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Characteristic of Bola DRB3.2 Gene in Taro Cattle

[1]I. Gede Soma, [2]Nyoman Mantik Astawa, [3]I. Dewa Made Sukrama and [4]I. Nengah Wandia

[1]Department of Physiology, Faculty of Veterinary Medicine, Udayana University, Denpasar Bali, Indonesia
[2]Pathobiology Laboratory, Faculty of Veterinary Medicine, Udayana University, Denpasar Bali, Indonesia
[3]Department of Microbiology, Medical Faculty, Udayana University, Denpasar Bali, Indonesia
[4]Department of Anatomy, Faculty of Veterinary Medicine, Udayana University, Denpasar Bali, Indonesia

Abstract: BoLa DRB3.2 gene plays an important role in immune response against an infectious disease in catlle. BoLA DRB3.2 gene encodes the β1unit of MHC molecule as a part of the receptor binding site that initiates the immune response. This research aimed to explore the characteristic of BoLA DRB3.2 gene in Taro cattle. Fifteen blood samples of Taro cattle were taken aseptically from jugular vein using vacutainer with 5% EDTA. The DNA was extracted and isolated from the whole blood and the segment of the BoLA DRB3.2 gene was amplified using primer F: 5'ATCCTCTCTCTGCAGCACATTTCC-3′ and R: 5′-TTTAAATTCGCGCTCACCTCGCCGCT-3′. The PCR products were then sequenced and analyzed descriptively using MEGA 5. The results showed that the BoLA DRB3.2 gene was 302 bp of length containing 17 bp of intron 5', 267 bp of exon 2 and 18 bp of intron 3'. Nucleotide translation started from 18 to 284 and produce 89 amino acids. Along the sequences there were 2 sites of single nucleotide polymorphism (SNP) 70A/G and 71G/C which producing 3 haplotypes of BoLA DRB3.2 gene in Taro cattle. In the case of amino acid sequences, there is a substitution at amino acid 24R/A/T that make a few changes of the hydrophobicity. It can be concluded that the polymorphism of BoLA DRB3.2 gene in Taro cattle is low.

Key words: Taro Cattle · Characteristic Bola DRB3.2 Gene

INTRODUCTION

Taro cattle is one of local Indonesia native cattle which is only found at a small isolated conservation forest in Taro village, Gianyar Bali. Local people treat them as a sacred animal, forbidden for meat consumption and not been used in farmer production or plantation. Local people use them in religion ceremony. Phenotypically, Taro cattle is similar to Bali cattle except of white coat covering all over the body. Morphometric study showed that average body measurement of Taro cattle lower than Bali cattle and Achai cattle from Pakistan also Red Chitagong cattle from Bangladesh [1]. Taro cattle could be a different breed from Bali cattle, but need more scientific data to support this hypothesis. Because of isolation in a small conservation forest, the Taro cattle tends to highly inbreed and prone to extinct. Recently, there were only 33 individuals in this population [1]. Taro cattle is one of genetic resources that have to be conserved. Improved raising management have been doing by local government as a part of conservation effort. Recording all of scientific data including genetic data base is importance to support a wise conservation management programs. The genetic characterization is essential prerequisite for a conservation program to be effective and meaningful [2]. However, the genetic study on the Taro cattle is lack and almost none.

Animal survival including cattle is much influenced by the gene in response to disease. MHC is a fundamental part and plays an important role to the immune system in vertebrata [3]. Genes that encode MHC molecules [4-6] are the most polymorphic genes in vertebrates [7]. The variation of the MHC genes influences the susceptibility of animal to a wide variety of infectious diseases [8]. BoLA DRB3.2 is a gene that encode MHC class II molecule of cattle [9] and is considered as a suitable marker for genetic diversity studies [10].

