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Genetic polymorphism analysis of 5' untranslated region of thyroglobulin gene in Bali cattle (*Bos javanicus*) from three different regions of Indonesia

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ABSTRAK

Variasi nukleotida g.422C>T pada gen *thyroglobulin* dibagian 5'untranslated region (5'UTR) (disebut TG5) telah dilaporkan mempunyai asosiasi dengan tingkat kandungan lemak intramuskuler atau *marbling* pada sapi potong. Penelitian ini ditujukan untuk mengkonfirmasi keragaman genetik gen TG5 pada sapi Bali dari tiga wilayah sumber bibit yang berbeda di Indonesia. Sebanyak 200 ekor sapi Bali telah dilakukan *genotyping* pada gen TG5 menggunakan metode *polymerase chain reaction-restriction fragment lenght polymorphism* (PCR-RFLP) dan analisis sekuen. Hasil penelitian menunjukkan bahwa TG5 terkonfirmasi monomorfik pada sapi Bali dimanapun wilayah asalnya. Selain itu, sebanyak sembilan kandidat SNP ditemukan di dalam gen TG bagian 5'UTR pada sapi Bali dibandingkan dengan sekuens Genbank, meskipun tidak ditemukan variasi SNP di dalam sampel sapi Bali yang diteliti. Marker genetik lain dari gen TG perlu dieksplorasi dan diverifikasi keragamannya pada sapi Bali. Sembilan kandidat SNP juga perlu diverifikasi dan divalidasi lebih lanjut dengan sampel yang lebih besar untuk dapat dikatakan sebagai SNP baru antara sapi Bali dengan referensi sekuen dari GenBank.

Kata kunci : gen thyroglobulin, marbling, polimorfisme genetik, sapi Bali, 5'-untranslated region

ABSTRACT

The g.422C>T nucleotide variations in the 5' untranslated region (5'UTR) of TG gene (called as TG5) has been reported to be associated with level in intramuscular fat (IMF) content or marbling in beef cattle. The objective of this study was to confirm genetic polymorphism of TG5 gene in Bali cattle populations from three different regions as the main resources of Bali cattle in Indonesia. A total of 200 head of Bali cattle have been performed genotyping on TG5 gene using *polymerase chain reaction-restriction fragment lenght polymorphism* (PCR-RFLP) method and sequence analysis. Results of the study confirmed that TG5 was monomorphic in Bali cattle wherever their origin regions. Moreover, nine candidate SNPs were detected within 5'UTR of TG gene in Bali cattle compared to Genbank reference sequences, although no SNP variations among Bali cattle sample studied. The new other genetic markers within an entire TG gene suggested to be explored and verified for their polymorphisms in Bali cattle.

The nine candidate SNPs were also required further verification and validation in a larger sample to be regarded as new SNPs between Bali cattle and Genbank reference sequences.
Keywords : Bali cattle, genetic polymorphism, marbling, thyroglobulin gene, 5'-untranslated region

INTRODUCTION

Beef palatability has becoming a major concern of consumers satisfaction in consuming beef. Intramuscular fat (IMF) or also called as marbling has been studied contributed to the beef palatability or eating quality of beef through its attributes such as flavour, juiciness and tenderness (Okumura *et al.,* 2007; Hudson *et al.,* 2015), even though several studies showed that the association between marbling score and beef palatability attributes was a positively small (Wheeler *et al.,* 1994; Platter *et al.,* 2003). However, marbling score has been used as one of major determinants for beef palatability in international markets especially in Japan, Australia and USA (Watson *et al.,* 2008; Gotoh *et al.,* 2014).

