Genotyping in the Insulin-like Growth Factor 1 (IGF1/*Sna*BI) Gene of Pasundan Cattle with PCR-RFLP Method

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ABSTRAK

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Gen *Insulin-like Growth Factor* 1 (IGF1) pada mamalia berfungsi untuk mengontrol pertumbuhan tulang dan otot. Oleh karena itu gen IGF1 banyak digunakan sebagai salah satu kandidat gen untuk seleksi ternak. Penelitian ini bertujuan untuk mengidentifikasi genotip gen IGF1 (ekson 1) menggunakan metode PCR-RFLP dengan enzim restriksi *Sna*BI (TAC*GTA). Sebanyak 90 ekor sampel DNA sapi Pasundan dari Kabupaten Ciamis dan Pangandaran, Jawa Barat telah digunakan pada penelitian ini. Hasil penelitian ini menunjukkan bahwa seluruh sampel yang dianalisis memiliki genotip CC dengan alel C sebagai alel yang umum pada gen IGF1/*Sna*BI. Genotip CC yang diperoleh pada penelitan ini disebabkan karena terdapat mutasi transisi pada posisi basa ke g.218T/C (GenBank: KF202095). Mutasi ini menyebabkan perubahan asam amino dari *methionine* (AUG) menjadi *valine* (GUG). Disimpulkan bahwa gen IGF1/*Sna*BI pada sapi Pasundan bersifat monomorfis dan tidak dapat digunakan untuk seleksi molekuler.

Kata Kunci: Gen IGF1/*Sna*BI, PCR-RFLP, Monomorfis, Mutasi, Sapi Pasundan

ABSTRACT

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The Insulin-like Growth Factor 1 (IGF1) gene is important to control sceleton and muscle development. Therefore, IGF1 gene was widely used as the candidate gene for livestock selection. This research was carried out to identify the genotype of IGF1 gene (exon 1) using PCR-RFLP method with *Sna*BI restriction enzyme (TAC*GTA). Total of 90 DNA samples of Pasundan cows from Ciamis and Pangandaran Regencies were used in the present study. Research reveals that all sample in the animal studied have CC genotype with C allele as the common allele in IGF1/*Sna*BI gene. The CC genotype that obtained in the present study was conducted by the transition mutation position g.218T/C (GenBank: KF202095). This mutation was changed the amino acid from methionine (AUG) to valine (GUG). It was concluded that IGF1/*Sna*B1 gene of Pasundan cattle is monomorphic and can not used for molecular selection.

Key Words: IGF1/*Sna*B1 Gene, , Monomorphic, Mutation, Pasundan Cattle, PCR-RFLP

INTRODUCTION

Pasundan cattle is one of Indonesian native cattle that adapt well in West Java province of Indonesia. This cattle was declared as native cattle of Indonesia through decision Ministry of Agriculture of Republic Indonesia No: 1051/Kpts/SR.120/10/2014. Indrijani et al. (2012) reported that the Pasundan cattle originates from crossbreeding batween Bali cattle (*Bos javanicus*) and Ongole or Madura cattle (*Bos indicus*) since a hundred years ago. The Pasundan cattle was kept by farmers in West Java as beef cattle. As the native beef cattle in Indonesia, the genetic improvement of Pasundan is important to increase productivity. Recently, the genetic improvement of Pasundan cattle can be obtained through molecular selection using some candidate genes that affecting the productivity.

Insulin-like Growth Factor 1 (IGF1) or somatomedin gene is one of the candidate that widely used as molecular selection in cattle (Szewczuk 2016). The bovine IGF1 is a small secreted peptide with 70-90 amino acids and molecular weight about 7500 kb and located on chromosome 5 with 6 exons and 5 introns (Miller et al. 1991; Rose 2002; Mullen et al. 2011). The IGF1 was produced in various body tissues, especially in the liver is produced mainly by influenced growth hormone. The IGF1 is a mediator of many biological effects, for example, it increases the absorbtion of glucose, stimulates myogenesis, inhibits apoptosis, participates in the activation of cell cycle genes,increases the synthesis of lipids, stimulates the production of progesterone in granular cells and intervenes in the synthesis (Etherton 2004). Hence, the

IGF1 gene is known to play an important role in various aspects of muscle growth and development.

