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Research article

Identification of TGFBR2 gene (SNP g.5112179A>G) associated with carcass characteristics and meat quality in sheep

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Abstract

This study aimed to examine the transforming growth factor, beta receptor II (TGFBR2) gene, for the association with carcass characteristics and meat quality. This study used 95 samples of rams consisting of 9 garut composite sheep (GCS), 12 garut sheep (GS), 9 compass agrinac sheep (CAS), 10 barbados cross sheep (BCS), 10 jonggol sheep (JS), and 45 javanese thin-tailed (JTT) with the same treatment and condition. The polymorphism identification of the TGFBR2 gene was performed by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) with SNP target g.5112179A>G. This study observed carcass characteristics and meat quality, including body weight, hot carcass, carcass percentage, carcass length, cold carcass, meat pH, tenderness, cooking loss, and water-holding capacity. The association between the TGFBR2 gene with carcass characteristics and meat quality was analyzed using a general linear model (GLM). The result showed that the TGFBR2 gene was significantly (P<0.05) associated with carcass length, carcass percentage, and tenderness. The TGFBR2 gene had two genotypes, AA and AG, with the highest genotype frequency being the AG genotype. The AA genotype was associated with a higher level of carcass percentage and tenderness, while the AG genotype was associated with a higher level of carcass length. It can be concluded that the TGFBR2 gene reveals a potential candidate gene to select sheep meat with high carcass characteristics and meat quality

Keywords: Carcass characteristics, Meat quality, Sheep, Tenderness, Transforming growth factor beta receptor II

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INTRODUCTION

Indonesia is the largest archipelago country with the world's fourth-most populous. The high population number in Indonesia creates a high need for protein. According to OECD/FAO (2020), the average lamb meat consumption between 2010 and 2019 is 0.4 kg per capita/ year. The production of lamb meats in Indonesia is 66,943.34 tons (BPS, 2020). The increasing population in Indonesia is creating increasing demand for lamb meat.

The quality of local sheep can be improved using conventional or molecular selection. Genetic selection can be driven by economic value traits. The quality improvement effort among local sheep in Indonesia uses markers selection based on genetic molecular that can be selected up to DNA level and resulting product for detection, profiling, and non-coding RNA discovery (Cánovas et al., 2010). Molecular-based studies for meat quality traits have been carried out since 1990, but only 789 studies on Quantitative Traits Loci (QTL) in sheep were found until 2013 (Zhang et al., 2013).

The TGFBR2 gene also already had a few correlations that have been identified, such as with differentiation, proliferation, and apoptosis in a mammal Kubiczkova et al. (2012), development of cancer (Derynck, 2008), gene candidate in meat quality on cattle (Sevane et al., 2015), gene candidate that correlated with fatty acid in sheep (Gunawan, 2021), and the gene that effected in apoptosis in cow (Nørgaard et al., 2008). The TGFBR2 gene has a broad spectrum of cellular functions such as apoptosis, proliferation, migration, and differentiation via the TGFB signaling pathway and osteoclast differentiation. However, there was no study investigating the polymorphism of TGFBR2 with carcass characteristics and meat quality in sheep, especially in Indonesian sheep. Functional studies suggested this gene could be important candidate genes for carcass characteristics and meat quality. Therefore, the objective of this study was to examine the effects of the TGFBR2 polymorphisms on carcass characteristics and meat quality in sheep.

MATERIALS AND METHODS

Ethics approval

This study was conducted after approval from the Animal Care and Use Committee of Bogor Agricultural University (No. 117 – 2018 IPB).

Animals and phenotypes

A total of 95 Indonesian rams are used to identify polymorphisms of the TGFBR2 gene. The rams consisted of 9 Garut Composite Sheep (GCS), 12 Garut Sheep (GS), 9 Compass Agrinac Sheep (CAS), 10 Barbados Cross Sheep (BCS), 10 Jonggol Sheep (JS), and 45 Javanese thin-tailed (JTT) with aged between 10-12 months and body weight 20-30 kg. All sheep were gathered from different farms but with the same treatment and conditions. A total of 95 rams were slaughtered in a commercial abattoir PT Pramana Pangan Utama Slaughter House. All procedures involving animals were approved by the Animal Ethics Commission of IPB University with approval number 117-2018 IPB. After being slaughtered, the carcass was frozen at -20 °C. Twenty-four hours after storing, the carcass was deboned and analyzed for carcass characteristics and meat quality.

Measurement of meat quality

Meat quality traits were analyzed including pH, tenderness, cooking loss, and water-holding capacity (WHC). The pH was measured using a pH meter in the longissimus dorsi muscle. The meat tenderness was measured using Warner-Bratzler shear force (WBSF). Cooking loss was measured by deducting the initial weight of the sample meat after being cooked in a water bath for one hour.

