Growth Hormone Receptor (GHR) gene and its applications in livestock: A review

Anamika, Dibyendu Chakraborty*, D. Kumar, Peer Mohd. Azhar, S. Gurdeep Singh, Simran singh and Aakriti Sudan

Division of Animal Genetics & Breeding, FVSc&AH, SKUAST-Jammu, R. S. Purana 181 102, Jammu, India.

Received: 29-02-2016 Accepted: 03-09-2016 DOI: 10.18805/ag.v37i3.3541

ABSTRACT

The Growth Hormone Receptor (GHR) gene provides instructions for making a protein called the growth hormone receptor. The GHR mediates biological actions of growth hormone on target cells by transducing the growth hormone (GH) signal across the cell membrane and inducing transcription of many genes, including insulin-like growth factor-1 (IGF1). The gene coding for bovine GHR gene consists of nine exons. In exon 8 of the bovine GHR gene, T/A nucleotide variation results in to change in tyrosine from phenylalanine in the transmembrane domain of the GHR protein, has been reported to be associated with a major effect on milk yield in cows. GHR (growth hormone receptor) gene has been shown to harbor a causal mutation of a QTL influencing milk yield and composition GHR gene is a polymorphic gene and the polymorphisms are related to different economic traits of different species. The GHR gene influences physical traits and helps to selection of animals. The lengths of the variable TG-repeats in the P1 promoter of the bovine GHR gene are associated with growth rates in young Angus cattle. Due to various functions of GHR are viewed as promising candidate markers for selection purposes in cattle. Thus GHR gene could be a candidate gene for application in marker assisted selection (MAS).

Key words: Economic traits, Growth hormone receptor gene (GHR), Polymorphism.

In mammals and birds, the growth and development are primarily regulated by the somatotropic axis. The somatotropic axis, also named neurocrine axis or hypothalamus-pituitary growth axis, consists of essential compounds such as growth hormone (GH), growth hormone releasing hormone (GHRH), insulin-like growth factors (IGF1 and 2), somatostatin (SS), their associated carrier proteins and receptors, and other hormones like insulin, leptin and glucocorticoids or thyroid hormones. GHR is a member of the cytokine/hematopoietin receptor superfamily (Maj et al., 2006) and consists of three functional domains of the extracellular (ligand-binding) domain, the trans-membrane domain and the cytoplasmic domain (signal transducing). The GHR gene provides instructions for making a protein called the growth hormone receptor. This receptor is embedded in the outer membrane of cells throughout the body and is most abundant in liver cells. The growth hormone receptor has three major parts: An extracellular region that sticks out from the surface of the cell, a trans-membrane region that anchors the receptor to the cell membrane, and an intracellular region that transmits signals to the interior of the cell. The extracellular region attaches (binds) to a substance called growth hormone, fitting together like a lock and it’s key. The binding of growth hormone triggers signalling via the intracellular region of the receptor that stimulates the growth and division of cells. This signalling also leads to the production, primarily by liver cells, of another important growth-promoting hormone called insulin-like growth factor I (IGF-1).

Organization of GHR gene: The bovine GHR gene has been mapped to BTA 20, between TGLA126 and GMBT41 (Moody et al., 1995).

The gene coding for bovine GHR consists of nine exons (numbered 2 to 10) in the translated part and a long 5'-noncoding region that includes nine un-translated exons-1A through 1I (Jiang and Lucy, 2001). Among them, only exons 1A, 1B and 1C are well characterised; the existence of exons 1D to 1I is based on RACE (Rapid Amplification of cDNA End) analyses only. Exons from the un-translated regions are spliced alternatively resulting in mRNAs differing in the 5'-untranslated region (5'-UTR). Exon 2 encodes a single peptide, exons 3-7 encode the extracellular GH-binding domain, exon 8 encodes the transmembrane domain and exons 9-10 encode an intracellular domain.

The GHR gene is used in animals as a nuclear DNA phylogenetic marker (Gonzalez et al., 2007). The exon 10 had first been experienced to explore the phylogeny of the major groups of Rodentia as reported by Adkins et al. (2001) and Blanga- Kanfi et al. (2009).

