Nucleotides Variability of Branched Chain Ketoacid Dehydrogenase E1-α Polypeptide (BCKDHA) Gene on Madura Cattle

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Abstract. Madura cattle is one of Indonesian native cattle which has physical strength and is highly adaptable under dry climates. Branched Chain α-Keto Dehydrogenase (BCKD) complex bound to mitochondrial inner membrane and catalyzes branched-chain amino acid catabolism into α-keto. Subunit E1-α of BCKD complex is encoded by the Branched Chain Ketoacid Dehydrogenase E1-α Polypeptide (BCKDHA) gene. This research was conducted to analyze the variability of the 3' end promoter and exon 1 of the BCKDHA gene. Variant analysis was done on 8 samples of Madura cattle, 1 sample of filial Ongole cattle, and 1 sample of Bali cattle. Missense substitution was found in one sample of madura cattle, i.e. G95A that changed polypeptide, i.e. arginine to glutamine (Arg28Glu); other substitution was found in all samples, i.e. T125C and changed polypeptide; i.e. phenylalanine to serine (Phe38Ser). Several silent mutations on promoter and exon 1 were observed in all samples. Apart from the point mutation above, there was a six-base deletion (ATGGCG) in the exon 1 segment that was identified in 4 samples of Madura cattle that shortened two amino acids of the signal peptide of BCKDHA.

1 Introduction

Madura cattle is a hybrid between banteng (*Bos javanicus*) and humped zebu (*Bos indicus*), while Bali cattle is a domestic form of banteng [1]. Madura cattle is one of Indonesian native cattle with prominent physical strength and excellent ability to adapt under dry climates [2]. The selection of Madura cattle by Madurese culture categorizes Madura cattle into three categories, i.e. dancing cattle (sonok), bull racing cattle (karapan), and the remaining common cattle. Sonok cattle require beauty and tameness of female cattle, while karapan cattle require the agility and speed of male cattle [3]. Madura cattle that are out of the qualifications are known as common cattle.

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Branched Chain α-Keto Dehydrogenase (BCKD) complex is a mitochondrial multienzyme complex that catalyzes the oxidative decarboxylation of α-keto. Branched-chain amino acids (BCAAs), such as leucine, isoleucine, and valine, need to be changed into α-keto to enter the Krebs cycle. These BCAAs are unable to be synthesized by animals and they have vital roles in protein synthesis and cell signalling. A metabolic condition known as Maple Syrup Urine Disease (MSUD) arises because of excessive amount of BCAAs in cells caused by BCKD deficiency [4]. BCKD has 3 catalytic components: E1, E2, and E3. The E1 subunit consists of two different subunits with molecular weights of approximately 46000 and 35000 [5]. The E1-α subunit is encoded by the Branched Chain Ketoacid Dehydrogenase E1-α Polypeptide (BCKDHA) gene. The E1-α subunit is located on chromosome 18, while E1-β subunit is on chromosome 9. BCKDHA gene has 9 exons and 8 introns with the gene length of approximately 20 kb [6]. Apart from catalytic components, the E1-α subunit has signal peptide site. The signal peptide is a peptide with 15 to 30 amino acids in the N terminal of most proteins working in organelle [7].

A common cause of BCKD deficiency is mutation in the BCKDHA gene. A single base mutation in exon 2 of the BCKDHA gene in Polled Hereford calves was caused by 248C/T substitution at codon 6 resulting in premature stop codon [8]. Furthermore, mutation of 1380C/T in BCKDHA gene was also found in Polled Shorthorn calves [9]. Tranversion mutation of G36T in exon 4 of BCKDHA gene was found in filial Ongole cattle and caused amino acid change from glutamic acid to stop codon. In Sonok cattle, simultaneous indel occurred at base positions 34 and 68 in intron 7 [10]. Mutation occurs other than exon 2 and 4 as well as intron 7 has not yet been reported. Madura common cattle and bull racing cattle (karapan) are predicted to have nucleotide variants in exon 1 of BCKDHA gene. Therefore, this research aimed to analyze variability of 3'end promoter and exon 1 of BCKDHA gene on Madura cattle.

2 Materials and methods

2.1 Samples

The samples used were 8 samples consisting of 6 blood samples of Madura cattle, 1 sample of filial Ongole cattle, and 1 sample of Bali cattle.

