A Single Nucleotide Polymorphism (SNP) c.20G>A in somatostatin gene (SST|BsrI) on Madura cattle

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Abstract. Madura cattle is one of the local cattle in Indonesia that have the various phenotype, especially in growth. The objective of this study was to identify SNP of Somatostatin gene on Madura cattle at Village Breeding Centre (VBC), Madura Island. A total of 66 Madura cattle was used in this study and 34 Ongole Grade cattle as a population reference. Blood samples were collected at VBC Waru, Pamekasan Regency. A potential SNP in exon 1 was analyzed using Polymerase Chain Reaction-Restricted Fragment Length Polymorphism (PCR-RFLP). A restriction enzyme of BsrI was used to identify the SNP. The results showed that a 464 bp of DNA fragment was successfully amplified and an SNP c.20G>A was identified in this study. This SNP was categorized as a missense mutation with the change of amino acid from serine (AGT) to asparagine (AAT). An A allele was only found on Madura cattle. The frequency of A allele was 1.00 in this population and was defined as monomorphic or monoallelic. This result probably indicated that Madura cattle at VBC Waru were selected in this locus.

1. Introduction

Madura cattle is one of which Indonesia domestic cattle. Madura cattle big population distribution on Madura island, East Java. In 2018, the population of Madura cattle is 1,004,226 heads in Madura island. Base on Animal Husbandry Departement No: 188/173/432.131/2015 deciding four sub-districts in Pamekasan as Village Breeding Center of Madura Cattle. One of which sub-districts Waru is Village Breeding Center for Madura cattle. Selection to get the best bull and heifer base on quantitative and qualitative phenotype and molecular selection. Selection bull and heifer of Madura cattle with quantitative and qualitative phenotype is based on Indonesian National Standard of Madura cattle. Molecular selection is to evaluate the profile of nucleotide sequences from genes in DNA that affect cattle productivity, which is the somatostatin gene.

Somatostatin is an inhibit hormone induced by low pH [1]. Somatostatin, a petide hormone, is a product of endocrine cells and the central nervous system. Ampofo et al. [2] stated that somatostatin is originated from prosomatostatin and have two forms in mammals; shorter form (somatostatin-14) and longer form (somatostatin-28). The release of somatostatin is inhibited by the vagus nerve [3]. Somatostatin regulates the endocrine system and influences nerve transmission and cell proliferation by interaction with the somatostatin receptors combined with G protein and inhibits the release of many secondary hormones.

Based on the description above it is important to study molecularly of the role of the somatostatin genes and their association to determine their effect on the qualitative and quantitative characters of...
Madura cattle. The purpose of this study was to identify the diversity of growth control genes Somatostatin in Madura cattle.

2. Materials and methods

2.1. Data collection and animal management
A total of 100 cattles were used in this study consisted of 66 Madura cattles and 34 Ongole Grade cattle (Table 1). The blood was used for DNA extraction. Blood of Madura cattle were collected from village breeding center of Waru, Pamekasan and Singsosari National Artificial Insemination Center, Malang. Blood of Ongole Grade cattle were collected from UPT-HMT Tuban and Singsosari National Artificial Insemination Center, Malang. Blood samples were collected from Vena jugularis to tubes containing Ethilenediaminetetraacetic Acid (EDTA). Blood samples were stored in cooler box immediately and transferred to a freezer at -20°C.

Table 1. Animal sample.

| Breed                  | Location                                      | Sex  | N  |
|------------------------|-----------------------------------------------|------|----|
| Madura cattle          | VBC Waru, Pamekasan, Madura                   | Female | 60 |
| Madura cattle          | Singsosari National Insemination Center (SNAIC)| Male | 6  |
| Ongole Grade cattle    | Singsosari National Insemination Center (SNAIC)| Male | 10 |
| Ongole Grade cattle    | UPT-HMT Tuban                                  | Male | 24 |

2.2. DNA isolation and amplification
Genomic DNA was isolated using Genomic DNA Mini Kit for blood and cultered cell (Geneaid Biotech Ltd., China). The quality of DNA was checked using 1.5% agarose gel electrophoresis. The concentration and purity of DNA were checked using NanoDrop ND-1000 Spectrophotometer (Thermo Scientific™, Massachusetts, USA) respectively.

