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Polymorphism of ADIPOQ and EDG1 genes in Indonesian beef cattle

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ABSTRAK

Gen ADIPOQ dan EDG1 berperan dalam deposisi lemak intramuskular dan skor *marbling*. Penelitian ini bertujuan untuk mengidentifikasi variasi indel g.81966364D>I di promotor gen ADIPOQ dan SNP c.-312A>G di 5'UTR gen EDG1 pada bangsa sapi potong Indonesia. Sampel darah diperoleh dari 211 ekor sapi, terdiri atas sapi Bali (44), Madura (20), Pesisir (18), Katingan (20), Peranakan *ongole* (PO) (22), Pasundan (20), Sumba Ongole (SO) (12), Brahman (20), Simmental (15), dan Limousin (18). Keragaman gen ADIPOQ dianalisis menggunakan metode PCR dan *direct sequencing*, sedangkan gen EDG1 dianlisis dengan PCR-RFLP (enzim *Msc*I) dan *direct sequencing*. Hasil genotiping indel g.81966364D>I adalah monomorfik (genotipe DD). Hasil SNP c.-312A>G adalah polimorfik (genotipe AA dan AG) pada sapi Madura, sapi Pesisir, sapi Pasundan, sapi Brahman dan sapi Limousin. Frekuensi alel A dan G masing-masing adalah 0.95, 0.92, 0.98, 0.95, 0.94 dan 0.05, 0.08, 0.02, 0.05, 0.06. Nilai Ho dan He masing-masing adalah 0.05-0.17 dan 0.05-0.15 serta dalam keseimbangan Hardy-Weinberg (P>0.05). Pada sapi Bali, Katingan, PO, SO, dan Simmental hasilya monomorfik (genotipe AA). Pada sapi Bali ditemukan dua kandidat SNP baru pada posisi c.-399C>T dan c.-273C>G yang potensial dijadikan marka genetik skor *marbling* untuk sapi Bali. Berdasarkan hasil dari penelitian ini, dapat disimpulkan bahwa gen ADIPOQ bersifat seragam sedangkan gen EDG1 bersifat beragam pada sapi potong Indonesia. Selain itu, ditemukan dua kandidat SNP potensial pada sapi Bali.

Kata Kunci: gen ADIPOQ, gen EDG1, indel g.81966364D>I, SNP c.-312A>G, sapi potong

ABSTRACT

The ADIPOQ and EDG1 genes were responsible in intramuscular fat deposition and marbling scores. This study was aimed to identify polymorphism of indel g.81966364D>I in promoter region of ADIPOQ gene and SNP c.-312A>G in 5' UTR of EDG1 gene in Indonesian beef cattle. Blood samples were collected from 211 cattle, including Bali (44), Madura (20), Pesisir (18), Katingan (20), PO (22), Pasundan (20), SO (12), Brahman (20), Simmental (15) and Limousin (18). Polymorphism of ADIPOQ gene was analyzed using PCR and direct sequencing methods, whereas EDG1 gene was analyzed using PCR-RFLP (*Msc*I enzyme) and direct sequencing methods. Results of genotyping indel g.81966364D>I was monomorphic (DD genotype). The SNP c.-312A>G was polymorphic (AA and AG genotype) in Madura, Pesisir, Pasundan, Brahman, and Limousine. The Frequencies of allele A and G were 0.95, 0.92, 0.98, 0.95, 0.94 and 0.05, 0.08, 0.02, 0.05, 0.06 respectively. The values of Ho and He were 0.05-

0.17 and 0.05-0.15 respectively and in Hardy-Weinberg equilibrium (P>0.05). In Bali, Katingan, PO, SO and Simmental were monomorphic (GG genotype). In Bali cattle, two novel SNP candidates were found in position of c.-399C>T and c.-273C>G which were potential to be used as genetic markers of marbling score for Bali cattle. As result this study, it can be concluded that ADIPOQ gene was similar while EDG1 gene was different in Indonesian beef cattle. in addition, found two candidates potential SNP in Bali cattle.

