A Variant of Insulin like Growth Factor 1 (IGF1) Gene Affects Body Conformation Traits in Madura Bulls

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Abstract. This study aimed to identify the DNA variants located in one of IGF1 gene region and to investigate the association between those variants with body conformation traits in Madura bulls. There were 58 Madura bulls used for this study and chest width; chest girth; body length; body height; hip width and hip height were measured. The DNA sample obtained from the bulls were amplified and sequenced. The result indicated that an identified variant located in the 5' upstream of IGF1 gene had effect on body height and hip height of Madura bulls. Since body conformation is one of phenotypic traits that generally used by the farmers to select for their cattle, this result suggested that this variant might be a candidate marker that could assist the selection based on molecular information for body conformation traits. However, another study with larger population needs to be conducted first.

Keywords: IGF1 · Madura cattle · polymorphisms · body height · hip height

1 Introduction

Madura cattle is one of Indonesian indigenous cattle. The coat of Madura cattle is brick-red or brownish red with a white pattern on the back, belly, and rump. They have little horns that bend upward [1]. Madura cattle serve a number of purposes, including cultural functions (such as participation in traditional ceremonies), prestige/social standing (as karapan and sonok cattle), agricultural support (as draught animals and fertilizer producers), and income/business (beef cattle). Sonok and karapan cattle have not only prestige value, but may also be used as a source of revenue due to their high selling values. Body conformation is one of important traits that commonly used by the farmers to select for their cattle. The farmers select both dam and sire with good body conformation in order to obtain calves that will also have good conformation.

The most prevalent strategy for improving cattle genetically is selection based on observable phenotypes (external performance). Because phenotype is the expression of both genes and environment, molecular information can help enhance the precision of

© The Author(s) 2023
E. Widodo et al. (Eds.): ICESAI 2022, ABSR 28, pp. 356–360, 2023.
https://doi.org/10.2991/978-94-6463-116-6_45

selection and genetic improvement, and so favorably influence phenotypic characteristics. In other words, for maximum precision, selection should be based on all available information, including phenotypic and genetic data. Marker assisted selection is the most popular strategy for improving qualities using molecular information [2].

Gene variations can be investigated to conduct selection based on molecular information. Gene variations are caused by a permanent alteration in a gene, causing the sequence to deviate from the "original" sequence. Point mutations are alterations in a single base pair, whereas in/dels are nucleotide insertions and deletions. Such changes can result in three types of mutations that can influence phenotypes [3]:

a. Missense mutations, which result in amino acid alterations.
b. Nonsense mutations that result in premature translation termination.
c. Frameshift mutations, which result in the insertion or deletion of a DNA nucleotide

A gene that is suggested to have role in body conformation traits is called IGF1 (Insulin like growth factor-1).

This study aimed to provide preliminary study in order to identify DNA variant(s) located in IGF1 gene and to investigate the association of the variant(s) with body conformation traits in Madura bulls.

2 Materials and Methods

2.1 Materials

There were 58 Madura bulls used for this study and chest width; chest girth; body length; body height; hip width and hip height were measured.

2.2 Designing Primers

Primers were designed with primer3 plus, which can be found at primer3plus.com. The gene sequences to be amplified were obtained from the website www.ensembl.org. Melting temperatures (TM) for forward and reverse primers were comparable. The optimal primer size is 16–20 bp, and there was no excessive GC content or hairpin loops in the primers.

2.3 PCR Condition Optimization

PCR conditions were optimised to ensure optimal temperature annealing (primary attachment temperature) and the production of a single product of expected size.The PCR was performed on a BIORAD T100 Thermal Cycler PCR equipment. The amount of DNA utilized is 1 μl. The reaction volume was 30 μl, which included 15 μl of go Taq green master mix, 0.3 μl of forward and reverse primers, and 14.4 μl of nuclease free water (NFW) and sample DNA.

2.4 PCR Product Sequencing Reaction

A sequencer machine (ABI Prims 3100-Avant Genetic Analyzer) was utilized to sequence gene fragments at 1st Base sequencing company in Selangor, Malaysia.

