The T945M Single Nucleotide Polymorphism of the Bovine Leptin Receptor Gene in Population of Slovak Spotted Bulls

Anna TRAKOVICKÁ
Nina MORAVČÍKOVÁ (✉)
Martina MILUCHOVÁ

Summary

The objective of this study was detection of DNA polymorphism of the leptin receptor gene using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The SNP T945M, which maps on bovine chromosome 3 at the exon 20 of the leptin receptor sequence and corresponds to a mutation in the intracellular region of the functional protein, was also analyzed. In exon 20, a T to C missense mutation was found at nucleotide 115, which causes an amino acid substitution at residue 945 (T954M). The polymorphism of leptin receptor gene was studied in a group of 57 bulls of Slovak spotted breed. A strategy employing PCR was used to amplify 197 bp products from blood samples. Digestion of PCR products with restriction enzyme *Bse*GI revealed two alleles: allele C was 130 and 67 fragments and allele T was 93, 67 and 37. Frequencies for allele C and T were 0.9737 and 0.0263, respectively and TT genotype was not detected.

Key words

bovine leptin receptor gene, cattle, polymorphism, SNP T945M

Slovak University of Agriculture in Nitra, Department of Animal Genetics and Breeding Biology, Tr. A. Hlinku 2, 949 76 Nitra, The Slovak Republic
✉ e-mail: nina.moravcikova1@gmail.com

Received: May 13, 2011 | Accepted: July 6, 2011

ACKNOWLEDGEMENTS
Authors acknowledge Scientific Agency of Slovak Republic for financial support under project VEGA No. 1/0061/10.

Introduction

Leptin is a protein hormone produced primarily by white adipose tissue and involved in regulation of feed intake, energy expenditure, growth and body composition, as well as immune system functions and several aspects of reproduction (Houseknecht et al., 1998). Leptin acts especially trough its receptor on the hypothalamus, the centre of energy homeostasis, as well as on ovarian follicular cells, on placenta and lactating mammary glands (Bartha et al., 2005; Chilliard et al., 2005). Effects of leptin are exerted trough six receptors isoforms, but only long form (LEPR-b) is fully functional and responsible for most hormone physiological functions (Tartaglia, 1997). A widespread expression of LEPR-b suggests that although most of leptin actions are mediated centrally, at the level of hypothalamus, it may also act in many peripheral tissues, including gonadal tissues (Silva et al., 2002).

The leptin receptor (LEPR) is a glycoprotein with a single transmembranespanning region. The isoforms that share identical extracellular and transmembrane domains are characterized by intracellular domains of variable length (Tartaglia, 1997). LEPR-b is the longest form and is thought to play an important role in signal transduction in the hypothalamus. LEPR-b consists of 1142 amino acids of which the first 816 form the extracellular part, the next 23 amino acids form the transmembrane region and the last 303 acids are located intracellular which play a role in signal transduction (Tartaglia, 1997). Amino acids 428-635 are important for leptin-binding (Fong et al., 1998). Leptin-binding to the LEPR results in the formation of a receptor complex leading to phosphorylation and activation of the JAK/STAT (Janus kinases/signal transducers and activators of transcription) signalling pathway. Two conserved tyrosines in the cytoplasmic domain of LEPR are important for the activation of this signalling pathway (White et al., 1997).

In ruminants, LEPR expression seems to be affected by high and low nutrition levels (Chilliard et al., 2005) and blood leptin concentrations seem to interfere in luteinizing hormone secretion and stimulate growth hormone release (Nonaka et al., 2006). Since leptin exerts its effect by interacting with receptors located in most bovine tissues (Silva et al., 2002), leptin receptor gene (LEPR) can also be considered as a candidate gene affecting productive traits.

