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Research paper

A SNP in the 3′-untranslated region of AMPKγ1 may associate with serum ketone body and milk production of Holstein dairy cows

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ABSTRACT

AMPK is the key switch for providing the energy balance between cellular anabolic and catabolic processes. In this study, we aimed to screen the PRKAG1 (AMPKγ1) gene in high, moderate, and low producing Holstein dairy cows. A sample of 100 pregnant dairy cows, comprising 41 high, 33 moderate, and 26 low milk yields were selected from three large dairy herds in Isfahan province of Iran. Body condition score (BCS) was estimated before parturition while beta hydroxyl butyric acid (BHBA) as a measure of ketone bodies was measured at the fifth day postpartum. In addition, using three primer pairs covering exons 2–11 and 3′-UTR of the PRKAG1 gene, a random sample of 10 high milk yield dairy cows were amplified and sequenced. The sequencing results showed the presence of a T12571C mutation in intron 6 and a T14280C mutation in the 3′-untranslated region (UTR) of the PRKAG1 gene. Following a PCR reaction for amplification of the 3′-UTR amplicons, single strand conformation polymorphism (SSCP) assay was implemented for discrimination of the mutation in the studied population. Then, we evaluated if the mutation associates with the BCS, serum BHBA level, and production traits. The experimental analysis showed that the mutated allele significantly increased the BHBA level, BCS, as well as milk and protein yield. Bioinformatic study revealed that this 3′-UTR mutation distorts the target site of mir-423-5p microRNA which is one of the most highly expressed microRNAs in the bovine mammary gland, liver, and kidney. Given the role of AMPK in energy metabolism, the newly identified 3′-UTR mutation highlights the importance of AMPK and suggests a role of miRNAs for regulation of cellular metabolism, metabolism disorders, and production traits in Holstein dairy cows.

1. Introduction

The transition period, three weeks pre- and post-calving, is the most decisive phase for the health and milk production of dairy cows (Drackley, 1999). Since energy requirements increase dramatically during this period, considering the ability of the dairy cow to cope with the metabolic needs is a very important factor for improving managerial issues of dairy cows, particularly some metabolic disorders such as ketosis and fatty liver syndromes (Block, 2010).

Adenosine mono-phosphate protein kinase gene (AMPK) was introduced as a "fuel gauge" which switches anabolic and catabolic pathways (Hardie and Carling, 1997) and is involved in cellular homeostasis, circadian oscillation, and physiological and subsequently seasonal behavioral events and daily environmental changes (Vieira et al., 2008). Therefore, the study of this gene family highlights AMPK's roles in the adaptation process, production, reproduction, and longevity attributes of animals as well as some human syndromes such as insulin resistance and abdominal obesity.

Mammalian AMPK comprises of two catalytic subunits (α1, α2) and five non-catalytic regulatory subunits (β1, β2, γ1, γ2, γ3) (Hardie and Carling, 1997; McKay et al., 2003; Xiao et al., 2007). Each of the three gamma components includes four cytathione beta-synthase domains which bind the adenosine part of AMP (Hardie and Hawley, 2001). Therefore, any mutation in the AMPKγ1 isoform leading to a small modification in the adenyl-binding site can severely affect AMP and ATP binding to AMPK (Xiao et al., 2007). AMPKγ1, PRKAG1 in bovine, consists of 12 exons and 11 introns and transcribes a 1619 bp mRNA which comprises of a coding DNA sequence (CDS: 993 nt) and a very long 3′-UTR (627 nt) (Benkel et al., 2005).

Apart from modifications in the protein structure through mutations in the coding sequence, administration of gene expression is the other important mechanism for the regulation of a protein function. Most of

Abbreviations: ABCI, animal breeding center of Iran; AMPK, adenosine mono phosphate protein kinase; BHBA, beta hydroxyl butyric acid; BCS, body condition score; CDS, coding DNA sequence; miRNA, microRNA; NCBI, National Center of Biotechnology Information; RPM, read per million; SNP, single nucleotide polymorphism; SSCP, single strand conformation polymorphism.

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the mammalian genes are transcribed from both DNA strands and translated into proteins whereas the remaining transcripts are considered non-coding RNAs (ncRNAs) (Carninci et al., 2005). These types of RNA can act as activators or repressors for other genes (Qu and Adelson, 2012). Among ncRNAs, microRNAs, known as miRNAs, are a class of small RNAs (19–25 nucleotides) with cap and polyA tail (Winter et al., 2009) which interfere with gene expression via either mRNA degradation or translational repression (Galio et al., 2013). Over the past decade, administration of miRNAs has been a nascent technology for the regulation of gene expression. These elements can down regulate the translation step by triggering RNAse endonucleases and enhancing mRNA decapping and de-adenylation (Valencia-Sanchez et al., 2006). So far, around 1000 miRNA genes have been discovered in mammalian genomes, though the function of the majority of these known miRNA still remains to be discovered (Glazov et al., 2009). In silico evaluation of bovine genome indicated a majority class of unknown miRNAs which are specific to ruminants (Glazov et al., 2009; Qu and Adelson, 2012). Nearly 60% of bovine ncRNAs are intergenic and highly corresponded to the 3′-UTRs of messenger RNAs (mRNAs) (Qu and Adelson, 2012). Moreover, there are a high number of intergenic ncRNAs which act through cis-regulation of mostly regulatory genes and locate in the 1 kb proximity of UTRs (Qu and Adelson, 2012). As the 3′-UTR associated/proximate ncRNAs are in highly conserved regions (Qu and Adelson, 2012), targeting the 3′-UTR of animal mRNA has been one of the most important pathways for regulation of gene expression (Glazov et al., 2009).

