Pakistan J. Zool., pp 1-6, 2024. **DOI**: https://dx.doi.org/10.17582/journal.pjz/20230310070334

Profile of Melanocortin-4 Receptor and Myogenic Factor 5 Genes in Pasundan Beef Cattle

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Article Information
Received 10 March 2023
Revised 25 April 2023
Accepted 16 May 2023
Available online 11 January 2024 (early access)

Authors' Contribution
AF, ETM designed and performed the study. AF, ETM, AI, providing materials and critical reviews. AF, ETM, AI, WPBP literature search and preparation of the manuscript. All authors read and approved the final draft of the manuscript.

Key words
Genes, MC4R, MYF5, Pasundan cattle, Polymorphism

ABSTRACT

Melanocortin-4 receptor (MC4R) and myogenic factor 5 (MYF5) play an important role in the growth trait, including regulating myogenesis. The study aimed to examine the polymorphism of MC4R and MYF5 genes as a reference for selecting growth traits in Pasundan beef cattle. Amount of 50 blood samples was collected from Breeding Center (?) of BPPT Cijeunjing, Ciamis, West Java, Indonesia. DNA samples were isolated and then purified following the extraction continued with polymerase chain reaction (PCR) and PCR-restriction fragment length polymorphism (RFLP). The Association of MYF5 genotypes with body weight and body size measurements was analyzed using General Linear Model by SAS 9.4 program. The result showed that MC4R gene with HpyCH41V restriction enzyme was polymorphic with three genotypes of CC, CG and GG with frequencies of 0.18 (9), 0.32 (16) and 0.5 (25), respectively. The MY5 gene also showed three genotypes of AA, AG and GG with frequencies of 0.08 (4), 0.38 (19) and 0.54 (27), respectively. Association of the polymorphism both MC4R and MYF5 genes indicated highest body weight with GG genotype. Both of these genes could be associated with growth trait, namely body weight markers. Thus, Further study needs to be conducted in association with more related genes for groth trait to get more comphrehensives results then can be applied for local Indonesian beef cattle.

INTRODUCTION

Indonesia has various types of cattle. Pasundan cattle is one of the original cattle based on the Ministry of Agriculture of the Republic of Indonesia's data in accordance continue to develop the local cattle. Pasundan cattle are reared and distributed in some areas of West Java Province, Indonesia, those are Kuningan, Pangandaran, Indramayu, Ciamis, Garut, Majalengka, Sukabumi, Purwakarta and Sumedang Regencies. Pasundan cattle adapt well to heat weather that is an important influencing factor in a farm is a genetic modification that can improve the whole quality of cattle in the market today (Susandani *et al*., 2021). Modern day breeds are a result of natural and artificial selection and adaptation to the local climate. Due to various reasons about 16% of the cattle breeds are already extinct and 30% face the risk of extinction. Preserving the local cattle breeds thus becomes necessary in order to prevent the depletion of the genetic resources of the country (McTavish *et al*., 2013).

Melanocortin-4 receptor (MC4R) is the primary gene responsible for regulating food intake and energy balance. MC4R is a pair of G protein receptors expressed in the hypothalamic nucleus, as it also plays an important role in the regulation of homeostasis (Zhang *et al*., 2009). The use of the MC4R gene as a molecular marker has been applied to optimize and improve the genetic quality of Madrasin cattle growth (Utomo *et al*., 2021). They reported that the GG alleles of MC4R gene were found to be good sources for breeding. While, myogenic factor 5 plays an active role in regulating myogenesis and pertains to the myogenic regulatory factor family, which includes MyoD, myogenic, MYF5, and MYF6 and all the MRF family members contain a helix-loop-helix domain structure, which can identify and bind to E-box (Li *et al*., 2004). Among the MRF Family members, MYF5 is expressed earliest in the

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0030-9923/2024/0001-0001 $ 9.00/0

precursor cells and played pivotal roles in skeletal muscle differentiation. Moreover, MYF5 can stabilize chromatin's open states, promoting transcription and myogenic (Singh and Dilworth, 2013).

Various studies reported there are SNPs in MC4R and MYF5 genes. These two genes are associated with economic traits in domestic animals. In cattle, polymorphism in MC4R gene affects body weight and intramuscular fat deposition in supporting efforts, which can increase productivity (Polini *et al*., 2016). Furthermore, the MYF5 gene was associated with growth traits, genetic indices, morphological scores, average daily gain and carcass traits, backfat thickness, and meat tenderness (Zhao *et al*., 2020).