Applications of GHR gene
- The GHR gene is used in animals as a nuclear DNA phylogenetic marker.
• GHR gene encodes a protein i.e., growth hormone receptor protein, is a transmembrane receptor for growth hormone.
• Growth hormone (GH) activity depends on the GH receptor (GHR). Binding of growth hormone to the growth hormone receptor leads to receptor dimerization and the activation of an intra-cellular and inter-cellular signal transduction pathway leading to growth.
• The GHR mediates biological actions of growth hormone on target cells by transducing the GH signal across the cell membrane and inducing transcription of many genes, including IGF1 (Rotwein et al., 1994; Argetsinger and Carter-Su, 1996).
• Growth hormone and IGF-I also influence metabolism, including how the body uses and stores carbohydrates, proteins, and fats from food.
• Growth hormone is the main regulator of postnatal growth and metabolism in mammals, stimulating anabolic processes such as cell proliferation, skeletal growth and protein synthesis, by modulating the expression of many genes (Burton et al., 1994).
• Growth hormone (GH) has a key role in mammary gland development and milk production (Akers et al., 1981). Also, GH has a tissue-specific action that is either direct or indirect via IGF-I, and the effect of this action depends on the GH receptor (GHR) and several other hormones.
• Curi et al. (2005) reported that Growth hormone (GH), insulin-like growth factors 1 and 2 (IGF1 and IGF2) and their associated binding proteins and transmembrane receptors (GHR, IGF1R and IGF2R) play an important role in the physiology of mammalian growth.
• A common alternate allele of GHR gene, called GHRd3, which lacks exon three. Mutations in this gene have been associated with Laron syndrome, also known as the growth hormone insensitivity syndrome (GHIS), a disorder characterized by short stature (proportional dwarfism).

Polymorphisms in Growth Hormone Receptor (GHR) gene in livestock: Several polymorphic sites in the 5’-noncoding region and in exon 10 of the bovine GHR gene have been identified (Aggrey et al., 1999; Blott et al., 2003; Falaki et al., 1996; Ge et al., 2000; Maj and Zwierzchowski, 2005). One of the sequence variations, the T/A transversion in exon 8, results in an amino acid F279Y substitution in the transmembrane domain of the receptor. The mutation was shown to have a strong effect on the yield and composition of milk in Holstein-Friesian and Jersey cattle (Blott et al., 2003). Till date, no polymorphisms have been reported in bovine species for exon 1A and exons 3-7 coding for the extracellular domain of GHR gene.

Lucy et al. (1998) reported TG-repeat (microsatellite) polymorphism in length of the of the bovine GHR gene P1 promoter, located 86 bp upstream from the start site of exon 1A. They found that an 11-TG-repeat allele commonly occurred in Bos indicus cattle while alleles with 16 to 20 consecutive TGs were the most common in Bos taurus breeds. Three mutations – A/G transition at position -154 (RFLP-NsiI), C/T transition at position 1104 (RFLP-Fnu4HI), and variable TGn repeat – were located in the regulatory sequences for the GHR gene, upstream to the alternative exon 1A.

Hale et al. (2000) reported correlation between the lengths of the variable TG-repeats in the P1 promoter of the bovine GHR gene and growth rates in young Angus cattle. Hale et al. (2000) reported that TG11 GHR allele was associated with lower growth rates in Angus steers.

Ge et al. (2000) reported SNPs were at positions 76 (T -> C), 200 (G -> A), 229 (T -> C), and 257 (A -> G) bp from the 52' end of the fragment (528-bp long) in exon 10 of the bovine GHR gene. Exon 10 of the GHR gene codes for the cytoplasmic domain of the growth hormone receptor. The SNP at 200 bp and 257 bp from the 52' end changed amino acid encoding from Ala (GCC) to Thr (ACC) and from Ser (AGC) to Gly (GGC), respectively. The other two SNPs did not cause amino acid change.

