Association of two SNPs in the coding region of the insulin-like growth factor 1 receptor (IGF1R) gene with growth-related traits in Angus cattle

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Abstract The insulin-like growth factor 1 (IGF-1) is considered to be a factor that mainly regulates growth, differentiation, and the maintenance of various function in numerous tissues through binding to a family of transmembrane tyrosine kinase receptors, signaling primarily through the insulin-like growth factor 1 receptor (IGF-1R) encoded by the IGF1R gene. The objectives of the present study were to estimate the allele and genotype frequencies of the IGF1R/MspI (silent mutation within exon 12) and the IGF1R/TaqI (within the 3′ untranslated region, 3′UTR) gene polymorphisms in beef cattle and to determine associations between these polymorphisms and growth traits. In a preliminary study on 310 Angus calves, association analyses with three production traits (birth weight, BWT; weaning weight adjusted to 210 days, WWT210; and average daily gain, ADG) were conducted. The GG genotype of the IGF1R/e12/MspI polymorphism was significantly associated (P≤0.05) with a higher WWT210 (+5.06 kg) compared to the AG genotype. Polymorphism within the 3′UTR had no significant effect on growth traits. The effect of combined genotypes was also examined. At WWT210, calves with the GG/AA and GG/AG combinations were heavier than calves with the AG/AA and AG/AG combined genotypes (P≤0.05). To our knowledge, this is the first report of a polymorphism within the coding region of the Bos taurus IGF1R gene.

Keywords Beef · Genetic markers · IGF1R · Quantitative trait loci

The insulin-like growth factor (IGF) family includes three ligands, three cell membrane receptors, six binding proteins, and a number of other associated proteins. In mammals, insulin regulates cell metabolism, whereas insulin-like growth factor 1 (IGF-1) is an important regulator of cell growth (Ullrich et al. 1986). The majority of circulating IGF-1 is produced by the liver, where its expression is stimulated by growth hormone (GH). However, other organs can also produce IGF-1 in an autocrine and paracrine manner, including bovine mammary gland and muscle (Plath-Gabler et al. 2001). The insulin-like growth factor 1 receptor (IGF-1R) is a receptor-tyrosine kinase that plays a crucial role in signaling that is important for cell survival and proliferation. After binding to the primary ligand (IGF-1), signal cascade activates the phosphoinositide 3-kinase (PI3K), promotes cell proliferation by activating the mitogen-activated protein kinase (MAPK), and blocks apoptosis by inducing the phosphorylation and inhibition of proapoptotic proteins (Galvan et al. 2003).

In view of the important role of genes coding for components of the somatotropic axis (GH-GHR-IGF1-IGF1R) in growth regulation, the objectives of the present study were to estimate the allele and genotype frequencies of the two SNPs (rs41640706 and rs41960562) within the coding sequence of the bovine IGF1R gene in beef cattle and to determine the effects of these markers on growth traits in animals under an intensive production system. Both of these SNPs were submitted in the year 2006, but they have not been validated so far. The study involved 310 purebred Angus female calves of 54 sires kept in the years 2005–2010 on a single farm located in the West Pomeranian province, Poland. The DNA was isolated using the MasterPure™ DNA Purification Kit.
of the et al. (1989) protocol was developed to introduce an artificial amplification-created restriction site (ACRS) (Haliassos et al., 1989) protocol was developed to introduce an artificial amplification-created restriction site (ACRS) (Haliassos et al., 1989). The TaqI recognition site of the Bos taurus IGF1R gene was used to amplify a 164-bp DNA fragment. The TaqI recognition site (T&lt;cg&gt;) was engineered by enforcing a base change within a new forward primer by replacing T with C (<i>T&lt;ca&gt;</i>→<i>C&lt;ga&gt;</i>); reverse: 5′-ctctgcattgtctgct-3′; mismatch is underlined). A 163-bp DNA fragment was amplified using 164-bp DNA fragment. The second polymorphic site (rs41960562; A→G transition) was located within the 3′ untranslated region (3′UTR) of the Bos taurus IGF1R gene. The TaqI recognition site (T&lt;cg&gt;) was engineered by enforcing a base change within a new forward primer by replacing T with C (<i>T&lt;ca&gt;</i>→<i>C&lt;ga&gt;</i>); reverse: 5′-ctctgcattgtctgct-3′; mismatch is underlined). A 163-bp DNA fragment was then amplified.

