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STUDY OF β2M GENE SEQUENCE VARIATION IN BUFFALO AND CATTLE

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Abstract: The beta 2 microglobulin (β2M) gene is a structural gene which acts as an integral component of FcRn (neonatal Fc receptor) heterodimer for its cell surface expression. The present investigation was carried out to study nucleotide sequencing and DNA polymorphism by PCR-SSCP of β2M gene in buffaloes and its comparison with cattle. Genomic DNA was isolated from a total of 80 animals from Livestock Production Management (LPM) Section of IVRI, India belonging to Murrah breed. Seven fragments of the gene, comprising of exonic and intronic regions of β2M gene were amplified. The PCR-SSCP analysis and sequencing of different patterns obtained by silver staining of various exonic and intronic fragments was done. Two insertions and one deletion of nucleotides in the intronic and exonic regions of β*2M* gene in buffalo were observed in comparison to β*2M* gene of cattle.
Keywords: FcRn, Murrah, NCBI, PCR-SSCP, polymorphism.

Introduction

Buffaloes play a pivotal role in livestock and agriculture economy of many countries across the globe. Domesticated species of buffalo are mainly river and swamp buffalo. The habitat of these animals is tropical, subtropical, wet grasslands, marshes and swamp regions throughout the world. Water buffaloes produce milk and meat and are used as draught animals in developing countries. Whole genome mapping technology and high throughput genomic sequencing have revolutionized the research associated with many domestic animal species (Miao & Luo 2013). Fuelled by the availability of the bovine genome sequence, genomics selection has revolutionized genetic selection for traits of interest. This may eventually facilitate on farm breeding and management decisions. Now genetic studies are only limited by the ability to collect high quality phenotypic data.

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FcRn are neonatal Fc receptors which play an important role in absorption of IgG and are also known to prolong their half life. FcRn is a heterodimer of two polypeptides i.e., a MHC class I homolog encoded by FCGRT gene and beta 2 microglobulin encoded by β2M gene. The β2M acts as an integral component of FcRn heterodimer for its cell surface expression (Roopenian and Akilesh, 2007) and in its absence, FcRn is retained in endoplasmic reticulum. Furthermore, in absence of β2M, IgG binding is decreased compared with that of native FcRn (Israel *et al*., 1995). More than 50 % cases of failure of passive transfer (FPT) are due to defective structure of FcRn which could be due to polymorphism in genes coding FcRn (Simister and Ahouse, 1996). As all the farm animal species genetic codes are yet unrevealed, although sequence data are available for some species, that is, sheep and buffalo, buffalo genome is sequenced but yet unexplored in terms of genes annotation and their functions. Therefore, it is a challenge for researchers associated with such species to successfully exploit the information on coding of these species and then application of that information for genetic improvement. A relatively simple approach to address this challenge is to take a comparative genomics approach to unravel information on related species. This approach has successfully been used in cross-species studies of microbes such as human and bovine (Richards et al. 2011). Beyond the interspecies microbial screening, this technique has also been applied to eukaryotic genomes such as sheep and human (Dalrymple et al. 2007), sheep and cattle (Kijas et al. 2006) and non-coding regions of cattle and human (Miziara et al. 2004). This approach of comparative genomics is applicable as demonstrated by the fact that 50% of the genetic markers of the first iteration of the ovine linkage map were in fact of bovine origin (Womack & Kata 1995).

Considering the importance of buffaloes in milk and meat production, the present study was undertaken to evaluate the possibility of successfully utilizing β*2M* sequence data of cattle to find out common markers for genetic studies, that is, genomic selection in buffaloes.

Material and Methods

Experimental animals: 80 Murrah buffaloes, maintained at Livestock Production Management (LPM) Section of IVRI, Izatnagar, were included in the present study.

Blood collection and DNA isolation: Approximately, 5ml venous blood was collected from each animal using 0.25 ml of 2.7% EDTA as an anticoagulant. The samples were brought to the laboratory in a double-walled icebox containing ice with cool pack and stored at −20 ◦C till the isolation of DNA. Genomic DNA was isolated from blood following standard protocol. The quality of DNA was checked by spectrophotometry taking ratio of optical

density (OD) value at 260 and 280 nm. Good quality DNA having OD ratio between 1.7 and 1.9 was used for further work.