Keywords: ADIPOQ gene, beef cattle, EDG1 gene, indel g.81966364D>I, SNP c.-312A>G

INTRODUCTION

Indonesia has a lot of animals genetic resources consisting beef cattle such as Bali, Madura, Aceh, Sumbawa, Pesisir, PO, Jabres, and Sumba Ongole (SO). Animals genetic resources were important to manage for increasing the farmer's income and welfare, leading to national food security as well as the vevelopment of security as a nation. The policies of management of the animal genetic resources referred to three approaches, those were: Pure-breeding and Conservation, Cross breeding, and the development of new breeds. Several of Indonesia beef cattle were potential to be developed into premium beef cattle.

The value of the economic traits in beef cattle included birth weight, weaning weight, and meat quality. Selection of these traits based on phenotype results in a slow genetic improvement. the potential alternative selection was the marker-assisted selection (MAS). Selection of livestock based on genetic markers could result in more accurate, effective and efficient. Selection could be decided based on candidate genes that control those traits (Van Werf and Kinghorn, 2003).

Several potential gene candidates related to meat quality included adiponectin (ADIPOQ) gene and endothelial differentiation sphingolipid G-protein-coupled receptor 1 (EDG1) gene. The ADIPOQ gene played a role in the process of lipogenesis, fatty acid oxidation, homeostatic energy, insulin sensitivity, and glucose utilization (Choi *et al.,* 2015; Kwon *et al.*, 2016). While the EDG1 gene played a role in intramuscular fat deposition (marbling) (Sasaki *et al.*, 2006). The ADIPOQ gene had a promoter region which was a region of DNA that initiates transcription of a particular gene (Gershon and Kadonaga, 2010; Ohler and Wassarman, 2010; Lenhard *et al.,* 2012). While the EDG1 gene had a 5' untranslated region (UTR) which has been recognized for the importance of regression of gene expression at the posttranscriptional level by affecting the mRNA stability, localization, and translational efficiency (Mignone *et al.,* 2002).

The promoter of ADIPOQ gene had been identified in some of the world's cattle such as Angus cattle (Morsci *et al.,* 2006), Chinese local cattle (Zhang *et al.,* 2013) and Hanwoo cattle (Kwon *et al.,* 2016). The 5'UTR of EDG1 gene was widely studied in wagyu cattle (Sasaki *et al.,* 2006; Yamada *et al.,* 2008; Sukegawa *et al.,* 2010). However, information polymorphism the promoter region of ADIPOQ gene and the 5' UTR of EDG1 gene had not been explored in Indonesian beef cattle. Thus, the objective of this study was to identify the variation in the ADIPOQ promoter region and 5' UTR of EDG1 gene in Indonesia beef cattle.

MATERIALS AND METHODS

Samples

The reseach was conducted in laboratory of molecular genetics of livestock, Faculty of Animal Science, Bogor Agricultural University. Blood samples were obtained from ten representative cattle breeds including Bali, Madura, Pesisir, Katingan, PO, Pasundan, SO, Brahman, Simmental and Limousin (Table 1).

DNA Isolation and PCR amplification

Blood samples were collected from jugular vein and kept into vaccuntainer tube containing ethanol absolut as anticoagulant. Genomic DNA was isolated using phenol-cloroform metods described by (Sambrook and Russel, 2001). Based on the bovine sequence (GenBank accession number JQ775868) and the cow sequence (ensembl accession number ENSBTAG00000005990), two pairs of primers (F: 5'-GCAGCTCTACTTGGCATCC-3' and R: 5'-TGAATCAGTCGTCCTTACCC-3') and (F: 5'-CGCAGATCTTTCCTGGACAG-3' and R: 5'-TTCTGCCTCTGAAGACCTCC-3') were designed to amplify in promoter region of the ADIPOQ gene and 5'UTR of the EDG1 gene, respectively. The primers were designed using MEGA7 and online evaluated using PCR *Primer Stats*