2.2 BCKDHA gene amplification using Polymerase Chain Reaction (PCR)

Nucleotides amplification within exon 1 of BCKDHA gene was performed on each sample using forward primer AF501 (5'TGGCATTTGAGTCAGGCTTA) and reverse primer AF502 (5'ATTCCCTTGCATGTCCTGTC) with amplicon length of 363 bp. PCR reaction used 1x *GoTaq® Green Master Mix* with buffer containing $MgCl_2$ and dNTPs, *nuclease-free water*, 10-100 ng DNA sample, and 25 pM for each primer AF501 and AF502. PCR was performed using ESCO Swift Maxi Thermal Cycler consisted of predenaturation at 95ºC for 2 minutes, then continued with 30 cycles including denaturation at 95ºC for 45 seconds, annealing at 60ºC for 1 minute, and elongation at 72ºC for 1 minute. After completing 30 cycles, it was continued with final elongation at 72ºC for 5 minutes. Amplified products were separated on 6% polyacrylamide gel electrophoresis (PAGE) (Bio-Rad) on 200 V for 40 minutes. DNA bands were visualized using silver staining [11].

2.3 Nucleotide sequencing of BCKDHA gene

Nucleotides sequencing within exon 1 of BCKDHA gene was determined on single and thick-banded amplicons as detected in polyacrylamide gel. Forward primer AF501 (5'-TCCGAGGGATGGTCAGCCAAG-3') and reverse primer AF502 (5'-CTGTCCTGTACGTTCCCTTA-3') were used in direct nucleotides sequencing on each sample with big dye terminator sequencing method.

2.4 DNA sequence analysis

Nucleotide sequences resulted from sequencing both from forward and reverse were edited using BioEdit version 7.1.11 [12] and compiled into one nucleotide sequence for each sample. The nucleotide sequence of each sample was aligned using MEGA (Molecular Evolutionary Genetics Analysis) version 5 [13].

3 Results

3.1 Nucleotides Substitution and Variant Analysis within Exon 1 of BCKDHA Gene

Exon and intron 1 of BCKDHA gene in Madura cattle, filial Ongole cattle, and Bali cattle were successfully amplified using two primers, AF501 and AF502, with amplicon length of approximately 307 bp. Forward primer AF501 started to attach on 5'UTR region, while reverse primer attached on intron 1 BCKDHA gene.

Nucleotides variability was shown by overlapping peaks on chromatograms resulted from sequencing. Exon 1 of BCKDHA gene had several base variants in various cattle. The further analysis was compiling nucleotides sequences of BCKDHA gene from both forward and reverse. The results of sequenced BCKDHA gene with the length of 307 bp were 82 nucleotides on 5' end untranslated region (5'UTR) region, 113 nucleotides on exon 1, and 112 nucleotides on intron 1. The difference between nucleotides number of amplified amplicon and analyzed nucleotides sequence as many as 56 nucleotides was because of sequence shortening due to nucleotides sequence on intron 1 was unable to be read after editing. Alignment of exon 1 of Madura cattle, filial Ongole cattle, and Bali cattle towards *Bos taurus* (NW1493616) showed that *B. taurus* and Madura cattle (4, 5, and 6) had C on nucleotide 42, while other Madura cattle (1, 2, and 3) and filial Ongole cattle had G. Filial Ongole cattle had C or G on nucleotide 42. Nucleotide variant of G or T was found on nucleotide 78 in Madura cattle 2 and 3, but *B. taurus* and other cattle had G base. Madura cattle 3 had nucleotide variant of G or A on nucleotide 95, while *B. taurus* and other cattle had G base. *B. taurus* had T base on nucleotide 125, while all Madura cattle, filial Ongole cattle, Bali cattle had C base. Nucleotide variant of T or A was found on nucleotide 126 in Madura cattle 3 (Table 1). There was no nucleotide variant found on intron 1 of BCKDHA gene. Deletion was also found on exon 1 of BCKDHA gene in Madura cattle (1, 2, 3, and 4) that were common cattle. Six nucleotides deletion, ATGGCG, on exon 1 of BCKDHA gene was found in Madura cattle (1, 2, 3, and 4) that shortened two amino acids of signal peptide of BCKDHA.