One single nucleotide polymorphism (SNP) of g.218T/C was detected in the bovine IGF1 gene and can be detected with PCR-RFLP method with *Sna*BI restriction enzyme (Ge et al. 2001). Since year 2000, this SNP was identified in the 5'UTR of bovine IGF1 gene (GenBank: AH009378.2) but in year 2013 this SNP was confirmed in the exon 1 (GenBank: KF202095). Polymorphism in IGF1/*Sna*BI was affected to the productivity traits of cattle i.e. body weight at three months in Hanwoo cattle (Chung & Kim 2005), serum concentration of IGF1 at 14 days post partum in Friesian Holstein (FH) cattle (Mirzaei et al. 2012), service per conception and days open in FH cattle (Ararouti et al. 2013), breeding value of milk and fat yields in Iranian Holstein bulls (Mehmannavaz et al. 2010), growth traits in beef cattle (Siadkowska et al. 2006; Reyna et al. 2010; Szewczuk et al. 2013), carcass traits (Reyna et al. 2010) and meat colour in FH bulls (Ardicli et al. 2018). This research was aimed to detect SNP in the exon 1 of IGF1/*Sna*BI gene in Pasundan cattle using PCR-RFLP method. The result of this study is important to find the candidate gene for productivity traits of Pasundan cattle in the future.

MATERIALS AND METHODS

Animals and bloods sample

Total of 90 DNA sample of Pasundan cows from Ciamis (57 heads) and Pangandaran (33 heads) Regencies were used in this study. The blood samples (3-5 ml) were taken from cocygeal vein using venoject tube and collected in vaccutainer tubes containing anticoagulant (K_2EDTA). The DNA extraction was obtained with Genomic DNA Mini Kit (Geneaid Biotech Ltd., Taiwan) following the producers instruction. The extracted DNA was appropriately recorded and stored at -20°C for next analysis.

Amplification and genotyping of IGF1/*Sna*BI gene

The DNA fragments of IGF1 gene in Pasundan cattle were successfully to amplify by primer forward: 5'- ATTACAAAGCTGCCTGCCCC -3' and reverse: 5'-ACCTTACCCGTATGAAAGGAATATACGT -3' (Ge et al. 2001). This primer was amplified of IGF1 gene fragments along 246 according to GenBank: KF202095 (Figure 1). The DNA samples for amplification were prepared by additing 0.60 µl ddH_2O; 4.0 µl DNA solution (10 - 20 ng) and 0.20 µl of primers (1 pmol) into tube of 5.0 µl of PCR kit Green Taq (Thermo Scientific, USA). The amplification of DNA fragments were performed by using mastercycler gradient machine (Eppendorf, Germany). The PCR machine was programmed for initial denaturation 94°C for 4 min., and followed by 36 cycles of denaturation at 94°C at 45 sec., annealing at 57°C for 45 sec., elongation at 72°C for 1 min. and final elongation at 72°C for 10 min. Genotyping of IGF1 gene was performed by RFLP analysis. The RFLP was performed with 4.2 µl of PCR product that consisted of 4.45 µl of ddH_2O; 0.25 µl of *Sna*BI restriction enzyme (TAC*GTA); 1.0 µl buffer 10x and 0.1 µl acetylated BSA. The mixture was incubated in waterbath at 37°C for 2 h and visualized using 2% agarose gel with GelRed staining (Biotium, USA) and captured in GBOX Documentation System (Syngene, UK).

Data analysis

Data analysis of the IGF1 gene were consisted of allele frequency, genotype frequency, expected heterozygosity (H_e), observed heterozygosity (H_o), number of effective allele (n_e), polymorphic informative content (PIC) and Chi-square value (χ^2) value based on Nei & Kumar (2000).