Table 1. The List of Indonesian Beef Cattle Classified Based on Breeds, Number of Samples, and Location

No	Breeds of cattle	N	Year of Collection	Location
1	Bali	44	2015	Bali Island
2	Madura	20	2011	Madura Island
3	Pesisir	20	2006	West Sumatera
4	Katingan	20	2010	Central Kalimantan
5	Ongole Grade	22	2006	West Java
6	Pasundan	20	2014	West Java
7	Sumba Ongole	12	2006	East Nusa Tenggara
8	Brahman	20	2006	West Java
9	Simmental	15	2006	East Java
10	Limousin	18	2006	East Java
	Total	211		

N = Number of samples

(*www.bioinformatics.org*) for reducing error. For both genes, the 15 µLPCR amplification mix contained 50 ng of DNA template, 1× promega green master mix (based on the protocol provided by manufacturer) and 5 pmol of each primer. The PCR protocol was 5 min at 95°C, followed by 35 cycles of 95°C for 20 s, annealing at 60°C for 30 s, 72°C for 30 s and a final extension at 72°C for 5 min. The expected amplified fragment size for ADIPOQ and EDG1 genes were about 265 (no insertion) or 331 bp (67 bp insertion) and 411 bp, respectively.

Genotyping and Sequencing

For the genotyping to determine insertion or deletion in promoter region of the ADIPOQ gene, 2% agarose gel (0.6 g of agarose was diluted in 30 ml of 0.5×TBE buffer) electrophoresis was conducted, and genotypes were determined based on banding patterns of DNA for length 265 bp as deletion and 331 bp as insertion. For the genotyping to determine singel nucleotide polymorphism (SNP) c.-312A>G in the 5'UTR of the EDG1 gene using PCR-RFLP method. PCR product was digested at 37°C for 2 h with restriction enzyme *Msc*I (TGG↓CCA) and electrophoresed on a 2.0% agarose gel. Agarose gels were stained with florosafe and photographed under an ultraviolet light. For restriction, the reaction mix contained 1.0 µLendonuclease free H_2O, 5 µLPCR product, 0.7 µL *Msc*I buffer, and 0.3 µL(3 U) *Msc*I restriction enzyme. 411 bp PCR fragments containing the SNP site were digested by *Msc*I into 186 and 225 bp fragments at the A allele, but not at the G allele, so the AA homozygotes, the GG homozygotes, and the AG heterozygotes resulted in two bands (186 and 225 bp), one band (411 bp) and three bands (186, 225 and 411 bp) respectively.

Sequencing was done only for Bali and Limousin cattle for promoter region of the ADIPOQ and 5'UTR of the EDG1 genes that had different genotypes (2 samples/genotype) using forward primer. Samples for sequencing were sent to commercial laboratory service at First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) using ABI PRISM 96-capillary 3730xl DNA Analyzer (Applied Biosystems, USA).

Data Analyses

Frequency of Genotype and Allele. Frequency of genotype and allele were calculated based on Nei and Kumar (2000) formula with the following statistical model: $\chi_{ii} = (n_{ii}/N)$ for genotype frequency and $\chi_i = (2n_{ii} + \Sigma n_{ij})/(2N)$ for allele frequency, where: χ_{ii} = frequency of ii genotype; χ_i = frequency of i allele; n_{ii} = number of individuals with ii genotype; n_{ij} = number of individuals with ij genotype; N = number of samples.

Observed Heterozygosity (Ho) and Expected Heterozygosity (He). Ho value was calculated based on Weir (1996) formula with the following statistical model: Ho = $(\Sigma n_{ij})/(N)$, where: Ho = observed heterozygosity; nij= number of heterozygot samples; N = number of samples. He value was calculated based on Nei and Kumar (2000) formula with the following statistical model:

$$He = 1 - \sum_{i=1}^{q} x_i^2$$

where: He = expected heterozygosity; χ_i = frequency of alleles; q = number of alleles.

