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Full Length Research Paper

Gene study within the 5′ flanking regions of growth hormone gene of first exon in *Bos indicus*

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Growth hormone (GH) is a main regulatory protein secreted by pituitary gland and placenta, involved in most anabolic processes in mammals. Expression of more than one gene for GH has been reported, indicating polymorphism at gene and protein level; apart from this, silent mutations has also been reported, relating to the level of expression of GH gene. The aim of this study is to identify silent mutations within the 5' flanking regions of GH2 gene of the first exon in *Bos indicus*. DNA was isolated from the blood of freshly slaughtered animal and a set of primer was used for gene amplification, binding at 5' flanking region of GH gene at chromosome 19. DNA was amplified and the resultant product of about 453 bp was sequenced. The results showed that there were 9 changes including 8 replacements and one addition, for GH 2.3 allele, as compared to Bos taurus Genome (Btau_4.0) and two earlier reported alleles 2.1 and 2.2 for GH2 gene in *B indicus*. As this region is related to higher milk production, growth regulation, carcass and immune response traits in livestock, these changes could be used as a genetic marker. The reported sequence has been deposited to the European Molecular Biology Laboratory (EMBL) with the Accession Number FN666263.

Key words: *Bos indicus*, growth hormone gene, silent mutation, growth hormone (GH) gene, allele.

INTRODUCTION

Growth hormone (GH) plays a fundamental role in the regulation of growth and metabolism in vertebrates, as reported by number of workers (Carnicella et al. 2003; Davidson 1987; Sami 2007; Wallis 1985). Growth hormone gene is a member of multi gene family about 1800 bp in length with 4 intervening sequences and consists of five exons of about 648 nucleotides (Gordon et al. 1983), with chromosome region 19q26 in bovine genome (Hediger et al. 1990; Fries et al. 1993). Bovine growth hormone (bGH) is synthesized as pre bGH with a signal peptide of 26 amino acids residues coded by exon I and part of exon II, contains short sequences of DNA that are recognized by binding sites, associated with the control of gene expression and initiation. It is important to investigate the promoter region and the region 5′ of untranslated gene, since these regions have several binding sites for transcription regulatory factors.

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As GH is involved in monitoring the milk production in farm animals, it has received considerable importance; during the last decade, there was huge span of publications on GH gene and its role in higher milk production by animals. Polymorphisms in the 5′flanking regions of the bovine GH gene have been described by Hecht and Gelderman (1996), Rodrigues et al. (1998), Suzuki (2001), Ge et al. (2003) and Ferraz et al. (2006). GH 1 and GH2 genes are identified in bovine species. Thus, GH gene is a candidate for genetic marker, related to identification of higher milk production, growth regulation, carcass and immune response traits in livestock (Yao et al. 1996; Ge et al. 2003). A number of studies have shown a close relationship between bGH gene polymorphisms and pro-ductive traits in dairy cattle (Lucy et al. 1993; Lagziel et al. 1996; Yao et al. 1996; Grochowska et al. 2001). Increased GH blood levels have been reported in dairy cattle selected for elevated milk yield (Peel and Bauman, 1987), lean-selected sheep (Fleming et al., 1997) and low backfat pigs (Althen and Gerritz, 1976), compared with divergently selected lineages. However, elevated GH plasmatic levels are

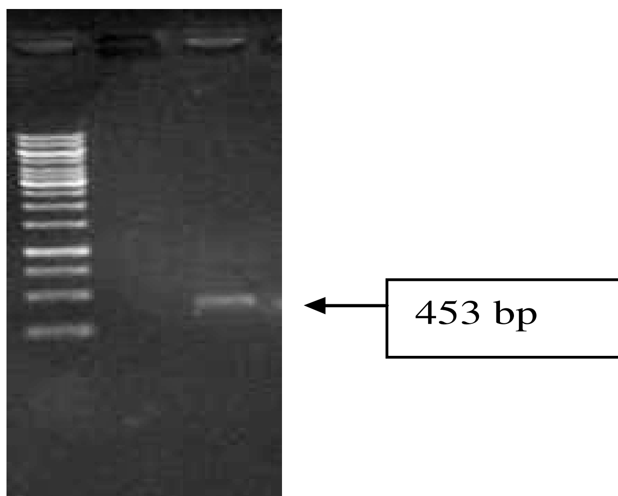

Figure 1. Lane 1 showing Marker; Lane 2 showing PCR product of 453 bp with cow DNA by using forward primer P3 and reverse primer P4.

apparently not dependent on GH gene transcription activity, since Fleming et al. (1997) found higher total GH in lean than in fat lamb pituitary glands, though GH mRNA concentrations were similar. Its connection with productive traits in beef cattle has also received attention of the researchers (Unanian et al. 2000; Ge et al. 2003). Silveira et al. (2008) reported that growth hormone 1 gene (GH1) polymorphisms are possible markers of the production potential of beef cattle using the Brazilian Canchim breed as a model.