This study was therefore conducted to determine the polymorphism profile of MC4R and MYF5 genes as a reference for growth traits and beef quality as an evaluation in Pasundan beef cattle.

MATERIALS AND METHODS

This study was conducted from September to December 2022 at Genomic Laboratory, National Research and Innovation Agency (BRIN), Indonesia.

Blood sample collection

Blood samples were collected at the BPPT Cijeunjing, Ciamis, West Java, Indonesia. The blood samples were collected from *Vena jugularis* in the volume of 5mL of 50 Pasundan cattle using an 18-G needle into a vacutainer containing EDTA and kept in an icebox (4℃) until further analysis.

DNA extraction

DNA was isolated and purified using QIAamp mini spin column DNA extraction kit. A total of 200 µL of blood samples were lysed by adding 200 µL of lysis buffer solution and 20 µL of proteinase K (10 mg/mL), the mixture was by incubation at 56℃ for 60 min in water bath shaker. After incubation, 200 µL of 96% ethanol were added to the solution and centrifuge at 8000x g for 1 min. DNA purification was undertaken using spin column method after adding 500 µL of wash buffer I washing solution followed by centrifugation at 8000 g for 1 min afterwards the supernatant was removed, the DNA was pellet (CHECK) then washed again with 500 µL of buffer II and centrifuged at 14.000 g for 3 min. The DNA solution (?) was then dissolved in 200 µL elution buffer, centrifuged at 8000x g for collecting DNA, and stored at -20℃ until further analysis.

PCR amplification

The primer was designed based on a National Center for Biotechnology Information (NCBI) data sequence. In this research, we used two pairs of primers, forward and reserve both MC4R and MY5 gene gene. The forward primer of MC4R gene was 5- GTCGGGCGTCTTGTTCATC -'3 and the reverse primer was 5- GCTTGTGTTTAGCATCGCGT -'3 while the forward primer of MYF5 gene was 5- ACGACCAACCCTAACC -'3 and the reverse primer was 5- CCAACTATCCACCAGTAA -'3. For the polymerase chain reaction, 10 µL of PCR amplification contained 1 µL DNA samples, 0.2 µL primer F, 0.2 primer R, 5 µL GoTag Green Master Mix, and 3.6 µL DDW. The PCR program consisted of pre-denaturation at 95º C for 5 min, denaturation at 95ºC for 15 sec, annealing at 52ºC for 15 sec, and extension at 72ºC for 20 sec with 45 repetitions of the cycle. The last cycle was ended with the final extension of 72ºC for 3 min, followed by a hold time at a temperature of 4ºC. The PCR products were analyzed by electrophoresis on 1 % agarose gel using SYBr dye (Invitrogen S7563, North America). Molecular markers (Ladder, Invitrogen) measuring 250 bp were electrophoresed together to determine the size of the PCR products. Gels were visualized under an ultraviolet transilluminator.

PCR-restriction fragment length polymorphism (PCR-RFLP)

50 PCR samples were incubated using HpyCH41V and MspI for MC4R and MYF5 genes, respectively. Each sample was incubated for 90 min at 37 ºC. The composition used for identification using the PCR-RFLP method were 0.2 µL MspI enzymes, 1 µL buffers, 6.8 µL DDW, and 2 µL PCR products. The products of DNA fragments from PCR-RFLP were visualized using agarose gel electrophoresis with a concentration of 2% agarose gel and added to 1 µL using SYBr dye (Invitrogen S7563, North America). Molecular markers (Ladder, Invitrogen) measuring 100 bp were electrophoresed together to determine the size of the PCR products. Gels were visualized under ultravioler transilluminator.

Data analysis

Each sample's allele and genotype frequencies were calculated by a simple allele counting method. Pearson's Chi-square test was used to verify the Hardy–Weinberg equilibrium status for the allele and genotype frequencies.

RESULTS

DNA isolated from 50 samples of Pasundan cattle blood wer for MC4R amplified (493 bp) and 285 (MYF5) genes (285bp). Fifty DNA samples of Pasundan cattle

were analyzed using the PCR-RFLP method to assess the *MC4R* and *MYF5* genes polymorphism. The *MC4R* gene was digested with HpyCH41V restriction enzyme and resulted in three genotypes designed as CC (a single, uncut fragment of 493 bp), CG (two fragments of 175 bp and 318 bp), and GG (three fragments of 175 bp, 318 bp, and 493 bp). Genotype frequencies of CC, CG, and GG are 0.18(9), 0.32(16), and 0.5(25), respectively, and allele frequencies are presented in Figure 1A and Table I.