Molecular markers could be used to identify the quantitative trait loci (QTLs) that affect desired quantitavive traits (Dekkers, 2004; Van Eenennaam *et al.,* 2007). Several studies have identified QTLs associated with fatness traits in cattle located on bovine chromosome 14 (BTA14) including thyroglobulin gene (TG) (Moore *et al.,* 2003; Casas *et al.,* 2005). Thyroglobulin gene encodes thyroglobulin, a glycoprotein hormone that is sinthesized by thyroid follicular cells and stored in thyroid gland. The TG acts as precursor for the thyroid hormone, the *triiodothyronine* (T3) and *thyroxine* (T4) hormones. Thyroid hormones are known to play an important role in influencing the lipid metabolism, adipocyte growth and differentiation (Ailhaud *et al.,* 1992; Casas *et al.,* 2005; Pannier *et al.,* 2010).

The nucleotide substitution cytosine (C) to thymine (T) at the position of 422 in the 5'-untranslated region (5'UTR) of TG gene (TG5) has been reported to be associated with level in IMF content or marbling score in beef cattle (Barendse, 1999). Therefore, this finding has been proposed as candidate gene for improving marbling level and eating quality in beef cattle. Recently, TG5 has been used as genetic marker to improve the marbling level and other fatness traits of beef in *Bos taurus* and *Bos indicus* cattle (Casas *et al*., 2005; Van Eenennaam *et al.*, 2007; Gan *et al*., 2008; Bonilla *et al,*. 2010; Anton *et al.,* 2011).

In previous study, Anwar *et al*. (2016) have identified that TG5 genetic marker tend to be monomorphic in 100 samples of Bali cattle at Maiwa Breeding Center (MBC), Enrekang District, South Sulawesi Province using PCR-RFLP method. For further confirmation of this result, more sample size from three different purebreeding regions of Bali cattle have been examined to investigate the possibility of changes in the distribution of alleles and genotypes in the sample studied by using PCR-RFLP method and sequence analysis. Therefore, the objective of this study was to confirm genetic variation of TG5 and to explore new candidate SNPs within 5'UTR of TG gene in Bali cattle (*Bos javanicus*) compared to GenBank reference sequences.

MATERIALS AND METHODS

Animals and Blood Collection

A total of 200 head of Bali cattle were used in this study. The animals were originated from three different regions as the main resources of Bali cattle in Indonesia, including : 1) South Sulawesi Province (SS) represented samples from Barru District (76 heads), 2) Bali Province (Bali) represented samples from island of Nusa Penida (52 heads) and West Nusa Tenggara (NTB) repesented from Banyumulek Sub-district (72 heads). All animals were unkown pedigree data. Blood sample of each animal was collected into vaccutainer tube containing K2EDTA as anticoagulant. The blood samples were kept at 4-5°C for further use.

DNA Isolation and PCR Amplification

Genomic DNA isolation was performed using Genomic DNA mini kit (Geneaid Biotech Ltd., Taiwan) according to the the manufacturer's instructions. DNA products were stored at -20°C for further use. A 545 base pairs (bp) of 5'UTR of TG gene was amplified using a pairs of primer designed by Barendse (1999) based on the GenBank reference sequence (accession no: X05380.1). The PCR reaction and program were performed according to Anwar *et al.*(2016).

PCR-RFLP Genotyping

Allele and genotype variations of TG5 gene were detected using PCR-RFLP method. The *BstY*I restriction enzyme (New England Biolabs, USA) with the restriction site at 5' R↓GATCY 3' were used to detect g.422C>T polymorphic site in 5'UTR of TG gene. The digestion mixture and procedure were performed according to Anwar *et al.* (2016).

Gene Sequencing and Sequence Analysis

In this study, DNA fragments of all samples have been found only one electrophoretic band pattern (CC genotype), while CT and TT genotype were not observed. However, in sequence analysis, we used seven samples consisting a total of six samples represent of CC genotype (four from SS, one from Bali and one from NTB regions). The three of four samples from SS were taken from our previous study (Enrekang District) while one of four samples was taken from Barru District. The one heterozygous CT animal found in Enrekang District was used as control to detect g.422C>T mutation. The two GenBank accession numbers (X05380.1 and AY615525) of TG gene of *Bos taurus* species were used to compare the different SNP (g.422C>T). The accession number of AY615525 was an updated sequence data from X05380.1 which contained two newly SNPs found in Korean cattle populations (g.257C>T and g.335A>G) and has been submitted to GenBank by Shin and Chung (2007).