Table 1. Alignment result of exon 1 of BCKDHA gene from Madura cattle, filial Ongole cattle and Bali cattle samples towards nucleotides of BCKDHA gene of *B. taurus* (NW1493616)

Breed	Nucleotide position number in exon 1										
	13	**14**	**15**	**16**	**17**	**18**	**42**	**78**	**95**	**125**	**126**
B. taurus (NW1493616)	A	T	G	G	C	G	C	G	G	T	T
Filial Ongole cattle	A	T	G	G	C	G	**C/G**	G	G	**C**	T
Bali cattle	A	T	G	G	C	G	**G**	G	G	**C**	T
Madura cattle 1 (common)	-	-	-	-	-	-	**G**	G	G	**C**	T
Madura cattle 2 (common)	-	-	-	-	-	-	**G**	**G/T**	G	**C**	T
Madura cattle 3 (common)	-	-	-	-	-	-	**G**	**G/T**	**G/A**	**C**	**T/A**
Madura cattle 4 (common)	-	-	-	-	-	-	C	G	G	**C**	T
Madura cattle 5 (common)	A	T	G	G	C	G	C	G	G	**C**	T
Madura cattle 6 (bull racing)	A	T	G	G	C	G	C	G	G	**C**	T

Nucleotide position numbers are counted starting from 5'UTR and identical with nucleotide 2012 of *B.taurus* (NW1493616). Bold letters show nucleotide substitution or variant.

3.2 Amino acids translation

Amino acids translation was performed after completing nucleotides alignment of exon and intron 1 of BCKDHA gene (Fig. 1). Amino acid repeats were observed in exon 1 of BCKDHA gene in *B. taurus* and all samples. Methionine, alanine, valine, and leucine were repeated amino acids in exon 1. Nucleotides variant of G95A on codon 28 in madura cattle 3 caused glutamine to be formed instead of arginine based on amino acids translation of BCKDHA gene on *B. taurus* (NW1493616). Nucleotide substitution of T125A on codon 38 in all Madura cattle, filial Ongole cattle, and Bali cattle caused serine to be formed instead of phenylalanine. Nucleotide substitution of C42G on codon 10 resulted in no changes in the dictated polypeptide. The C42G nucleotide substitution was observed in Madura cattle (1, 2, and 3), filial Ongole cattle, and Bali cattle. The nucleotide variant of G78T on codon 22 in Madura cattle 2 and 3 also caused no changes in a dictated polypeptide (Table 2).

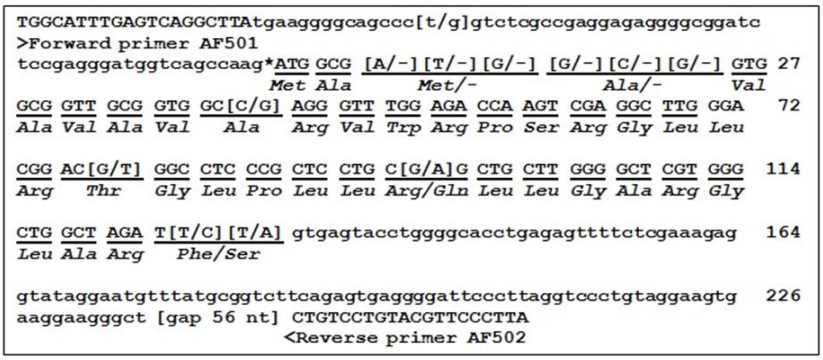

Fig. 1. Nucleotides sequence in exon 1 of BCKDHA gene started from primer AF501 and ended by primer AF502 alongside with translation result. Underlined codon=exon 1. (*): codon 1. Nucleotide position numbers are determined from 5'UTR and identical with nucleotide position of 2012 in *B.taurus* (NW1493616).

Table 2. Summary of amino acids translation in exon 1 of BCKDHA gene in madura cattle

Breed	Codon position number in exon 1							
	1	**2**	**3**	**4**	**10**	**22**	**28**	**38**
B. taurus (NW1493616)	M	A	M	A	A	T	R	F
Filial ongole cattle	M	A	M	A	A	T	R	**S**
Bali cattle	M	A	M	A	A	T	R	**S**
Madura cattle 1 (common)	M	A	**-**	**-**	A	T	R	**S**
Madura cattle 2 (common)	M	A	**-**	**-**	A	T	R	**S**
Madura cattle 3 (common)	M	A	**-**	**-**	A	T	**Q**	**S**
Madura cattle 4 (common)	M	A	**-**	**-**	A	T	R	F
Madura cattle 5 (common)	M	A	M	A	A	T	R	**S**
Madura cattle 6 (bull racing)	M	A	M	A	A	T	R	**S**

Nucleotide position numbers are counted from the start codon. Bold letters show mutation in exon 1. M=Methionine (start codon); A=Alanine; T=Threonine; R=Arginine; Q=Glutamine; F=Phenylalanin; S=Serine; -=Absent amino acid due to nucleotide deletion.