RESULTS AND DISCUSSION

The IGF1 gene fragment was successfully amplified along 246 bp (Figure 2). The RFLP analysis showed that the IGF1/*Sna*B1 of Pasundan cattle was monomorphic with CC genotype as the common genotype (Table 1). The SNP of g.218T/C in the IGF1 gene can be detected with *Sna*BI restriction enzyme. All cattle in the present study are homozygote CC animals and signed by one fragments along 246 bp on the agarose gel. The n_e value in SNP of g.218T/C was 1.00 and indicated that only one common allele that found in this SNP. The PIC value in the IGF1/*Sna*B1 gene was 0.00 and indicated that this gene can not be used for molecular selection. In addition, this mutation was changed the amino acid from methionine (AUG) to valine (GUG). Previous studies reported that Ongole grade cattle (*Bos indicus*) and Bali cattle (*Bos javanicus*) was monomorphic with CC genotype as the common genotype (Table 2). Despite, the C allele was the dominant allele in some Bos indicus cattle such as Pesisir, Najdi and Kedah-Kelantan cattle. It was concluded that monomorphism in the IGF1/SnaB1 gene in this study can be caused by the evidence of material genetics of *Bos javanicus* and *Bos indicus* in the Pasundan cattle. This finding is important to clarify the origin of Pasundan cattle according to the previous study. The IGF1/*Sna*BI gene did not used as molecular selection in this study because of monomorphic. Absence of T allele in Pasundan cattle can be caused by selection, migration and inbreeding (Bourdon 2000).

Table 1. The statistical analysis of IGF1/*Sna*B1 gene in Pasundan cattle

Genotype frequency (N)			Allele frequency		H_o	H_e	n_e	PIC	χ^2
TT	TC	CC	T	C					
0.00 (0)	0.00 (0)	1.00 (90)	0.00	1.00	0.00	0.00	1.00	0.00	-

N: number of observation; H_o: observed heterozygosity; H_e: expected heterozygosity; n_e: number of effective allele; PIC: polymorphic informative content; χ^2: Chi-square value

Table 2. The genotype and allele frequencies of IGF1/*Sna*B1 gene in the several breeds cattle

Breeds	Species	N	Genotype frequency			Allele frequency		Reference
			TT	TC	CC	T	C	
Angus	*Bos taurus*	760	0.43	0.41	0.16	0.64	0.36	Ge et al. (2001)
		204	0.19	0.52	0.29	0.45	0.55	Islam et al. (2009)
Charolais	*Bos taurus*	186	0.30	0.52	0.18	0.56	0.44	Islam et al. (2009)
Angus × Charolais	*Bos taurus*	455	0.36	0.51	0.13	0.62	0.38	Islam et al. (2009)
Hanwoo	*Bos taurus*	280	0.59	0.26	0.15	0.72	0.28	Chung & Kim (2005)
Montbeliarde	*Bos taurus*	316	0.44	0.47	0.09	0.67	0.33	Szewczuk (2016)
Friesian Holstein	*Bos taurus*	662	0.29	0.47	0.24	0.52	0.48	Siadkowska et al. (2006)
		282	0.16	0.56	0.28	0.44	0.56	Mehmannavaz et al. (2010)
		201	0.26	0.56	0.18	0.54	0.46	Szewczuk et al. (2012)
		191	0.27	0.55	0.18	0.54	0.46	Szewczuk et al. (2013)
		37	0.19	0.59	0.22	0.49	0.51	Mirzaei et al. (2012)
		848	0.33	0.48	0.19	0.57	0.43	Mullen et al. (2011)
		70	0.31	0.54	0.14	0.59	0.41	Nicolini et al. (2013)
		50	0.12	0.80	0.08	0.48	0.52	Ardicli et al. (2018)
South Anatolian Red	*Bos taurus*	50	0.08	0.30	0.62	0.23	0.77	Akis et al. (2010)
East Anatolian Red	*Bos taurus*	50	0.18	0.40	0.42	0.38	0.62	Akis et al. (2010)
Beefmaster	*Bos taurus*	25	0.00	0.07	0.93	0.03	0.97	Reyna et al. (2010)
Najdi	*Bos indicus*	84	0.02	0.83	0.15	0.10	0.90	Yazdanpanah et al. (2013)
Ongole grade	*Bos indicus*	55	0.00	0.00	1.00	0.00	1.00	Anggraeni et al. (2017)
Pesisir	*Bos indicus*	183	0.01	0.01	0.98	0.02	0.98	Yurnalis et al. (2017)
Kedah-Kelantan	*Bos indicus*	46	0.07	0.13	0.80	0.13	0.87	Suriaty et al. (2010)
Bali	*Bos javanicus*	242	0.00	0.00	1.00	0.00	1.00	Mu'in (2010)
Brahman × Charolais	*Bos ind* × *Bos tau*	55	0.02	0.44	0.54	0.24	0.76	Jeanmas et al. (2013)