Blott et al. (2003) fine mapped QTL on chromosome 20 in Holstein–Friesian cattle by using a dense marker map and by exploiting linkage disequilibrium resulted in a relatively narrow region including the GHR gene. They reported two missense mutations in GHR gene and F279Y polymorphism, was associated with strong effect on milk yield and composition. The first (SNP1) was a phenylalanine–tyrosine substitution (F279Y) in the transmembrane domain of the receptor (exon 8) and the second (SNP2) substitution was a replacement of a polar asparagine with a polar threonine (N528T) in the cytoplasmic domain (exon 10). However, they concluded that two or more QTL could exist within the region and in addition to GHR gene another candidate genes may also play a key role in lactation.
Maj et al. (2004) reported polymorphisms in GHR gene and its association with both the IGF1 expression in liver and its level in blood in Polish Holstein-Friesian cattle. They concluded that GHR and IGF1 genes were associated with milk production and meat production of cattle. They also found that GHR genotype significantly influenced the IGF1 expression in the liver and the highest expression for the genotypes was found in: RFLP-AluI (AT), RFLP-Fnu4HI(CC), and RFLP-NsiI(GA), and for the combined GHR genotype: AluI(AT)/Fnu4HI(CC)/NsiI(GA). They reported GHR haplotypes significantly affected the IGF1 blood level and combined GHR genotypes AluI(AA)/AccI(CC)/Fnu4HI(CC)/NsiI(AG) and AluI(AA)/AccI(CT)/Fnu4HI(CC)/NsiI(AG) animals had a higher IGF1 concentration in blood than other genotype carriers.

Curi et al. (2005) genotyped 384 bulls including 79 Nellore, 30 Canchim (5/8 Charolais + 3/8 Zebu) and 275 crossbred animals originating from crosses of Simmental (1/2 Simmental, n = 30) and Angus (1/2 Angus, n = 245) sires with Nellore females. The effects of substituting L allele for S allele of GHR microsatellite across Nellore, Canchim and 1/2 Angus were significant for weight gain and body weight (P < 0.05).

Di Stasio et al. (2005) reported polymorphisms at GHR locus at position 257 in exon 10 (G/A substitution causing change of amino acid Ser to Gly) detected by PCR-AluI, which was associated with meat quality in Piemontese breed of cattle. They also reported that GHR variant was associated with unfavourable effect on meat quality with higher drip losses.

Pariset et al. (2006) reported one SNP (AY292283:g. 122A.G) in exon 8 European ovine breeds.

Viitala et al. (2006) studied by multi-marker regression analysis in Finnish Ayrshire into two QTL segregated on the chromosomal region 20 including GHR gene. By sequencing the coding sequences of GHR and the sequence of three GHR promoters from the pooled samples of individuals of known QTL genotype, F-to-Y substitution in the transmembrane domain of GHR gene was identified that was associated with milk production traits. They concluded that the GHR F279Y had the highest influence on protein percentage and fat percentage. The coding sequences of GHR (exons 2–10) and the sequence of three well-characterized GHR promoters were screened and a total of five exonic SNPs were detected in GHR, four of which (sp1, -2, -3, and -4) lead to an amino acid substitution (Table 1).

Maj and Zwierzchowski (2008) studied gene coding for bovine GHR consists of nine protein-coding exons and untranslated, alternative exons 1A, 1B, and 1C in its 5′-region in one-hundred-and-fifty-three young Black-and-White (BW) bulls and the animals were genotyped for GHR RFLP-NsiI, RFLP-Fnu4HI, and variable TG microsatellite. They concluded that P1 promoter, responsible for growth hormone receptor expression in the liver was associated with exon 1A.

Three conformational patterns by SSCP were found in Baluchi sheep at exon 10 of GHR gene (Valeh et al., 2009).

Rahbar et al. (2010) developed a PCR-based method for detecting AluI RFLP in promoter region of the bovine growth hormone receptor (GHR) gene and it has been tested for association with milk-related traits in Holstein cows.

Oleński et al. (2010) reported polymorphisms in GHR gene exon 10 (S555G) by using PCR-RFLP method in 395 Polish Holstein-Friesian and 477 Polish Holstein-Friesian bulls with the frequencies of alleles: A – 0.832 and 0.891 and G – 0.168 and 0.109 for cows and bulls, respectively. They reported that A allele was significantly related to fat yield, protein yield and fat content whereas, A allele significantly increased bulls’ breeding value for protein content, although, the results showed inconsistency of associations between cow and bull data.