DNA fragments of each sample were sequenced for both directions using PCR primers forward and reverse performed by commercial laboratory service at First BASE Laboratories Sdn. Bhd. (Selangor, Malaysia) using ABI PRISM 96-capillary 3730xl DNA Analyzer (Applied Biosystems, USA). Sequence analysis begins with a contig analysis which combines both the sequencing results (forward and reverse) to obtain a complete single sequence of each sample. The nucleotide sequence obtained were then aligned with the GenBank reference sequences (Accession number : X05380.1 and AY615525) and analyzed by using Bioedit sequence alignment editor (Hall, 1999) and ClustalW alignment method in Molecular Evolutionary Genetic Analysis (MEGA6) software (Tamura *et al.,* 2013).

RESULTS AND DISCUSSION

PCR Product and Genotype Variants

The TG5 is code used for genetic marker found in the 5'UTR of TG gene which has nucleotide substitution (C>T) at the position of 422. In this study, a 545 bp targeted DNA sequence of 5'UTR of TG gene was succesfully amplified using a pairs of primer designed by Barendse (1999) as shown by the result of a single clear band (Figure 1).

Detection of genotype variants of TG5 was performed using PCR-RFLP method and then confirmed using sequence analysis. The genotype found in this study only CC genotype whereas CT and TT genotypes were not detected in 200 sample studied. Therefore, in order to know the position of g.422C>T mutation, one DNA sample represent CT genotype in previous study was used

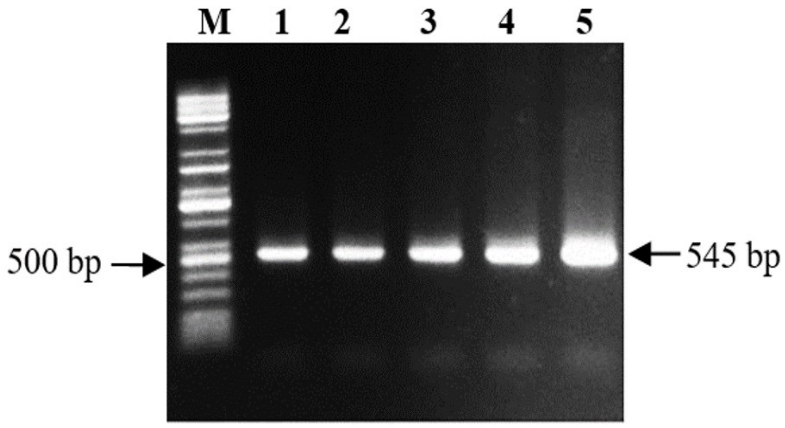

Figure 1. Electrophoretic PCR Product Visualization of TG5 Gene (545 bp) in a 1.2% Agarose Gel (w/v). Lane M: marker with 100 pb DNA ladder; lane 1,2,3,4,5: number of sample.

to be sequenced (Anwar *et al.,* 2016) as a control in sequence analysis. The genotype variants (CC and CT) corresponding to g.422C>T mutation of TG5 were succesfully confirmed through DNA sequencing. On the C allele, the base C at the position of 422 were not changed so that the *BstY*I restriction enzyme recognize the restriction site and cuts the DNA into three fragments (72, 295 dan 178 pb). While on the T allele, *BstY*I could not recognize the restriction site at the position of 422 because the base C was replaced by base T and generates only two fragments (72 dan 473 pb). The position of TG5 gene within GenBank reference sequence (accession no : X05380.1), its restriction map and chromatogram visual representation of SNP (g.422C>T) are shown in Figure 2.