Hardy-Weinberg Equilibrium (HWE). HWE was calculated according Hartl and Clark (1997) formula with the following statistical model:

$$x^2 = \sum \frac{(O - E)^2}{E}$$

where: χ^2 = HWE test; O = observed number of genotype; E = expected number of genotype. Degree of freedom (df) for HWE test was defined according to Allendorf *et al.* (2013) where: df = number of genotype probabilities – number of alleles.

Sequence Analysis

Data of ADIPOQ and EDG1 genes sequences were analyzed by FinchTV and Bioedit program (Hall, 2011). The determination of SNP (single nucleotide polymorphism) was identified using Molecular Evolutionary Genetics Analysis 5 (MEGA5) (Tamura *et al.,* 2011).

RESULTS AND DISCUSSION

Amplification and Polymorphism of ADIPOQ Gene

The promoter region of ADIPOQ gene was amplified successfully at annealing temperature 60°C for 30 seconds. PCR product was 265 bp as shown in Figure 1, confirming the sizes using agarose gel electrophoresis and sequencing analyses. The present analysis determined genotypes according to length of DNA banding patterns, showing that the length 265 bp was assigned to D alleles (Deletion). The present PCR analysis did not find any cattle with insertion (331 bp), whereas 211 samples possessed D alleles, showing that allele frequency for D was 1.0. That the promoter region of ADIPOQ gene was monomorphic, when a locus in a population found only one allele or if the most common allele was known to be a high frequency (more than 95% or 99%), the locus was considered as monomorphic (Nei and Kumar, 2000; Allendorf *et al.,* 2013).

Allele frequency of indel g.81966364D>I in promoter region of ADIPOQ gene had been reported in several studies in Bos taurus, Bos indicus and its crossbreeds cattle as presented in Table 2. SNP indel g.81966364D>I in promoter region of ADIPOQ gene had been reported under significant effects on heart girth and huckle-bone in three Chinese cattle breeds (Zhang *et al.,* 2013), marbling scores in Hanwoo cattle (Choi *et al.* 2015; Kwon *et al.,* 2016). The sequences from promoter region of ADIPOQ gene were aligned with a reference sequence from NCBI (JQ775868.1), indicating that no insertion was found in Indonesian beef cattle.

Amplification and Polymorphism of EDG1 Gene

The 5'UTR of EDG1 gene was amplified successfully at annealing temperature 60°C for 30 seconds. Genotyping by restriction enzim *Msc*I resulted in two genotypes: AA (225 and 186 bp) and AG (411, 225 and 186 bp) as presented in Figure 2. The present RFLP analysis did not find any cattle with GG genotype. Genotyping analysis

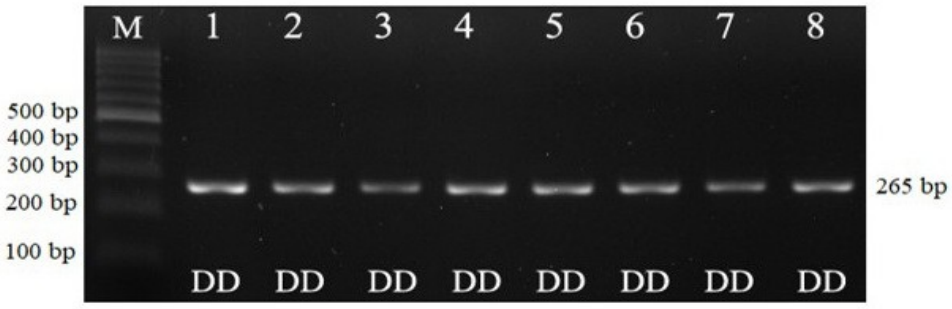

Figure 1. Amplification product of promoter region *ADIPOQ* gene in 2% agarose gel (w/v). Lane M = marker with 100 bp DNA ladder; Lane 1-8 = DD genotype

Table 2. Allele Frequency of Indel g.81966364D>I in Promoter Region of *ADIPOQ* Gene in Numerous Cattle Breeds

