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Association between the *Melanocortin-4 Receptor* (*MC4R*) Gene Polymorphisms and Growth Trait in Sumba Ongole Cattle

Research Article

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ABSTRACT

Melanocortin-4 receptor (*MC4R*) gene has been known as a candidate gene for growth traits in livestock. This research was aimed to identify the polymorphism of *MC4R* gene and its association with growth traits in Sumba Ongole (SO) cattle. The growth traits data consist of body weight and body size. Eighty four blood samples were collected and used for DNA isolation. The results showed one SNP (g.1133C>G) resulting in two alleles (C and G) and three genotypes (CC, CG, and GG) in SO cattle. The frequency of G allele (0.59) was higher than C allele (0.41). The CG genotype had the highest value of genotype frequency (0.48). The allele and genotype distributions followed the Hardy-Weinberg equilibrium. The single nucleotide polymorphism (SNP) g.1133C > G revealed having no significant association with growth traits in SO cattle. In conclusion, the SNP g.1133C > G may not useful as selection tool in Sumba Ongole cattle. It can be suggested that the future study is needed to find out the effect of the *MC4R* gene in Sumba Ongole cattle in different locus.

KEY WORDS growth traits, *MC4R* gene, polymorphism, Sumba Ongole cattle.

INTRODUCTION

Sumba Ongole (SO) cattle is one of the potential beef cattle in Indonesia. This cattle are widely bred on Sumba Island (East Nusa Tenggara Province) and well adapted in tropical climate (Sutarno and Setyawan, 2016). The SO cattle have been registered by the Ministry of Agriculture, Republic of Indonesia (427/Kpts/Sr.120/ 3/2014) as a local breed cattle. The characteristics of SO cattle were white-grayish colored fur, black nose, black tail fur, black colored fur around the eyes, shorter male horns and loose wattle hanging from the neck (Ministry of Agriculture of the Republic of Indonesia, 2014). Putra *et al.* (2018) reported that the birth weight, weaning weight and yearling weight in male and female cattle were 24.29 ± 5.30 / 21.89 ± 5.18 kg; 121.53 ± 38.57 / 105.25 ± 29.61 kg and 181.34 ± 44.69 / 157.92 ± 34.93, respectively. The SO cattle also have slaughter weight from 267.80 ± 8.00 to 635.50 ± 6.91 kg with dressing percentage ranging from 51.42 to 56.34% (Agung *et al.* 2017; Said *et al.* 2016a). Growth traits can be used as one of the animal selection parameters in livestock breeding programs. Animals having good productivity can be identified by the DNA information (Allan and Smith, 2008). The information about genetic polymorphisms based on a marker in different quantitative traits of animals is essential (Cheong *et al.* 2006). The use of genetic markers associated with economic traits can contribute to evaluating the genetic diversity and genetic improvement in cattle breeding companies (Kong *et al.* 2012; Singh *et al.* 2014). Studies on molecular selection markers has recently been carried out in

SO and several Bos indicus cattle (Agung *et al.* 2017; Agung *et al.* 2018; Hilmia *et al.* 2018; Maharani *et al.* 2018; Fathoni *et al.* 2019). The *melanocortin-4 receptor* (*MC4R*) gene is one of a candidate gene associated with economic traits in cattle (Liu *et al.* 2009; Huang *et al.* 2010; Kong *et al.* 2012). The *MC4R* gene was located in chromosome 24 in cattle (*Bos indicus*) based on the GenBank acc no. NC_032673.1 (position 61723201..61725110). The gene sized 1910 bp containing one exon that codes 333 amino acids. Activation of the *MC4R* gene will affect feed intake, energy balance, and body weight (Liu *et al.* 2009). The POMC-neurons and *MC4R* receptors were activated by leptin and insulin to produce the α-MSH. The *MC4R*-AGRP bond produces an anorexigenic signal which increases the feed intake and *MC4R*-α-MSH bond produces anorexigenic signals which decrease feed intake (Delgado *et al.* 2017). In humans and mice, the mutation of *MC4R* gene affects the obesity (Switonski *et al.* 2013; Girardet and Butler, 2014; Yazdi *et al.* 2015). Moreover, polymorphisms in the *MC4R* gene have been reported to be significantly associated with economics in the cattle (Liu *et al.* 2009; Huang *et al.* 2010; Lee *et al.* 2013). The previous study reported the SNP g.1133C > G of *MC4R* was detected in Kebumen Ongole Grade cattle and associated with birth body length (Maharani *et al.* 2018). The study of *MC4R* gene in SO cattle was not performed yet. Therefore, this study aimed to identify the polymorphism within the *MC4R* gene and its association with growth traits in SO cattle.