Genetic Polymorphism Analysis

Genetic polymorphism could be measured at various levels, e.g. allelic and genotypic variation at certain locus or loci within a populations. In previous study, Anwar *et al.* (2016), showed that the frequency of C allele and CC genotype of TG5 gene from 100 samples derived from Maiwa Breeding Center (MBC), Enrekang District, South Sulawesi (SS) were 0.995 and 0.990, respectively, and suggested to be monomorphic. In this study, the different sample locations have been used. A total of 200 head of Bali cattle were taken from three different regions that designated by Indonesian government as purebreeding locations for Bali cattle including South Sulawesi (SS), Bali and West Nusa Tenggara (NTB) Province to confirms the polymorphism of TG5 gene.

The allelic and genotypic frequencies for the TG5 gene studied in Bali cattle from three regions are shown in Table 1. The C allele and CC genotype were found to be fixed in all three region samples (1.000 and 1.000, respectively), whereas the T allele did not observed. As the consequent of the absence of T allele in total samples, CT and TT genotypes were not observed in this study (0.000 and 0.000, respectively).

The results in this study indicate that C allele and CC genotype of TG5 gene were found to be fixed in Bali cattle (*Bos javanicus*) wherever their origin region. According to Nei (1987), Frankham *et al.* (2004) and Allendorf and Luikart (2007), when a locus in a population found only one allele or if the most common allele is known to be a high frequency (more than 95% or 99%), the locus was considered as monomorphic. This finding confirms on Anwar *et al.* (2016) study, that the TG5 gene was monomorphic in Bali cattle in which the most common allele (C allele) was 0.995 or 99.5%.

The TG5 marker was located in the 5' leader sequence or 5' UTR of the TG gene. Barendse *et al.* (2004) stated that TG5 was chosen for marker

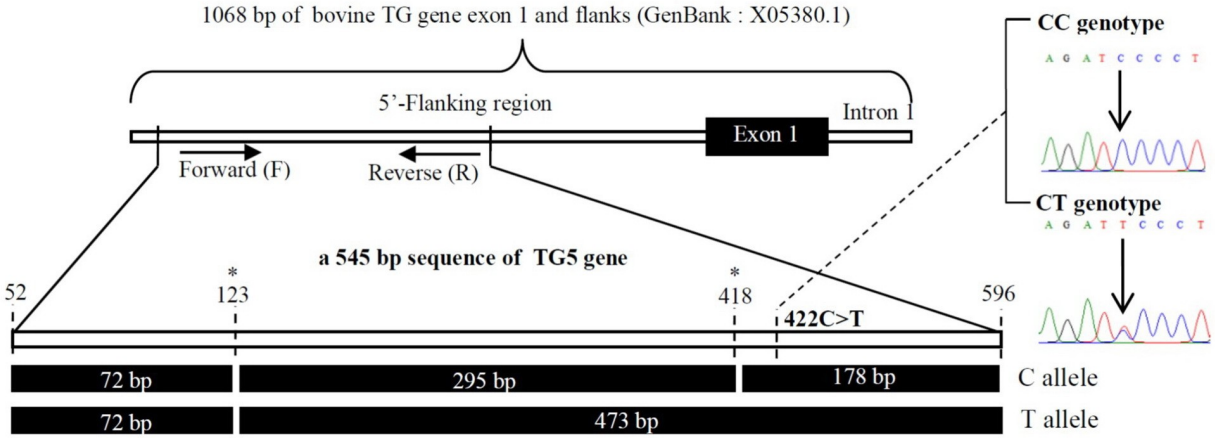

Figure 2. Schematic Representation of the Position of TG5 Gene, Restriction Map and Chromatogram Visual Representation of SNP within GenBank Reference Sequence (accession no : X05380.1). The arrows indicate a pairs of primer position (forward and reverse) to initiated a process of amplification. The restriction site of *BstY*I (5' R↓GATCY 3') are indicated with an asterisk. In the T allele, nucleotide subtitution (C>T) at the position of 422 causing *BstY*I restriction enzyme do not recognize the restriction site at this position.

