ISSN 1819-1878

Asian Journal of Animal Sciences

http://knowledgiascientific.com

Knowledgia SCIENTIFIC
A Place to Publish Outstanding Research

OPEN ACCESS Asian Journal of Animal Sciences

ISSN 1819-1878
DOI: 10.3923/ajas.2016.219.223

Research Article
Identification of Single Nucleotide Polymorphisms and Allele Distribution of MC1R Gene in Different Head and Neck Color of Ettawa Grade Goat

Dyah Maharani, I Gede Suparta Budisatria and Panjono

Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3, Bulaksumur, 55281 Yogyakarta, Indonesia

Abstract

Objective: The melanocortin-1-receptor (MC1R) gene is known as an important candidate gene for the coat color trait. The present study identified the polymorphisms and tested whether a functional polymorphism in exon 1 of MC1R gene was related to different head and neck color in Ettawa grade goat based on their allele distribution. **Methodology:** Total 30 Ettawa grade goats were divided in three group, CP: Brown head and neck color with white body color, RP: Brown or black head and neck color with various body color and HP: Black head and neck color with white body color were used in this study. The blood samples from all groups were collected for DNA isolation. Some samples were used to identify the polymorphisms by direct sequencing. Three Single Nucleotide Polymorphisms (SNPs) of MC1R gene were identified: SNP 676A>G, SNP 748T>G and SNP 801C>G. The SNP 676A<G was performed for genotyping by using *Ear*I restriction enzyme. **Results:** The A and G allele frequencies were similar 50% in both CP and RP group. However, the A allele frequency (55%) was slightly higher than G allele (45%) in HP group. The A and G allele distribution in all the group population was nearly the same ($X^2 = 0.016$, df = 1, p = 0.898). All of the goats in CP and RP groups had heterozygote (AG) genotype. In HP group only had one goat with homozygote animal (GG genotype). Interestingly, none AA genotype found in this study. **Conclusion:** In conclusion, the allele distributions were equal in all groups and almost all the goats with different head and neck color have similar heterozygote genotype.

Key words: Melanocortin-1-receptor (MC1R), single nucleotide polymorphism, allele distribution, Ettawa grade goat

Received: February 09, 2016 Accepted: March 03, 2016 Published: April 15, 2016

Citation: Dyah Maharani, I Gede Suparta Budisatria and Panjono, 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: 219-223.

Corresponding Author: Dyah Maharani, Faculty of Animal Science, Universitas Gadjah Mada, Jalan Fauna No. 3, Bulaksumur, 55281 Yogyakarta, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

The contribution of goats to the national meat supply in Indonesia is only 2.9% while goats population increase from 2003-2007 was just around 2-3% per annum. The goats population in Indonesia is approximately 13.491 million and involves 2.7 million farmers (Anonymous, 2015). Among local goat in Indonesia, Ettawa grade goat is one of potential asset that can be developed due to their dual purpose in milk and meat production. The Ettawa grade goats originally descended from cross-breeding between Kacang and Ettawa goats. This breed consists of three groups: Black head hair with white body, brown head hair with white body and mix color. To increase the income, the farmers prefer keep the goat with black head and neck color for competition purpose rather than for milk purpose due to the high price compare to brown color. Even though, farmers believed that color differences will not have impact on the productivity of goats. Initially, these perception need to be confirmed based on genetic molecular basis, which can describe the distribution of allele of gene, which responsible to the hair color differences. In order to detect the genotype variability of the type of both goats, an analysis of gene polymorphism need to be conducted. Furthermore, this molecular approach is of major significance for many fundamental knowledge and applied areas of animal genetics. This advent of gene technology leads to give basic evidence to prove the group of farmer perceptions. This study also gives an implication of socio-economic policy to gain the benefit of farmers.

The melanocortin-1-receptor (MC1R) gene plays a central role in regulation of animal coat color formation. The gene has been widely used to identify the coat color in various ruminants such as in sheep, cattle, goat and rabbit. The haplotype AATGT in MC1R was uniquely associated with black coat color in minxian black-fur breed (Yang *et al*., 2013). The coat color extension gene (E), which encoded the transmembrane domain MC1R have been indicated affecting the coat color in French cattle breed (Rouzaud *et al*., 2000). In goat, the p.267W missence mutation located in coding region of MC1R was present in all Murciano-Granadina black goats, whereas it was never identified in the brown ones (Fontanesi *et al*., 2009). The c.[124A, 125_130del6] was suggested responsible for a MC1R variant determining eumelanin production in the black area of the rabbit (Fontanesi *et al*., 2010). Moreover, the mutation at the position 676 bp of MC1R gene had been detected in Boer goat with red head and neck color (Wu *et al*., 2006). Therefore, the objective of this study was to identify the genetic profile of Ettawa grade goat with different head color based on MC1R gene. Due to the cross bred nature of Ettawa grade goats, the research hypothesis is that the genotype is heterozygote.