Species	Breeds of Cattle	N	Allele Frequency		References
			D	I	
Bos taurus	Angus	1669	0.81	0.19	Morsci *et al.* (2006)
B. taurus x B. indicus	Nayang	224	0.96	0.04	Zhang *et al.* (2013)
B. taurus x B. indicus	Jiaxian	142	0.91	0.09	Zhang *et al.* (2013)
B. taurus x B. indicus	Qinchuan	318	0.87	0.13	Zhang *et al.* (2013)
B. taurus x B. indicus	Luxi	142	0.95	0.05	Zhang *et al.* (2013)
B. taurus x B. indicus	Jinnan	72	0.94	0.06	Zhang *et al.* (2013)
B. taurus x B. indicus	Chinese Rep Steppe	216	0.91	0.09	Zhang *et al.* (2013)
Bos taurus	Chinese Holstein	97	1.00	0.00	Zhang *et al.* (2013)
Bos taurus	Hanwoo	1952	0.84	0.16	Choi *et al.* (2015)
Bos taurus	Hanwoo	8378	0.86	0.14	Kwon *et al.* (2016)
Bos javanicus	Bali cattle	44	1.00	0.00	This study
Bos taurus	Simmental	15	1.00	0.00	This study
Bos taurus	Limousin	18	1.00	0.00	This study
Bos indicus	Pesisir	20	1.00	0.00	This study
Bos indicus	Ongole Grade	22	1.00	0.00	This study
Bos indicus	Katingan	20	1.00	0.00	This study
Bos indicus	Brahman	20	1.00	0.00	This study
Bos indicus	Pasundan	20	1.00	0.00	This study
Bos indicus	Madura	20	1.00	0.00	This study
Bos indicus	Sumba Ongole	12	1.00	0.00	This study

N = Number of sample; D = Allele of deletion; I = Allele of insertion

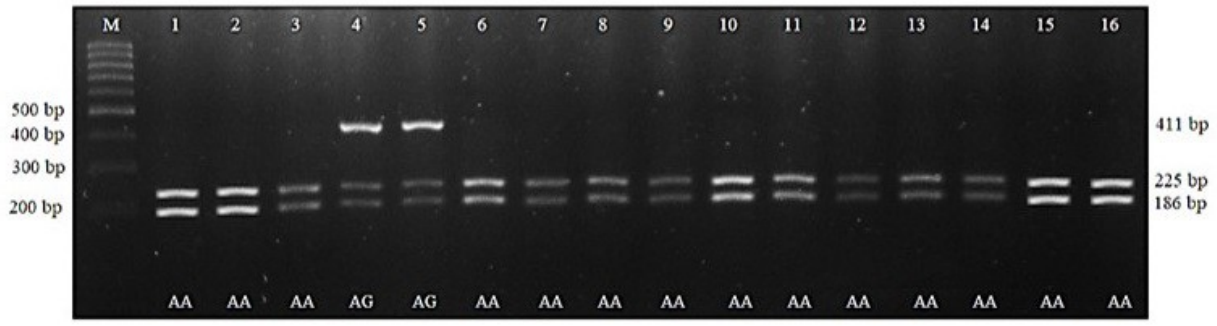

Figure 2. Digestion pattern of 5'UTR *EDG*1 gene by *Msc*I in 2% agarose gel (w/v). Lane M = marker with 100 bp DNA ladder; Lane 1-3 and 6-16 = AA genotype; Lane 4-5 = AG genotype

on 211 Indonesia beef cattle revealed that allele frequency of c.-312A>G gene fragment was high for A allele (digested by *Msc*I), while for G allele (not digested by *Msc*I) was considerably low frequency (Table 3). additions in some cattle populations had only A alleles as in Bali,

Table 3. Genotype and Allele Frequency, Heterozygosity Values and HWE of SNP c.-312A>G in 5'UTR *EDG*1 Gene in Indonesian Beef Cattle

