Polymorphisms in the Promoter Region of the Chinese Bovine PPARGC1A Gene

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ABSTRACT: The peroxisome proliferator-activated receptor gamma coactivator-1 alpha protein, encoded by the PPARGC1A gene, plays an important role in energy homeostasis. The genetic variations within the PPARGC1A gene promoter region were scanned in 808 Chinese native bovines belonging to three cattle breeds and yaks. A total of 6 SNPs and one 4 bp insertion variation in the promoter region of the bovine PPARGC1A gene were identified: SNP -259 T>A, -301 C>T, -298insCTTT, -915 A>G, -1175 T>G, -1590 C>T, -1665 C>T and -1690 G>A, which are in the binding sites of some important transcription factors: sex-determining region Y (SRY), myeloid-specific zinc-finger-1 (MZF-1) and octamer factor 1 (Oct-1). It is expected that these polymorphisms may regulate PPARGC1A gene transcription and might have consequences at a regulatory level. (Key Words: Bovine, PPARGC1A Gene, Promoter Region, Polymorphisms)

INTRODUCTION

The peroxisome proliferator-activated receptor gamma coactivator-1 alpha (PPARGC1A, also known as PGC-1α) protein, encoded by the PPARGC1A gene, plays an important role in energy homeostasis. PGC-1α is a metabolic switch, which convergently regulate metabolic pathways through its pleiotropic interactions with nuclear receptors (NRs) and other non-NR transcription factors. PGC-1α transcriptionally activates a complex pathway of mitochondrial biogenesis, lipid and glucose metabolism (Puigserver et al., 1998). The expression of PGC-1α is enriched in tissues where mitochondria are abundant and oxidative metabolism is active, such as brown adipose tissue (BAT), the cardiac and skeletal muscles (Puigserver et al., 1998).

Independent studies in humans have identified a common hPPARGC1A polymorphism, Gly482Ser, associated with type 2 diabetes (Barroso et al., 2006; Bhat et al., 2008; Povel et al., 2010; Geloneze et al., 2012). The most common polymorphism c.1892-19T>C in bovine PPARGC1A intron 9 was studied for the associations with milk production and milk-fat composition traits (Weikard et al., 2005; Khatib et al., 2007; Komisarek and Dorynek, 2009; Schennink et al., 2009; Kowalewska-Luczak et al., 2011). Several other polymorphisms in the bovine PPARGC1A gene have been identified and studied for the association with meat quality and carcass phenotypes (Soria et al., 2009; Ryu et al., 2012).

As an important component of the world’s bovine population, the Chinese native population possesses very abundant genetic resources. However, up to now, there was no information about the genetic variation of PPARGC1A gene promoter in Chinese indigenous bovine breeds. Therefore, a scan of the genetic variations within the PPARGC1A gene promoter in Chinese native bovines, should provide important information on bovine genetic resources that could be useful for selection and breeding through marker assisted selection (MAS).

MATERIAL AND METHODS

Bovine populations, genomic DNA isolation and pooling

Genomic DNA samples were obtained from 808 bovines belonging to three cattle breeds and yaks. Three Chinese indigenous cattle breeds, Jiaxian (JX, n = 148), Nanyang (NY, n = 278), Qinchuan (QC, n = 325) were
investigated. The Jiaxian cattle were from the breeding farm of Jiaxian cattle (Jiaxian county, Henan Province, China); the Nanyang cattle were from the breeding farm of Nanyang cattle (Nanyang city, Henan Province, China); the Qinchuan cattle were from the reserved farm (Fufeng county, Shaanxi Province, China), the breeding and seed centre of Qinchuan cattle (Yangling District, Shaanxi Province, China). The yaks (n = 57) were from the breeding farm of yaks (Datong county, Qinghai province, China). DNA was isolated from 1 ml whole blood samples according to Sambrook and Russell (2001). DNA of 50 to 100 randomly chosen individuals from the same breed were mixed to one DNA pool (Bansal et al., 2002).

**Primers designing**

Six primer pairs were designed with the Primer V5.0 software based on NCBI database (GenBank accession number AC_000163) to amplify the promoter region of the bovine PPARGC1A gene. Detailed information about oligonucleotide primers, amplicon sizes and corresponding annealing temperatures is given in Table 1.