Amplification of β2M gene: Seven pairs of primers for amplification of both exonic and intronic regions of β2M gene in the DNA samples of Murrah buffaloes were designed on the basis of sequences of cattle (*Bos taurus*) in public database at NCBI (AC_000167.1) with the help of Primer3 online computer software. Amplication was done using following sets of primers. Fragment I (F: 5'GAGTGCGGACTATAAAGGCGAGCG3'; R: 5'CAGGGTGCCAAGTGGGTGTGAT3'); Fragment II (F: 5'CACTGAAGCTCGGATTTCTCTT3'; R: 5'CCTACACAGGGAAAGAGCTGA3'); Fragment III (F: 5'CGCTCCTCAGGTCCTCCAAAGATT3'; R: 5'CCCCACTTAACTATCCGGGGTTGTT3'); Fragment IV (F: 5'GGATCTCAGGCTGGTAGGAAAGGTC3'; R: 5'GCTGTCTGTAATGGTCCCCAGG3'); Fragment V(F: 5'GGCTTTCCCAGCATCACTAA3'; R: 5'GGTGTCTCTGCAGAACCATGT3'); Fragment VI (F: 5'CCCCCAGGTTTGAAGATGCC3'; R: 5'CCTCCACTGCGGCTTACTGC3'); Fragment VII (F: 5'GGTCTGTGTTGGCAGTAAGCCGC3'; R: 5'CACTTCCCTGTTTGAACGAAGGC3'. The annealing temperature used after standardization was 57, 59.3, 55, 55, 51, 55 and 55 °C for fragments I through VII respectively.

PCR-SSCP and nucleotide sequencing: Single strand conformation polymorphism (SSCP) analysis was performed on all PCR products/DNA samples for determination of nucleotide variability by standard procedure with slight modifications (Orita *et. al.,* 1989), followed by sequencing. The gels were stained with silver nitrate (Bassam *et. al.,* 1991) and the band patterns were scored for each amplified product. Representative samples from variants detected by PCR-SSCP of seven fragments of β_2M gene were sequenced by outsourcing. Both forward and reverse sequencing was carried out for each allelic pattern from three animals which was sent as triplicate for each pattern. Resulting sequences were analysed by DNASTAR (Lasergene, USA) software.

Results and discussion

Nucleotide sequencing of amplified fragments of β2*M* gene of buffalo were done and submitted to NCBI GenBank accession no. KM261591 to KM261602.

Comparison of reported cattle (*Bos taurus*) sequence GenBank resource (AC_000167.1) with observed buffalo sequence of fragment I revealed two single nucleotide

changes viz., G174T and T184C. However, no changes in buffalo *β2M* gene could be observed when compared to cattle with respect to fragment II and fragment III. In a similar comparison with respect to fragment IV revealed 7 single nucleotide changes viz., A31T, T71G, C81T, C89T, T100A, T111C and T198C. However, a deletion of 1 nucleotide at 23rd position in buffalo was also observed. We also observed an insertion of 10 bp nucleotides viz., GCTTAAGAAG at 186th to 195th position in buffaloes as compared to cattle (*Bos taurus*). Comparison of fragment V and VI of observed sequence of buffalo to reported cattle sequence revealed 8 single nucleotide changes viz., C108T, A114G, G122A, G147A, GG185,186AA, G219A and G221A and 5 single nucleotide changes viz., A23T, C67T, G81T, C107T and G139A, respectively. Comparison of observed sequence in buffalo of fragment VII with cattle sequence revealed 7 single nucleotide changes viz., A36G, A131G, G142C, CA148,149 TG, A169G, T256G and A303T and 1 insertion (7 bp) TTCTTTC at 277th to 283rd position. These SNPs have been presented in Table 1. Similar SNPs were observed in neonatal buffalo calves by Chaudhary *et. al.,* 2016.

Conclusion

In conclusion, the length of amplified product of β*2M* gene varied with that of cattle and buffalo. There were two insertions and one deletion of nucleotides in the intronic and exonic region of buffalo β*2M* gene in comparison to β*2M* gene of cattle.