Breeds of Cattle	N	Genotype Frequency			Allele Frequency		Diversity Parameters		
		AA	AG	GG	A	G	Ho	He	HWE
Pesisir	18	0.83	0.17	0.00	0.92	0.08	0.17	0.15	0.149 ^{ns}
Madura	20	0.90	0.10	0.00	0.95	0.05	0.10	0.10	0.055 ^{ns}
Brahman	20	0.90	0.10	0.00	0.95	0.05	0.10	0.10	0.055 ^{ns}
Limousin	18	0.89	0.11	0.00	0.94	0.06	0.11	0.10	0.062 ^{ns}
Pasundan	20	0.95	0.05	0.00	0.98	0.03	0.05	0.05	0.013 ^{ns}
Bali	44	1.00	0.00	0.00	1.00	0.00	0.00	0.00	-
Simmental	15	1.00	0.00	0.00	1.00	0.00	0.00	0.00	-
Ongole Grade	22	1.00	0.00	0.00	1.00	0.00	0.00	0.00	-
Katingan	20	1.00	0.00	0.00	1.00	0.00	0.00	0.00	-
Sumba Ongole	12	1.00	0.00	0.00	1.00	0.00	0.00	0.00	-

N = Number of sample; Ho = observed heterozygosity; He = expected heterozygosity; HWE = Hardy-Weinberg Equilibrium; ns = non significant.

Simmental, PO, katingan, and SO.

The results of this study indicating SNP c.-312A>G EDG1 gene in Limousine, Pesisir, Brahman, Pasundan, and Madura were polymorphic because the allele frequency was obtained more than 0.01 (Nei and Kumar 2000; Allendroft *et al.,* 2013). While for Bali, Simmental, PO, Katingan and SO were monomorphic due to the frequency of alleles obtained less than 0.01 (Nei and Kumar 2000; Allendroft *et al.,* 2013). The highest frequency of allele A in samples was estimated to be due to selection and mating control managed by farmers. The selection conducted by the breeder was to maintain cattle with A allele rather than with G allele. According to Noor (2010), factors affecting gene frequency were selection, mutation, inbreeding, crossbreeding and genetic drift.

The EDG1 gene encoded 383 amino acids and was associated with the traits of intramuscular fat deposition (marbling) in Wagyu cattle (Sasaki *et al.,* 2006). Mutations in c.-312A>G was associated with marbling in Japanese Black cattle. Cattle with G alleles had higher marbling scores than cattle with A allele (Yamada *et al.,* 2008; Sukegawa *et al.,* 2010). The frequency of EDG1 gene alleles in various cattle in the world is presented in Table 4.

The heterozygosity value was the mean percentage of individual heterozygot or the percentage of heterozygot individuals in the population (Nei and Kumar, 2000). Observed and expected heterozygocity (Ho and He, respectively) values indicated that diversities of Indonesian beef cattle were remarkably low. The values were 0.05-0.17 and 0.05-0.15 for Ho and He, respectively (Table 3). Table 3 also showed that Ho and He values among the cattle breeds in this experiment were statistically similar. This indicated gene frequency in each population was in equilibrium state as supported by Hardy-Weinberg test in this experiment (P>0.05). Yet, Bali, Simmental, PO, Katingan and SO were an exception in which the gene frequencies in this population were considerably not in equilibrium state based on the test (P<0.05).

In general, population of Indonesian beef cattle was in dynamic equilibrium, with exception for Bali, Simmental, PO, Katingan and SO cattle population. This discrepancy might be due to limited sample number in this experiment. As Allendrof *et al.* (2013) reported that population size was one of constraint in Hardy-Weinberg equilibrium status. Other constraints were random matting, the absence of mutation, the absence of selection as well as the absence of migration.

