Short communication

Association of LEPR gene Polymorphism with milk yield and age at first calving in the Iranian Holstein dairy cows

S. Ghanbari Baghenoey, S. Ansari Mahyari*, H. Asadollahpour Nanaei**, M. Rostami, M.A. Edriss

Department of Animal Sciences, Isfahan University of Technology, College of Agriculture, Isfahan 84156-83111, Iran.

* Corresponding author, E-mail address: s.ansari@cc.iut.ac.ir

**Present address: Department of Animal Science, Faculty of Agriculture, Shahid Bahonar University of Kerman, Kerman, Iran.

Abstract The LEPR is a glycoprotein expressed mainly in hypothalamus, where it takes part in energy homeostasis and in regulation of the activity of the secretory organs. A transition mutation in this gene results in substitution of cytosine by thymine leading to the substitution of threonine by methionine in the intracellular domain of the LEPR-b isoform. This study investigated the impact of polymorphism located in the LEPR (T945M) gene on milk yield and age at first calving traits. The analysis was conducted on 395 randomly Holstein dairy cows. In the association studies, traits of interest were analyzed using the GLM procedure of SAS; means of the LEPR genotypes were compared by the LSMeans test. Statistical analyses showed no significant difference between the SNP and selected traits. Regarding the association revealed, theT945M SNP may not be as a possible candidate for marker – assisted selection in the Iranian dairy cattle breeding program.

Keywords: *LEPR* gene, polymorphism, milk yield, Holstein cows

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Introduction

Leptin (LEP) is a polypeptide hormone synthesized and secreted primarily in the adipose cells and also secreted by placenta, skeletal muscle and mammary gland (Houseknecht et al., 1998; Masuzaki et al., 1997 and Wang et al., 1998). In bovine cattle, LEP gene is located on chromosome 4. This hormone involve in regulation of food intake, energy expenditure, modulation of reproduction, immune and stress responses, blood pressure, as well as cell differentiation and proliferation (Fruhbeck 2001). Effects of LEP exerted trough at least five membrane and one soluble isoforms of LEP receptor (LEPR). LEPR gene is located in the chromosome 3q33 and consists of 20 exons divided over 1.75 Mb (Pfister-Genskow et al., 1997). The isoforms are divided into three classes: long, short and secretory. The long form isoforms (LEPR-b) has the complete cytoplasmic domain and is expressed mainly in hypothalamus, where it responsible for most of the physiological effects of LEP hormone (energy homeostasis and regulation of the activity of the secretory organs) (Tartaglia 1997). Due LEP exerts its effect by interacting with receptors located in most bovine tissues, LEPR gene considered as a candidate marker for energy homeostasis and milk production traits. Inside LEPRgene, the C → T SNP is located in exon 20 at position 115, and causes the threonine, T to methionine, M amino acid substitution in the intracellular domain of the LEPR-b isoform. This mutation (T945M) may have induced a structural change in the intracellular domain of LEPR, and have possible impact on milk composition in dairy cattle (Liefers et al., 2004). The aim of current study was to determine whether the LEPR T945M polymorphism influences milk yield and age at first calving in Holstein dairy cows.

Material and methods

For this experiment, genomic DNA samples were extracted from 395 Holstein dairy cows belonging to five different herds. The LEPR genotypes were identified with the polymerase chain reaction fragment length polymorphism (PCR-RFLP) technique, PCR conditions and primers were described by Asadollahpour Nanaei et al. (2014). Eight microliters PCR products was digested with five unites of *Taq*I (Fermentase Co.) in 20 μL of reaction volume at 60°C for 6 h for RFLP of the LEPR gene.

Polymorphic variants of the LEPR gene on recorded traits, milk yield and age at first calving, were analyzed

Table 1. Means and their standard deviations (SD) for milk yield and age at the first calving in Iranian Holstein dairy cows with different T945M LEPR genotypes.

