The association of SNP g.880A/G with body weight in F1 cross Dorper x Garut sheep

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Abstract. Single nucleotide polymorphism (SNP) in the MC4R gene has been known to be associated with feed intake and growth performance. Our objective was to analyze the association of SNP g.880A>G to birth weight (BW), weaning weight (WW), and 6-month body weight (MW) in F1 cross Dorper x Garut sheep. In forty-one F1 cross Dorper x Garut sheep with phenotypic records, genotyping based on SNP g.880A>G was achieved using the direct-sequencing process. As a result, the homozygous AA genotype was absent in the samples. The frequency of the G allele (90%) was higher than the A allele (10%), followed by GG (80%) and AG (20%) genotypes. The population did not deviate from Hardy Weinberg Equilibrium (p > 0.05) based on SNP g.880A>G. The SNP g.880A>G was significantly associated with MW but not significant in BW and WW. The GG genotype (32.33 ± 4.81 kg) was higher MW than the AG genotype (27.19 ± 1.86 kg). In conclusion, the findings suggested that SNP g.880A>G of the MC4R gene could be used as a potential selection tool for high MW in F1 cross Dorper x Garut sheep.

Keywords: association, MC4R gene, body weight, F1 cross Dorper x Garut sheep

1. Introduction
Growth and meat production traits were part of sheep's economic traits [1]. There has been an escalation of concern to identify and characterize markers correlated with meat traits in recent years. Recently, polymorphisms in sheep have been recognized in less than 5 percent of gene screening of sheep's whole genome. Most of the polymorphisms were found when the research for methods to increase sheep productivity was carried out [2]. A few studies reported on the non-coding regions of genes, which could be good candidates for assessing the potential use of the new variants as growth-related trait markers in sheep. One of the candidate genes for growth traits is the Melanocortin-4 Receptor (MC4R). MC4R is a peptide generated by the mammalian hypothalamus. According to the previous study, MC4R regulates the energy balance and leptin hormone, controlling animal food intake and body weight [3, 4]. The ovine MC4R gene is located in chromosome 23, 3869 base pairs long, and has two exons (Gene ID: 100147707). The polymorphism and association study within the non-coding region of the MC4R gene have been reported in Hu sheep [8, 9, 11, 12], German Merino sheep [10], Chinese Merino sheep [13], and Tibetan sheep [14].

Garut sheep is a local sheep breed distributed mostly in West Java Province, Indonesia [15]. This sheep is currently being crossed with Dorper sheep to improve meat production. Based on its molecular
approaches, the genetic profile of F1 cross Dorper x Garut sheep has not been reported yet. Hence, the purpose of this study was to examine the relationship between MC4R gene polymorphisms on body weight (birth weight, weaning weight, and 6-months weight) in F1 cross Dorper x Garut sheep.

2. Materials and methods

2.1 Animals and DNA extraction

In total, 41 F1 cross Dorper x Garut sheep were genotyped in this study. All studied animals were under similar feeding and environmental management in PT. Agro Investama Malangbong, West Java. Three milliliters of blood samples were collected using a 21G venoject needle from the jugular vein. It was preserved in EDTA vacutainer tubes during transport to the laboratory. The DNA was extracted using the gSYNC DNA Extraction Kit (Geneaid, New Taipei City, Taiwan).

2.2 Genotyping the samples

A polymerase chain reaction (PCR) technique was used to get the partial fragment of sheep's MC4R gene. The 25 µL mixture reaction consists of 9.5 µL double-distilled water (DDW), 12.5 µL MyTaq HS Red Mix (Bioline, UK), 0.5 µL of each primer (forward: 5'-TTCGTGTGGGGCAAGTCAAG-3' and reverse: 5'-GGAAACGCTCACAAACATGT-3'), and 2 µL of DNA. The amplification conditions consist of initial denaturation 95 °C 5 min, followed by 35 cycles of denaturation 94 °C 30 s, annealing 59 °C 30 s, and the final extension for 72 °C 10 min. Two percent of agarose gel (1st BASE, Singapore) was added with 100bp molecular weight (New England Biolabs, United States) used for verification of the PCR product's size (approximately 377 bp). The PCR products were then sequenced (one-direction sequencing) to get the nucleotide's structure at Central University Laboratory of Universitas Gadjah Mada. Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and BioEdit programs were used to analyze the raw sequence data and genotype the samples.

2.3 Statistical analysis

POPGENE program ver. 1.32 were used to analyze the genetic diversity (allele and genotype frequencies, heterozygosity, and chi-square test for Hardy-Weinberg’s equilibrium) within the F1 cross Dorper x Garut sheep population. The effect of genotype based on SNP g.880A/G on body weight (birth weight, weaning weight, and 6-months weight) was analyzed using an independent sample t-test with a mathematical model as follows:

\[ Y_{ij} = \mu + T_i + \epsilon_{ij} \]

where: \( \mu \): average of the population. \( T_i \): effect of K-individual genotype, \( \epsilon_{ij} \): effect of random error [6]. The effect of genotype was considered statistically significant if the \( P \)-value was less than 0.05.

