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Genetic Variability in Integrin Beta-1 (ITGB1) Gene of Buffaloes

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Abstract

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A fragment of 151 bp corresponding to exon-5 of ITGB1 gene was amplified in 45 buffaloes ***(Bubalus bubalis)*** *of murrah breed. The RFLP analysis showed the absence of polymorphism in this fragment with respect to* Tru*1I restriction enzyme and showed only one restriction site, which produced two fragments of 21 and 130 bp. The putative metal ion binding D-X-S-X-S sequence motif in the I-like domain of b1 subunit was highly conserved in these buffaloes. Three and nine nucleotide sequence variations were found in buffalo sequences when compared to that of cattle and human, respectively. The sequence of the amplicon which was the first report on buffalo ITGB1, was submitted to GenBank.*

Keywords: Buffalo, exon-5, integrin β–1, murrah, *Tru*1I, RFLP.

Introduction

The development of disease resistance in animals is through immune system, which regulates various immune effector functions. Integrins are one of the important cell adhesion molecules (CAMs) which govern the immune function. Each integrin molecule which is heterodimeric glycoprotein in nature, comprises of one α and one β subunit. There are 19 different types of α and 8 different types of β subunits described, forming at least 25 types of combinations (Arnaout, 2002). β1 integrin is encoded by a single gene called ITGB1 comprising of 16 exons. The relative preservation of lymphocyte-dependent functions *in vivo* in leukocyte adhesion deficiency (LAD) infections has been attributed to the normal expression of β1 integrins which mediate the adhesive functions and signaling of lymphocytes (Harris *et al.*, 2001). β1 integrin is critical for lymphocyte trafficking in general and transvascular migration in particular (Iwata, 2002). β1 integrin-ligand binding is regulated by a metal ion dependent adhesion site (MIDAS) in the I-like domain of β1 subunit. Exon-5 is a very important region of ITGB1 gene as it codes for the metal binding D-X-S-X-S sequence motif (where X denotes any amino acid) in the I-like domain of β1 subunit, which is similar to that of the MIDAS in the I domain of the a subunit of integrin molecule and is

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very important for integrin-ligand binding. The present study is an attempt to characterize and to ascertain the genetic variability in the exon-5 of ITGB1 gene in murrah breed of buffaloes.

Materials and Methods

Genomic DNA was isolated from 45 buffaloes (*Bubalus bubalis*) of murrah breed, randomly selected from livestock farm at LPM section of IVRI, Izatnagar. Fifteen ml of venous blood was collected from each animal in a sterile centrifuge tube containing 0.5 ml of 2.7% EDTA as anticoagulant. Isolation of genomic DNA was done as per the standard protocol (Sambrook *et al.,* 1989). A set of primers (5' GGG AGC CAC AGA CAT TTA CAT TA 3' as forward primer and 5' ATC CTC CTC ATT TCA TTC ATC AGA 3' as reverse primer) was designed on the basis of genomic sequence of human (Zhang *et al.,* 2003, GenBank Accession No. NM002211) to amplify the 151 bp fragment corresponding to exon-5 of ITGB1 gene. The PCR was carried out in a total volume of 25 µl containing 100 ng DNA, 2.5 µl of 10X PCR buffer (500 mM KCl, 100 mM Tris-HCl, pH 8.3), 0.2 mM of dNTP mix, 1.5 mM $MgCl_2$, 12 pM of each primer and 0.5 U Taq DNA polymerase. Samples were amplified for 35 cycles of 94C for 25s, 51C for 30s and 72C for 30s; followed by 72C for 5 min. For genotyping, 12 µl of the PCR reaction mixture along with 1.5 µl 10X buffer R was digested for 16h at 37C with 10 U of *Tru*1I (*Mse* I) with restriction site as 5'...T↓TAA...3'. Restriction fragments were resolved in 1.5% agarose gel stained with ethedium bromide in TAE buffer and visualized under UV light. The amplicon was later sequenced using dedeoxy chain termination method. This sequence was also submitted to the GenBank (www.ncbi.nlm.nih.gov). The sequence was compared with sequences of other species available in the GenBank database using DNASTAR software.

Results and Discussion

This is the first report on *Tru*1I PCR-RFLP of ITGB1 gene in buffalo in which the 151 bp fragment corresponding to exon-5 of ITGB1 gene of murrah buffalo on digestion with *Tru*1I restriction enzyme produced two bands of 21 bp and 130 bp. However, the smaller fragment of 21 bp could not be resolved in the gel (Fig 1). This suggested that amplified fragment of exon-5 contained only one *Tru*1I RE site and no polymorphism was found with respect to this restriction enzyme. The PCR product was sequenced and the sequence was submitted to GenBank (Accession number DQ251725). The nucleotide sequence of the buffalo ITGB1 gene revealed highest homology with cattle

