their third cytoplasmic domain, respectively, were identified. Similarly, 5 SNPs were identified in Yak's sequences and defined in three haplotypes (Chen *et al.* 2009). In French cattle breed revealed four SNPs, among them, one SNP was indicated to change amino acid from leucine to proline (Rouzaud *et al.* 2000). Moreover they reported that the other SNPs have a deletion and lead to change the amino acid. Three SNPs were also identified in Ettawa Grade goat with different neck color (Maharani *et al.* 2016).

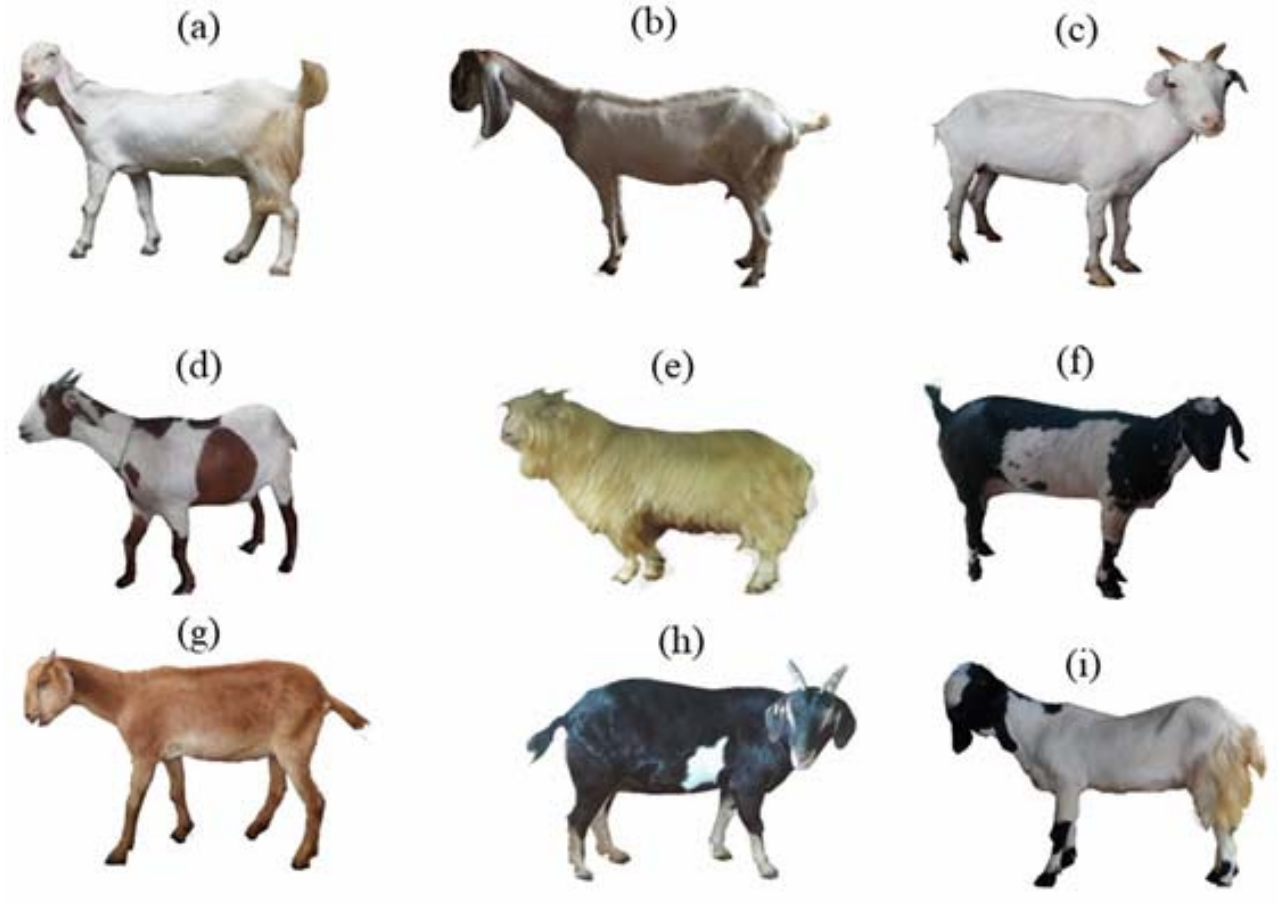


**Figure 1** The profile of some Indonesian goat breeds based on their coat color
(a) Senduro goat; (b) Boerawa goat; (c) Samosir goat; (d) Boerka goat; (e) Gembrong goat; (f) Muara goat; (g) Kacang goat; (h) Kosta goat and (i) Ettawa grade goat

