Genetic polymorphisms within coding region of insulin like growth factor-1 gene in six indigenous draught cattle

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ABSTRACT

The study was undertaken to detect genetic polymorphisms in the coding regions of bovine insulin-like growth factor (IGF-1) gene in six indigenous draught cattle breeds viz., Bargur, Hallikar, Kangayam, Ongole, Pullikulum and Umblachery. A total of 312 blood samples (52 samples from each breed) were collected and genomic DNA was isolated. Four sets of primers were designed for the expressed regions of the IGF-1 gene. The presence of six single nucleotide polymorphisms (SNPs) was detected after sequencing and analysis. The sizes of amplicons (607, 454, 518 and 671 bp) obtained covered the exons one to four with intronic sequences on either sides. The SNPs in exon 1 were at positions g. 213G>A (transition) and g. 244C>A (transversion) between Bos taurus and Bos indicus cattle. The variation at first position resulted in non-synonymous mutation, replacing the amino acid 'Serine' with 'Asparagine'. Whereas the mutation at position g. 244C>A, resulted in a stop codon (TGA). At position g. 4827 in exon 2, a 'G' to 'A' transition was observed, which resulted in synonymous mutation. In exon 3, three SNPs were observed at positions g. 56233G>A (transition), g. 56317G>T (transversion) and g. 56354A>T (transversion). These variations resulted in non-synonymous changes in amino acid sequences, i.e. from 'Arginine' to 'Lysine', 'Lysine' to 'Asparagine' and 'Methionine' to 'Leucine' respectively. This region exhibited maximum polymorphisms at different loci whereas; the fourth exon exhibited the greatest homology between Bos taurus and Bos indicus cattle, showing no variation in any of the positions. Genotyping these SNPs in larger number will give significant information on the role of these SNPs among Indian draught cattle.

Key Words: Genetic polymorphism, draught cattle, Insulin-like growth factor 1 gene, SNPs

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INTRODUCTION

The Indian cattle, popularly known as zebu cattle have specific merits like disease resistance, heat tolerance, endurance and ability to produce under stress and low feed input. At present around 100 million hectares of farm land are ploughed by draught animals, which form 55 per cent of total cultivable area (Singh, 1999). According to 19th Livestock Census Report (2012) it becomes obvious that draught animal population in India has been steadily declining. The population of draught animals in field operations has shown a negative annual growth rate from 54.32 million in 2003 - 2007 to 39.85 million in 2012. The working group on Animal Husbandry and Dairying, 11th five year plan (2007- 12) revealed that although use of mechanical and electrical power has increased over the years, the draught cattle shall continue to be a major source of farm power in India in future for small and marginal farmers. However, critical review of literature revealed that extensive studies had been carried out on physical characteristics, work performance and biochemical parameters of work bullocks. But the work on genetic improvement of draught cattle and molecular markers related to draught power has not been attempted so far. Hence the present study was planned to characterise the bovine Insulin-like growth factor – 1 (IGF-1) gene and to explore the polymorphisms of the gene involved in the main metabolic pathway related to physical performance of draught cattle. In the research, six popular draught breeds of south India *viz.* Bargur, Hallikar, Kangayam, Ongole, Pullikulum and Umblachery were selected. Insulin like Growth Factor-1 (IGF-1) is considered as one of the potential candidate markers for muscle strength and muscle mass in cattle due to their role in regulation of cell proliferation and animal growth (Siadkowska et al. 2006). It is also known as Somatomedin C, a member of insulin superfamily. The primary source of IGF-1 is the liver, from which it is released into the blood and acts as an endocrine mode on other tissues. IGF-1 is a polypeptide of molecular weight 7.5 kDa, built of 70 amino acids (Daughaday and Rotwein, 1989) and is identical in human, cattle, dogs and pigs (Nixon et al. 1999). The mature IGF-1 in cattle is expressed from a gene consisting of 4 exons (exon 1-4) and spanning more than 71 kb of genomic DNA. The IGF -1 gene was localized on chromosome 5 in cattle by Bishop