Measurement of carcass characteristics

Carcass characteristic traits were analyzed, including body weight, hot carcass, carcass percentage, carcass length, and cold carcass. Body weight was calculated before the rams were slaughtered. Hot carcass weight and cold carcass weight were measured after all non-carcass components were removed and after the carcass was stored at –20 °C for 24 hours, respectively. Carcass percentage was calculated based on empty body weight. Carcass length was measured from the point of the shoulder to the distal end of the tarsus.

DNA extraction, PCR amplification, and genotyping using PCR-RFLP

Genomic DNA was extracted from longissimus dorsi muscle samples using the Geneaid gSYNC DNA Extraction Kit (Geneaid, Taiwan) based on the manufacturing protocol. The SNP of the TGFBR2 gene (g.5112179A>G) resulted from RNA sequencing in sheep. The primer sequences are introduced by Gunawan et al. (2018) (Table 1).

Table 1 Primer sequences of TGFBR2 gene

Accession Number	Primer Sequence	Size (bp)	Tm (°C)	Enzyme	SNP	Digested fragments length (bp)
NC_019476.2	F: 5'- CAG AGA TAA GGC AGT TTG GC-3' R: 5'- GCA AAA GTA CTC AGG ACA GC-3'	488	55	*Taq*1	g.5112179A>G	GG : 303, 152, and 32 AA : 456 and 32 AG : 456, 303, 153, and 32

The DNA amplification using PCR was carried out in a microtube containing 15 µL of reaction (1 µL of DNA and 14 µL of premix solution containing 0.2 µL of forward and reverse primers, 7.5 µL of Green Master Mix, and 6.1 µL of ddH_2O). The amplification process of PCR began the denaturation stage at 94 °C for five minutes. The second phase consists of 35 cycles, each cycle consisting of a denaturation process at 94 °C for 10 seconds, primer annealing at temperatures of 55 °C, and DNA extension at 72 °C for 30 seconds. The final stage was the primer extension at 72 °C for ten minutes.

Genotyping was carried out using the PCR-RFLP method with the restriction enzyme *Taq*1 (Thermo Fisher Scientific, USA). This method was carried out in a 5 µL reaction consisting of 1 µL ddH_2O, 0.3 µL of *Taq*1 enzyme, and 0.7 µL of *Taq*1 enzyme buffer. The reaction was incubated at 65 °C for 4 hours. The genotyping product was analyzed using 2% agarose gel.

Data analysis

The allele and genotype frequency were calculated according to Nei and Kumar (2000). Hardy-Weinberg equilibrium value was calculated according to Brooker (2018). The association of the TGFBR2 gene with carcass characteristics and meat quality was analyzed using SAS software version 9.2 (SAS Institute Inc., Cary, USA). The effect of genotype on carcass characteristics and meat quality were assessed using general linear models (GLM) refers to Listyarini et al. (2018) and Lan et al. (2017).

$$Y_{ijk}=\mu + G_j + E_{ijk}$$

Where: Y_{ijk}=the carcass characteristics and meat quality; μ=the population mean; G_j=the fixed effect of j-th genotype; E_{ijk}=the residual error.

RESULTS

Polymorphism of the TGFBR2 gene

The 488 base pairs (bp) fragment of the TGFBR2 gene was successfully amplified as shown in Figure 1. A single nucleotide polymorphism (SNP) was identified in the TGFBR2 gene (g.5112179A>G). Two genotypes were detected and defined as AA and AG genotypes (Figure 2). The PCR-RFLP products with *Taq*1 enzyme genotypes were: AA genotype (456 and 32 bp) and AG (456, 303, 153, and 32 bp). The SNP of the TGFBR2 gene was not detected in Hardy Weinberg equilibrium (Table 2).