3. Result and discussion

3.1 SNP identification

A 377 bp partial fragment of the MC4R gene has successfully amplified in F1 cross Dorper x Garut sheep population (Figure 1). This partial fragment consists of nucleotide sequences from 5'UTR, coding sequence (CDS) 1, and intron regions. Alignment among sequences from 41 samples disclosed no polymorphic site in the CDS 1 and one SNP (g.880A>G) in the intron region. The electropherogram showed clear peaks of GG and AG genotypes of SNP g.880A>G (Figure 2). No homozygous AA animal was detected in this F1 cross Dorper x Garut population. The SNP g.880A>G was previously reported to be polymorphic in Hu and East-Friesian sheep [12], German Merino sheep [10], and Tibetan sheep [14]. This SNP was thought to affect the biological processes of the studied sheep. This suspicion was confirmed by Nakaya et al. [17], which states that intron polymorphisms can affect phenotypic changes because introns or non-coding RNAs (micro RNAs) play a role in various biological processes control of transcriptional and post-transcriptional gene expression.
3.2 Genotype and allele frequency
The result of genetic diversity analysis (allele and genotype frequencies, chi-square test, and expected and heterozygosity values) of SNP g.880A>G in F1 cross Dorper x Garut sheep are shown in Table 1. The frequency of the G allele (90%) is higher than the A allele (10%), followed by GG (80%) and AG (20%) genotypes. Zuo et al. [10] reported that allele G in the same SNP is also the most frequent in German Merino sheep. The SNP g.880A>G in F1 cross Dorper x Garut sheep have not deviated from HWE (P<0.05). The heterozygosity (He) value was obtained from the frequency of heterozygous genotype at each locus. In this study, the heterozygosity was low based on the SNP g.880A>G. The low genetic variation in the F1 cross Dorper x Garut population might be due to the absence of the AA genotype. This finding is supported by Wang et al. [12] that the degree of variation in genetic structure can be reflected in the He value. The higher the He value indicates that the genetic variation is high and rich in genetic diversity. Nei [18] states that the He value ranges from 0 (zero) to 1 (one). If the He value is close to zero or equal to 0 (zero), the measured population has a very close kinship. Another reason might be the mating arrangement of the Dorper and Garut sheep was not in control. Only 12 male Dorper were used in the mating system without mating records, leading to a close relationship among the offspring.

Table 1. Allele and genotype frequencies, observed and expected heterozygosity, and chi-square analysis of SNP g.880A>G in F1 cross Dorper x Garut sheep.

| Locus  | Genotype frequencies | Allele frequencies | Obs Het | Exp Het | χ² |
|--------|----------------------|-------------------|--------|--------|----|
| g.880A>G | AA  | GG  | AG  | A     | G     | 0.80 | 0.20 | 0.10 | 0.90 | 0.20 | 0.19 | 0.41 |

3.3 The effect of SNP g.880A>G on body weights
The relationship between genotypes in F1 cross Dorper x Garut sheep based on SNP g.880A>G obtained in this study and phenotypes (birth weight, weaning weight, and 6-months body weight) were analyzed using an independent sample t-test in the SPPS Program ver 20. Based on Table 2, the genotype
by SNP g.880A> G had no significant impact on birth weight and weaning weight but had a substantial effect on 6-months weight in F1 cross Dorper x Garut sheep. This finding is consistent with Zuo et al. [10], which reported that the same locus (g.1016G/A) had a significant effect on body weight aged 120 and 180 days (6 months) in German Merino sheep. Song et al. [8] reported that different SNP (g.1016G>A), which was located in the non-coding region (3’UTR position), had a significant effect on the weaning weight of 45 days in Hu sheep. In the 5’UTR region, Shishay et al. [9] found that the SNP g.-103C>G was associated with body weight in Hu sheep.

Table 2. The association of SNP g.880A>G with body weights in F1 cross Dorper x Garut sheep.

| Locus         | Phenotype                      | n  | Genotype | p-value |
|---------------|--------------------------------|----|----------|---------|
| g.480A/G      | Birth weight (kg)              | 41 | GG       | 3,59 ± 0,63 | 3,52 ± 0,96 | 0,778 |
|               | Weaning weight (kg)            | 41 | AG       | 21,41 ± 5,14 | 23,74 ± 6,15 | 0,274 |
|               | 6-month market weight (kg)     | 25 | GG       | 32,33 ± 4,81^a | 27,19 ± 1,86^b | 0,049 |

The means within the same row with a different letter have significant differences (P>0.05).

4. Conclusion
One SNP (g.880A>G) was detected in intronic region of the ovine MC4R gene in F1 cross Dorper x Garut population. The GG genotype and G allele were most frequent in the samples. The relationship between genotypes (GG and AG) based on SNP g.880A>G significantly impact the 6-month bodyweight. Hence, the SNP g.880A>G of the MC4R gene could be a potential selection tool for a high 6-month bodyweight in F1 cross Dorper x Garut sheep.