Table 1. Allelic and Genotypic Frequencies of TG5 Gene in Bali Cattle (*Bos javanicus*) from Three Different Regions

Marker	Region	Total (n)	Genotypic Frequencies			Allelic Frequencies	
			CC (n)	CT (n)	TT (n)	C	T
TG5	SS	76	1.000 (76)	0.000 (0)	0.000 (0)	1.000	0.000
	Bali	52	1.000 (52)	0.000 (0)	0.000 (0)	1.000	0.000
	NTB	72	1.000 (72)	0.000 (0)	0.000 (0)	1.000	0.000
	Total	200	1.000 (200)	0.000 (0)	0.000 (0)	1.000	0.000

SS = South Sulawesi Province, Bali = Bali Province, NTB = West Nusa Tenggara Province

genetic test, due to the known low level of polymorphism in the TG coding sequence and more importantly to the features present in that DNA sequence. Furthermore, TG5 is in the leader sequence of the gene affects for the RNA polymerase III binding site which important to initiate transcription in regulation of gene expression, although its functional effect to circulating level of thyroid hormones (T3 and T4) were not undertaken yet.

The T allele in TG5 marker has been proposed by Barendse *et al.* (2004), Casas *et al.* (2005), Bonilla *et al.* (2010) and Anton *et al.* (2011), as the favourable allele because the homozygous TT cattle tend to be higher in IMF fat deposition or marbling score than heterozygous CT and homozygous CC cattle. This TG5 marker was then launched as a DNA marker test for commercial use and has been performed a validation studies although genetic association analysis with marbling traits still varied results in some cattle breeds (Casas *et al.,* 2005; Rincker *et al.,* 2006; Shin dan Chung, 2007; Van Eenennaam *et al.,* 2007; Johnston and Graser, 2010 and Pannier *et al.,* 2010).

The allelic and genotypic frequencies of TG5 gene has been reported in several studies in *Bos taurus*, *Bos indicus* and its crossbreeds cattle as presented in Table 2. It showed that C allele of TG5 was the most common allele found in all cattle breeds. However, C allele was more frequent found in *Bos indicus* breeds such as Brahman cattle (Casas *et al.,* 2005) and Nelore cattle (Fortes *et al.,* 2009) and tend to be fixed as well as in *Bos javanicus* than in *Bos taurus* breeds. Conversely, the T allele was mostly frequent in *Bos taurus* breeds (0.229) compared to *Bos indicus* (0.020) and *Bos javanicus* (0.002), although the frequency of T allele in *Bos taurus* was varied (0.016-0.487). Wagyu cattle breeds were regarded as *Bos taurus* type that carrying T allele, and the consequently on its inheritances (Casas *et al.,* 2007; Van Eenennaam *et al.*, 2007). This occurance may caused by strong selection in Wagyu breed for marbling traits as well as on alleles of other candidate genes associated with high marbling (Watanabe *et al.,* 2011).

In this study, the C alel was fixed in Bali cattle populations represented from three selected regions (SS, Bali and NTB). These regions were established by Indonesian government for Bali cattle purebreeding since 1976 as well as in East Nusa Tenggara (NTT) (Handiwirawan and Subandriyo, 2004). Then, in 2013, Barru District, South Sulawesi Province, designated as a new location for Bali cattle purebreeding through Ministerial Decree No. 4437/Kpts/SR.120/7/2013 (Ministry of Agriculture of the Republic of Indonesia, 2013). This results also indicate that if C allele was fixed in Bali cattle, additional samples of Bali cattle should not be increasing the frequency of T allele with the exception in case of mutation or when it crossed with other breeds confirmed the T allele carriers. Morever, crosbreeding may still be difficult to increase the frequency of T allele because the action of TG5 suggested as recessive mode of inheritance (Casas *et al.,* 2007). This is the possible reason for previous study by Anwar *et al.* (2016), that has found only one heterozygous cattle (CT genotype) in Enrekang District, which is not included as location of purebreeding of Bali cattle. However, it needs to be further in-depth investigation. Frankham *et al.* (2004), stated that the other possible factors that allow allele or genotype to be fixed were inbreeding, genetic drift, small, isolated and sustained reduction population size.