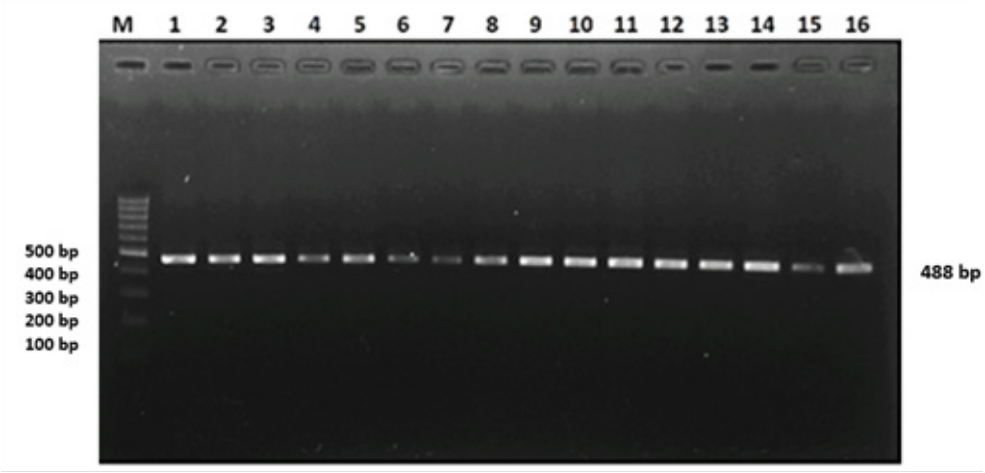

Figure 1 PCR product of the SNP g.5112179A>G in the TGFBR2 gene (Accession number: NC_019476.2). M= 100 bp marker.

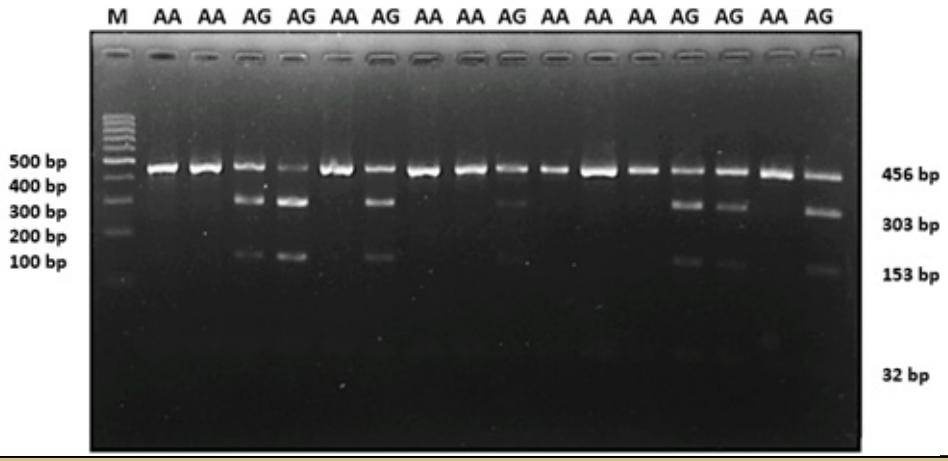

Figure 2 PCR product of the SNP g.5112179A>G in the TGFBR2 gene. M=100 bp marker.

Table 2 Total sample of animals per genotype and allele frequency

Sample	N	Genotype Frequency			Allele Frequency		χ^2
		AA	AG	GG	A	G	
Indonesian sheep	95	0.27 (25)	0.73 (70)	0.00 (0)	0.63	0.37	32.33

The association of the TGFBR2 gene with carcass characteristics and meat quality

The association analysis of the TGFBR2 gene (g.5112179A>G) with carcass characteristics revealed a significant association ($P<0.05$) to carcass length and carcass percentage (Table 3). Rams with the AA genotype were associated with higher carcass percentages. The AG genotype was associated with carcass length, whereas rams bearing the AG genotype showed higher trait values. However, the TGFBR2 gene had no significant effect ($P>0.05$) on live weight, hot carcass, and cold carcass. Furthermore, the TGBR2 gene also had a significantly associated ($p\leq0.05$) with tenderness for meat quality traits (Table 4).

Table 3 Genotype and association analysis of TGFBR2 gene with carcass characteristics

Carcass characteristic	Genotype (μ±SD)			*P*-value
	AA (n=20)	AG (n=63)	GG (n=0)	
Live weight (kg)	23.03 ± 2.76	22.62 ± 4.44		0.700
Hot carcass (kg)	9.17 ± 1.13	8.44 ± 2.14		0.147
Carcass percentage (%)	41.98 ± 3.88	38.36 ± 4.73		0.003*
Carcass length (cm)	64.55 ± 6.17	77.10 ± 18.99		0.005*
Cold carcass (kg)	9.04 ± 1.09	8.23 ± 2.26		0.127

*Note: *=significantly different at 5%*

Table 4 Genotype and association analysis of the TGFBR2 gene with meat quality

Meat Quality	Genotype (μ±SD)			*P*-value
	AA (n=25)	AG (n=61)	GG (n=0)	
pH	5.99 ± 0.66	6.05 ± 0.57		0.702
Tenderness	3.49 ± 0.73	3.91 ± 0.87		0.038*
Cooking loss	48.13 ± 5.9	46.95 ± 8.05		0.510
WHC (mgH_2O)	84.15 ± 9.26	84.25 ± 9.68		0.964
WHC (%mgH_2O)	28.05 ± 3.09	28.08 ± 3.27		0.964