MATERIALS AND METHODS

Animal and sampling: Thirty Ettawa grade goats, which divided in three groups, CP: Brown head and neck color with white body color, RP: Brown or black head and neck color with various body color and HP: Black head and neck color with white body color were reared in the field laboratory in Faculty of Animal Science, Universitas Gadjah Mada (FAS UGM) with the same environments condition. The blood samples were collected for genomic DNA isolation using gSYNC DNA Extraction Kit (Genetika Science, Indonesia).

DNA amplification and genotyping: The primer sequences according to Wu *et al*. (2006), annealing temperature for PCR amplification and the restriction enzyme for PCR-RFLP are shown in Table 1. Amplifications were performed in 10 min at 94°C for pre-denaturation, 30 sec at 94°C for denaturation, annealing at 64°C for 30 sec, elongation (extension) at 72°C for 30 sec and a final extension at 72°C for 10 min. The PCR products were visualized in 1.5% standard agarose gels stained with ethidium bromide.

The PCR products were sequenced using the same primers for PCR by PT Genetika Science, Indonesia. The DNA sequences were analyzed with the BioEdit program ver. 7.00 (Tom Hall, Ibis Therapeutics, California, USA) and the SNPs was confirmed based on the electrophoregram results. The SNP G.676A>G was used for genotyping using PCR-restriction fragment length polymorphism (PCR-RFLP) method. The restriction enzyme digestion was performed in 20 μL reaction volumes with approximately 15 μL of PCR products and 2 units of each restriction enzyme. The digested products were run on 3% agarose gels.

Statistical analysis: A chi-square test was performed to test the allele and genotype frequencies for Hardy Weinberg equilibrium. The following mathematical model was:

Table 1: Primers, annealing temperature and restriction enzyme for genotype of Ettawa grade goat

GenBank accession No.	Primer	PCR product size	Restriction enzyme
Y13958	E1-F: 5' gtggaccgctacatctccat 3' E1-R: 5' ttgaagatgcagccacagg 3'	416 bp	*Eah*

$$\chi^2 = \sum_{i=1}^{n} \frac{(Oi - Ei)}{Ei}$$

where, χ^2 is chi-square value, Oi is observed frequency, Ei is expected frequency and n is the number of possible outcomes of each event.

RESULTS AND DISCUSSION

The SNPs in MC1R gene was initially identified by direct sequencing using PCR product pool. The SNP was confirmed by BioEdit program. As the results, three SNPs of MC1R gene were identified: SNP 676A>G, SNP 748T>G and SNP 801C>G (Fig. 1a, b). Among three SNPs, the SNP 676A>G and SNP 748T>G were found to have amino acid variants. The amino acid variant K-E (lysine to glutamic) was detected in SNP 676A>G and variant F-V (phenylalanine to valine) in SNP 748T>G. However, no amino acid variant have been detected in SNP 801C>T. Similar result have been observed in Boer goat that the amino acid variant K-E detected in SNP 676A>G (Wu *et al*., 2006). In sheep, two nonsynnonymous mutations previously associated with coat color (c.218 T>A, p.73 Met>Lys c.361 G>A, p.121 Asp>Asn) and three synonymous mutations (c.429 C>T, p.143 Tyr>Tyr; c.600 T>G, p.200 Leu>Leu, c.735 C>T, p.245 Ile>Ile) were identified in the CDS of MC1R gene (Yang *et al*., 2013).