Several mutations have been identified in the bovinegrowth hormone gene (Chikuni et al. 1991; Zhang et al. 1993; Unanian et al. 1994; Lagziel and Soller, 1999). Sequence variations of the region involved in bovine GH gene regulation have also been reported. Hecht and Geldermann (1996) identified six variable nucleotide sites in the 5′ flanking region, some of them potentially binding sites for trans-acting factors possibly involved in genetic expression. Rodrigues et al. (1998) identified an AAG deletion nine nucleotides upstream from TATA box in beef but not in dairy cattle. Ferraz et al. (2006) identified six new polymorphisms of the bovine GH gene (one INDEL and five SNPs), which can be used as molecular markers in genetic studies. Within the GH1 fragment, five polymorphisms were identified, corresponding to three different alleles: GH1.1, GH1.2 and GH1.3. In the GH2 fragment two alleles were identified, GH2.1 and GH2.2. Goal of the present study was to identify the silent mutation within the 5′ flanking regions of bovine GH gene in local species of *B. indicus*.

MATERIALS AND METHODS

Blood sample was collected form freshly slaughterd animal from the Bukkar Mandi, Multan road, Lahore and DNA was isolated by using DNA extraction Kit (Fermantas) according to the instructions.

A set of primers was synthesized, as reported by Ferraz et al. (2006), to amplify a fragment of genomic DNA, which covers a part of the 5'-Flanking the first exon and the beginning of the first intron of GH gene P3: 5 '- TCTCAAGCTGAGACCCTGTGT - 3', P4: 5 - CAAATGGGCTCTGGGTGTAGA – 3. Primers bind at the position 4900746-4901198. (Bos taurus chromosome 19 genomic contig, reference assembly, based on Btau_4.0 ref | NW_001493688.2 | Bt19_WGA1736_4).

Reaction mixture consisted of 25 µl of master mix for PCR as provided by Fermantas, 10 µl of DNA 4 µl of each forward and reverse primer and finally 7 µl of distilled water was added, to make a total volume of 40 µl. Conditions were set as follows: Denaturation at 94°C for 30 s, annealing at 60°C for 30 s, extension at 72°C for 30 s and then final extension at 72°C for 5 min, for 45 cycles. The amplified product was visualized on 1.0% agarose gel after electrophoresis using ethidium bromide.

RESULTS AND DISCUSSION

Variation at the 5′ coding sequence on level of expression of bovine growth hormone has been previously studied (Sami et al. 1990; Wallis et al. 1995). Hecht and Geldermann (1996) reported that the region (5′-untranslated and the signal peptide coding regions) of GH gene is potentially involved in the regulation and expression of the bovine growth hormone gene. They identified six sites of variable nucleotides in the 5′ flanking region of the GH gene and one in the intron I. Therefore, the region of GH gene is important to study the possible polymorphisms in the 5′ flanking first exon and part of the first intron of GH gene in local species of *B. indicus.*

Genomic DNA was isolated from blood collected from freshly slaughtered cow from a local slaughter house. 2 µg of freshly prepared genomic DNA was used to amplify the 5' flanking region of growth hormone gene at positions 4900746 - 4901198, Bos taurus chromosome 19 genome based on Batu 4.0. This position is considered to be important by a number of workers for polymorphism in GH gene (Ferraz et al., 2003, 2006; Hediger et al., 1990; Carnicela et al., 2003; Chikuni, 1991; Lagziel and Soller, 1999; Unanian et al., 1994). Ferraz et al. (2003, 2006) reported a number of variations in the region, which provide a ground for studying the variations in the region of GH 2 gene. Ferraz et al. (2003, 2006) also reported two allele with the variations as 2.1 and 2.2 allele for GH gene. We are able to amplify the gene in the region 4900746 – 4901198 and a product of 453 base pairs was obtained, (Figure 1). The amplified part of GH gene was sequenced and it was revealed that the region is susceptible to mutation, as we found out 9 variations, including 5transversions, 3 transitions and one addition with reference to GH allelele 2.1 (AY662651.1) and 2.2 (AY662652.1) (Ferraz et al., 2006). All the three sequences aligned in Clustal W, with reference to Batu 4.0 are shown in turquoise color, yellow shows the position of mutation in all four sequences (Figure 2). Figure 2 results are summarized in Table 1, as first replacement was at position 80 with reference to binding