BoLA DRB3 genes are highly polymorphic [2, 7, 11, 12]. The polymorphism of BoLA DRB3.2 genes confines mainly to second exon that responsible for

Corresponding Author: I. Gede Soma, Department of Physiology, Faculty of Veterinary Medicine, Udayana University, Denpasar Bali, Indonesia.

peptide binding site [10, 7]. The synonimous and nonsynonimous substitutions of nucleotides of DRB3 genes occur in bovine. The polymorphism at amino acid levels tends to be clustered around the site of peptide binding region, especially amino acid residues at position 11 and 37 [4]. The present study aimed to characterize the BoLA DRB3.2 gene in Taro cattle.

MATERIALS AND METHODS

A total of 15 blood samples approximately 10 ml each was taken aseptically by vacutainer with EDTA 5% from jugular vein of Taro cattle. DNA was then isolated from the whole blood and extracted using DNA extraction kit (PureLink™ Genomic Mini Kit Invitrogen). The BoLA *DRB3*.2 gene was amplified by PCR using primer (F) 5´-ATCCTCTCTCTGCAGCACATTTCC-3´ and (R):5´-TTTAAATTCGCGCTCACCTCGCCGCT-3´ proposed by Van Eijk *et al.* [13]. The reaction was carried out with final volume about 25 µL. Each reaction contained 1x PCR buffer, 1.5 mM $MgCL_2$, 0.2 mM dNTP, 0.2 µM each primer, 1 unit Taq DNA polymerase, 2 µL DNA template and deionized water. The PCR reactions were done using Applied Biosystems 2720 Thermal Cycler. The PCR contained 3 holdes, that were pre PCR: denaturation at 94°C for 5 minute, PCR30 cycles including the denaturation at 94° C for 1 minute, annealing at 56°C for 55 seconds and elongation at 72° C for 1 minute, post PCR: elongation at 72° C for 5 minute. The PCR products were separated on 1.5% agarose gel electrophoresis migrated at 50 V for 30 minutes and stained with ethidium bromide. The PCR products were then sequenced. The sequence results were analyzed using MEGA [14].

RESULTS AND DISCUSSION

The PCR product of DNA segment of BoLA DRB3.2 gene of Taro cattle is 302 bp, consisted of 17 bp intron 5', 267 pb of exon 2 and 18 bp of intron 3'. There were 3 haplotypes of BoLA DRB3.2 gene in Taro cattle that have been registered to the Genebank with the access number KT722740, KT722741 and KT722742 [15, 17].

Table 1: Nucleotide sequence alignment of the three haplotype of BoLA DRB3.2

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       111 111 111 122 222 222 223 333 333 333 444 444 444 ]
[         123 456 789 012 345 678 901 234 567 890 123 456 789 012 345 678 ]
#KT722741 CAT TTC CTG GAG TAT TCT ACG AGC GAG TGT CAT TTC TTC AAC GGG ACC
#KT722740 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KT722742 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

[         455 555 555 556 666 666 666 777 777 777 788 888 888 889 999 999 ]
[         901 234 567 890 123 456 789 012 345 678 901 234 567 890 123 456 ]
#KT722741 GAG CGG GTG CGG TAC CTG GAC AGA TAC TTC CAT AAT GGA GAA GAG TTC
#KT722740 ... ... ... ... ... ... ... GC. ... ... ... ... ... ... ... ...
#KT722742 ... ... ... ... ... ... ... .C. ... ... ... ... ... ... ... ...

[             111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 ]
[         999 000 000 000 011 111 111 112 222 222 222 333 333 333 344 444 ]
[         789 012 345 678 901 234 567 890 123 456 789 012 345 678 901 234 ]
#KT722741 GTG CGC TTC GAC AGC GAC TGG GGC GAG TAC CGG GCG GTG ACC GAG CTA
#KT722740 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KT722742 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

[         111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 111 ]
[         444 445 555 555 555 666 666 666 677 777 777 778 888 888 888 999 ]
[         567 890 123 456 789 012 345 678 901 234 567 890 123 456 789 012 ]
#KT722741 GGG CGG CGG GTC GCC GAG CAG TTG AAC GGC CAG AAG GAC ACC CTG GAG
#KT722740 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KT722742 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...