The LEPR gene is located on bovine chromosome 3q33 (Pfister - Genskow et al., 1997) and several polymorphisms have been mapped in this chromosome, such as the short tandem repeats BM7225 at 101.7 cM, BMS694 at 94.6 cM, and BMS2145 at 93.8 (Kappes et al., 1997). The leptin receptor gene consists of 20 exons divided over 1.75 Mb. Inside the LEPR gene, Liefers et al. (2004) described a missense mutation T945M. Single nucleotide polymorphism T945M is located on chromosome 3 in the leptin receptor gene. It is a cytosine to thymine base substitution at position 115 in exon 20, which results in a substitution of the aimino acid threonine by methionine at residue 945 of leptin. The T945M polymorphism may have induced a structural change in the intracellular domain of the LEPR (Liefers et al., 2004).

The aim of this study was conducted in order to identify the polymorphism of bovine leptin receptor gene (SNP LEPR T945) in population of Slovak spotted bulls.

Material and methods

The total numbers of blood samples were taken from 57 samples of Slovak spotted bulls. Genomic DNA was extracted from whole blood samples with isolation kit NucleoSpin Blood (Macherey-Nagel). The SNP T945M, which maps at the exon 20 on bovine chromosome 3 of the LEPR sequence was analyzed using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. This mutation was previously investigated by DNA sequencing (Liefers et al., 2004). A 197 bp fragment in bovine LEPR gene was amplified by PCR using forward and reverse primers according to Almeida et al. (2008). DNA was amplified in a total volume of 25 µl containing: 10 x PCR reaction buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs, 6 pM primers (Generi-Biotech), 1 U Tag DNA polymerase (Fermentas) and 50 ng genomic DNA. PCR amplification was carried out in C1000^{TM} thermal cycler (Biorad). PCR conditions were at 94°C for 3 min, followed by 35 cycles of 94°C for 30 s, 51°C for 50 s and 72°C for 30 s. After 35 cycles, reactions were completed by an extension at 72°C for 5 min.

Table 1. Primer sequences of LEPR *Bse*GI loci

Locus	Primer sequence
LEPR *Bse*GI [1]	F 5′-ACTACAGATGCTCTACTTTGG-3′
	R 5′-TGCTCCTCCTCAGTTT-3′

Note: F= Forward, R= Reverse. [1]Almeida et al. (2008)

The PCR product for each sample was digested with 1 µl of FastDigest *Bse*GI (Fermentas) restriction enzyme at 37°C in time of 5 min. The digestion products were separated by horizontal electrophoresis in 3% agarose gels in 0.5 x TBE (160 V for 40 min) stained with GelRed (Biotium) and fragments presented T allele (93 bp, 67 bp, and 37 bp) and C allele (130 bp, and 67 bp) were visualized under UV light.

Results and discussion

Single nucleotide polymorphism T945M in exon 20 on chromosome 3 in the bovine leptin receptor gene based on the use of restriction fragment length polymorphism was detected. The digested CC PCR product exhibited two fragments of 130 and 67 bp. For the CT genotype was exhibited 130, 93, 67 and 37 bp. The TT genotype was not observed. Figure 1 shows PCR product size and the restriction patterns of the two genotypes CC and CT.

The results show, that the most frequent genotype for leptin receptor gene in observed population was CC. The frequency of the C allele was 0.9737 and for T allele was 0.0263 in group of 57 Slovak spotted bulls. Alleles and genotypes frequencies for SNP T945M in population Slovak spotted bulls are described in Table 2. We detected the presence of two genotypes CC (n=54) and CT (n=3) in studied population.

Similar results of allele and genotype frequencies were reported in study Szyda et al. (2011), Giblin et al. (2010) and Komisarek (2010). A higher frequency of C allele agree with those of Liefers et al. (2004), who verified a frequency of 0.93 in population of 323