Table 1. Primer sequences designed for amplifying IGF – 1 gene

Region		Primer Sequence (5'-3'end)	Annealing Temperature (^{0}C)
1	Forward	ttt gcc aga aga ggg aga ga	62.0
	Reverse	caa gcc ctg aag aag tgg ag	
2	Forward	tag cat gat gcc aag acc tg	53.8
	Reverse	gct cgc att aag gtg agg aa	
3	Forward	gaa aaa cct ggg agg gtc a	59.9
	Reverse	cct ctc agg gga gaa tgg a	
4	Forward	cca tgc cat caa ggg aaa	52.4
	Reverse	caa gcc tgc tga atg aat g	

et al. (1991) and Miller et al. (1991). A recent study in humans suggested that polymorphism in IGF-1 gene might influence the muscle strength in response to prolonged physical exercise (Kostek et al., 2005). Thus, bovine IGF-1 gene was considered to contribute to exercise tolerance in these draught breeds.

MATERIALS AND METHODS

A total of 312 blood samples (52 samples from each breed) were collected from respective breeding tracts of the six breeds in sterile vacutainers, containing EDTA as an anticoagulant and stored at 4^{0}C till further processing. Genomic DNA was isolated using standard phenol-chloroform extraction procedure (Sambrook et al., 1989) with slight modifications using DNAzol reagent for lysis prior to phenol-chloroform extraction and then DNA was diluted to 50 ng/µl. The purity and concentration of DNA samples were estimated by Biospectrophotometer (Eppendorf, USA). Based on the bands observed in the agarose gel and concentration determined by spectrophotometer measurement, DNA was diluted using Tris EDTA buffer in 1 in 25 or 50 or 100 dilutions to obtain the template DNA (working DNA) concentration of approximately 20 to 50 ng per µl and stored at -20^{0}C till further processing. Using "Primer3" online software tool (http://primer3.wi.mit.edu/), four sets of primers were designed to amplify the expressed regions of the IGF-1 gene (1 to 262, 4735 to 4894, 56190 to 56371 and 71601 to 71821 nucleotide positions corresponding to GenBank accession No. AC_000162.1) (Table 1). The most critical variables considered while designing the primers were primer length (18-24 bp), melting temperature (55^{0}C to 80^{0}C), specificity, complementary primer sequence, GC content (40 per cent to 60 per cent) and 3'-end sequence. PCR was performed by following the protocol given in Table 2. The PCR amplicons were analysed on a 2% agarose gel and bands were documented. The bands developed were observed in a GelDoc (Bio-Rad,USA) system. The amplicons were sequenced in both forward and reverse directions at M/s. Ocimum Biosolutions, Hyderabad. The instrument used for sequencing was ABI

Table 2. PCR protocol for IGF -1 gene amplification

Step	Process	Temperature	Duration
1	Initial denaturation	95^{0}C	5 min
2	Denaturation	95^{0}C	45 sec
3	Annealing : Exon 1	62.2^{0}C	1 min 30 sec
	Exon 2	53.8^{0}C	40 sec
	Exon 3	59.9^{0}C	1 min 30 sec
	Exon 4	52.4^{0}C	45 sec
4	Extension : Exon 1	72^{0}C	1 min 15sec
	Exon 2	72^{0}C	40 sec
	Exon 3	72^{0}C	1 min
	Exon 4	72^{0}C	1 min 15 sec
5	Back to steps 2 to 4		35 cycles
6	Final extension	72^{0}C	10 min
7	Hold	4^{0}C	Until the samples are removed

3730XL DNA analyser (Applied Biosystems, U.S.A). The variations in sequences among the six cattle breeds and individual animals within a breed were determined using DNA Lasergene Version 2.1 software. The *.ab1 files obtained were fed to "Seqman module" of Lasergene software for multiple sequence analysis. The *Bos taurus* sequence was considered as the reference sequence and was aligned with the query sequences of *Bos indicus*. This software created the consensus sequence and highlighted the SNPs, which were verified by base calling using chromatogram. The SNP position was noted down from the reference sequence and marked as a SNP.