[         111 111 122 222 222 222 222 222 222 222 222 222 222 222 222 222 ]
[         999 999 900 000 000 001 111 111 111 222 222 222 233 333 333 334 ]
[         345 678 901 234 567 890 123 456 789 012 345 678 901 234 567 890 ]
#KT722741 CGG GAG CGG GCC TAT GTG GAC ACG TAC TGC AGA CAC AAC TAC GGG GTC
#KT722740 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...
#KT722742 ... ... ... ... ... ... ... ... ... ... ... ... ... ... ... ...


[         222 222 222 222 222 222 222 222 222 ]
[         444 444 444 555 555 555 566 666 666 ]
[         123 456 789 012 345 678 901 234 567 ]
#KT722741 GTT GAG AGT TTC ACT GTG CAG CGG CGA
#KT722740 ... ... ... ... ... ... ... ...| ...
#KT722742 ... ... ... ... ... ... ... ... ...
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Table 2: Amino acid sequence alignment of the three haplotype of BoLA

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[                  1 1111111112 2222222223 3333333334 4444444445 ]
[         1234567890 1234567890 1234567890 1234567890 1234567890 ]
#KT722741 HFLEYSTSEC HFFNGTERVR YLDRYFHNGE EFVRFDSDWG EYRAVTELGR
#KT722740 .......... .......... ...A...... .......... ..........
#KT722742 .......... .......... ...T...... .......... ..........