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Batu 4.0       TCTCAAGCTGAGACCCTGTGTGCACAGCCCTCTGGCTGGTGGCAGTGGAG 50
AY662651.1     TCTCAAGCTGAGACCCTGTGTGCACAGCCCTCTGGCTGGTGGCAGTGGAG 50
AY662652.1     TCTCAAGCTGAGACCCTGTGTGCACAGCCCTCTGGCTGGTGGCAGTGGAG 50
FN666263       TCTCAAGCTGAGACCCTGTGTGCACAGCCCTCTGGCTGGTGGCAGTGGAG 50
               **************************************************

Batu 4.0       ACGGGATGATGACAAGCCTGGGGGACATGACCCCAGAGAAGGAACGGGAA 100
AY662651.1     ACGGGATGATGACAAGCCTGGGGGACATGACCCCAGAGAAGGAACGGGAA 100
AY662652.1     ACGGGATGATGACAAGCCTGGGGGACATGACCCCAGAGAAGGAATGGGAA 100
FN666263       ACGGGATGATGACAAGCCTGGGGGACATGGCCCCAGAGAAGGAATGGGAA 100
               ***************************** ************** *****

Batu 4.0       CAGGATGAGTGAGAGGAGGTTCTAAATTATCCATTAGCACAGGCTGCCAG 150
AY662651.1     CAGGATGAGTGAGAGGAGGTTCTAAATTATCCATTAGCACAGGCTGCCAG 150
AY662652.1     CAGGATGAGTGAGAGGAGGTTCTAAATTATCCATTAGCACAGGCTGCCAG 150
FN666263       CAGGATGAGTGAGAGGAGGTTCTAATTTATCCATTAGCCCAGGCTCCCAG 150
               ************************* ************ ****** ****

Batu 4.0       TGGTCCTTGCATAAATGTATAGAGCACACAGGTGGGGGGAAAGGGAGAGA 200
AY662651.1     TGGTCCTTGCATAAATGTATAGAGCACACAGGTGGGGGGAAAGGGAGAGA 200
AY662652.1     TGGTCCTTGCATAAATGTATAGAGCACACAGGTGGGGGGAAAGGGAGAGA 200
FN666263       TGGTCCTTGCATAAATGTATAGAGCACACAGGTGGGGGGAAAGGGAGAGA 200
               **************************************************

Batu 4.0       GAGAAGAAGCCAGGGTATAAAAATGGCCCAGCAGGGACCAATTCCAGGAT 250
AY662651.1     GAGA---AGCCAGGGTATAAAAATGGCCCAGCAGGGACCAATTCCAGGAT 247
AY662652.1     GAGAAGAAGCCAGGGTATAAAAATGGCCCAGCAGGGACCAATTCCAGGAT 250
FN666263       GAGAAGAAGCCAGGGTATAAAAATGGCCCAGCAGGGACCAATTCCAGGAT 250
               ****   *******************************************

Batu 4.0       CCCAGGACCCAGTTCACCAGACGACTCAGGGTCCTGTGGACAGCTCACCA 300
AY662651.1     CCCAGGACCCAGTTCACCAGACGACTCAGGGTCCTGTGGACAGCTCACCA 297
AY662652.1     CCCAGGACCCAGTTCACCAGACGACTCAGGGTCCTGTGGACAGCTCACCA 300
FN666263       CCCAGGACCCAGTTCACCAGACGACTCAGGGTCCTGTGGACAGCTCACCA 300
               **************************************************

Batu 4.0       GCTATGATGGCTGCAGGTAAGCTCGCTAAAATCCCCTCCATTCGCGTGTC 350
AY662651.1     GCTATGATGGCTGCAGGTAAGCTCGCTAAAATCCCCTCCATTCGCGTGTC 347
AY662652.1     GCTATGATGGCTGCAGGTAAGCTCTCTAAAATCCCCTCCATTCGCGTGTC 350
FN666263       GCTATGATGGCTGCAGGTAAGCTCGCTAAAATCCCCTCCATTCGCGTGTC 350
               ************************ *************************