Table 4. Allele Frequency of SNP c.-312A>G in 5'UTR of *EDG*1 Gene in Numerous Cattle Breeds

Species	Breeds of Cattle	N	Allele Frequency		Reference
			A	G	
Bos taurus	Japanese black sires	96	0.42	0.58	Yamada *et al.* (2008)
Bos taurus	Japanese Black progeny steers	1049	0.43	0.57	Yamada *et al.* (2008)
Bos taurus	Japanese Black progeny steers	681	0.22	0.78	Yamada *et al.* 2(008)
Bos taurus	Japanese Black sires	101	0.41	0.59	Watanabe *et al.* (2009)
Bos taurus	Japanese Black progeny steers	1730	0.39	0.61	(Watanabe *et al.* (2009)
Bos taurus	Japanese Brown sires	85	0.95	0.05	Watanabe *et al.* (2009)
Bos taurus	Japanese Brown progeny steers	27	0.96	0.04	Watanabe *et al.* (2009)
Bos taurus	Japanese Short Horn sires	79	0.97	0.03	Watanabe *et al.* (2009)
Bos taurus	Japanese Short Horn progeny steers	264	0.95	0.05	Watanabe *et al.* (2009)
Bos taurus	Holstein	274	0.99	0.01	Watanabe *et al.* (2009)
Bos taurus	Brown Swiss	117	0.80	0.20	Watanabe *et al.* (2009)
Bos taurus	Japanese Black (Kagoshima)	489	0.41	0.59	Sukegawa *et al.* (2010)
Bos taurus	Japanese Black (Miyazaki)	160	0.44	0.56	Sukegawa *et al.* (2010)
Bos taurus	Japanese Black (Nagasaki)	191	0.58	0.42	Sukegawa *et al.* (2010)
Bos taurus	Japanese Black (Niigata)	130	0.57	0.43	Tong *et al.* (2013)
Bos javanicus	Bali	44	1.00	0.00	This study
Bos taurus	Simmental	15	1.00	0.00	This study
Bos taurus	Limosin	18	0.94	0.06	This study
Bos indicus	Pesisir	18	0.92	0.08	This study
Bos indicus	Ongole Grade	22	1.00	0.00	This study
Bos indicus	Katingan	20	1.00	0.00	This study
Bos indicus	Brahman	20	0.95	0.05	This study
Bos indicus	Pasundan	20	0.98	0.03	This study
Bos indicus	Madura	20	0.95	0.05	This study
Bos indicus	Sumba Ongole	12	1.00	0.00	This study

N = number of sample

Hardy-Weinberg equilibrium status was also found in population of Jiaxian, Jinnan, Luxi, Nanyang, Red Steppe Cattle (Zhang *et al.,* 2013), Hanwoo (Choi *et al.,* 2015; Kwon *et al.,* 2016). The large difference between Ho and He values could be an indicator of imbalance genotype in population (Tambasco *et al.,* 2003).

The population of animals was expressed in equilibrium if the genotype and allele frequencies were constant from generation to generation (Allendorf *et al.,* 2013). Large populations would not change from one generation to another if there was no selection, migration, mutation, and genetic drift (Noor, 2008)

Sequence Analysis of SNP c.-312A>G

Sequences analysis on A and G allele polymorphism in Bali and Limousin samples used reference from GenBank (access code NW_003103868 region: 12578072-12578482) and Ensembl (access code ENSBTAG00000005990). The result of alignment verified nucleotide transition at positions 189 (A>G) from forward PCR product 411 bp or at the

position to c. -312 (nucleotide positions relative to the transcription initiation site of the EDG1 gene). The SNP c.-312A>G cause cut by *Msc*I restriction enzyme for A allele and could not recognize G allele. The results of this study found two new SNP candidates in Bali that were C>T and C>G at positions 102 and 228 (nucleotide positions relative to PCR product) or at position c.-399C>T and c.-273C>G in 5'UTR of EDG1 gene (ENSBTAG00000005990). Details of sequence are shown in Table 5 and Figure 3.