Trait		Genotype		
		TT	CT	CC
Milk yield (Kg)	Mean	10870.04	10939.73	10800.76
	SD	315.92	167.12	188.92
Age at first calving (day)	Mean	768.426	772.75	772.299
	SD	6.11	3.62	3.72

Regarding the P value, all numbers in each row are not significantly different.

using SAS package (Statistical Analysis System, 2003) and significance of differences based on genotypes effect of traits were tested by following general linear models:

$Y_{ijkl}^{1} = \mu+G_i+HYS_j+S_k+b1(N_{ijkl} + N)+b2(z_{ijkl}+Z)+e_{ijkl}$
$Y_{ijkl}^{2} = \mu + G_i+ HYS_j +S_k+ + e_{ijkl}$

Where, Y_{ijkl}^{1} –milk yield,Y_{ijkl}^{2}– age at first calving, μ – overall mean, G_i – effect of genotype, HYS_j– fixed effect of herd (1, 2, 3, 4, and 5), S_k– random effect of sire (1,…,155),b_1– the linear regression coefficient ofopen days trait, N_{ijk}– effect of open days, b_2– the linear regression coefficient of dry period, z_{ijk}– effect of dry period, e_{ijkl}– random error.

Results

In this study, the DNA restriction fragments were obtained for LEPR gene using *Taq*I enzyme. The fragments were 400 bp (no digestion) for the TT genotype, 375 and 25 bp for the CC, 400 and 375 or 25 bp for the CT (The PCR products and restriction fragments are shown in fig. 1 and 2, respectively). As previously reported the genotypic frequencies were 0.4 for CC, 0.49 for CT and 0.11 for TT as followed by 0.65 for C allele and 0.35 for T allele which were in linkage disequilibrium (Asadollahpour Nanaei et al., 2014).
Molecular basis of this polymorphism was the missense mutation (C → T) located inexon 20 at position 115 of the LEPR gene (Liefers et al., 2004). The genotypes were considered to be in the association analysis between LEPR T945M polymorphism and milk yield and age at first calving in the Iranian Holstein dairy cows (Table1). Results showed that there was no association between genotypes and selected traits in this study.

Discussion

Investigation of the polymorphism for LEPR gene first reported by Liefers et al. (2004), who found two alleles T and C, which encoded three possible genotypes: TT, TC, and CC in Holstein-Friesian cows. They revealed that this SNP was correlated with the plasma LEP concentration and might influence the signal transduction pathway of the hormone. In cattle, the effect of LEPR gene on some economic traits were evaluated in different breeds. Suchocki et al. (2010) showed a weak association between T945M and milk yield and days to first service in the Holstein dairy cattle. The result of study by Komisarek and Dorynek (2006) showed an effect of this SNP on fat and protein content in Jersey cattle. In their study, animals with the TT genotype were characterized by the lowest values for fat and protein percentages. Similarly, one study on the Jersey and Polish Holstein-Friesian has shown an association of this SNP with milk composition traits (Suchocki et al., 2010). However, according to Asadollahpour Nanaei et al. (2014), the T945M SNP had not an effect on several economic

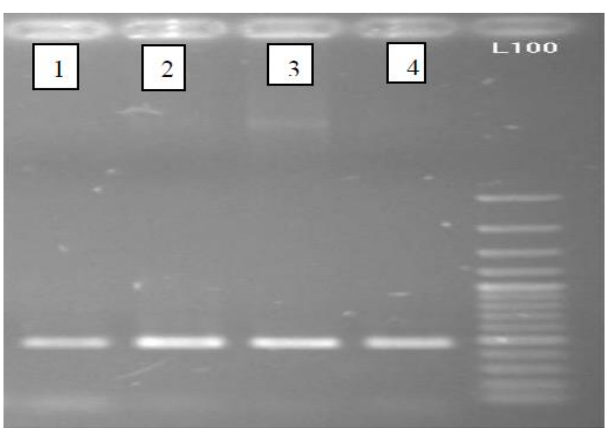

Fig. 1. Agarose gel electrophoresis of PCR products.

