International Journal of Science, Environment and Technology, Vol. 8, No 4, 2019, 795 – 798

ISSN 2278-3687 (O)
2277-663X (P)

IN-SILICO COMPARATIVE ANALYSIS OF THE PROMOTER REGIONS OF OVINE AND BOVINE LEPTIN (*LEP*) GENE

Cauveri, D.
Department of Animal Genetics and Breeding
Veterinary College and Research Institute, Orathanadu
Tamil Nadu Veterinary and Animal Sciences University, Chennai, Tamil Nadu, India

Abstract: The promoter region of the *LEP* gene approximately 2500 bp was screened for the CCAAT/enhancer-binding protein (C/EBP) motifs using the online software 'TFSEARCH' (*http://www.cbrc.jp/research/db/TFSEARCH.html*). The promoter region of Ovine *LEP* gene revealed nine binding sites for the C-EBP of which two were for C/EBPα. The binding sites for ovine were identical to that of bovine as *LEP* is highly homologous in these species. Four elements in the proximal 109 bp contribute to leptin promoter activity: the TATA box at -30, a C/EBP motif at -53, LP1 region at -87, and a Sp1 motif at -97. The analysis revealed that site of the leptin promoter is conserved in evolution, binds Sp1 present in adipocyte nuclear extracts, and contributes to promoter activity.
Keywords: Sheep, Leptin gene promoter region, *in-silico* analysis.

Introduction

In silico is an expression used to mean "performed on computer or *via* computer simulation." The term "*in silico*" characterizes biological experiments carried out entirely in a computer. The leptin (*LEP*) gene could be a potential candidate gene controlling some proportion of adipose and lean accretion in sheep. The present study was designed to compare the genomic organization and promoter activity of the ovine *LEP* gene to that of the bovine. The binding of C/EBP (especially C/EBPα) was reported to enhance the leptin receptor mRNA expression. The C/EBPα is a basic region/leucine zipper transcription factor important for the transcription of most adipocyte genes and of other genes involved in energy metabolism.

Materials and Methods

The complete sequence of the ovine *LEP* genes was obtained from the online database, the National Centre for Biotechnological Information (NCBI) (*www.ncbi.nlm.nih.gov*) which provides access to biomedical and genomic information. The gene accession number for the *LEP* gene is NC_019461 and GeneID 443534. The sequence was obtained in FASTA format.

Sequence data were analysed using the SeqMan program of LASERGENE software (DNASTAR Inc., USA). The *LEP* gene sequence obtained from NCBI were used as

*Received June 12, 2019 * Published Aug 2, 2019 * www.ijset.net*

reference sequences for analysis. The promoter region was screened for transcription factors using the free online software 'TF search' (*http://www.cbrc.jp/research/db/TFSEARCH.html*) (Heinemeyer *et al*., 1998).

Results and Discussion

The expression of *LEP* gene is controlled by cis- and trans-acting elements and factors. Analysis on approximately 2500 bp of the promoter region of the *LEP* gene screened for the CCAAT/enhancer-binding protein (C/EBP) motifs since binding of C/EBP (especially C/EBPα) was reported to enhance the leptin receptor mRNA expression. The promoter region of Ovine *LEP* gene revealed nine binding sites for the C-EBP of which two were for C/EBPα. As bovine *LEP* is highly homologous to the ovine *LEP* gene, the binding sites were identical. The results of this *in-silico* analysis are shown in Fig. 1. Four elements in the proximal 109 bp contribute to leptin promoter activity: the TATA box at -30, a C/EBP motif at -53, LP1 region at -87, and an Sp1 motif at -97. The C/EBPα is a basic region/leucine zipper transcription factor important for the transcription of most adipocyte genes and of other genes involved in energy metabolism (Fukuda and Iritani, 1999; Liefers *et al*., 2005). The site of the leptin promoter is conserved in evolution, binds Sp1 present in adipocyte nuclear extracts, and contributes to promoter activity. The regulation of leptin promoter by Sp1 and C/EBP in bovines was predicted by Mullis *et al.,* (2005) and was confirmed *via* cotransfection with C/EBP caused that resulted 24-fold activation of leptin reporter expression, as compared to cotransfection with control plasmid by Taniguchi *et al*. (2002). The leptin gene expression has been known to respond to hormones and metabolic state. The *ob/ob* mice, which have a nonsense mutation, exhibit a 20-fold increased leptin RNA level, suggesting existence of an intact mechanism to sense adiposity and to transcribe the leptin gene.

Conclusion

The transcription factor binding motifs in the leptin promoter, such as the putative C/EBP site, seem to play a role in mediating gene regulations. The active binding sites predicted need to be assessed by promoter assay

Acknowledgment

The author thanks Madras Veterinary College, TANUVAS, Chennai for providing the facilities and financial assistance to carry out this research work

Figure 1. *In-silico* analysis of the Ovine Leptin Promoter The TATA box (highlighted green), the exons (highlighted grey) and start codon (red and bold) are shown. *Nine C-EBP binding sites were predicted in the Ovine Leptin promoter of which three are C-EBP(highlighted blue), two are C-EBPα (underlined red) and four are C-EBPβ (highlighted yellow) sites. The SP1 sites are also shown (bold underlined).*

References

[1] Fukuda, H. and N. Iritani, 1999. Transcriptional regulation of leptin gene promoter in rat. *FEBS Letters*, 455: 165-169.

[2] Heinmeyer T., E. Wingender, I. Reuter, H. Hermjakob, A.E. Kel, O.V. Kel, E.V. Ignatieva, E.A. Ananko, O.A. Podkolodnaya, F.A. Kolpakov, N.L. Podkolodny and N.A. Kolchanov, 1998. Databases on transcription regulation: TRANSFAC, TRRD and COMPEL. *Nucleic Acids Research*, 26: 362-367

[3] Liefers, S.C., R.F. Veerkamp, M.F. te Pas, C. Delavaud and Y. Chilliard, 2005. Leptin promoter mutations affect leptin levels and performance traits in dairy cows. *Animal Genetics*, 36: 111-118.

[4] Mullis, P.E., 2005. Genetic control of growth *European Journal of Endocrinology*, 152: 11–31.

[5] Taniguchi, Y., T. Itoh, T. Yamada and Y. Sasaki, 2002. Genomic structure and promoter analysis of the bovine leptin gene. *Life*, 53: 131-135.