[         5555555556 6666666667 7777777778 8888888881
[         1234567890 1234567890 1234567890 1234567891]
#KT722741 RVAEQLNGQK DTLERERAYV DTYCRHNYGV VESFTVQRR
#KT722740 .......... .......... .......... .........
#KT722742 .......... .......... .......... .........
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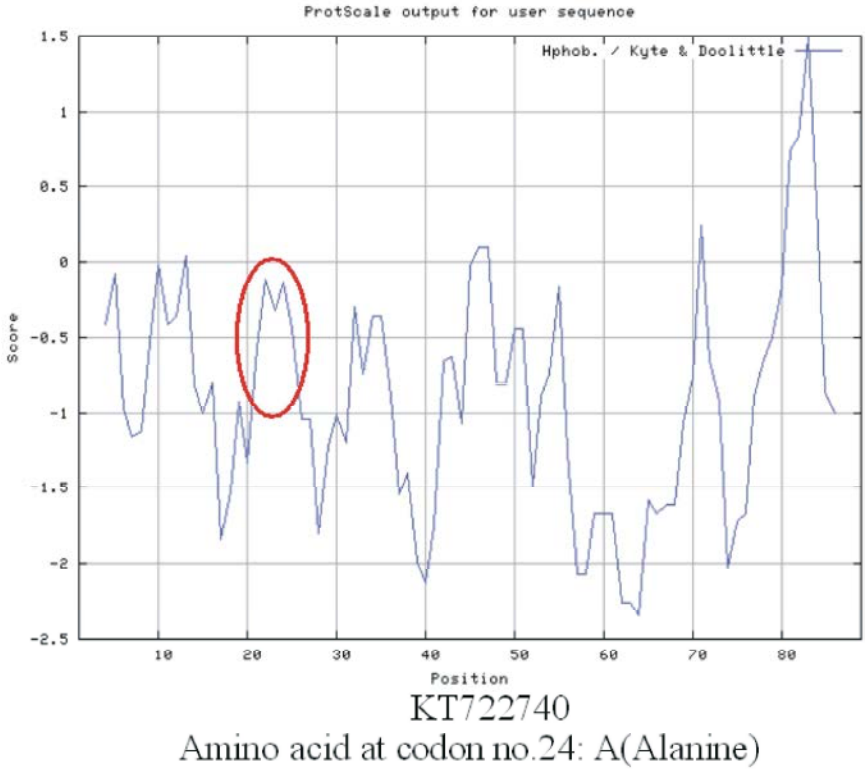
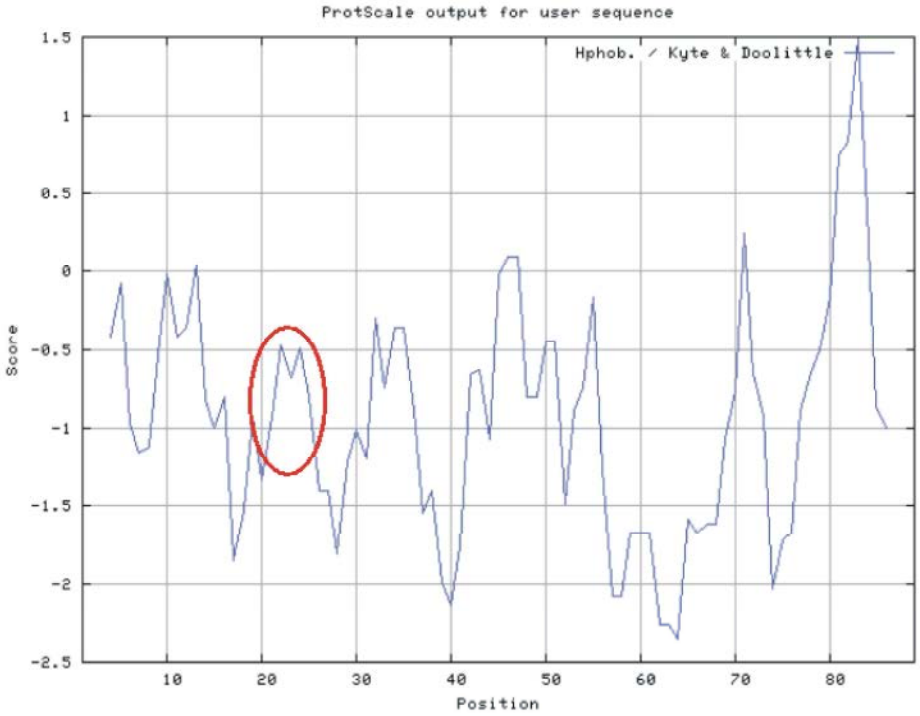
Haplotype KT722741 is the common haplotype of BoLA DRB3.2 allele of Taro cattle with the frequency of 75%. The frequency of KT722740 and KT722742 are 12, 5% each. There were two sites of single nucleotide polymorphisms (SNP) at position 70A/G, 71G/C presented in Table 1. Substitution of nucleotide at position 70 and 71 caused amino acid changes at position 24R/A/T presented in Table 2. Amino acid substitution at position 24 caused hydrophobicity changes presented in Table 3.

DNA fragment of BoLA DRB3.2 gene of Taro cattle was 302 bp containing of 17bp of intron 5', 267 bp of exon 2 and 18 bp of intron 3'. Similar PCR product (302bp) of BoLA DRB3.2 in *Bos taurus* was reported by Sun *et al*. [18]. Aravandakshan and Nainar [19] reported that PCR product was 304 bp in Ongole cattle. Wu *et al*. [20] reported that the PCR product of BoLA DRB3 in Chinese Holstein was 267 bp of exon 2, 14 bp of intron 5' and 3 bp of intron 3'. In contrast, Oprzadek *et al*. [21] found the size of PCR product of BoLA DRB3 gene 284 bp in Polish Holstein Friesian cattle. Chakraborty, *et al.* [22] also reported that the PCR product of BoLA DRB3.2 in Sahiwal cattle was 284 bp composed of 17 bp intron 5' and 267 exon. The size of the PCR product of the BoLA DRB3 gene varies, depends on the primer uses. They are different in intron 5' and intron 3', but not for the second exon.