4 Discussion

Sequencing of exon 1 of BCKDHA gene showed G95A only in madura cattle 3, while T125C was found on all analyzed cattle samples. The nucleotide variant of G95A in madura cattle 3 caused amino acid to change from arginine (CGG) to glutamine (CAG). The change from arginine to glutamine was caused by difference on the second base. This mutation was called transition mutation, which meant changing the purine base to another. Nucleotide substitution of T125C caused amino acid to change from phenylalanine (TTT) to serine (TCT). This mutation was also called transition mutation. Mutations found on G95A and T125C were also called missense mutation, caused changes in amino acid from arginine to glutamine and phenylalanine to serine, respectively. Nucleotide substitution of C42G was found in madura cattle (1, 2, and 3), which were categorized into common cattle and Bali cattle. A nucleotide variant of C42G was found in filial Ongole cattle. A nucleotide variant of G78T was found in Madura cattle 2 and 3. The nucleotide substitution and variant are called silent mutation because there was no change in the amino acid sequence [14]. A study on intron 7, exon 8, and intron 8 of the BCKDHA gene reported no specific mutation found in Madura cattle designation. This suggested that the selection pressure on the BCKDHA gene was not differentiated in Madura cattle [10]. It was also reported that nucleotide substitution and heterozygous allele were found in exon 2 and 3 of BCKDHA gene and its flanking region of Madura cattle [15] which is similar to this present study.

Genetic variations present in the form of single nucleotide polymorphisms, insertion or deletion of nucleotide or genes, gene or chromosomal rearrangements, and gene duplications. These variations carry different effects, such as amino acids substitution for another, shift in reading frame, premature termination of translation, and deletion of whole exon or gene in diseased individuals because of genetic variations [16]. Deletion of 6 nucleotides, ATGGCG, in exon 1 was predicted to cause methionine (ATG) and alanine (GCG) absence in dictated polypeptide. It also meant that the deletion shortened 2 amino acids in signal peptide of BCKDHA gene. Signal peptide is peptide with 15 to 30 amino acids in N terminal of most proteins working in organelle [7]. The deletion was found in Madura cattle (1, 2, 3, and 4) which were categorized into common cattle. The deletion never occurs in exon 1 of BCKDHA gene in *B. mutus* (XM5890618), *Bison bison* (XM10849339), *Capra hircus*

(XM5692437), *Ovis aries* (NM1126344), dan *Bubalus bubalis* (XM6052057) based on the result of BLASTn in GenBank.

Amino acid repeats were observed in exon 1 of BCKDHA gene in *B. taurus* and all madura cattle samples. Methionine, alanine, valine, and leucine were repeated amino acids in exon 1. Nucleotide repeats in the RNA coding region are translated into several repeat types, such as single amino acid repeats (SAARs). Besides, the result of amino acid repeats affects function and structure of signal peptide. The repeat pattern is predicted as a mechanism for interacting with target molecules [17]. One of the most significant evolutionary strategies used by species to adapt to their surroundings is internal duplication in their genomes [18]. Amino acid homorepeats are discrete components that are frequently found in proteins that are under strict regulation. Their presence promotes adaptation and fitness by enabling quick exploration of a population's genotype-phenotype landscape [19].

5 Conclusion

Nucleotides sequence of exon 1 of BCKDHA gene in Madura cattle, filial Ongole cattle, and Bali cattle showed nucleotide variant of G95A in one sample of Madura cattle and caused amino acid change from arginine to glutamine. Nucleotide substitution of T125C in all samples resulted in amino acid change from phenylalanine to serine. Nucleotide substitution of C42G in Madura common cattle (1, 2, and 3) and Bali cattle caused no change in dictated polypeptide, as well as the nucleotide variant of G78T in two samples of Madura cattle. Six nucleotides deletion, ATGGCG, in exon 1 was found in Madura common cattle with a percentage of 80% from all Madura cattle samples which were categorized into common cattle. The deletion was absent in other several members of Bovidae.

Acknowledgements

The study was funded by Ministry of Research, Technology, and Higher Education within World Class Research (WCR) programme (Grant No. 1538/IT#L1/PN/2019) to Dyah Perwitasari.

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