RESULTS AND DISCUSSION

All together, four exons of IGF-1 gene were amplified generating amplicons of sizes 607, 454, 518 and 671 bp. However, the actual sizes of exons were 262, 160, 182 and 221 bp (NCBI; Gene ID 281237; http://www.ncbi.nlm.nih.gov/). The four numbers of expressed regions of bovine IGF-1 gene sequenced in the present study, based on NCBI website, concurred with findings of Francis et al*.* (1986) and Honneger and Humbel, (1986). The SNPs found in the nucleotide sequences of expressed regions among the south Indian cattle breeds and *Bos taurus* cattle are presented in Table 3. The genotype and gene frequencies of SNPs obtained through population genetic analysis are tabulated in Table 4. In exon 1, the allele frequencies of 'A' allele at SNP positions g.213 and g.244 were 15.00 percent and 11.92 percent respectively. Whereas, the frequencies of SNPs 3, 4, 5 and 6 were less than 10 per cent. Moreover, all the three possible genotypes were also observed in the huge number of samples screened for genotyping of SNPs. In general, the alleles 'A' and 'T' were found replacing the other alleles in the SNPs detected. The exon 1 (1 to 262 nucleotide) was amplified along with -89 bases upstream and +256 bases down the exon which forms the part of second intron. Two polymorphisms detected in this region were g. 213G>A (transition) and g. 244C>A (transversion); the former (g.213 G>A) resulted in a non-synonymous variation and latter (g. 244 C>A) resulted in a nonsense mutation. The consequence of g.244 C>A would be the termination of protein, since this region with 42 nucleotides could only code for 14 amino acids as against 154 amino acids by the normal IGF-1 gene. The variation of g. 244C>A ensuing a stop codon was also detected in Kangayam cattle, which is an excellent draught breed of south India. No phenotypic difference was found in animals harbouring this SNP that resulted in premature termination of this protein. This could be explained by the heterozygous nature at this locus and possibility of differential expression levels of bovine IGF-1 gene as class 1 and class 2 mRNAs (Wang et al*.,* 2003). The second exon of 160 bp was amplified and a variation at position g. 4827G>A (transition) was observed. However, this variation was synonymous and it did not result in any amino acid change. Presence or absence of this mutation will not change the functional property of the gene, even though such variations have been detected in Bargur, Ongole and Pullikulum cattle. On the contrary, three different mutations were detected by Gao et al. (2009) who sequenced the bovine IGF-1 gene and analysed a 357 bp fragment of exon 2 in Chinese beef cattle. Two mutations of A-to-G transition at positions 3620 bp and 3842 bp and one C-to-T transitions at 3628 bp were observed. The mutations at 3620 and 3628 were non-sense mutations; whereas the mutation at 3842 resulted in change of amino acid ('Glutamic acid' to 'Glycine'). The third exon, which was highly polymorphic, exhibited three variations at position g. 56233G>A (transition), g. 56317G>T (transversion) and g. 56354A>T

Table 3. SNPs found in expressed regions of IGF-1 gene between Bos taurus and Bos indicus cattle

Locus (Position in bp)	*Bos taurus*	*Bos indicus* Bargur	Hallikar	Kangayam	Ongole	Pulikulum	Umblachery	Type of variation
Exon 1								
213	G	AG	AG	AG	AG	AG	AG	Non-synonymous (Ser to Asn)
244	C	AC	AC	AC	AC	AC	AC	Protein terminated
Exon 2								
4827	G	AG	AG	AG	AG	AG	AG	Synonymous (Glu)
Exon 3								
56233	G	AG	AG	AG	AG	AG	AG	Non-synonymous (Arg to Lys)
56317	G	GT	GT	GT	GT	GT	GT	Non-synonymous (Lys to Asn)
56354	A	AT	AT	AT	AT	AT	AT	Non-synonymous (Met to Leu)