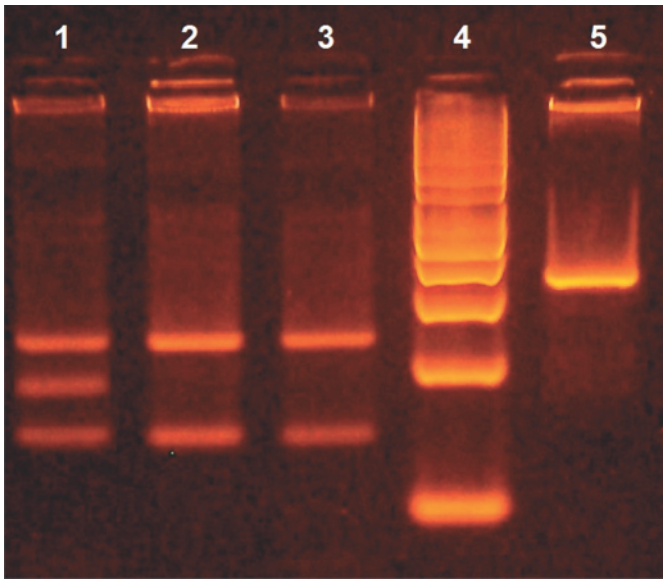

Figure 1. Representative results PCR-RFLP analysis SNP LEPR T945M on 3% agarose gel. Line1 is CT genotype (130, 93 and, 67 bp), line 2 and 3 is CC genotype (130 and, 67 bp), line 4 is a marker of molecular weight (Fermentas, 50 bp) and line 5 is PCR product (197 bp)

Table 2. Allele and genotype frequencies of SNP LEPR T945M in population of Slovak spotted bulls

Bulls (n=57)	Genotype			Allele	
	CC	CT	TT	C	T
Number	54	3	-	0.9737	0.0263
Frequency	0.9474	0.0526	-		

Holstein-Friesian cows and did not detect TT animals. Liefers et al. (2004) verified an association between the T945M mutations with circulating leptin concentrations during late pregnancy. Similar, the T allele was present at a low frequency in population Brangus Ibage, Charolais and Aberdeen Angus breeds (Almeida et al., 2008). Ferraz et al. (2009) study showed the associations between SNP T945M with carcass traits in Nellore cattle, when identified only three animals for TT genotype and additive effect were observed for T945M on longissimus dorsi muscle area and backfat thickness. Komisarek and Dorynek (2006) found significant effect of LEPR T945M polymorphism on fat and protein content in milk, when animals with TT genotype were characterized by lowest values of both traits in population of 219 Jersey cows (frequency of C and T allele were 0.79 and 0.21, respectively). According to Chen et al. (2004), Mackowski et al. (2005) and Schenkel et al. (2006), genetic variations of the bovine LEPR gene are associated with milk production traits and fatness traits. The bovine LEPR gene is associated with balance of energy; it may be a potential candidate gene for traits in animals. The genetic diversity of the Slovak spotted breed was also evaluated using the polymorphic proteins of milk (Žitný et al., 2009).

Conclusions

Genetic polymorphisms have been detected by PCR-RFLP method in the bovine leptin receptor gene. The PCR-RFLP method employed to screening the T945M SNP permitted the identification of both alleles. In the studied population of 57 Slovak spotted bulls only two genotypes were detected: the CC and CT genotype. The TT genotype was not observed. The most frequent allele in studied population was allele C with observed frequency of 0.9737. The T allele was present at a low frequency.

References

Almeida S. E. M., Santos L. B. S., Passos D. T., Corbellini A. O., Lopes B. M. T., Kirst C., Terra G., Neves J. P., Goncalves P. B. D., Moraes J. C. F., Azevedo Weimer T. (2008). Genetic polymorphsims at the leptin receptor gene in three beef cattle breeds. Genet Mol Biol 31: 680- 685.

Bartha T., Sayed-Ahmeda A., Rudas P. (2005). Expression of leptin and its receptors in various tissues of ruminants. Domest Anim Endocrinol 29: 193- 202.

Chen C. C., Chang T., Su H. Y. (2004). Characterization of porcine leptin receptor polymorphisms and their association with reproduction and production traits. Anim Biotechnol 15: 89- 102.

Chilliard Y., Delavaud C., Bonnet M. (2005). Leptin expression in ruminants: Nutritional and physiological regulations in relation with energy metabolism. Domest Anim Endocrinol 29: 3- 22.