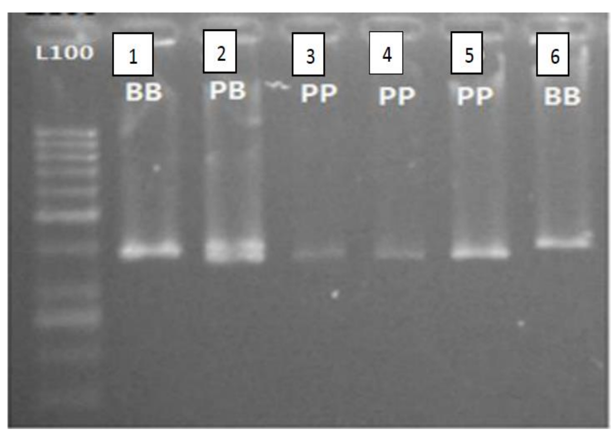

Fig. 2. Electrophoretic separation of LEPR gene PCR products digestion with *Taq*I.

reproductive traits such as pregnancy length, open days, services per conception, dray days, milk days and calving interval in the Iranian Holstein cattle.

Recently De Matteis et al. (2012) reported that four new SNPs of LEPR gene were identified throughout the sequence of the bovine LEPR gene and suggested that these SNPs may be a potential candidate for milk production traits.

In the current study, the results from analysis did not reveal any significant effect of the LEPR-T945M polymorphism on milk yield and age at first calving in Iranian Holstein dairy cows. In agreement with our results Glantz et al. (2011) and Trakovická et al. (2013), reported no significant effect of the LEPR-T945M polymorphism on milk yield and age at first calvingin the dairy cattle, respectively. In conclusion, the associated analysis suggested that no significant difference were detected between the one single SNP of LEPR gene and selected traits in cattle. Further investigations are needed to confirm or refute the revealed results in this study.

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ارتباط چندشکلی ژن LEPR با تولید شیر و سن اولین زایش در گاوهای هلشتاین ایران

س. قنبری باغنویی، س. انصاری مهیاری*، ح. اسدالله پور نعنایی، م. رستمی، م.ع. ادریس

نویسنده مسئول، پست الکترونیک: s.ansari@cc.iut.ac.ir

چکیده LEPR یک گلیکوپروتئین است که به طور عمده از هیپوتالاموس، مکانی که بخشی از هموستازی انرژی و تنظیم فعالیت ارگان های ترشحی بدن را تنظیم می کند، ترشح می شود. یک جهش انتقالی موجود در ناحیه داخل سلولی ایزوفرم LEPR-b این ژن باعث جایگزینی باز سیتوزین بوسیله تیمین و در نتیجه باعث تغییر اسیدآمینه ترئونین به متیونین می شود. در این پژوهش تاثیر چند شکلی (T945M) موجود در ژن LEPR روی تولید شیر و سن اولین زایش بررسی شده است. در این رابطه از ۳۹۵ راس گاو شیری هلشتاین که به صورت تصادفی انتخاب شده بودند جهت انجام محاسبات آماری استفاده شده است. برای محاسبه میانگین ژنوتیپ های صفات مورد مطالعه از نرم افزار SAS، روش GLM استفاده شد و نیز مقایسه میانگین ژنوتیپ ها با استفاده از آزمون LSMeans انجام شده است. محاسبات آماری تفاوت معنی داری را بین چند شکلی موجود در این ژن و صفات مورد مطالعه نشان نداد. بر اساس محاسبات انجام شده موجود در این تحقیق می توان نتیجه گرفت که ممکن است نتوان از چند شکلی موجود در این ژن (T945M) به عنوان یک نشانگر در جهت انتخاب و بهبود صفات مذکور در برنامه های اصلاح نژادی گاو هلشتاین ایران استفاده کرد.