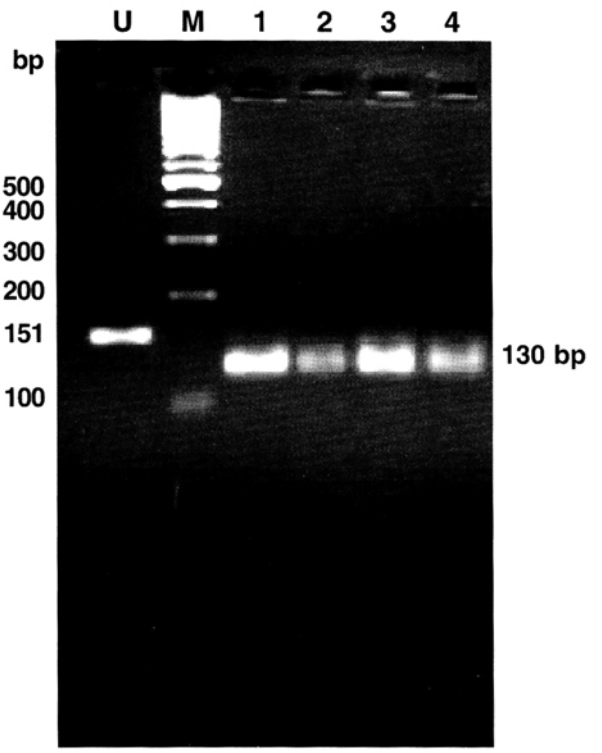

Fig. 1 : *Tru*1I restriction patterm of Exon-5 of ITGB1

Lane U: Undigested 151 bp product of ITGB1 gene.

Lane M: Molecular size marker (100 bp DNA ladder plus)

Lane 1-4: Digested product of 130 bp size.

(98.0%) followed by human (92.6%), pig (92.6%), cat (88.6%), mouse (88.6%) and chicken (79.2%).

Single nucleotide polymorphisms (SNPs) of ITGB1 gene have been reported only in 3 species viz. chicken (*G. gallus),* human *(H. sapiens)* and mouse *(M. musculus).* No SNPs have been reported from buffalo and cattle. Alignment report indicated that only 3 single nucleotide differences existed in buffalo sequences when compared to that of cattle which were identified to be C393T (C→T), C397T (C→T) and T505C (T→C). A similar mutation in human, from C→T at 571 bp was reported in the vicinity of exon-5 that maps to the I-like domain in keratinocytes with squamous cell carcinoma (Evans *et al.,* 2003). But in our studies the amino acid sequences showed 100% homology despite the differences in nucleotides, indicating that variations in these nucleotides did not affect the amino acid coding with respect to exon-5.

Nine single nucleotide differences were noticed in buffalo sequences when compared to that of human sequences. No mutant allele was observed in the buffaloes of murrah breed under study. Studies have revealed that some mutations in the important residues of β1 subunit, which are known to be critical for ligand binding of integrins (e.g. Asp130) have adverse effect on α-β association (Lee *et al.,* 1993). Our study revealed that there was a sequence homology in the I-like domain around Asp130 in β1 subunit of integrin molecule. The β1 and β2 subunits share a similar MIDAS like sequence that when mutated, would result in loss of integrin-ligand binding capacity (Loftus *et al.,* 1994). The β1 MIDAS sequence in our study showed sequence homology with earlier reported β2 MIDAS sequences in buffaloes (Kumar, 2005). This study also revealed that the D-X-S-X-S (Asp–X–Ser–X–Ser) sequence motif in the I-like domain of β1 subunit was highly conserved in murrah buffaloes and was D-L-S-Y-S. Amino acid sequence homology in the vicinity of D-L-S-Y-S site was nearly 100% as compared to different species.

Absence of any mutation at MIDAS site in buffalo ITGB1 gene strengthens its conserved nature across the species and could be one of the factors developing or controlling the disease resistance. Identification of novel critical residues for ligand binding as well as α-β association of β1 subunit and their significance in cell signaling mechanism suggest the need of sequencing the entire gene of β1 integrin (ITGB1) and polymorphism studies with more number of restriction enzymes for better understanding of variability in disease resistance.

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बिजोय जॉन, सुबोध कुमार, एस.एम.देव, अभिजीत मिश्र, एस. के.निरंजन, सौमेन नस्कर, आर्जव शर्मा। भैंस के इन्टेग्रिन बीटा-1 (आईटीजीबी 1) का जीन में आनुवंशिक विविधता।

मुर्रा नस्ल की 45 भैसों के आईटीजीबी 1 जीन के एक्सान-5 के संगत 151 बीपी के एक खंड का प्रवर्धन किया गया। आरएफएलपी विश्लेषण द्वारा इस खंड में II संकोचन इन्जाइम से बहुरूपता नहीं मिली और केवल एक संकाचन स्थल मिला, जिससे 21 और 130 बीपी के दो खंड बने। इन भैसों में बीटा-1 उप ईकाई के आई सद्दश प्रक्षेत्र में प्रसिद्ध धातु आयन बंधन डी-एक्स-एस-एक्स-एस अनुक्रम रूपांकन अति संरक्षी था। गौ और मनुष्यों की तुलना में भैसों में क्रमशः तीन और नौ न्यूक्लीयोटाइड अनुक्रम विविधताएं पाई गयीं। भैंस के आईटीजीबी-1 के एम्लीकान अनुक्रम की प्रथम रिपोर्ट को जीन बैंक में जमा किया गया।