PCR was carried out in a total volume of 20 μl containing 60–80 ng of DNA template, 0.5 U of Taq DNA polymerase, buffer with (NH₄)₂SO₄, MgCl₂, dNTP mix (MBI Fermentas/ABO, Gdansk, Poland), 10 pmol of each primer (Oligo, IBB PAN, Warsaw), and nuclease-free deionized water (Epicentre Technologies™, AKOR Gdansk, Poland). The reaction conditions were as follows: an initial DNA template denaturation at 94 °C for 5 min, followed by 33 cycles of: denaturation of DNA template at 94 °C for 50 s, annealing (<i>T<sub>a</sub></i> = 59.5 °C; forward: 5′-ttctgcattgtctgct-3′; reverse: 5′-ctctgcattgtctgct-3′) was used to amplify a 164-bp DNA fragment. The 164-bp fragment amplified to identify rs41640706 polymorphism was cleaved by TaqI in the presence of the IGF1R<sup>A</sup> allele into two fragments of 145 bp and 18 bp; when the IGF1R<sup>G</sup> allele was present, no cleavage was observed (163 bp) (Fig. 1). Among the 310 calves that were genotyped the 163-bp fragment, the PCR product was digested for 2 h at 65 °C with 5 units of the TaqI restriction enzyme (10 U/μl, TΔCGA; MBI Fermentas/ABO, Gdansk, Poland). After digestion, 10 μl of each of the products was then separated by electrophoresis in 2 % ethidium bromide-stained agarose gels (Basica Prona™ Agarose, ABO, Gdansk, Poland).

The association of birth weight (BWT), weaning weight adjusted to 210 days of age (WWT<sub>210</sub>), as well as average daily gains between birth and weaning (ADG), with the selected SNPs within the bovine IGF1R gene was analyzed based on the data obtained from the official recordings.

Statistical calculations were performed using a general linear model (GLM) incorporated in the procedure of the STATISTICA 9.0 PL software package (StatSoft, Inc., 2009). The following statistical model was used:

\[ Y_{ijkl} = \mu + G_i + s_j + BYS_k + e_{ijkl} \]

where \( Y_{ijkl} \) is the analyzed trait, \( \mu \) the overall mean, \( G_i \) the fixed effect of the IGF1R genotype or combination of genotypes, \( s_j \) the random effect of the sire, \( BYS_k \) the fixed effect of the year/season of birth, and \( e_{ijkl} \) is the random error. The 164-bp fragment amplified to identify rs41640706 SNP contained a single Mspl recognition site. The digestion resulted in two fragments (51 and 113 bp) in the case of the IGF1R<sup>Δ</sup> allele and one uncut 164-bp-long fragment carrying the IGF1R<sup>G</sup> allele (Fig. 1). The GG genotype had the highest frequency (0.593), followed by the AG genotype (0.371). The least frequent genotype was AA (n = 11, only 3.6 % of the analyzed population). The frequency of the rare IGF1R<sup>A</sup> allele was 0.22.

The 3′UTR PCR product carrying the rs41960562 polymorphism was cleaved by TaqI in the presence of the IGF1R<sup>A</sup> allele into two fragments of 145 bp and 18 bp; when the IGF1R<sup>G</sup> allele was present, no cleavage was observed (163 bp) (Fig. 1). Among the 310 calves that were genotyped for this polymorphism, 196 (63.2 %) were of the AA genotype, 95 (30.7 %) were of the AG genotype, and only 19 individuals (6.1 %) possessed the GG genotype (allele IGF1R<sup>G</sup>; 79 %; IGF1R<sup>A</sup>; 21 %).

