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

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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, Etawa 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 (Ditjenphk, 2017). Indonesia has various local goat breeds including Gembrong, Senduro, Etawa 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 (Fontanesi 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™ 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 1st 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 > G. 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).
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. Furthermore, 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.
Allelic and Genotypic Distribution of MC1R Gene

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

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

<table>
<thead>
<tr>
<th>Breed</th>
<th>Allelic frequency</th>
<th>Genotypic frequency</th>
<th>²χ</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall</td>
<td>0.83 0.17</td>
<td>0.66 0.34</td>
<td>0.18</td>
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</tr>
<tr>
<td>Gembrong</td>
<td>0.90 0.10</td>
<td>0.80 0.20</td>
<td>0.01</td>
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</tr>
<tr>
<td>Senduro</td>
<td>0.50 0.50</td>
<td>0.00 1.00</td>
<td>2.25</td>
<td></td>
</tr>
<tr>
<td>PE</td>
<td>0.83 0.17</td>
<td>0.67 0.33</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Boerawa</td>
<td>0.79 0.21</td>
<td>0.57 0.43</td>
<td>0.20</td>
<td></td>
</tr>
<tr>
<td>Boerka</td>
<td>0.94 0.06</td>
<td>0.88 0.12</td>
<td>0.01</td>
<td></td>
</tr>
<tr>
<td>Kosta</td>
<td>0.63 0.38</td>
<td>0.25 0.75</td>
<td>0.88</td>
<td></td>
</tr>
<tr>
<td>Samosir</td>
<td>0.88 0.13</td>
<td>0.75 0.25</td>
<td>0.04</td>
<td></td>
</tr>
<tr>
<td>Boer</td>
<td>0.50 0.50</td>
<td>0.00 1.00</td>
<td>5.25*</td>
<td></td>
</tr>
<tr>
<td>Kacang</td>
<td>0.95 0.05</td>
<td>0.90 0.10</td>
<td>0.00</td>
<td></td>
</tr>
</tbody>
</table>

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 Etawa 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 be 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 activate the enzyme and related proteins which are responsible for melanogenesis. In case in Etawa 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 melanocyte analysis. For example: the keratin genes, melano- noma inhibitory activity (MIA) family genes, fatty acid related genes, and melanoma-associated genes may play important roles in coat color formation (Li B. et al. 2017). Moreover, they indicated top 10 the 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 synthesis (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 χ² 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, Etawa Grade and Samosir goats which indicated having white hair color in whole (Gembrong) and some parts of their body (Etawa 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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