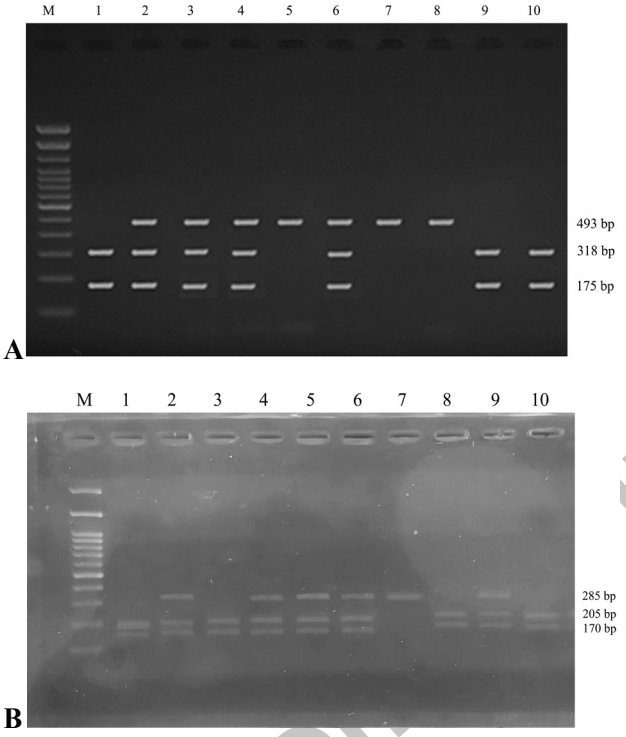

Fig. 1. PCR-RFLP product of MC4R gene. M: Ladder bp; Genotype CC: 493 bp; CG: 175 bp, 318 bp, 493 bp; GG: 175 bp, 318 bp. B: PCR-RFLP product of MYF5 gene. M: Ladder bp; Genotype AA: 285 bp; GA: 115 bp, 170 bp, 285 bp; GG: 115 bp, 170 bp

Table I. Genotype frequency and allele frequency in *MC4R* gene in Pasundan cattle.

Populations	N	Genotype frequency			Allele frequency	
		CC (n)	CG (n)	GG (n)	C	G
Pasundan cattle	50	0.18(9)	0.32(16)	0.5(25)	0.34	0.66

n, number of individuals population.

The MYF5 gene was digested with MspI restriction enzyme and resulted in three genotypes designed as AA (a single, uncut fragment of 285 bp), GG (two fragments of 115 bp and 170 bp), and GA (three fragments of 115 bp, 170 bp, and 285 bp). Genotype frequencies of AA, AG and GG are 0.08(4), 0.38(19), and 0.54(27), respectively, and allele frequencies are presented in Figure 1B and Table II. Association of MC4R and MYF5 genes for body weight measurement in Pasundan cattle is presented in Table III.

Table II. Genotype frequency and allele frequency in *MYF5* gene in Pasundan cattle.

Populations	N	Genotype frequency			Allele frequency	
		AA (n)	AG (n)	GG (n)	A	G
Pasundan cattle	50	0.08 (4)	0.38(19)	0.54(27)	0.27	0.73

n, number of individuals population.

Table III. Association of MC4R and MYF5 genes for body weight measurements in Pasundan cattle.

Gene	Genotype	
MC4R	CC (n=9)	186.06±1.4[a]
	CG (n=16)	192.18±0.7[a]
	GG (n=25)	220.25±0.6[b]
MYF5	AA (n=4)	181.31±3.4[a]
	AG (n=19)	193.20±1.2[a]
	GG (n=27)	221.11±2.7[b]

n, number of individuals; [a,b], different superscripts in the same row indicate significantly different ($p<0.05$).

DISCUSSION

Genotyping is identifying individuals' genotypes through DNA markers at a specific locus within the genome (Dai and Long, 2015). Genotyping results are often used to describe the distribution of alleles within a population based on observations of genetic diversity. Genotyping is also used to determine the population balance, according to the Hardy-Weinberg equilibrium (Sha and Zhang, 2011). Genotypic determinations are often conducted using PCR-RFLP. The RFLP method has been applied to detect quantitative traits loci in livestock. RFLP detection has been developed and used for linkage studies in cattle, chickens, and pigs (Han *et al*., 2013).