MATERIALS AND METHODS

Sample collection and DNA extraction

The animals used in this study originated from East Sumba Regency, East Nusa Tenggara Province. Three milliliters of 84 blood samples were taken from the jugular vein. Blood samples were stored at vacutainer containing K3EDTA (anticoagulant). Genomic DNA was extracted using a gSYNC DNA Extraction Kit (Geneaid, Taiwan). Growth traits such as body weight and body size were measured in age 2 to 3 years according to SNI (The Indonesian National Standard) protocol (Figure 1).

Polymerase chain reaction (PCR)

Genbank, primer sequences, target position and SNP g.1133 C > G in according to Kong *et al.* (2012) and Maharani *et al.* (2018) are shown in Table 1. PCR amplification was performed in a total volume of 20 µL containing 12.6 µL double distillated water (DDW), 12.5 µL MyTaq™ HS Red Mix (Bioline, UK), 0.8 µL (10 pmol/µL) of each primer, and 2 µL DNA product (30 ng/µL). The cycling conditions were carried out as followed: pre-denaturation at 94 ˚C for 5 min, denaturation at 94 ˚C with 35 cycles of 30 s, annealing at 58 ˚C for 30 s, extension at 72 ˚C for 30 s, and a final extension at 72 ˚C for 10 min using a Parkin Elmer Thermal Cycler PCR system. The PCR products were visualized by 1.5% standard agarose gels stained with ethidium bromide.

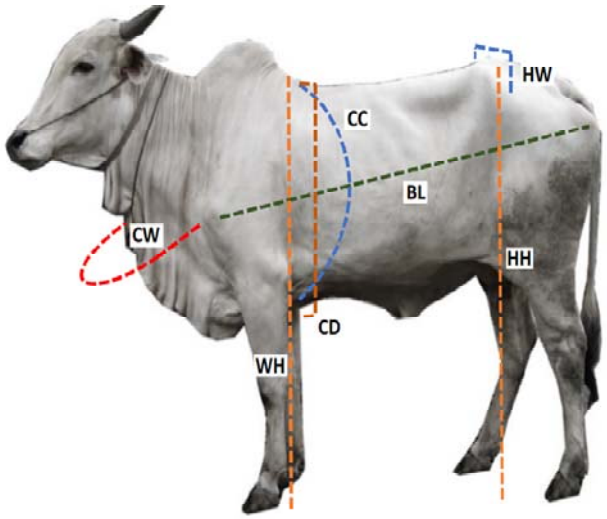

Figure 1 The body measurements protocol based on the Indonesian National Standard

Table 1 Genbank, target location, primer, SNP, region, fragment size of *MC4R* gene

Genbank	Target location	Primer	SNP	Fragment size
EU366350.1	813-1306	F:5'-GTCGGGCGTCTTGTTCATC-3' R:5'-GCTTGTGTTTAGCATCGCGT-3'	g.1133 C > G	493 bp

PCR-RFLP and genotyping

The SNP g.1133C > G was used for genotyping by the PCR-RFLP method. *HpyCH4IV* was used to digest the product 493 bp of the *MC4R* gene with a recognition site of 5'-A'CG'T-3' (Figure 2). The PCR-RFLP was performed in 20 µL reaction volumes containing 2.8 µL DDW, 2 µL 10 × buffer, 0.5 µL restriction enzyme, and 15 µL of PCR products. The digested products were visualized on 3% agarose gels.