*Note: *=significantly different at 5%*

DISCUSSION

The polymorphism study of the TGFBR2 gene successfully amplified the target fragments using PCR. The genotype and allele frequencies were calculated in Indonesian sheep (Table 2). The TGFBR2 had two genotypes including AA and AG. The genotype frequency of AA and AG genotypes were 0.27 and 0.73, respectively (Table 2). According to Nei and Kumar (2000), the polymorphism in a population is two alleles or more have 1% gene variety. Gene variety can be a reference for breeding selection by doing population-based and cross-breed-based selection if the population is diverse (Noor, 2010). The SNP of the TGFBR2 gene was not detected in Hardy Weinberg equilibrium (χ^2>3.84). Hardy Weinberg equilibrium is affected by mutation, migration, selection, and genetic drift (Noor, 2010; Gunawan et al., 2017).

The TGFBR2 gene polymorphism (g.5112179A>G) is found to be significantly associated with carcass percentage (Table 3). The carcass percentage typically ranged between 48 to 56%, with 52% considered an average for shorn lamb (Greiner, 2005). The carcass percentage in this study was lower than the typical carcass percentage in lamb, with the highest average being in the AA genotype with a carcass percentage of 41.98 ± 3.88. According to Assan (2015), factors influencing carcass percentage include slaughter weight, age, nutrition, management system, genotype, gender, and castration.

This study showed that the TGFBR2 gene polymorphism (g.5112179A>G) is associated with carcass length (Table 3). Lamb with genotype AA and AG had carcass lengths of 64.55 ± 6.17 cm and 77.10 ± 18.99 cm, respectively. According to Stanford et al. (1997), the average carcass length of rams is 105.1 cm. Both genotypes (AA and AG) had lower carcass lengths. The TGFBR2 gene is also related to developing long bones and joints. Seo and Serra (2007) also found that TGFBR2 plays an essential role in developing Mice's long bones and joints. Mice without the TGFBR2 gene had results with shortened limbs. These happen because the TGFBR2 gene is required for maintaining Interzone by preventing mesenchymal cells of the Interzone from differentiating into cartilage by forming a boundary between cartilage and Interzone. Mice with the TGFBR2 gene deleted also had a defect in the skull's parietal bones, suggesting a defect in intramembranous bone formation. The TGFBR2 gene had a significant effect (P<0.05) on tenderness. The effect of TGFBR2 on tenderness occurred during the growth period. According to Wang et al. (2020), the TGFBR2 gene has a vital role in tendon development, healing, and adaptation because the TGFBR2 gene has a role in tenogenesis.

The tenderness of the meat is also affected by the post-mortem process in the muscles. In post-mortem conditions, muscle is unstable in storing calcium in the muscle, thereby making calcium leak out of the muscle cells and sending a signal to the muscle to contract. Contracted muscles make the meat less tender (Huff-Lonergan et al., 2006).

Increasing complex traits in sheep associated with meat quality and carcass characteristics have experienced several problems, such as measuring difficult and expensive to standardize the important traits (Simm et al., 2009). Therefore, these traits have low heritability. These factors make genomic selection impractical. However, consumer demand for increased quality lamb meat makes the economic value commensurate with the reciprocity. With the TGFBR2 gene becoming a candidate in sheep's meat quality, in the future, it can be possible to improve a local sheep with high-quality meat adapting to market demand.

CONCLUSIONS

The TGFBR2 gene (g.5112179A>G) was polymorphic in Indonesia sheep. The population in the sample was not in Hardy-Weinberg equilibrium due to the high AG genotype frequency. The polymorphism of the TGFBR2 gene is associated with carcass length, carcass percentage, and meat tenderness. The AA genotype was associated with a higher level of carcass percentage and tenderness, while the AG genotype was associated with a higher level of carcass length. It can be concluded that the TGFBR2 gene reveals a potential candidate gene to select sheep meat with high carcass characteristics and meat quality.

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AUTHOR CONTRIBUTIONS

Mohammad Gilang Fauzan; Original Draft Preparation, Methodology
Ratna Sholatia Harahap; Formal Analysis, Review and Editing
Kasita Listyarini; Visualization, Review and Editing
Efani Gustia; Project Administration, Resources
Cece Sumantri; Investigation, Supervision, Review and Editing
Imam Mujahidin Fahmid; Data Curation, Validation
Farakka Sari; Data Curation, Validation
Ivan Mangaratua Siburian; Data Curation, Validation
Wahyudi; Data Curation, Validation
Asep Gunawan; Conceptualization and design the experiment, Review and Editing, Supervision, Funding Acquisition, Resources

CONFLICT OF INTEREST

We have no conflict of interest.

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