In the sequence analysis between six

Table 2. Allelic and Genotypic Frequencies of TG5 Gene in Numerous Cattle Breeds

Species	Breeds	n	Genotypic Frequencies			Allelic Frequencies		References
			CC (n)	CT (n)	TT (n)	C	T	
Bos taurus	Limousin	123	0.667 (82)	0.317 (39)	0.016 (2)	0.825	0.175	1
	Charolais	80	0.563 (45)	0.350 (28)	0.088 (7)	0.738	0.263	1
	Simmental	58	0.397 (23)	0.552 (32)	0.052 (3)	0.672	0.328	1
	Simmental	438	0.534 (234)	0.390 (171)	0.075 (33)	0.729	0.271	2
	Angus	819	0.643 (527)	0.32 (265)	0.033 (27)	0.805	0.195	3
	Angus	39	0.205 (8)	0.615 (24)	0.179 (7)	0.513	0.487	1
	Angus	173	0.457 (79)	0.410 (71)	0.133 (23)	0.662	0.338	2
	Hereford	32	0.969 (31)	0.031 (1)	0.000 (0)	0.984	0.016	1
	Belgian blue	19	0.737 (14)	0.158 (3)	0.105 (2)	0.816	0.184	1
	Friesian Holstein	76	0.737 (56)	0.263 (20)	0.000 (0)	0.868	0.132	1
	Blonde d'Aquitaine	13	0.615 (8)	0.308 (4)	0.077 (1)	0.769	0.231	1
	Friesian Holstein	415	0.745 (309)	0.241 (100)	0.014 (6)	0.865	0.135	2
	Friesian Holstein	1279	0.580 (742)	0.390 (499)	0.030 (38)	0.775	0.225	4
	Jersey	283	0.601 (170)	0.350 (99)	0.049 (14)	0.776	0.224	2
	Korean	309	0.411 (127)	0.460 (142)	0.129 (40)	0.641	0.359	5
	TOTAL	4156	0.59 (2455)	0.360(1498)	0.049(203)	0.771	0.229	
Bos indicus	Brahman	467	0.946 (442)	0.039 (18)	0.015 (7)	0.966	0.034	6
	Brahman	383	0.992 (380)	0.008 (3)	0.000 (0)	0.996	0.004	7
	Nellore	46	1.000 (46)	0.000 (0)	0.000 (0)	1.000	0.000	8
	TOTAL	896	0.969 (868)	0.023 (21)	0.008 (7)	0.980	0.020	
Bos javanicus	Bali	100	0.990 (99)	0.010 (1)	0.000 (0)	0.995	0.005	9
	Bali	200	1.000 (200)	0.000 (0)	0.000 (0)	1.000	0.000	*
	TOTAL	300	0.997 (299)	0.003 (1)	0.000 (0)	0.998	0.002	
Crossbreed	British-GPE6	211	0.564 (119)	0.341 (72)	0.095 (20)	0.735	0.265	10
	Norwegian-GPE6	72	0.653 (47)	0.306 (22)	0.042 (3)	0.806	0.194	10
	Swedish-GPE6	68	0.574 (39)	0.324 (22)	0.103 (7)	0.735	0.265	10
	Friesian- GPE6	149	0.631 (94)	0.349 (52)	0.020 (3)	0.805	0.195	10
	Wagyu-GPE6	153	0.484 (74)	0.405 (62)	0.111 (17)	0.686	0.314	10
	Angus cross	40	0.650 (26)	0.275 (11)	0.075 (3)	0.788	0.213	11
	RGxN	26	0.962 (25)	0.038 (1)	0.000 (0)	0.981	0.019	8
	Canchim	41	0.610 (25)	0.341 (14)	0.049 (2)	0.780	0.220	8
	B3x	19	0.684 (13)	0.316 (6)	0.000 (0)	0.842	0.158	8
	BV3x	15	0.467 (7)	0.400 (6)	0.133 (2)	0.667	0.333	8
	TOTAL	794	0.591 (469)	0.338 (268)	0.072 (57)	0.759	0.241	

n: number of sample;