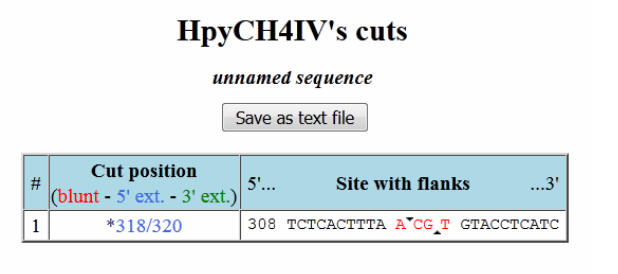

Figure 2 The site and cut position of HpyCH4IV

Statistical analysis

The frequency of allele and genotype is calculated by the following formula (Maharani *et al.* 2018):

Alelle frequency C= ∑ locus C / ∑ (locus C+locus G)
Alelle frequency G= ∑ locus G / ∑ (locus C+locus G)
Genotype frequency CC= ∑ locus CC / ∑ sample in population) × 100%
Genotype frequency CG= ∑ locus CG / ∑ sample in population) × 100%
Genotype frequency GG= ∑ locus GG / ∑ sample in population) × 100%

The allele and genotype frequencies were identified for the Hardy-Weinberg equilibrium status by Pearson's Chi-square test with the mathematical model according to Moonesinghe *et al.* (2010) and Kang and Shin, (2004):

$$X^2 = \sum_{i=1}^{n} \frac{(O_i - E_i)^2}{E_i}$$

Where:
X^2: Chi-square test value.
O_i: observed frequency.
E_i: expected frequency.
n: number of compared data.

The Chi-square test values (X^2) for Hardy-Weinberg equilibrium were calculated using Pop-Gene 1.32 program (Yeh *et al.* 1997).

The general linear model was performed to verify the association of SNP g.1133C > G of *MC4R* gene genotypes with body weight and body size using R program with the following:

$Y_{ij} = \mu + \tau_i + \varepsilon_{ij}$
Where:
Y_{ij}: analyzed trait.
μ: general mean.
τ_i: genotype effect.
ε_{ij}: random error effect.

The $P < 0.05$ was regarded as statistically significant.

RESULTS AND DISCUSSION

Growth traits profile of Sumba Ongole cattle

The growth traits were figured by body weight (BW) and body size including body length (BL), shoulder height (SH), hip height (HH), chest circumference (CC), chest width (CW), chest depth (CD), waist width (WW), hip-width (HW) and scrotum circumference (SS). The mean value and standard deviation of each growth parameters were shown in Table 2.

Genotype and allele frequency

The digestion product of 493 bp by *HpyCH4IV* generated three genotypes: CC, GG, and CG. Animals with CC genotype are defined when the fragment size is recognized at 493, homozygote GG is characterized by fragment size of 175 and 318 bp, while the heterozygote CG has 175, 318, and 493 bp of fragment size (Figure 3). Allelic frequency of G (0.59) was higher than C (0.41) while the genotype frequency of CG was more dominant than CC and GG genotype (Figure 4).

The chi-square tests showed that the allelic and genotypic frequencies in SO cattle did not deviate from HWE (P>0.05) (Table 3).

The effect of SNP g.1133 C > G to growth parameters in Sumba Ongole cattle

The genotypes in this study associated with the growth traits in cattle (Maharani *et al.* 2018). However, the SNP g.1133 C > G did not affect significantly the growth traits in Sumba Ongole cattle as shown in Table 4.

Sumba Ongole cattle were potential cattle in Indonesia. Previously, many researchers reported that the birth weight and weaning weight of Sumba Ongole were higher than the other Indonesian native cattle and the other *Bos indicus* breed such as Bali, Ongole grade, Red Chitagong, and Malawi Zebu (Kaswati *et al.* 2013; Paputungan *et al.* 2015; Nandolo *et al.* 2016; Hossain *et al.* 2018).