**Table 1** The primers, annealing temperature and restriction enzyme for genotyping analysis of Indonesian goats using SNP g.676A > G

| GenBank Acc. No | Primer | PCR product size | Restriction enzyme |
|---|---|---|---|
| Y13958 | E1-F:5'GTGGACCGCTACATCTCCAT 3'<br>E1-R:5'TTGAAGATGCAGCCACAGG 3' | 416 bp | *EarI* |

**Genotyping and allele distribution**

Genotyping of animals was performed by PCR-RFLP method using SNP g. 676A > G in *MC1R* gene. Initially, the 416 bp of PCR product was digested using *EarI* restriction enzyme. Animals having AA genotype exhibited 163 bp and 253 bp fragments (Figure 3). The heterozygous AG animals revealed three fragments, i.e. 163, 253 and 416 bp. If the restriction enzyme did not digest the target gene, it meant that the animals would be classified to have GG genotype.

As a result, there were no homozygous GG animals detected in this study. Only homozygous AA and heterozygous AG animals were found in all breeds. These findings differ with Maharani *et al.* (2016) who reported no AA genotype in Ettawa Grade goat using the same SNP. The differences might be due to different rearing location. In this study, the goats were sampled from goat research center with controlled breeding system, while the samples in the study of Maharani *et al.* (2016) were collected from individual farmers.

As shown in Table 2, in all of the investigated breeds, genetic diversity indices including observed heterozygosity, Nei's (1973) expected heterozygosity, and Shannon index were found to be 0.3425, 0.2858 and 0.4578, respectively, indicating low level of genetic diversity. Futhermore, A and G alleles belonging to *MC1R* gene were identified. The AA genotype had the highest frequency (0.66) than AG genotype (0.34) in overall breed, as presented in Table 2.

The A allele frequency was higher than G allele. The frequency of homozygous AA genotype was the highest (83%) in Kacang goat, while that of heterozygous AG genotype was fixed in Senduro and Boer goats. The AA genotype frequency was lower than the AG genotype frequency in Kosta goat.