Ferraz J. B. S., Pinto L. F. B., Meirelles F. V., Eler J. P., De Rezende F. M., Oliveira E. C. M., Almeida H. B., Woodward B., Nkrumah D. (2009). Association of single nucleotide polymorphisms with carcass traits in Nellore cattle. Genet Mol Res 8: 1360- 1366.

Fong T. M., Huang R. R., Tota M. R., Mao C., Smith T., Varnerin J., Karpitskiy V. V., Krause J. E., Van der Ploeg L. H. (1998). Localization of leptin binding domain in the leptin receptor. Molecular Pharmacology 53: 234- 240.

Giblin L., Butler S. T., Kearney B. M., Waters S. M., Callanan M. J., Berry D. P. (2010). Association of bovine leptin polymorphisms with energy output and energy storage in progeny tested Holstein-Friesian dairy cattle sires. BMC Genetics 11: 73.

Hauseknecht K. L., Baile C. A., Matteri R. L., Spurlock M. E. (1998). The biology of leptin: a review. J Anim Sci 76: 1405- 1420.

Kappes S. M., Keele J. W., Stone R. T. McGraw R. A., Sonstegard T. S., Smith T. P., Lopez-Corrales N. L., Beattie C. W. (1997). A second-generation linkage map of the bovine genome. Genome Res 7: 235- 249.

Komisarek J. (2010). Imapct of LEP and LEPR gene polymorphisms on functional traits in Polish Holstein-Friesian cattle. Anim Sci Pap Rep 28: 133- 141.

Komisarek J., Dorynek Z. (2006). The relationship between the T945M single nucleotide polymorphism in the leptin receptor gene (LEPR) and milk production traits in Jersey cows. Anim Sci Pap Rep 24: 271- 277.

Liefers S. C., Veerkamp R. W., Te Pas M. F. W., Delavaud C., Chilliard Y., Van der Lende T. (2004). A missense mutation in the bovine leptin receptor gene is associated with leptin concentrations during late pregnancy. Anim Genet 35: 138- 141.

Mackowski M., Szymoniak K., Szydlowski M., Kamyczek M., Eckert R., Rozycki M., Switonski M. (2005). Missense mutations in exon 4 of the porcine LEPR gene encoding extracellular domain and their association with fatness traits. Anim Genet 36: 135- 137.

Nonaka S., Hashizume T., Kasuya E. (2006). Effect of intracerebroventricular injection of leptin on the release of luteinizing hormone and growth hormone in castrated calves. J Anim Sci 77: 196- 200.

Pfister-Genkow M., Hayes H., Eggen A., Bishop M. D. (1997). The leptin receptor (LEPR) gene maps to bovine chromosome 3q33. Mamm Genome 8: 227.

Schenkel F. S., Miller S. P., Moore S. S., Li C., Fu A., Lobo S., Mandell I. B., Wilton J. W. (2006). Association of SNPs in the leptin and leptin receptor genes with different fat depots in beef cattle. In: Proccedings of the 8th Word Congress on genetics applied to livestock production, Belo Horizonte, Minas Gerais, Brazil, 13-18 August.

Silva L. F. P., Vanderhaar M. J., Weber Nielsen M. S., Smith G. W. (2002). Evidence for a local effect of leptin in bovine mammary gland. J Dairy Sci 85: 3277- 3286.

Szyda J., Morek-Kopéc M., Komisarek J., Zarnecki A. (2011). Evaluating markers in selected genes for association with functional longevity of dairy cattle. BMC Genetics 12: 30.

Tartaglia L. A. (1997). The leptin receptor. J Biol Chem 272: 6093- 6096.

White D. W., Kuropatwinski K. K., Devos R., Baumann H., Tartaglia L. A. (1997). Leptin receptor (OB-R) signalling. Cytoplasmic domain mutational analysis and evidence for receptor homo-oligomerization. J Biol Chem 272: 4065- 4071.

Žitný J., Bujko, J., Kasarda, R., Candrák J. (2009). Evaluation of genetic diversity between bulls of Slovak spotted breed. Acta fytot zoot Special edition 12: 108- 110.

acs76_26