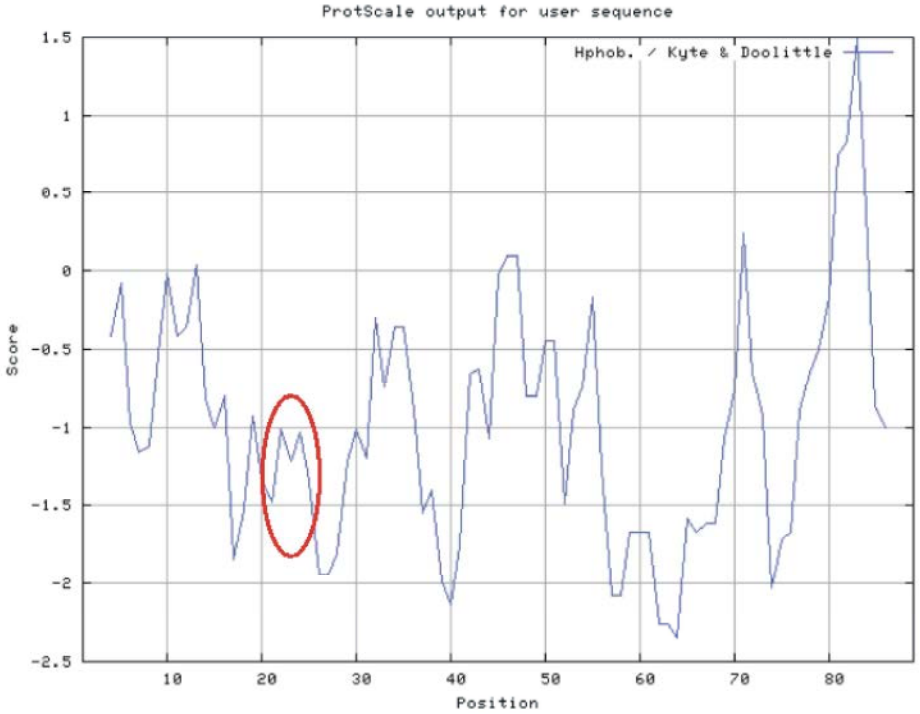
Table 3: Hydrophobicity of amino acid sequence of the three haplotype of BoLA DRB3.2 gene in Taro cattle





KT722742

Amino acid at codon no.24:T(Threonine)



KT722741

Amino acid at codon no.24:R(Arginine)

Note: Red circles show changes in hydrophobicity scale

Along 302 bp of BoLA DRB3.2 Taro cattle, codon sequences (CDS) start from nucleotide number 18 to 284 which deduces 89 amino acids (Table 2). Nucleotide position at 70 and 71 were the variable site of BoLA DRB3.2 in Taro cattle, in which nucleotide substitutions occur (Table 1). The nucleotide substitutions at position 70 and 71 causes changes of amino acid at number 24 from R (Arginine) in haplotype KT722741 [17] to A (Alalnine) in haplotype KT722740 [16] and T (Threonine) in haplotype KT722742 [18]. The substitution amino acid at 24 is common for BoLA alleles. Two different amino acids have been recorded at position 24 in BoLA alleles namely L (Leucine) and V (Valine) [23, 7], but the present study found that at position 24 was occupied by Arginine (R), Alanine (A) and Threonine (T). Almost all of the amino acids comprise the BoLA molecules in Taro cattle are hydrophilic, with some of them form hydrophobic groove. Amino acids changes cause changes in hydrophobicity of the groove, but do not much change the entire amino acids hydrophobicity (Table 3).

CONCLUSIONS

BoLA DRB3.2 gene of Taro cattle was 302 bp containing 17 bp of intron 5', 267 bp of exon 2 and 18 bp of intron 3'. The codon sequences start from nucleotide number 18 to 284 and deduce 89 amino acids. There were 2 variable site of BoLA DRB3.2 gene in Taro cattle with Single Nucleotide Polymorphism (SNP) at positions 70 A/G and 71 G/C, with caused amino acids substitutions at position 24 R/A/T. Polymorphism of BoLA DRB3.2 gene in Taro cattle is low.

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