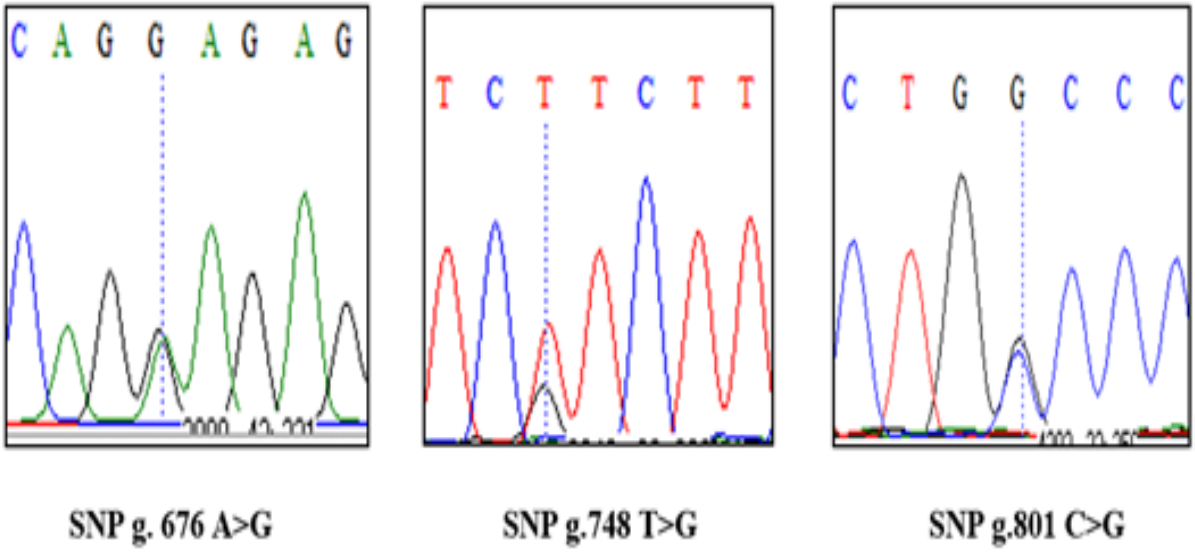


**Figure 2** Identification of three SNPs of MC1R gene of Indonesian goat breeds based on electrophoregram

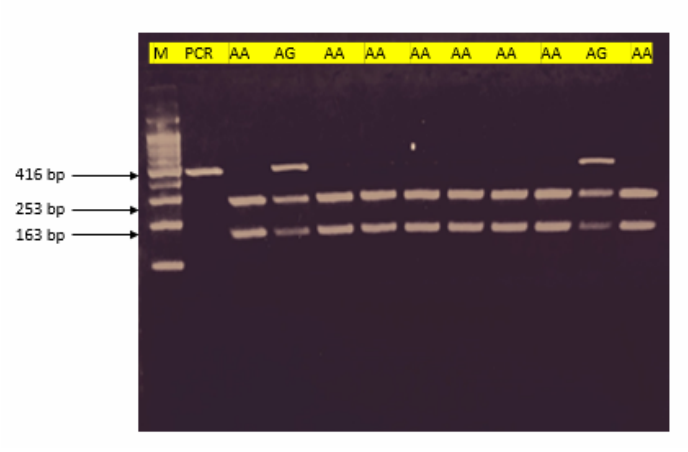


**Figure 3** Genotyping resuls for SNP g.676A > G of MC1R using PCR-RFLP methods in various Indonesian goat breed

**Table 2** The allelic and genotypic frequency of MC1R gene in nine goat breeds

| Breed | Allelic frequency | | Genotypic frequency | | $\chi^2$ |
|---|---|---|---|---|---|
| | A | G | AA | AG | |
| Overall breed | 0.83 | 0.17 | 0.66 | 0.34 | 0.18 |
| Gembrong | 0.90 | 0.10 | 0.80 | 0.20 | 0.01 |
| Senduro | 0.50 | 0.50 | 0.00 | 1.00 | 2.25 |
| PE | 0.83 | 0.17 | 0.67 | 0.33 | 0.04 |
| Boerawa | 0.79 | 0.21 | 0.57 | 0.43 | 0.20 |
| Boerka | 0.94 | 0.06 | 0.88 | 0.12 | 0.01 |
| Kosta | 0.63 | 0.38 | 0.25 | 0.75 | 0.88 |
| Samosir | 0.88 | 0.13 | 0.75 | 0.25 | 0.04 |
| Boer | 0.50 | 0.50 | 0.00 | 1.00 | 5.25* |
| Kacang | 0.95 | 0.05 | 0.90 | 0.10 | 0.00 |

* significant deviation from HWE expectation, with Chi-Square table score= 3.84.

The AA genotype may indicate in accordance with the white color of Gembrong and Samosir but not in Kosta, Kacang and Ettawa Grade goats. However, AG genotype may indicate the black color especially in Kosta which had a higher AG genotype frequency than other breeds. The differences may because some genes inhibit the melanocytes deposit melanosomes in white hair of Gembrong and Samosir goats. However, in fact, some gembrong goats have black hair color. This indicated that the *MC1R* gene may active the enzyme and related proteins which are responsible for melanogenesis. In case in Ettawa Grade goats, the head and neck color are black or brown but their body is white.

The white color in their body may be controlled by another gene or the *MC1R* gene may inactivate the melanocyt analysis. For example: the keratin genes, melanoma inhibitory activity (MIA) family genes, fatty acid realted genes, and melanoma-associated genes may play important roles in in coat color formation (Li B. *et al.* 2017). Moreover, they indicated top 10 up and down-regulated differentially expressed genes in white coat color *vs*. black coat color. The tyrosinase-related protein 1 (TYRP1) have been assessed acts in chocolate ("dark chestnut, liver chestnut, silver, seal brown") coat color family and be restricted to a non-chestnut background of the individual horse (Rieder *et al.* 2001). Similarly, as reported by Wu *et al.* (2006), the Boer goats were indicated to have two colors in their body, red color in head and neck, and white color found in their body. The MC1R is known to play an important role in controlling melanin syntesis (Jackson *et al.* 1997). The receptor of MC1R is activated by melanocyte stimulating hormone which leads to black or brown eumelanin production in melanosome. In human, the variant of MC1R had been reported having correlation with red hair color (Valverde *et al.* 1995). Interestingly, the highest AA genotype frequency in Kacang goat may indicate that the *MC1R* gene activates the enzyme strongly to synthesize melanocyte to produce eumelanin which increase the brown hair color. The $\chi 2$ test showed that allele and genotype distribution of the SNP g.676A > G of *MC1R* gene confirmed with Hardy-Weinberg equilibrium ($P>0.05$), indicating that there is no strong selection in favor of any genotype regarding the coat color in the investigated goat breeds.