2.5 Polymorphism Identification

When the sequencing results obtained, the process of identifying gene polymorphisms began. BioEdit was used to examine the sequencing results. Following the interpretation of the sequencing results with BioEdit, the results will be validated using Molecular Evolutionary Genetic Analysis 5 (MEGA 5) to detect the polymorphisms.

2.6 Data Analysis

The effects of the SNPs on phenotypic variables were investigated using an unbalanced design analysis of variance with fixed effects (Genstat). For each SNP in a given gene, the fixed effects were the age of the cattle, the cohort (place), and the genotype of the cattle. The model was:

$$\mathbf{Yijk} = \boldsymbol{\mu} + \boldsymbol{\alpha i} + \boldsymbol{\beta j} + \boldsymbol{\gamma k} + \boldsymbol{\varepsilon ij}$$

Where: Yij is the response variable (phenotypic traits); μ is the overall mean; αi is the effect of ith age; βj is the effect of jth cohort; γk is SNP/polymorphisms genotype and ϵij is the residual effect.

The variance of each detected "significant" SNP was calculated using a "linear mixed model" (Genstat). The SNP genotypes were included as random effects, whereas age was fitted as a fixed effect.

3 Results and Discussion

On the PCR product obtained from the designed primers, IGF1 had only one SNP which was discovered at 5' upstream. The IGF1 SNP was found to have significant relationships with body height ($P < 0.05$) and hip height ($P < 0.01$). The IGF1 SNP accounted for 31.70% of the phenotypic variance in hip height and 14.01% in body height. Bulls with the GG and GT genotypes exhibited bigger body and hip height than bulls with the TT genotype (Fig. 1).

Insulin-like growth factor 1 (IGF1) is a major regulator of numerous physiological and metabolic processes in vertebrates, as well as a mediator of many biological effects. Many studies have found that IGF1 gene mutations affect growth traits (such as birth weight and weaning weight) [4–6]. IGF1 is also involved in the development and growth of skeletal muscle and bone, which is fundamental for body conformation.

IGF1 regulates the synthesis of skeletal muscle protein via complicated PI3K/Akt/mTOR and PI3K/Akt/GSK3 pathways [7]. The pathways are activated by IGF1 binding to its receptor (IGF-1R), which phosphorylates phosphoinositide 3-kinase (PI3K) and then Akt. The PI3K/Akt signaling pathway is essential in myotube hypertrophy [8]. IGF1 is also involved in the formation and development of skeletal bones. IGF1

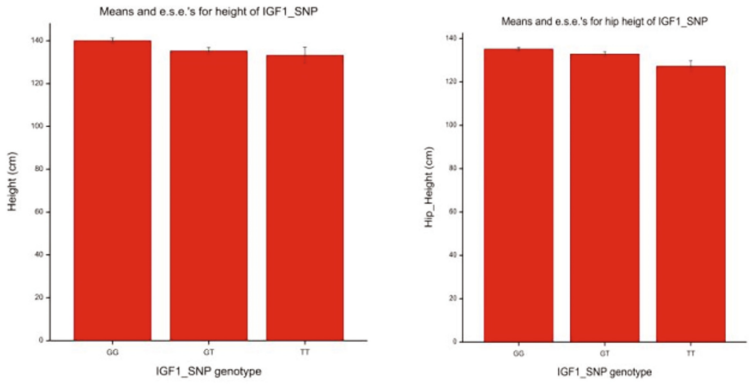

Fig. 1. Effect of IGF1 SNP on body height and hip height

is a key anabolic signal for both embryonic and postnatal skeletal development [9], and IGF1 signaling is required for chondrocyte differentiation, osteoblast maturation and function, and proper bone turnover.

The IGF1 SNP was discovered in the promoter region of the gene in this investigation. SNPs in the promoter region have no effect on the protein's amino acids.They may, however, inhibit gene transcription or modify the binding of transcription factors. Changes in gene expression levels are directly related to protein levels, and so such SNPs may impact phenotypic characteristics.

4 Conclusions

Single Nucleotide Polymorphims identified at the 5' promoter region might be a candidate gene marker for body conformation in Madura cattle. However, it is suggested to conduct the study using larger population.

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