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References

[1] Bassam, B.J., Annoles, G.C. and Greshoff, P.M. 1991. Fast and sensitive silver staining of DNA in polyacrylamide gels. *Ani. Biochem*. 196: 80-83.

[2] Chaudhary, R., Kumar, S., Yathish, H.M., Sivakumar, A., Mishra, C., Kumar, A., Chauhan, A., Sivamani, B., Sahoo, N.R. 2016. Nucleotide variability in Beta 2 Microglobulin (*β_2M*) gene and its association with colostral IgG levels in buffaloes (*Bubalus bubalis*). *Indian J Anim Res*. Accepted.

[3] Dalrymple, B.P., Kirkness, E.F., Nefedov, M., McWilliam, S., Ratnakumar, A., Barris, W., Zhao, S., Shetty, J., Maddox, J.F., O'Grady, M., Nicholas, F., Crawford, A.M., Smith, T., de Jong, P.J., McEwan, J., Oddy, V.H., Cockett, N. E. 2007. Using comparative genomics to reorder the human genome sequence into a virtual sheep genome. *Genome Biol*. 8: 152.

[4] Israel, E.J., Patel, V.K., Taylor, S.F., Marshak-Rothstein, A. & Simister, N.E. 1995. Requirement for a β2 microglobulin associated Fc receptor for acquisition of maternal IgG by fetal and neonatal mice. *J. Immunol.* 154: 6246–6251.

[5] Kijas, J.W., Menzies, M., Ingham, A. 2006. Sequence diversity and rates of molecular evolution between sheep and cattle genes. *Anim. Genet*. 37: 171–174.

[6] Miao, X., Luo, Q. 2013. Genome-wide transcriptome analysis between small-tail Han sheep and the Surabaya fur sheep using high-throughput RNA sequencing. *Reproduction* 145: 587–596.

[7] Miziara, M.N., Riggs, P.K., Amaral, M.E. 2004. Comparative analysis of noncoding sequences of orthologous bovine and human gene pairs. *Genet. Mol. Res*. 3: 465– 473.

[8] Orita, M., Iwahana, H., Kanazawa, H., Hayashi, K, Sekiya T (1989) Detection of polymorphism of human DNA by gel electrophoresis as single strand conformation polymorphisms. *Proc Natl Acad Sci USA*. 86:2766-2770.

[9] Richards, V.P., Lang, P., Bitar, P.D., Lefebure, T., Schukken, Y.H., Zadoks, R.N., Stanhope, M.J. 2011. Comparative genomics and the role of lateral gene transfer in the evolution of bovine adapted Streptococcus agalactiae. *Infect. Genet. Evol*. 11: 1263–1275.

[10] Roopenian, D.C. and Akilesh, S. 2007. FcRn: the neonatal Fc receptor comes of age. *Nature Review Immunol*. 7: 715-725.

[11] Simister, N.E. and Ahouse, J.C. 1996. The structure and evolution of FcRn. *Res. Immunol.* 147: 333-337.

[12] Womack, J.E., Kata, S.R. 1995. Bovine genome mapping: evolutionary inference and the power of comparative genomics. *Curr. Opin. Genet. Dev*. 5: 725–733.

Table 1: SNP comparison of cattle and buffalo

Fragment	*Bos taurus* (Cattle) AC_000167.1	Position	*Bubalis bubalis* (Buffalo)
Exon 1 (Fragment I)	G	174	A
	T	184	C
Exon 3 (Fragment IV)	G	23	-
	A	31	T
	T	71	G
	C	81	T
	C	89	T
	T	100	A
	T	111	C
	-	186-195	GCTTAAGAAG
	T	198	C
Intron 3 (Fragment V)	C	108	T
	A	114	G
	G	122	A
	G	147	A
	GG	185-186	AA
	G	219	A
	G	221	A
Exon 4a (Fragment VI)	A	23	T
	C	67	T
	G	81	T
	C	107	T
	G	139	A
Exon 4b (Fragment VII)	A	36	G
	A	131	G
	G	142	C
	CA	148-149	TG
	A	169	G
	T	256	G
	-	277-283	TTCTTTC
	A	303	T