The SNP 676A>G was used for genotyping using PCR-RFLP. Homozygote AA and GG were defined when the fragments size being recognized at 163, 253 and 416 bp, respectively. The heterozygote AG existed by PCR-RFLP method at the same position of the homologous chromosome with 163, 253 and 416 bp of fragments size (Fig. 2). The results showed most of animals in three groups have heterozygote (AG) genotype. Only one animal have GG genotype in HP group. Based on Table 2, the results indicated the spread of both alleles A and G were equal in all groups. The A and G allele frequencies were similar 0.5 in both CP and RP group. However, the A allele frequency (0.55) was slightly higher than G allele (0.50) in HP group. The AG genotype frequencies were

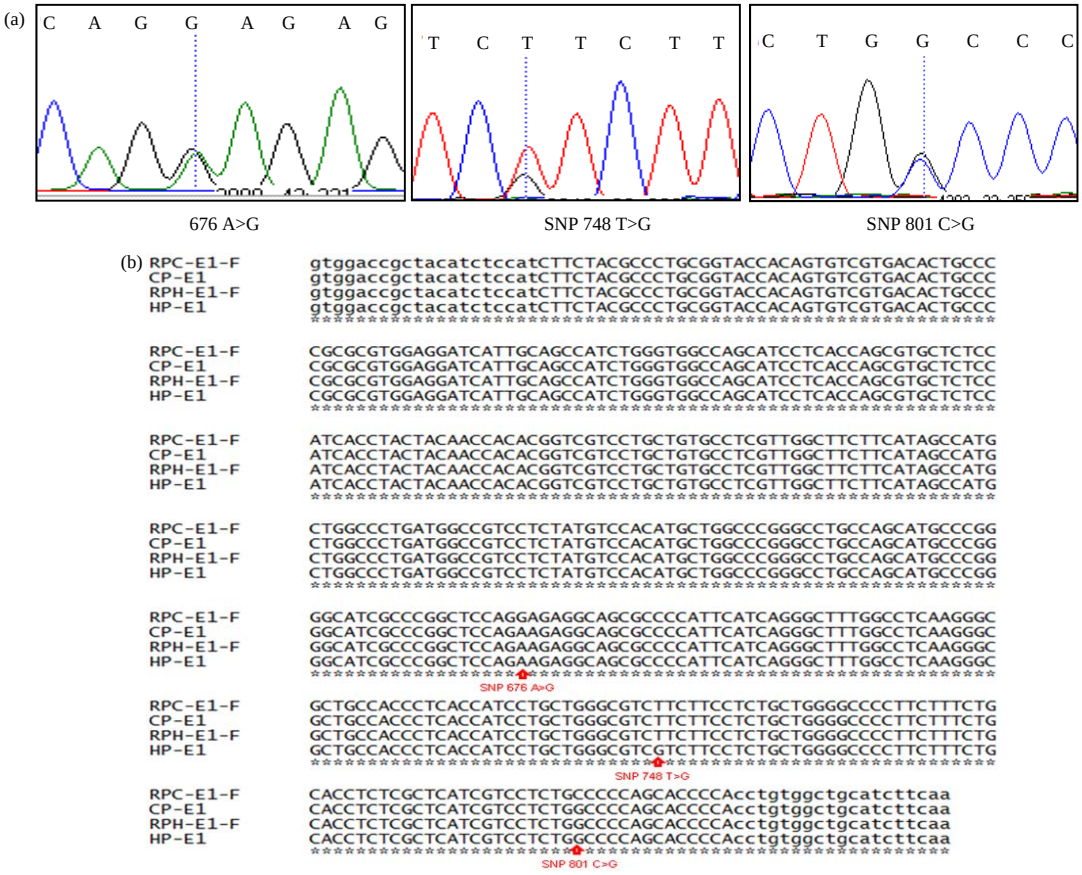

Fig. 1(a-b): Identification of three SNPs based on (a) Electrophoregram and (b) Alignment sequence of PCR products

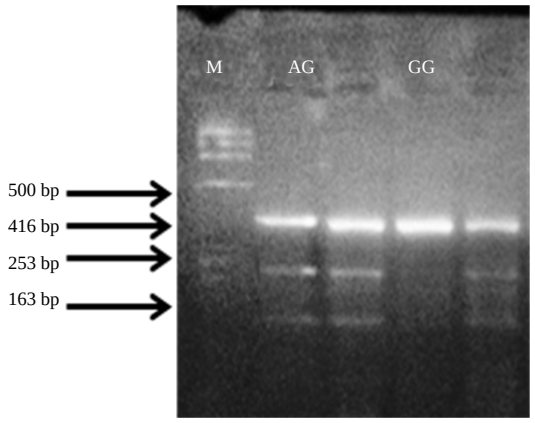

Fig. 2: PCR-RFLP genotyping results for SNP 676A>G of MC1R in Ettawa grade goats, heterozygote animal with AG genotype was defined when the fragment size being recognized with 163, 253 and 416 bp and GG genotype with 416 bp