1: Pannier *et al.* (2010); 2: Anton *et al.* (2012); 3: Barendse *et al.* (2004); 4: Khatib *et al.* (2007); 5: Shin and Chung (2007); 6: Casas *et al.* (2005); 7: Smith *et al.* (2009); 8: Fortes *et al.* (2009); 9: Anwar *et al.* (2016); 10: Casas *et al*. (2007); 11: Anton *et al.* (2013); *: this study.

GPE6 = crossbred genetic group by maternal grandsire. RGxN = Rubia Gallega X Nelore,Canchim =(5/8 *Bos taurus* + 3/8 *Bos indicus*), B3x = (9/16 *Bos taurus* + 7/16 *Bos indicus*), BV3x =(3/4 *Bos taurus* + 1/4 *Bos indicus*).

consensus sequence samples in this study compared to GenBank reference sequences (X05380.1 and AY615525), obtained only 523 bp of 545 bp targeted sequence which is located on the base pair of 63 to 585 (Figure 3). Individual sample represent CT genotype that found by Anwar *et al.* (2016) was not regarded as polymorphic, thus it could not be included in the sequence analysis.

In alignment analysis, besides g.422C>T mutation, the total of nine new candidate SNPs were also identified in Bali cattle compared to Genbank reference sequences (X05380.1 and AY615525). The two SNPs were known as transversion nucleotide substitutions, while seven SNPs were known as transitions nucleotide substitutions. This findings supports the statement of Nei and Kumar (2000), in which transitionals were more frequent than transversions nucleotide substitutions in most DNA segments. However, nine new candidate SNPs found in this study could not regarded as novel SNPs because the sequence analysis conducted only in small studied samples. The candidate SNPs, its locations and the type of nucleotide subtitutions are shown in Table 3.

In this study, the six of nine candidate SNPs compared to both the X05380.1 and AY615525 were located in the base position of 240, 274, 387, 416, 537 and 552, respectively. The two of nine candidate SNPs compared only with X05380.1 were in the base position of 257 and 335, while one candidate SNP compared only with AY615525 was in the base position of 404 (Table 3). However, there are no SNP variations in all those nine candidate SNPs among six samples studied and were not considered as polymorphic site within Bali cattle or *Bos javanicus* populations.

As the results in this study, the other new genetic markers may be explored as well as Fortes *et al.* (2009) suggestion from their study in Nelore cattle. Gan *et al.* (2008) and Zang *et al.* (2015) have reported any SNPs within TG gene in beef cattle. Gan *et al.* (2008), have found six SNPs in the 3'-flanking region and one of six SNPs significantly associated with marbling score in *Bos taurus* breeds. Zang *et al.* (2015), have identified four SNPs in the 5'-flanking region of the TG gene and two of four SNPs significanlty associated with some carcass and meat quality traits in Chinese steers. For further studies, those SNPs entire TG gene can be verified for their polymorphisms in Bali cattle. Furthermore, to ensure the nine nucleotide variations that found in this study to be regarded as SNPs between Bali cattle and Genbank reference sequences, those required further verification and validation in a larger sample.