In this study, we aimed to screen the bovine PRKAG1 exons 3–7 and 12 as well as the 3′-UTR and evaluate the effect of polymorphisms on 305-day production traits, serum BHBA level, and BCS of Holstein dairy cows.

2. Materials and methods

2.1. Animals and records

In this study, based on the first parity adjusted milk yield records for a 305-day production period, 41 high- (from 13,000 to 20,000 kg), 33 moderate- (from 11,500 to 12,900 kg), and 26 low- (from 7500 to 11,400 kg) producing dairy cows were selected among available pregnant dairy cows from three large dairy herds in Isfahan province of Iran. Since assessment of the ketosis prevalence was one of the main agendas of this research, the selected dairy cows were restricted to those having more than three parities. Evaluation of BCS was carried out at the late stage of the pregnancy, during the dry period. At the fifth day post-partum, at 7 am, blood samples were collected and quickly sent to the laboratory for the measurement of serum BHBA level using the D-3-hydroxybutyrate (RANBUT) assay, RB-1007 kit (Randox, UK). A fraction of the collected blood underwent the DNA extraction process. Moreover, first parity records of production traits such as milk, fat, and protein yield which were previously adjusted for 305-days of production period were gathered from Animal Breeding Center of Iran (ABCI). Because the maximum heritability for production traits is attributed to the first parity records, the genetic polymorphism effects were evaluated on the first parity productive traits.

2.2. Genotyping assays

The polymerase chain reactions (PCR) assay was performed using three primer pairs which were designed to amplify exons 3–7 which are closely located with quite short introns, as well as exon 12, which is the last exon of PRKAG1, and the early part of the 3′-UTR of PRKAG1 (Table 1) based on the AC_000162.1 reference sequence in National Center for Biotechnology Information (NCBI). The first, second and third primer pairs cover exons 3–5, exons 5–7, and exon 12 along with the early part of 3′-UTR, respectively. PCR productions of the amplified fragments from 10 randomly selected high-yield dairy cows were sequenced (BioBasic, Canada). The sequencing results were aligned to the reference sequence and possible mutations were looked for by the CLC Workbench 6 software. All of the DNA samples were genotyped for the detected mutation using the single strands conformation polymorphism (SSCP) technique considering the sequenced samples as positive controls. Since the detected mutation was in the 3′-UTR of PRKAG1, we used the MiRbase database (http://www.mirbase.org/search.shtml) to search for miRNAs which target the mutated sequence.

Table 1
Primer pairs for amplification of exons 3–7, 12 and 3′-untranslated region (UTR) of bovine *PRKAG1*.

Primer sequence	Amplicon length
F1: 5′-AATAGCAACAAAACAGAATAC-3′ R1: 5′-CAACAGAACTCGAGACTCA-3′	847 bp: from 11,654 to 12,500
F2: 5′-TTATTGGCATCTTGTACTGGG-3′ R2: 5′-GGAAGGCAGGGATTAATGG-3′	757 bp: from 12,245 to 13,001
F3: 5′-AGGGTGGGGACTGACGG-3′ R3: 5′-GATGGGCTGGGTGAGGATAAG-3′	384 bp: from 14,053 to 14,436

2.3. Statistical analysis

Data were analyzed using the General Linear Model (GLM) procedure in SAS package. Mean comparison among different genotypes for the afore-mentioned traits were carried out using the LSD test. Allelic and genotypic frequencies were compared using Chi-square test. The P-value <0.05 was considered as the significant level for comparison-wise error rate.

3. Results

3.1. Detection of two mutations in the PRKAG1 of Holstein dairy cows

Alignment of the sequenced samples to the NC_007303.5 reference number, *Bos taurus* breed Hereford chromosome 5 alternate assembly, in NCBI showed the presence of two mutations (Fig. 1). The first single nucleotide polymorphism (SNP) was T12571C which is located in intron 6 of PRKAG1. The second SNP was T14280C which resides in the 3′-UTR of PRKAG1; or T1043C compared to the NM_174586.2 mRNA sequence in NCBI.

Then, the genotype of all 100 dairy cows for this 3′-UTR polymorphism was detected via the SSCP technique. Allelic and genotypic frequencies for the T1043C mutation are presented in Table 2. Allelic frequency of the SNP was 45.5% in the whole selected population. The genotypic results indicated higher frequencies for both wild- and mutant-type homozygotes whereas the heterozygote genotype was considerably lower than the expected frequencies based on the Hardy–Weinberg equilibrium. The majority of high producing dairy cows were mutant type homozygous for the 3′-UTR SNP. The genotypic distribution was significantly different among high-, moderate-, and low-yield classes.

3.2. The 3′-UTR mutation associates with variation in serum BHBA level, BCS, and production traits

The mean body condition score of pregnant dairy cows at the late dry period was significantly higher in CC genotypes versus both TC and TT classes (Fig. 2). Then we tested if the serum BHBA level at the 5th day following the parturition was different among the PRKAG1 genotypes (Fig. 2). A highly significant difference was observed between the homozygous mutant type (0.77 mmol/L) compared to both heterozygous and wild type homozygous genotypes (0.56 mmol/L). Moreover, mean comparison among different genotypes for the 305-day production traits are presented in Fig. 3. In agreement with the observed significant differences in the genotypic frequency distribution among high-,