N: Number of observation

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   Forward >>>
  1 attacaaagc tgcctgcccc ccaggttcta ggaaatgaga tcatttccct cacttggcac
 61 caggacgagg ggtcatccca gcgctgtctt ccattctagt ttaccccagt cgtttgaggg
121 ttaaaatcat agagtaggct tgagatggtc ttttttttcat ttcttgtttt ttaaattttg
181 tgttggctct ggaatataaa attgctcgcc catcctcyac*gtatattcct ttcatacggg
241 taaggt
    <<< Reverse
```

Figure 1. The target sequence of IGF1 gene in the exon 1 along 246 bp (GenBank: KF202095) and primer position (underlines). *) *Sna*BI restriction point; Y= T/C.

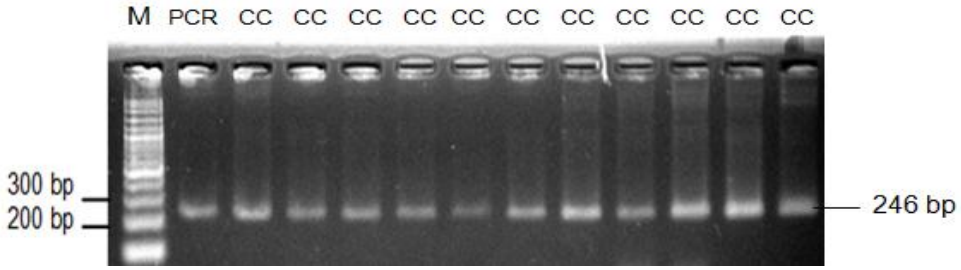

Figure 2. Visualization of IGF1/*Sna*B1 genotype in Pasundan cattle on 2% agarose gel was monomorphic with CC genotype (246 bp) as the common allele. M: DNA ladder 100 bp; PCR: DNA amplification / PCR product (246 bp).

According to Table 2, most of the IGF1/*Sna*BI gene in *Bos taurus* cattle was polymorphic and can be used for molecular selection. Chung & Kim (2005) reported that the CC genotype in Hanwoo cattle had lowest of weaning weight rather than other genotypes. Mirzaei et al. (2012) reported that the CC genotype in FH cattle had highest of serum concentration of IGF1 at 14 days prepartum in rather than other genotypes. Despite, CC genotype in FH cattle had lowest of service per conception (S/C) and days open (DO) values (Ararouti et al. 2013). Li et al. (2004) reported that CC genotype in Angus cattle had highest of body weight and weight gain rather than other genotypes. In addition, CC genotype in Polish Holstein had higest of body weight at two months and average daily gain from one to two months (Szewczuk et al. 2013). Ardicli et al (2018) reported that the CC genotype had the best of meat colour score at 24 hours post-slaughtering in FH bull. The CC genotype in the present study was detected in all samples and can be suggested as the common genotype for Pasundan cattle.

The further study regarding to detect SNP along IGF1 gene is important for developing marker assisted selection (MAS) to improve productivity traits of Pasundan cattle in the future. Several studies reported that the polymorphism of bovine IGF1 gene were occured in exon 4 and had associated with production traits in Bali cattle (Maskur et al. 2012). Mullen et al. (2011) reported that polymorphisms of bovine IGF1 gene were occured in 5' UTR, intron and 3' UTR. Polymorphism in the intron 4 of IGF1 gene was not associated with production traits in mixed population of Charolais and Beefmaster cattle (Reyna et al. 2010). Morever, polymorphism in the 5'UTR of IGF1 gene was associated with milk yield and milk quality in Polish Friesian Holstein cows (Szewczuk et al. 2013). Despite, Szewczuk (2016) reported that exon 7, exon 12 and exon 21 of bovine insulin-like growth hormon factor 1 receptor (IGF1R) was polymorphic and potential as molecular selection.

CONCLUSION

The IGF1/*Sna*BI gene of Pasundan cattle was monomorphic with C allele as the common allele in the animal studied. Therefore, this gene can not used as molecular selection for productivity traits of Pasundan cattle.

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