Table 2: Frequencies allele and genotype of three groups Ettawa grade goats based on PCR-RFLP results using SNP g.676A>G

	Allele frequency		Genotype frequency		
Groups	A	G	AA	AG	GG
CP	0.50	0.50	0.00	1.00	0.00
RP	0.50	0.50	0.00	1.00	0.00
HP	0.55	0.45	0.00	0.90	0.10

CP: Brown head and neck color with white body color, RP: Brown or black head and neck color with various body color, HP: Black head and neck color with white body color

similar 1.00 in both CP and RP group. This suggested that both allele A and G of the MC1R gene may control the black and red color in head and neck of Ettawa grade goat since, they are descended originally from crossing between the Kacang and Ettawa goats. Most of Kacang goats have red (brown) color and Ettawa have mix black and white color. Thus, most of goats in CP and RP groups are heterozygote genotypes. However, no AA genotype was identified in the study. Compare to Wu *et al*. (2006) using similar SNP, three genotypes of Boer goats have been detected. The AA genotype indicated has association with red head and neck color in Boar goats. Breed differences may due to pattern genotype variances.

The results of Pearson's chi-square (χ^2) test indicated that the genotypes of the goats were deviated from the Hardy-Weinberg equilibrium (HWE) since χ^2>3.84 (5% significance level for 1 degree of freedom). The deviation may due to variation of causes. Mutation, gene flow, non-random mating (Assortative mating), genetic drift and selection may lead to deviate from HWE (Falconer and Mackay, 1996). In case of this study, non-random mating and small sample size may due to the deviation.

CONCLUSION

In conclusion, all the groups of Ettawa grade goats with different head and neck color have equal allele distribution and same heterozygote genotypes based on MC1R gene. These results may give evidence to group of farmer perceptions in order to select their animal. Furthermore, these results also suggest that the SNP 676A<G of MC1R may control the genotype profile of Ettawa grade goats.

SIGNIFICANT STATEMENT

This molecular approach is of major significance for many fundamental knowledge and applied areas of animal genetics. This advent of gene technology leads to give basic evidence to prove the group of farmer perceptions. This study also gives an implication of socio-economic policy to gain the benefit of farmers.

ACKNOWLEDGMENT

This study was supported by a grant namely "Competency Research Grant 2015" from The Indonesian Directorate General of Higher Education (DIKTI) with contract No. 203/LPPM/2015. Furthermore, all the authors report no financial interests or potential conflicts of interest in this study.

REFERENCES

Anonymous, 2015. Agriculture statistic census 2013. Central Bureau of Statistic, Indonesia. http://st2013.bps.go.id/dev2/index.php/site/topik?kid=5&kategori=Peternakan.

Falconer, D.S. and T.F.C. Mackay, 1996. Hardy-Weinberg Equilibrium. In: Introduction to Quantitative Genetics, Falconer, D.S. and T.F.C. Mackay (Eds.). 4th Edn., Pearson, Essex, UK., ISBN: 978-0582243026, pp: 5-18.

Fontanesi, L., F. Beretti, V. Riggio, S. Dall'Olio and E.G. Gonzalez *et al*., 2009. Missense and nonsense mutations in melanocortin 1 receptor (*MC1R*) gene of different goat breeds: Association with red and black coat colour phenotypes but with unexpected evidences. BMC Genet., Vol. 10. 10.1186/1471-2156-10-47.

Fontanesi, L., E. Scotti, M. Colombo, F. Beretti and L. Forestier *et al*., 2010. A composite six bp in-frame deletion in the melanocortin 1 receptor (*MC1R*) gene is associated with the Japanese brindling coat colour in rabbits (*Oryctolagus cuniculus*). BMC Genet., Vol. 11. 10.1186/1471-2156-11-59.

Rouzaud, F., J. Martin, P.F. Gallet, D. Delourme and V. Goulemot-Leger *et al*., 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.

Wu, Z.L., X.L. Li, Y.Q. Liu, Y.F. Gong and Z.Z. Liu *et al*., 2006. The red head and neck of Boer goats may be controlled by the recessive allele of the *MC1R* gene. Anim. Res., 55: 313-322.

Yang, G.L., D.L. Fu, X. Lang, Y.T. Wang, S.R. Cheng, S.L. Fang and Y.Z. Luo, 2013. Mutations in MC1R gene determine black coat color phenotype in Chinese sheep. Scient. World J. 10.1155/2013/675382