Table 1 shows the effect of the RFLP-Mspl and ACRS-TaqI polymorphisms in the IGF1R gene on growth performance. The IGF1R/e12/Mspl polymorphism had a significant effect only on WWT<sub>210</sub> (P≤0.05). The GG genotype was associated with a higher WWT<sub>210</sub> (+5.06 kg) when compared to GG genotypes.
Table 1  Mean values of growth traits of Angus cows with the different IGF1R gene variants (standard errors in parentheses)

| SNP      | Genotype or combined genotype | n   | f    | BWT (kg) | ADG (g) | WWT210 (kg) |
|----------|-------------------------------|-----|------|----------|---------|-------------|
| IGF1R/MspI | GG                            | 184 | 0.593| 36.49 (0.23) | 994.5 (4.9) | 247.61a (1.45) |
|          | AG                            | 115 | 0.371| 36.88 (0.32) | 984.7 (7.0) | 242.55a (1.77) |
| IGF1R/TaqI | AA                            | 196 | 0.632| 36.68 (0.22) | 988.6 (4.8) | 245.84 (1.42) |
|          | AG                            | 95  | 0.307| 36.80 (0.34) | 994.9 (7.7) | 246.31 (2.13) |
| IGF1R MspI/TaqI | GG/AA                       | 109 | 0.352| 36.46 (0.28) | 993.6 (6.2) | 247.98b (1.91) |
|          | GG/AG                         | 60  | 0.193| 36.67 (0.44) | 1000.7 (9.5) | 248.13c (2.68) |
|          | AG/AA                         | 78  | 0.252| 36.97 (0.37) | 982.8 (8.3) | 242.59b (2.22) |
|          | AG/AG                         | 34  | 0.110| 37.09 (0.56) | 982.1 (13.0) | 241.18b (2.93) |

Values within columns bearing the same letters differ significantly at P≤0.05
n number of animals in the group; f frequency; BWT birth weight; ADG average daily gains between birth and weaning; WWT210 weaning weight adjusted to 210 days of age

The bovine IGF1R gene was mapped to chromosome 21 (Moody et al. 1996). It consists of at least 21 exons encoding 1367 aa of unprocessed protein chain, including a 30-residue signal peptide. Like in humans, gene and protein organization is structurally related to the insulin receptor (Ullrich et al. 1986). Davis et al. (1998) and Casas et al. (2003) reported significant quantitative trait loci (QTL) for birth weight in the centromeric region of BTA21. The most significant QTL was at 4 centimorgans (cM) from the beginning of the linkage group on chromosome 21. Therefore, the gene(s) of interest should be located in the candidate region up to 10 cM. With respect to this criterion, we have identified several genes located in the candidate region, based on the Bos taurus UMD_3.1/Btau 4.6.1 genome assemblies (http://www.ncbi.nlm.nih.gov/assembly/; RefSeq ID AC_000178.1/NC_007319.5). One of the most promising candidates is the IGF1R gene (relative position: 8.278 cM), closely related to IGFI, located in the QTL for birth weight on BTA5 (Kim et al. 2003).

Little is known about the associations between the polymorphisms within the bovine IGF1R gene and meat production traits. The IGF1R/TaqI polymorphism within intron 12, detected originally by Moody et al. (1996), seems to be exclusive to Bos indicus. To date, very few data are available regarding the effect of this polymorphism on growth and carcass traits in Bos indicus (Curi et al. 2005) and Chinese indigenous beef cattle (Zhang and Li 2011).

In the present study, we described a novel protocol to detect silent mutation within exon 12 (rs41640706; GenBank accession no. JQ924783) that does not affect the protein sequence. The proline-807+30 residue is localized exactly in the middle of the extracellular sequence of the IGF-1R β subunit (residues 711 to 905 upstream from the transmembrane domain; amino acids of the pro-receptor are numbered starting at Glu 1, preceded by a 30-residue signal peptide). The second polymorphic site, annotated as rs41960562 (see also GenBank accession no. JQ957797), is one of the several SNPs detected within the long 3’UTR (exon 21) and, therefore, it is not related to the amino acid substitution either. However, point mutations within the 3’ UTR can be associated with the determination of mRNA stability/instability and, in special cases, with diseases. MicroRNAs (miRNAs) function post-transcriptionally by base-pairing to the mRNA 3’UTRs to repress protein synthesis by mechanisms that have not yet been fully understood. Current evidence suggests that the target mRNA degradation provides a major contribution to silencing by miRNAs (Huntzinger and Izaurralde 2011). To evaluate the
potential role of the aforementioned SNP, we compared the nucleotide sequences of a full-length \textit{IGF1R} 3′UTR (about 6.75 kb) of several species (incl. \textit{Bos taurus}, \textit{Homo sapiens}, \textit{Mus musculus}, and \textit{Rattus norvegicus}; TargetScanHuman rel. 6.2; http://www.targetscan.org/), and then the location of the JQ957797 sequence with the rs41960562 SNP was established. A total of 66 miRNAs families conserved only among mammals were used to predict the biological targets of these miRNAs by searching for the presence of conserved 8- and 7-mer sites within the 3′ UTR regions that would match the seed region of each miRNA (Lewis et al. 2005). The results did not indicate the presence of any conserved miRNAs overlapping the rs41960562 polymorphism. The nearest miRNA 7-mer (bta-miR-328) was located just 80-bp downstream.