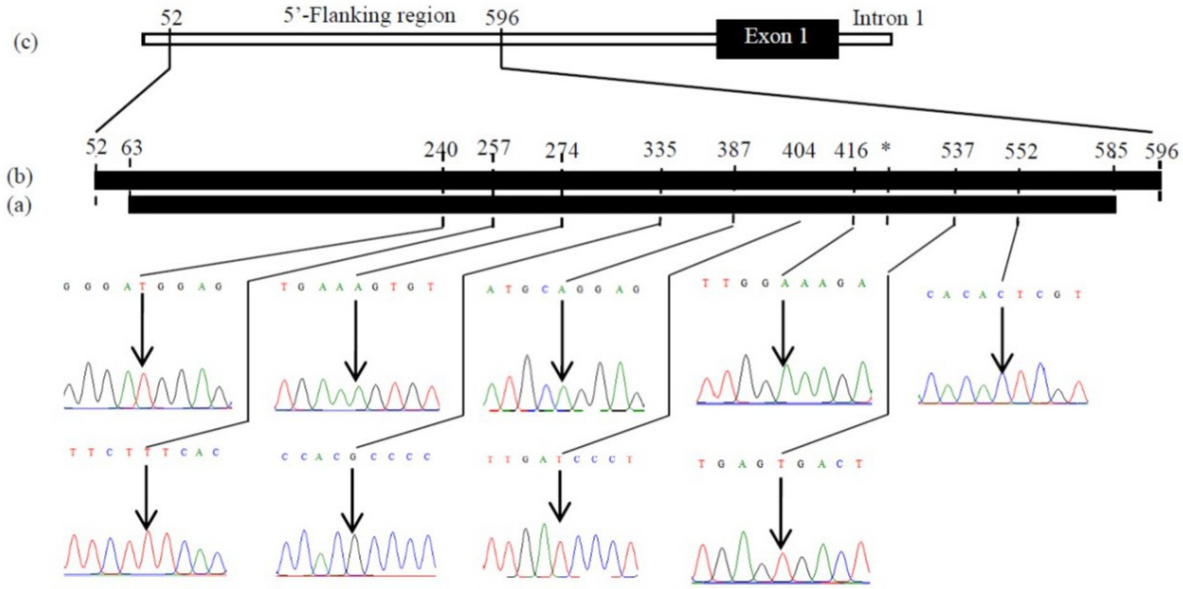

Figure 3. Schematic representation of the position of nine candidate SNPs within (a) a 523 bp of consensus sequence of 5'UTR of TG gene in Bali cattle compared to b) AY615525 and c) X05380.1 of GenBank reference sequences. The g.422C>T indicated with an asterisk.

Table 3. Candidate SNPs, Locations and the Type of Nucleotide Subtitutions in 5'UTR of TG Gene in Bali Cattle Compared to GenBank Reference Sequences

Samples	Candidate SNPs and Base Locations								
	240	257	274	335	387	404	416	537	552
X05380.1[1]	G	C	C	A	G	T	G	C	T
AY6155[1]	G	T	C	G	G	C	G	C	T
SS1[2]	T	T	A	G	A	T	A	T	C
SS2[2]	T	T	A	G	A	T	A	T	C
SS3[2]	T	T	A	G	A	T	A	T	C
SS4[2]	T	T	A	G	A	T	A	T	C
Bali[3]	T	T	A	G	A	T	A	T	C
NTB[4]	T	T	A	G	A	T	A	T	C
Type of subtitutions[5]	tv	ts	tv	ts	ts	ts	ts	ts	ts

[1]GenBank reference sequences; [2,3,4]DNA samples from South Sulawesi, Bali and West Nusa Tenggara Region, respectively; [5]Type of nucleotide substitutions, tv = transversion, ts = transition.

CONCLUSION

It can be concluded that TG5 was confirmed to be monomorphic in Bali cattle (*Bos javanicus*). Nine candidate SNPs has been found within 5'UTR of TG gene in Bali cattle compared to Genbank reference sequences, although there were no SNP variations within Bali cattle studied. The new other genetic markers within TG gene may be explored and verified for their polymorphisms on Bali cattle. The nine candidate SNPs were also required further verification and validation in a larger sample to be regarded as SNPs between Bali cattle and Genbank reference sequences.

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