Zulkharnaim et al. (2010) estimated genetic diversity of the GHR|AluI gene in different cattle breeds. Single nucleotide polymorphisms (SNP) had been found in exon 10, coding for the cytoplasmic domain of GHR, which was located at position 81 bp (A/G) induced amino acid substitutions Ser/Gly.

Bai et al. (2011) investigated single nucleotide polymorphism (SNP) of growth hormone receptor (GHR) gene exon 10, in one hundred seventy-eight goats from Liaoning Cashmere (96), Inner Mongolia White Cashmere (40), and Chengdu Grey (42) breeds in China by polymerase chain reaction-single strand conformation polymorphism (PCR-SSCP). In all goat breeds investigated, a SNP in exon 10 of GHR gene consisting of a single nucleotide substitution A → G were identified and found that the allele A was more

### Table 1: GHR polymorphisms and allele frequencies in Finnish Ayrshire cattle as reported by Viitala et al. (2006)

<table>
<thead>
<tr>
<th>SNP</th>
<th>Polymorphism</th>
<th>Exon</th>
<th>Substitution</th>
<th>Freq.</th>
<th>Flanking sequence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Snpl</td>
<td>F279Y</td>
<td>8</td>
<td>T/A</td>
<td>0.89/0.11</td>
<td>TTATT/ATTTA</td>
</tr>
<tr>
<td>Spi2</td>
<td>N528T</td>
<td>10</td>
<td>A/C</td>
<td>0.63/0.37</td>
<td>GACAA/CCGCT</td>
</tr>
<tr>
<td>Spi3</td>
<td>A541S</td>
<td>10</td>
<td>G/T</td>
<td>0.90/0.10</td>
<td>CATTG/TCCCC</td>
</tr>
<tr>
<td>Spi4</td>
<td>S555G</td>
<td>10</td>
<td>A/G</td>
<td>0.87/0.13</td>
<td>GCCAA/GGCTT</td>
</tr>
</tbody>
</table>
common in the animals investigated. They found no significant association between the polymorphism revealed and the cashmere traits analyzed.

Akad et al. (2012) reported several polymorphic sequences in the bovine GHR gene by PCR-RFLP using AluI, AccI, StuI, NsiI, and Fnu4HI restriction enzymes in East Anatolian Red cattle, South Anatolian Red cattle and Turkish Grey cattle and they were found to be associated with milk and meat traits.

Othman et al. (2012) reported monomorphic (GG genotype only) GHR gene in Egyptian river Buffalo in PCR-RFLP technique by AluI endonuclease.

Deepika and Salar (2013) studied GHR gene polymorphisms in exon 10 and 5’-noncoding regions of 453 animals belonging to 10 indigenous grey cattle breeds (Hariana, Kankrej, Mewati, Nagori, Tharparkar, Ghumusari, Hill cattle, Kangayam, Binjharpuri and Punganur) from different agro-climatic regions of India. Target regions of GHR gene were amplified and digested by AluI and NsiI restriction enzymes. They reported 79.2% animals were homozygous for AA genotype and 20.8% of animals were heterozygous AG genotype. And no animals were homozygous for GG genotype.

Bahrami et al. (2013) reported monomorphon exon 10 of GHR gene in Mehraban sheep by using PCR-SSCP technique.

Janmeda and Vataliya (2014) reported GHRI locus as monomorphon for MueHI restriction enzyme by PCR-RFLP technique in Mehsana buffalo and only one type of genotype i.e. RR was found in all the buffaloes.

Hussain (2015) reported only GG genotypes in 100 Mesopotamian Buffaloes for exons 10 of GHR gene in PCR-RFLP technique by using AluI endonuclease.

CONCLUSION

GHR gene is a polymorphic gene and the polymorphisms are related to different economic traits of different species e.g. higher body weights in small ruminants, higher production in dairy animals etc. There is a significant association between the detected SNPs and different economic traits of livestock and are effective in selection for better animals with better performance. Growth hormone receptor (GHR) gene is significantly associated with the productive traits in livestock like growth, carcass, milk yield and composition. Thus it can be concluded that GHR gene could be a candidate gene for application in marker assisted selection (MAS).

REFERENCES