## CONCLUSION

The AA genotype was highly distributed in Kacang, Gembrong, Ettawa Grade and Samosir goats which indicated having white hair color in whole (Gembrong) and some parts of their body (Ettawa Grade and Samosir). The distribution of both allele and genotype of *MC1R* gene confirmed with the expectation of Hardy-Weinberg equilibrium.

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## REFERENCES

Ditjenpkh. (2017). Livestock and Animal Health Statistics. Directorate General of Livestock and Animal Health Service, Jakarta, Indonesia.

Chen S., Huang Y., Zhu Q., Fontanesi L., Yao Y. and Liu Y. (2009). Sequence characterization of the *MC1R* gene in yak (*Poephagus grunniens*) breeds with different coat colors. *J. Biomed. Biotechnol*. **2009,** 1-6.

Fontanesi L., Barreti F., Riggio V., Dall'Olio S., Gonzales E.G., Finocchiaro R., Davoli R., Russo V. and Partolano B. (2009). Missense and nonsense mutations in melanocortin 1 receptor (*MC1R*) gene of different goat breeds: Association with red and black colour phenotypes but with unpected evidences. *BMC Genet*. **10,** 1-12.

Jackson I.J. (1997). Homologous pigmentation mutations in human, mouse and other model organisms. *Hum. Mol. Genet.* **6,** 1613-1624.

Kijas J.M., Wales R., Tornsten A., Chardon P., Moller M. and Andersson L. (1998). Melanocortin 1 receptor (MC1R) mutations and coat color in pigs. *Genetics*. **150,** 1177-1185.

Li B., He X., Zhao Y., Bai D., Shiraigo W., Zhao Q. and Manglai D. (2017). Regulatory pathway analysis of coat color genes in Mongolian horses. *Hereditas.* **155,** 13-23.

Maharani D., Budisatria I.G.S. and Panjono P. (2016). Identification of single nucleotide polymorphisms and allele distribution of *MC1R* gene in different head and neck color of Ettawa grade goat. *Asian J. Anim. Sci*. **10,** 219-233.

Nei M. (1973). Analysis of gene diversity in subdivided populations. *Proc. Natl. Acad. Sci. USA*. **70,** 3321-3323.

Rieder S., Taourit S., Mariat D., Langlois B. and Guérin G. (2001). Mutations in the agouti (ASIP), the extension (MC1R), and the brown (TYRP1) loci and their association to coat color phenotypes in horses (*Equus caballus*). *Mamm. Genome*. **12,** 450-455.

Rouzaud F., Martin J., Gallet P.F., Delourme D., Goulemotleger V., Amigues Y., Menissier F., Levezie H., Julien R. and Oulmouden A. (2000). A first genotyping assay of French cattle breeds based on a new allele of the extension gene encoding the melanocortin-1 receptor (*MC1R*). *Genet. Sel. Evol.* **32,** 511-520.

Valverde P., Healy E., Jackson I.J., Rees J.L. and Thody A.J. (1995). Variants of the melanocyte stimulating hormone receptor gene are associated with red hair and fair skin in humans. *Nat. Genet.* **11,** 328-330.

Vage D.I., Klungland H., Lu D. and Cone R.D. (1999). Molecular and pharmacological characterization of dominant black coat color in sheep. *Mamm. Genome*. **10,** 39-43.

Wu Z.L., Li X.L., Liu Y.Q., Gong Y.F., Liu Z.Z., Wang X.J., Xin T.R. and Ji Q. (2006). The red head and neck of Boer goats may be controlled by recessive allele of the *MC1R* gene. *Anim. Res*. **55,** 313-323.

Yeh F.C., Rong-Cai Y. and Tim B. (1999). POPGENE VERSION Microsoft Window-based Freeware for Population Genetic Analysis. University of Alberta and Tim Boyle, Centre for International Forestry Research, Bogo.