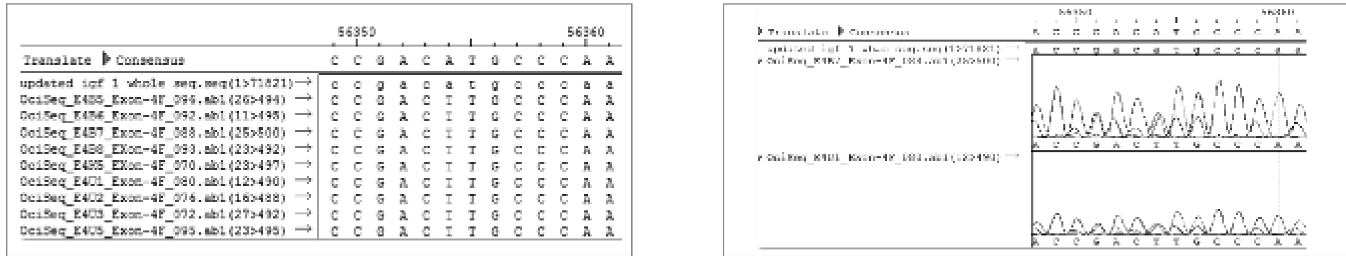

Figure 1. Chromatogram displaying SNP 6 (56354A>T) of exon 3 in Bos taurus and Bos indicus cattle

(transversion). All these variations resulted in non-synonymous changes in the amino acid sequence of IGF-1. The coding domain of exon 3 codes for 28 amino acids; of which, three amino acids are changed. The first variation, which caused the change in amino acid from 'Arginine' to 'Lysine' is replacement of basic amino acid with a basic one; the second change from 'Lysine' to 'Asparagine' is a replacement of basic with neutral; and the third change from 'Methionine' to 'Leucine' is a replacement of neutral with neutral type of amino acid. Though, Hallikar and Ongole exhibited no variation in sequence at position g. 56233, they seem to differ in the nucleotide sequences from the rest of *Bos indicus* breeds (Bargur, Kangayam, Pullikulum and Umblachery) of Tamil Nadu. Similarity in sequences of fourth exon between *Bos indicus* and *Bos taurus* cattle indicates that this region is highly conserved across different breeds of these two bovine species. There was no earlier study pertaining to exon 3 and exon 4 in bovines. But two mutations were reported in exon 4 of caprine IGF-1 by Wang et al. (2011) in Chinese goat breeds (Xinjiang, Bogeda Cashmere and Nanjiang Cashmere goats), which were of silent in nature.

Overall six SNPs in four exons of IGF – 1 gene were found to be characteristics of *Bos indicus* cattle. This study is first of its kind in India to characterise the IGF-1 gene in *Bos indicus* cattle and to explore the polymorphisms of IGF-1 gene involved in the main metabolic pathway related to physical performance of draught cattle. A candidate gene approach would pave the way for selecting animals with better draught quality, *i.e.* selection based on molecular tool. Further, it will also help in implementing rational decisions for conservation and improvement of our treasured genetic resources.

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Table 4. Genotype frequency of SNPs in exons of IGF-1 gene among six breeds of south Indian cattle

Locus (position in bp)	**Genotype**	**Cattle breeds and genotype frequency**					
		Bargur	**Hallikar**	**Kangayam**	**Ongole**	**Pulikulum**	**Umblachery**
Exon 1							
213	GA	0.40	0.00	0.25	0.33	0.40	0.60
	GG	0.60	1.00	0.75	0.66	0.60	0.40
244	CA	0.20	0.00	0.66	0.33	0.20	0.20
	CC	0.80	1.00	0.33	0.66	0.80	0.80
Exon 2							
4827	GA	0.37	0.37	0.00	0.20	0.50	0.00
	GG	0.62	0.62	1.00	0.80	0.50	1.00
Exon 3							
56233	GA	0.57	0.00	0.50	0.00	0.57	1.00
	GG	0.42	1.00	0.50	1.00	0.42	0.00
56317	GT	0.57	0.00	0.25	0.00	0.57	0.66
	TT	0.42	1.00	0.75	1.00	0.42	0.33
56354	AT	0.42	0.00	0.25	0.00	0.42	0.66
	TT	0.57	1.00	0.75	1.00	0.57	0.33

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