DNA sequence of SST gene exon 1 was amplified using PCR technique. Forward and reverse primers used in this study were 5’-CTGCTTTAGGAGGCAAGGAC-3’ and 5’-CCTGAAGTCGTGACC TACTGC-3’ respectively (accession number FJ609300). The primers were designed to amplify a 464 bp of DNA fragment. A total of 1 µL DNA template was used with 50 ng/µL concentration. Pre-mixed solution consisted of 0.3 µL of each primer (10 pmol/µL), 7.5 µL Go Taq Green Master Mix (Promega, USA), and 6.9 µL Nucleus Free Water (NFW). The DNA amplification conducted using Bio-Rad T100™ Thermal Cycler (Bio-Rad, USA). The machine conditions followed Furqon et al (2017) using a pre-denaturation temperature at 94°C for 5 min, 35 cycles of 94°C for 10 s (denaturation), 60°C for 20 s (annealing), 72°C for 30 s (extension), and a final extension at 72°C for 5 min [4].

2.3. Genotyping
Genotyping was determined by results of PCR-RFLP. A total of 5 µL PCR product was mixed with 0.4 µL BsrI enzyme, 0.6 µL buffer and 1 µL NFW. The mixing solutions were incubated at 37°C for 4–16 hr. The incubated products were electrophoresed at 100V on 1.5% agarose gel for 35 min. Furthermore, the agarose gel was stained using Diamond™ Nucleic Acid Dye (Promega, USA). Genotypes were determined through the visualization under blue light of Glite 965 GW imaging system (Pacific Image Electronics Co., Ltd.).

2.4. Data analysis
The genotype and allele frequency were calculated using the following formula:
Genotype frequency:

\[ x_{ii} = \frac{n_{ii}}{N} \]

Allele frequency:
\[ x_i = \frac{2n_{ii} + \sum n_{ij}}{2N} \]

Where:
- \( \chi_{ii} \) = frequency of ii genotype
- \( \chi_i \) = frequency of i allele
- \( n_{ii} \) = number of individuals with ii genotype
- \( n_{ij} \) = number of individuals with ij genotype
- \( N \) = number of samples

3. Result and discussion

DNA genome was successfully isolated from blood samples. The DNA concentrations of all samples were adjusted to 50 ng/µL for amplification process. According to the ratio of 260/280 nm absorbance, DNA purity was around 1.7–1.8 in this research. DNA purity was assessed by the ratio of absorbance at 260 and 280 nm. The pure DNA is generally accepted if the ratio of absorbance 260/280 nm is 1.8 [5]. The DNA purity can be secondary measured by the ratio of absorbance at 260/230 nm [6,7]. The pure DNA have range between 2.0 and 2.2 ratio of absorbance at 260/230 nm [8].

In this research, A pair of primers successfully amplified DNA fragment of SST gene exon 1. These primers have 50% GC content, no hairpin formation and no primer dimer. Primer dimers was caused by primer-primer binding and subsequent elongation that resulted off-target amplification artefacts. The primer dimer can be predicted using ROC analysis [9]. The annealing temperature was 60°C. This temperature was widely used on previous study for DNA amplification on cattle and chicken [4,10]. A total of 464 bp DNA fragment were successfully amplified using PCR technique (figure 1). The PCR is a technique to amplify a specific DNA sequence. This technique consisted of three steps; denaturation, annealing, and elongation. Now, PCR is widely spread and innumerable used in genetics, forensic, bacteriology, virology and many other studies [11].

In this study, genotyping was conducted using PCR-RFLP technique. This technique used a restriction enzyme. This technique has advantages including high simplicity, specificity, sensitivity and commonly used for genotyping and species identification [12]. The BsrI enzyme was used to identified SNP c.20G>A. This enzyme have restriction site ACTGGN. There was a mutation point from guanine (G) to adenine (A). This mutation was well known as transition; purine-purine. The change of nitrogen base from G to A changed amino acid from serine (AGT) to asparagine (AAT).
Figure 2. The visualization of restricted product by BsrI on SST gene exon 1 in 2% agarose gel. M: marker; 1-11: samples.