Batu 4.0       CTAAAGGGGTAATGCGGGGGGCCCTGCCGATGGATGTGTTCAGAGCTTTG 400
AY662651.1     CTAAAGGGGTAATGCGGGGGGCCCTGCTGATGGATGTGTTCACAGCTTTG 397
AY662652.1     CTAAAGGGGTAATGCGGGGGGCCCTGCCGATGGATGTGTTCAGAGCTTTG 400
FN666263       CTAAAGGGGTAATGCGGGGGGCCCTGCCGATGGATGTGTTCAGAGCTTTG 400
               *************************** ************** *******

Batu 4.0       GGCTTTAGGGCTTCCGAATGTGAACATAGGTATCTACACCCAGACATTTG 450
AY662651.1     GGCTTTAGGGCTTCCGAATGTGAACATAGGTATCTACACCCAGACATTTG 447
AY662652.1     GGCTTTAGGGCTTCCGAATGTGAACATAGGTATCTACACCCAGACATTTG 450
FN666263       GGCTTTAGGGCTTCCGAATGTGAACATAGGTATCTACACCCAGACATTTG 450
               **************************************************

Batu 4.0       GCC 453
AY662651.1     GCC 450
AY662652.1     GCC 453
FN666263       GCC 453
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Figure 2. Alignment of nucleotide sequences of 5' flanking regions of growth hormone gene (with reference to Bos taurus Genome (Btau_4.0), 4900746-4901198) of first exon in *Bos indicus* for Bos taurus Genome (Btau_4.0), GH allele 2.1 and GH allele 2.2 (accession no. AY662651.1, AY662652.1), GH allele 2.3 (Accession no. FN666263) (shown in red) using CLUSTAL W 2.0.12 multiple sequence alignment. Mutation with reference to Bos taurus Genome (Btau_4.0), are shown in turquoise color, yellow shows the position of mutation in all four sequences. TATA box shown in orange while exon 1 shown in blue color.

site of primer P3 (at 4900746), G replaced A (Transition),second change was a replacement of T with C at position 95 (transition), third change was a transversion where T replaced A at position 126, fourth change was observed for position 139 where C replaced A and fifth change was for position 146 where C replaced G for a transversion. All these changes were upstream TATA box. Changes at position 80, 126, 139 and 146 are not reported with reference to Bos taurus Genome (Btau_4.0).

Some of these variable sites were reported to be potential binding sites for transacting factors and

Table 1. Table shows the Mutation (transversion/transition) recorded in GH allele 2.3 gene of Bos indicus, as compared to Batu 4.0, GH allele 2.1 and 2.2 (accession no. AY662651.1, AY662652.1).

S/N	Comparison to GH gene	Position	Replacement	Transition / Transversion
1	GH allele 2.1; GH allele 2.2; Batu 4.0	80	G replace A	Transition
2	GH allele 2.1; Batu 4.0	95	T replace C	Transition
3	GH allele 2.1; GH allele 2.2; Batu 4.0	126	T replace A	Transversion
4	GH allele 2.1; GH allele 2.2; Batu 4.0	139	C replace A	Transition
5	GH allele 2.1; GH allele 2.2; Batu 4.0	146	C replace G	Transversion
6	GH allele 2.1	325	G replace T	Transition
7	GH allele 2.1	378	C replace T	Transition
8	GH allele 2.1	393	G replace C	Transversion
9	GH allele 2.1	Addition at 205 to 207	Addition of AGA	

therefore possibly involved in the expression of the growth hormone gene (Hecht and Geldermann, 1996). Further, 3 replacements were found for GH allele 2.1 and 2.2 between the exon I and exon II, for position 325 (G replaced T, a transition), position 378 (where C replaced T, a transition) while the third change was the replacement of G with C at position 393 for a transversion. These changes recorded are with reference to GH allele 2.1 and GH allele 2.2. The sequence was deposited for GH allele 2.3 to EMBL under the Accession No. FN666263.

The region between the exon I and exon II is important, as GH is synthesized as pre GH with a signal peptide of 26 amino acid residues. The signal peptide is coded by exon I and part of exon II, comprising of short DNA sequences, recognized by binding sites, associated with the control of gene expression and initiation. Garrido et al. (2006) summarized that it is important to explore the knowledge of the promoter region and the region 5' untranslated gene, since these regions have several binding sites for transcription regulators and inhibitors for gene expression.

Silveira et al. (2008) reported that growth hormone 1 gene (GH1) polymorphisms are possible markers for beef cattle, using the Brazilian Canchim breed as a model. The mutations reported are in the region important for the productivity of farm animals. It is suggested that the frequency of these mutations may be checked which could be related to the animal productivity in terms of milk yield, growth regulation and carcass composition.

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