The somatotropic axis contains the most promising candidate genes associated with meat production traits. Based on the knowledge of the polygenetic control of the studied physiological processes, we agree that further studies investigating the functional biology of the bovine \textit{IGF1R} gene are necessary, in order to be able to consider the polymorphisms studied in this work as the causative mutations.

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**References**

Casas E, Shackelford SD, Keele JW, Koohmaraie M, Smith TPL, Stone RT (2003) Detection of quantitative trait loci for growth and carcass composition in cattle. \textit{J Anim Sci} 81:2976–2983

Curi RA, Oliveira HN, Silveira AC, Lopes CR (2005) Association between IGF-I, IGF-IR and GHRH gene polymorphisms and growth and carcass traits in beef cattle. \textit{Livest Prod Sci} 94:159–167

Davis GP, Hetzel DJS, Corbet NJ, Scacheri S, Lowden S, Renaud J, Mayne C, Stevenson R, Moore SS, Byrne K (1998) The mapping of QTL for birth weight in a tropical beef herd. In: Proceedings of the 6th World Congress on Genetics Applied to Livestock Production, Armidale, NSW, Australia, January 1998, vol 26, pp 441–444

Galvan V, Logvinova A, Sperandio S, Ichijo H, Bredesen DE (2003) Type 1 insulin-like growth factor receptor (IGF-IR) signaling inhibits apoptosis signal-regulating kinase 1 (ASK1). \textit{J Biol Chem} 278:13325–13332

Hallassos A, Chomel JC, Tesson L, Baudis M, Kruh J, Kaplan JC, Kitzis A (1989) Modification of enzymatically amplified DNA for the detection of point mutations. \textit{Nucleic Acids Res} 17:3606

Huntzinger E, Izaurralde E (2011) Gene silencing by microRNAs: contributions of translational repression and mRNA decay. \textit{Nat Rev Genet} 12:99–110

Kim JJ, Farnir F, Savell J, Taylor JF (2003) Detection of quantitative trait loci for growth and beef carcass fatness traits in a cross between \textit{Bos taurus} (Angus) and \textit{Bos indicus} (Brahman) cattle. \textit{J Anim Sci} 81:1933–1942

Lewis BP, Burge CB, Bartel DP (2005) Conserved seed pairing, often flanked by adenosines, indicates that thousands of human genes are microRNA targets. \textit{Cell} 120:15–20

Moody DE, Pomp D, Barendse W (1996) Linkage mapping of the bovine insulin-like growth factor-1 receptor gene. \textit{Mamm Genome} 7:168–169

Plath-Gabler A, Gabler C, Sinowatz F, Berisha B, Schams D (2001) The expression of the IGF family and GH receptor in the bovine mammary gland. \textit{J Endocrinol} 168:39–48

StatSoft, Inc.: \textit{STATISTICA} (data analysis software system) 2009, version 9.0 PL. http://www.statsoft.com

Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Henzel W, Le Bon T, Kathuria S, Chen E, Jacobs S, Francke U, Ramachandran J, Fujita-Yamaguchi Y (1986) Insulin-like growth factor I receptor primary structure: comparison with insulin receptor suggests structural determinants that define functional specificity. \textit{EMBO J} 5:2503–2512

Zhang R, Li X (2011) Association between IGF-IR, \textit{m-calpain} and \textit{UCP-3} gene polymorphisms and growth traits in Nanyang cattle. \textit{Mol Biol Rep} 38:2179–2184