In this research, only AA genotype was found. The AA genotype was shown by two bands (Figure 2). The frequency of A allele was 1.00 in all cattle (Table 2). Previous study reported that there were 2 genotypes (AA and AG) of SST gene on Chinese cattle [13]. All cattle population were defined as monomorphic or monoallelic. It probably indicated that cattle were selected by farmers. In this study, the population of Madura cattle in VBC Waru was categorized as Sonok cattle. Sonok cattle is the selected cattle for a contest in Madura. Another breed cattle in SNAIC and UPT-HMT Tuban were intensively bred as superior breed where selection program was conducted to support their goals.

Table 2. Frequency of allele and genotype on SST|BsrI gene exon 1.

| Breed                                      | n  | Genotype frequency | Allele frequency |
|--------------------------------------------|----|--------------------|------------------|
| Madura cattle in VBC Waru                  | 60 | 1.00 0.00 0.00     | 1.00 0.00        |
| Madura cattle in SNAIC                     | 6  | 1.00 0.00 0.00     | 1.00 0.00        |
| Ongole Grade cattle in SNAIC               | 10 | 1.00 0.00 0.00     | 1.00 0.00        |
| Ongole Grade cattle in UPT-HMT Tuban       | 24 | 1.00 0.00 0.00     | 1.00 0.00        |

4. Conclusion
The SNP c.20G>A of SST gene exon 1 was successfully identified using PCR-RFLP technique as the mutation of transition. Only AA genotype and A allele were found in locus of SST|BsrI on all cattle population. All populations were monomorphic.

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References
[1] Costoff A 2008 Sect. 5, Ch. 4: Structure, Synthesis, and Secretion of Somatostatin. Endocrinology: The Endocrine Pancreas (Medical College of Georgia) p 16
[2] Ampufo E, Nalbach L, Menger M D and Laschke M W 2020 Regulatory mechanism of somatostatin expression Int. J. Mol. Sci. 21 4170
[3] Holst J J, Skak-Nielsen T, Orskov C and Seier-Poulsen S 1992 Vagal control of the release of somatostatin, vasoactive intestinal polypeptide, gastrin-releasing peptide, and HCl from porcine non-antral stomach Scand. J. Gastroenterol. 27 677–85
[4] Furqon A, Gunawan A, Ulupi N, Suryati T and Sumantri C 2017 Expression and association of SCD gene polymorphism and fatty acid composition in chicken cross Trop. Anim. Sci. J. 40
[5] Hassan R, Husin A, Sulong S, Yusoff S, Johan M F, Yahaya B H, Ang C Y, Ghazali S and Cheong S K 2015 Guidelines for nucleic acid detection and analysis in hematological disorders *Malaysian J. Pathol.* **37** 165–73

[6] Usman T, Yu Y, Liu C, Fan Z and Wang Y 2014 Comparison of methods for high quantity and quality genomic DNA extraction from raw cow milk *Genet. Mol. Res.* **13** 3319–28

[7] Aleksic J M, D Stojanovic, B Banovic and R Jancic 2012 *Biochem. Genet.* **50** 881–92

[8] Liu P, Avramova L V and Park C 2009 Revisiting absorbance at 230 nm as a protein unfolding probe *Anal. Biochem.* **398** 165–70

[9] Andrew D, Jhonston, Lu J, Ru K, Korbie D and Trau M 2019 Primer ROC: accurate condition-independent dimer prediction using ROC analysis *Sci. Rep.* **9** 1–14

[10] Jakaria, Ulum M F, Lestari D, Akwila S, Sihite D E W T, Priyanto R, Muladno and Sumantri C 2020 Investigating new SNPs of CAST, CAPN, and SCD genes in 5‘UTR of Bali cattle *Biodiversitas* **2** 2971–6

[11] Verma K, Dalal J and Sharma S 2014 Scientific concepts of polymerase chain reaction (PCR) *Int. J. Pharm. Sci. Res.* **5** 3086–95

[12] Guan F, Jin Y, Zhao J, Xu A and Luo Y 2018 A PCR method that can be further developed into PCR-RFLP assay for eight species identification *J. Anal. Methods. Chem.* **18** 1–6

[13] Gao L, Zan L S, Wang H B, Hao R J and Zhong X 2011 Polymorphism of somatostatin gene and its association with growth traits in Chinese cattle *Genet. Mol. Res.* **10** 703–11