Bali cattle (Bos javanicus) as one of native beef cattle in Indonesia was a cattle domestication from bull (Bibos banteng) (Purwantara *et al.,* 2012). Many reseach in bali cattle were found new SNP such as in calpain-1 (CAPN1) gene (Pratiwi *et al.,* 2016), stearoyl-CoA denaturase (SCD) gene (Alwiyah *et al.,* 2016), 5'UTR

Table 5. Position of SNP and Type of Substitutions of *EDG*1 Gene in Bali and Limousin Cattle

No	SNP Position	Allele	Type Substitutions	Beef Sample
1	102th (c.-399C>T)	C	Transition	Bali cattle
		T		Limousin
2	189 th (c.-312A>G)	A	Transition	Bali cattle
		G		Limousin
3	228 th (c.-273C>G)	C	Transversion	Bali cattle
		G		Limousin

102th, 189th and 228th = SNP positions from primers forward; (c.-399C>T), (c.-312A>G), (c.-273C>G) = SNP positions based reference ENSBTAG00000005990

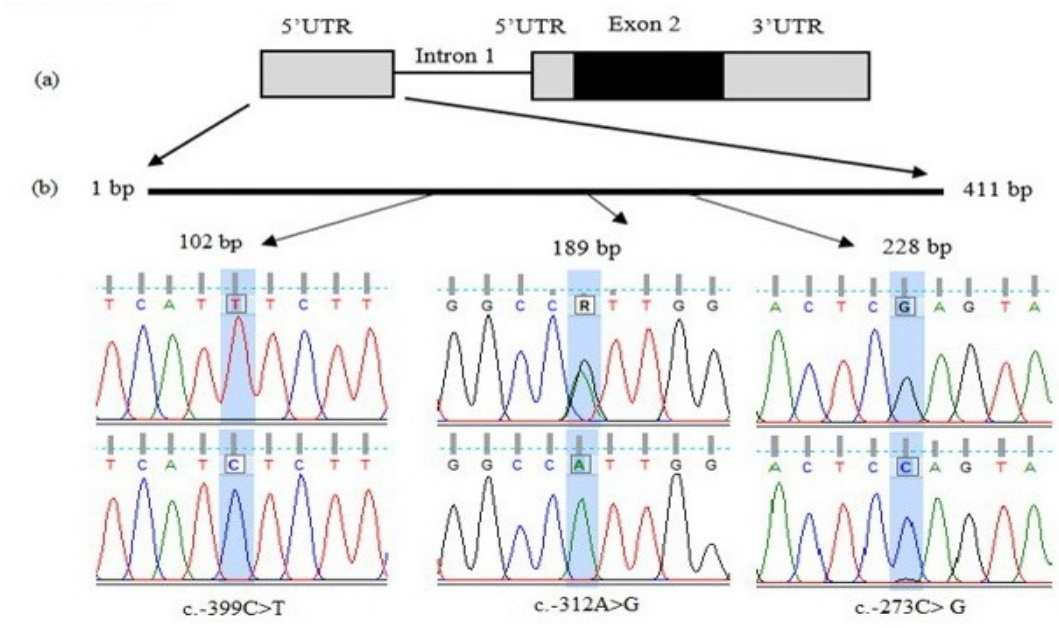

Figure 3. Schematic of SNP position located in 5'UTR of *EDG*1 gene. (a) sequences reference of *EDG*1 gen (ENSBTAG00000005990); (b) SNP position in PCR product 411 bp; c.-399C> T and c.-273C> G = candidat novel SNP in Bali cattle; c.-312A>G = SNP was recognized by *Msc*I restriction enzyme (A allele); R = purine base (adenine or guanine)

thyroglobulin (TG5) gene (Anwar *et al.,* 2017), and 5'UTR of EDG1 gen (this study). Therefore, Bali beef cattle could be potential to be developed into premium beef cattle.

CONCLUSION

The indel g.81966364D>I of ADIPOQ gene was monomorphic in Indonesian beef cattle. While the SNP c.-312A>G of EDG1 gene was polymorphic in Pesisir, Brahman, Pasundan, Madura and Limousine cattle. In Bali cattle, two new SNP candidates were found in position of c.-399C>T and c.-273C>G which were potential to be used as genetic markers of marbling score.

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