MC4R gene plays an important role in regulating food intake and body weight. In another study, MC4R gene also acts on energy homeostasis mediated by alpha-MSH. Meanwhile, the gene can encode this receptor due to mutations and contributes to obesity (Arslan *et al*., 2017). Our data showed that the allele frequencies showed that the G allele (0.66) frequency was higher than the C allele (0.34), and it resulted in polymorphic. Those polymorphic

are because the frequency of the G allele was not more than 0.99 (Singh *et al*., 2014). The data of genotypes in the present study showed that GG and CG genotypes had two and three DNA fragments, whereas the CC genotype had one large fragment. GG genotype has significant results compared to CG and CC genotypes. The Pearson's Chi-square test results indicate that the cattle genotypes have not deviated from the Hardy-Weinberg equilibrium. This study gives information that the allele and genotype frequencies will be constant in generations if there were no unconditional factors, selection mutation, migration, and inbreeding in these populations (Dar *et al*., 2018).

The association data of body weight also showed that GG genotypes were associated with the heavier body weight group, among others. The previous study showed that cattle with the GG genotype had higher economic characteristics and better growth than GC and CC genotype (Sharma *et al*., 2016). In Korean cattle, the GG genotype of the Luxi breed also has better economic features than those with the CC genotype (Choi *et al*., 2015). The live and carcass weights and the backfat thickness of cattle with the GG genotype were higher than those of the CG and CC genotypes (Strucken at al., 2015). Also, in native Korean cattle (brown, striped, and black), GG genotypes have higher body weight measurements (Weller *et al*., 2016). Similar results have also been noted in adult Hanwoo cattle, with higher live and body weight values attributed to the GG genotype (Kim *et al*., 2015). In addition, in local cattle in Indonesia, the GG genotype of the PO Kebumen breed has a higher birth length (66.34±6.73 cm) than the CC (65.43±7.21 cm) and GC (59.47±5.77 cm) genotypes, also in Madrasin cattle MC4R gene be used as a molecular marker for the selection of superior Madrasin cattle (Maharani *et al*., 2016; Utomo *et al*., 2021).

The fragment length of MYF 5 gene was successfully amplified in Pasundan cattle in this study. The MYF5 gene has been considered to play an important role in the growth and development of mammals (Zammit, 2017). Our data showed that the allele frequencies showed the G allele (0.73), this frequency was higher than the A allele (0.27), resulting in polymorphic. The data of genotypes showed that GG and AG genotypes had two and three DNA fragments, whereas the AA genotype had one large fragment. GG genotype has significant results compared to AG and AA genotypes. The results were also not deviated from the Hardy-Weinberg equilibrium). The association data of body weight also showed that GG genotypes were associated with heavier body weight groups among groups. As results in previous studies found, the MYF5 gene played a pivotal role in muscle development and growth Ref need). In the meanwhile, it is ubiquitously expressed in a variety of tissue, such as the liver, kidneys, heart, spleen, lungs, stomach, and intestines (Hou *et al*., 2015). MYF5 gene also significantly affects growth performance and beef quality traits of Qinchuan cattle (Zhao *et al*., 2020), and MYF5 genes are strong candidate genes that influence growth traits in other Chinese cattle (Zhang *et al*., 2007). Several studies stated MYF5 gene was significantly associated with yearly body weight and body gain, including Hanwoo and Canada cattle (Li *et al*., 2004). In pigs, MYF5 polymorphism was associated with meat quality traits (Liu at al., 2007) and in knockout mice, experiments demonstrated that the MYF5 gene affected muscle development (Lynes *et al*., 2015). However, the MYF5 gene with SNP g.643G>A had different results in Bali cattle in that it could not be used as a genetic marker for body weight and still needs to be evaluated in a comprehensive study (Saputra *et al*., 2016).

The population of Pasundan cattle at BPPT Cijeunjing, Ciamis was in Hardy-Weinberg equilibrium. As a result, the MC4R and MYF5 gene polymorphism are associated with the highest body weight with the GG genotype. Both of these genes could be associated with growth traits like body weight markers. Thus, further detailed investigations are necessary to be performed on the associated genes to the economic value and give more comprehensive results. This finding can then be applied in local Indonesian beef cattle.

ACKNOWLEDGMENTS

This study was supported by the Postdoctoral Program of the National Research and Innovation Agency (BRIN) of Indonesia.

Funding

This research was supported by National Research and Innovative Agency (BRIN) of Indonesia with refrence number 169/HK/2022.

IRB approval

This research was approved by National Research and Innovative Agency (BRIN) of Indonesia and School of Biomedical Sciences, IPB University, Indonesia.

Ethical approval

All procedures performed in this study were approved by the Animal Ethics Committee of the School of Veterinary Medicine and Biomedical Sciences, IPB University (reference number: 013/KEH/SKE/II/2022).

Statement of conflict of interest

The authors have declared no conflict of interest.

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