Table 2 The growth profile of Sumba Ongole cattle

Variable	n	Mean ± SD	Minimum	Maximum
Body weight (kg)	84	359.7±64.6	205.00	492.00
Body length (cm)	84	131.76±8.15	110.00	150.00
Shoulder height (cm)	84	126.77±5.83	111.00	137.00
Chest circumference (cm)	84	168.02±10.98	142.00	191.00
Chest width (cm)	83	35.76±5.37	28.00	45.00
Chest depth (cm)	83	60.31±7.96	51.00	70.00
Waist width (cm)	35	38.85±17.34	29.00	49.00
Hip height (cm)	84	135.17±5.47	120.00	145.00
Hip width (cm)	41	38.85±17.34	21.00	33.00
Scrotum circumference (cm)	63	38.85±17.34	22.00	33.00

n: number of samples.
SD: standard deviation.

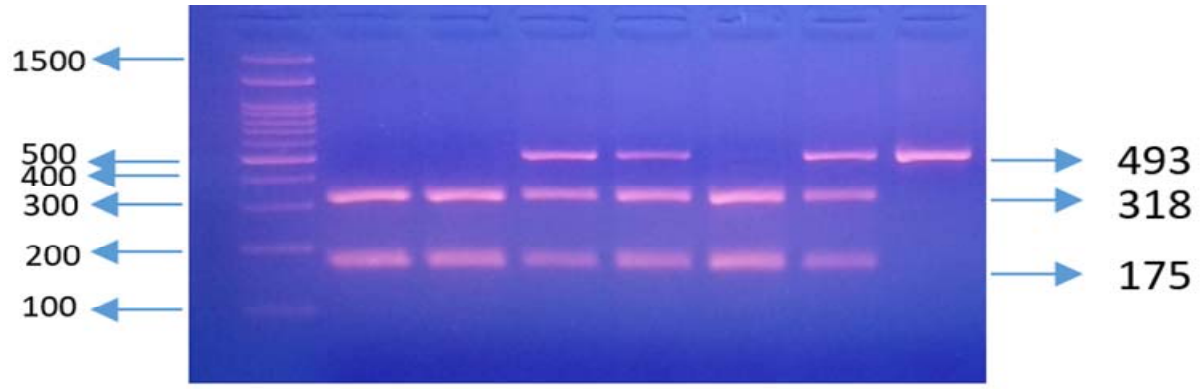

Figure 3 The results of PCR-RFLP with *HpyCH4IV* restriction enzyme (M: marker, CC, GG, CG: genotype sample)

Table 3 Pearson's Chi-square test variable

Population	Total	Genotype			X^2
		CC (n=18)	GC (n=51)	GG (n=38)	
SO cattle	Observed	18.00	51.00	38.00	0.02
	Expected	17.68	51.63	37.68	
	D	0.32	-0.63	0.32	
	D^2/e	0.01	0.01	0.00	

$X^2_{0.05,\,2} = 5.99$.

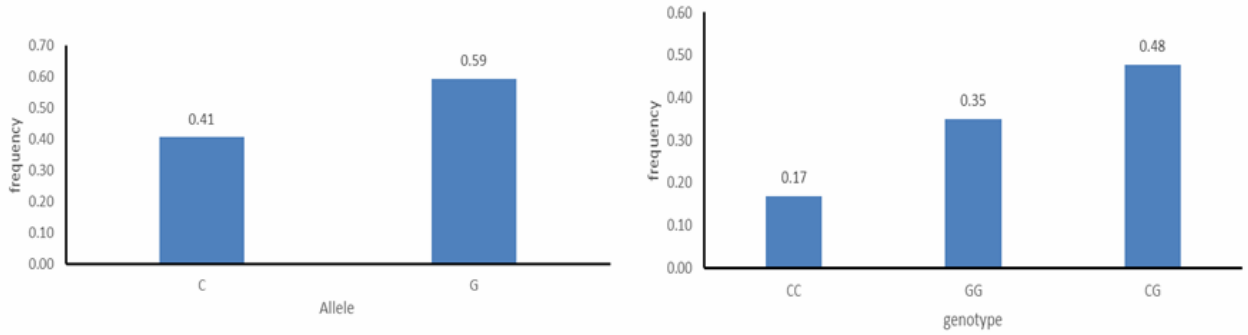

Figure 4 Frequency of alleles and genotypes based on *MC4R* Gene with SNP g.1133C > G in Sumba Ongole cattle