5. References
[1] Zhang L, Liu J, Zhao F, Ren H, Xu L, Lu J, Zhang S, Zhang X, Wei C, Lu G, Zheng Y, and Du L 2013 Genome-Wide Association Studies for Growth and Meat Production Traits in Sheep PLoS One 8 1–12
[2] Darlay R J, McCarthy A J, Illot N E, Smith J E and Shaw M A 2011 Novel polymorphisms in ovine immune response genes and their association with abortion Anim. Genet. 42 535–43
[3] Markison S and Foster A C 2006 Targeting melanocortin receptors for the treatment of obesity Drug Discov. Today Ther. Strateg. 3 569–76
[4] Yeo G S H, Farooqi I S, Aminian S, Halsall D J, Stanhope R G and O’Rahilly S 1998 A frameshift mutation in MC4R associated with dominantly inherited human obesity [1] Nat. Genet. 20 111–2
[5] Prihandini P W, Sumadi, Suparta G and Maharani D 2019 Melanocortin-4 receptor (MC4R) gene polymorphism and its effect on growth traits in Madura cattle J. Indones. Trop. Anim. Agric. 44 38–46
[6] Maharani D, Fathoni A, Sumadi, Hartatik T and Khusnudin M 2018 Identification of MC4R gene and its association with body weight in Kebumen Ongole Grade cattle J. Indones. Trop. Anim. Agric. 43 87–93
[7] Latifah L, Maharani D, Kustantinah A and Hartatik T 2018 Association of Melanocortin 4 Receptor gene polymorphism with growth traits in Bligon goat J. Indones. Trop. Anim. Agric. 43 343–51
[8] Song X M, Jiang J F, Zhang G Z, Shi F X and Jiang Y Q 2012 DNA polymorphisms of the Hu sheep melanocortin-4 receptor gene associated with birth weight and 45-day weaning weight Genet. Mol. Res. 11 4432–41
[9] Shishay G, Liu G, Jiang X, Yu Y, Teketay W, Du D, Jing H and Liu C 2019 Variation in the Promoter Region of the MC4R Gene Elucidates the Association of Body Measurement Traits in Hu Sheep Int. J. Mol. Sci. 20 1–18
[10] Zuo B, Liu G, Peng Y, Qian H, Liu J, Jiang X and Mara A 2014 Melanocortin-4 receptor (MC4R) polymorphisms are associated with growth and meat quality traits in sheep Mol. Biol. Rep.
41 6967–74

[11] Shan H, Song X, Cao Y, Xiong P, Wu J, Jiang J and Jiang Y 2020 Association of the melanocortin 4 receptor (MC4R) gene polymorphism with growth traits of Hu sheep Small Rumin. Res. 192 1–7

[12] Wang Y, Wang C, Zhang J, Meng C, Zhang X, Wang Z, Fang Y, Mao D and Cao S 2015 Three novel MC4R SNPs associated with growth traits in Hu sheep and East Friesian × Hu crossbred sheep Small Rumin. Res. 125 26–33

[13] Zeng X, Chen H, Jia B, Zhao Z, Hui W, Zhang W, Wu H and Wang Z 2011 Effects of Single and Combined Genotypes of MC4R and PROP1 Genes on Growth Traits in Chinese Merino Sheep Acta Vet. Zootech. Sin. 42 1227–32

[14] Zhao X L, Cao S J and Wang J Q 2018 Association Analysis of MC4R Gene Polymorphisms and Genotype Combination with Growth Traits of Tibetan Sheep (Ovis aries) J. Agric. Biotechnol. 26 429–36

[15] Tawaf R, Heriyadi D, Anang A, Sulaeman M and Hidayat R 2011 Empowerment of small farmer business ‘Garut sheep’ in West Java International Conference on Sustainable Agricultural and Food Security: Challenges and Opportunities pp 1–8

[16] Maharani D, Elieser S, Budisatria I G S, Batubara A, Hariyono D N H and Sari A P Z N L 2019 Allelic and Genotypic Distribution in Single Nucleotide Polymorphism (SNP) G .676A>G of Melanocortin -1 Receptor (MC1R) Gene in Indonesian Goat Breeds Iran. J. Appl. Anim. Sci. 9 687–92

[17] Nakaya H I, Amaral P P, Louro R, Lopes A, Fachel A A, Moreira Y B, Tarik A E-J, da Silva A M, Reis E M and Verjovski-Almeida S 2007 Genome mapping and expression analyses of human intronic non-coding RNAs reveal tissue-specific patterns and enrichment in genes related to regulation of transcription Genome Biol. 8

[18] Nei M 1978 Estimation of average heterozygosity and genetic distance from a small number of individuals Genetics 89 583–90

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