Table 4 The level of significance for the *MC4R* gene to the growth parameters using SNP 1133 g. C > G

Variable	n	Genotype		
		CC	CG	GG
Body weight (kg)	84	344.73±67.88	363.15±72.96	362.8±52.6
Body length (cm)	84	130.20±10.39	132.95±9.03	131.13±5.58
Shoulder height (cm)	84	126.53±5.74	126.41±5.64	127.31±6.22
Chest circumference (cm)	84	166.27±12.65	168.62±12.00	168.16±9.01
Chest width (cm)	83	34.93±3.73	36.42±3.72	35.41±7.31
Chest inside (cm)	83	61.13±5.10	60.92±4.46	59.25±11.47
Waist width (cm)	35	25.67±2.80	28.25±3.02	25.23±7.83
Hip height (cm)	84	135.40±5.84	134.86±5.56	135.41±5.34
Hip width (cm)	41	36.13±3.31	43.35±24.65	35.44±10.43
Scrotum circumference (cm)	63	26.00±8.72	28.44±2.67	27.52±3.25

n: number of samples.

The profile of growth traits in Sumba Ongole cattle in this study was higher than that reported by Said *et al.* (2016b) and Fauzyah *et al.* (2017) but lower than Yantika *et al.* (2016). The BW found in this study was higher than Nguni and lower than Bonsmara, Limousin Charolais, Hungarian Simmental, Hereford, Angus, and Charolais cattle (Zahrádková *et al.* 2010; Mashiloane *et al.* 2012; Bene *et al.* 2007). The BL and SH of Sumba Ongole cattle also higher than Bali cattle of the same age (Agung *et al.* 2018a). In general, the average body weight and body size in *Bos taurus* breed were higher than *Bos indicus*, that differences may be caused by diversity in genetics, management, and environment of animals.

In this study, the *MC4R* gene indicated to be polymorphic. The SNP 1133 g. C > G in Sumba Ongole cattle resulting two alleles (C and G) and three genotypes (CC, CG and GG) (Figure 3). The frequency of G allele (0.59) was higher than C allele (0.41). The CG genotype had the highest value of genotype frequency (Figure 3). The same results were occured in Kebumen Ongole Grade, Hanwoo and Nanyang cattle reported by Maharani *et al.* (2018), Kong *et al.* (2012) and Liu *et al.* (2009), respectively. The different results of allelic frequencies was reported in Qinchuan, Anxi and Angus cattle (Liu *et al.* 2009; Kong *et al.* 2012).

The association analysis revealed that there was no significantly effect of SNP g. 1133 C > G to the growth traits in Sumba Ongole cattle. The results were different with the previously study.

Maharani *et al.* (2018) reported that the SNP g. 1133 C > G has significant effect on the birth body length of the calf in Kebumen Ongole grade cattle. Kong *et al.* (2012) also reported that variation in the *MC4R* gene influenced the marbling score, backfat thickness, and and marbling score in Hanwoo cattle. However, in the several studies, *MC4R* gene was not associated with economic traits in Holstein cattle (Lee and Kong, 2011). In the pig, the missense mutation of *MC4R* gene effected the fat deposition and carcass composition (Óvilo *et al.* 2006).

Shishay *et al.* (2019) also reported that the polymorphism of *MC4R* gene has a significant effect on the body measurement of Hu Sheep. The difference in these results could be caused by several factors such as cattle breed, the number of samples, and environmental diversity at the farmer level. Future research is needed using different markers to find out the effect of *MC4R* gene in Sumba Ongole cattle.

CONCLUSION

The association study of SNP g.1133 C > G revealed having no significant effect on the growth traits parameter. The SNP may not useful for a selection tool in Sumba Ongole cattle, therefore the future study is needed to find out the effect of *MC4R* gene in Sumba Ongole cattle with the different markers.

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