**Single nucleotide polymorphisms (SNPs) detection**

The genomic DNA pool samples were used as templates. Each amplification reaction was carried out in a total volume of 25 μl in the presence of 50 ng bovine genomic DNA pool as template, 0.5 μM of each primer, 1× buffer (including 1.5 mM MgCl2), 200 μM dNTPs (dATP, dTTP, dCTP and dGTP), and 0.625 U Taq DNA polymerase (MBI, Vilnius, Lithuania). The cycling protocol was 4 min at 95°C, 35 cycles of denaturing at 94°C for 30 s, annealing at the optimized temperature for 30 s, extending at 72°C for 30 s, with a final extension at 72°C for 10 min. All PCR products were then sequenced using ABI 3730 sequencer (ABI, Foster City, CA, USA) and analyzed with BioXM software (version 2.6) to search for SNPs.

**SNP genotyping**

The genomic DNA samples were used as templates to individually and specifically amplify the promoter region of PPARGC1A. SSCP analysis of PCR fragments obtained above was performed on electrophoresis units (Bio-Rad, Amersham), coupled with a refrigerated system. Aliquots of 5 μl PCR products were mixed with 5 μl denaturing solution (95% formamide, 25 mM EDTA, 0.025% xylene cyanol and 0.025% bromophenol blue), heated for 10 min at 98°C and chilled on ice. Denatured DNA was subjected to PAGE (80×73×0.75 mm) in 1×TBE buffer and constant voltage (200 V) for 2.5 to 3.0 h. After electrophoresis, gels were stained with 0.1% silver nitrate and visualized with 2% NaOH solution (supplied with 0.1% formaldehyde) according to Zhang et al. (2007). After the polymorphisms were detected, three PCR products of each different electrophoresis pattern were sequenced in both directions in an ABI 3730 sequencer (ABI, Foster City, CA, USA) and analyzed with BioXM software (version 2.6).

**Data analyzing**

Allele frequencies were determined for each breed by direct counting. The program MatInspector (http://www.genomatrix.de) was performed to identify any known transcription factors that may potentially bind to polymorphic promoter sequences in the TRANSFAC database.

**RESULTS AND DISCUSSION**

In the present study, genomic DNA of all bovine breeds was successfully amplified using primer pairs for the promoter region of the bovine PPARGC1A gene. A total of 6 SNPs and one 4 bp insertion variation in the promoter region of bovine PPARGC1A gene were identified. In the

| Table 1. Primer information of bovine PPARGC1A gene |
|---|---|---|---|
| Primer | Primer sequence (5’ to 3’) | Size (bp) | Tm (°C) | Note (ref. AC_000163) |
| P1 | 1F: TATTATATCGCCAGGGCCTC | 432 | 49.1 | -385 to -47 |
| | 1R: TCGATGTCACTCCATACAGA | | | |
| P2 | 2F: GGTATTTTGTGCTTGGTCTC | 417 | 48.0 | -798 to -382 |
| | 2R: AATAAATTTATTTCCAATGCTC | | | |
| P3 | 3F: TGGTGTTAGTCTCTACATTGGTCTC | 363 | 50.5 | -1143 to -781 |
| | 3R: ACAAAGCACAAATACCATCC | | | |
| P4 | 4F: TGATAAAGGGAAGGACAAAT | 347 | 48.0 | -1461 to -1115 |
| | 4R: TCGAGCCTGACATGACATT | | | |
| P5 | 5F: TTCTCTGGTTCTTCTTCTTTG | 352 | 52.4 | -1791 to -1440 |
| | 5R: TCTATAGTCCCTTCTTACCTAT | | | |
| P6 | 6F: TCAGTAGAACAGGAATATAA | 290 | 46.9 | -2072 to -1783 |
| | 6R: GAAAGAACAGGAAAATACAA | | | |

PCR primers were designed according to the bovine PPARGC1A gene (GenBank accession No. AC_000163). Location of primers referred to the translation start site, where +1 corresponding to the adenine of the translation initiation codon ATG.
PCR product primer1, the SNP -259 T>A and -301_-298insCTTT were found; in primer3, -915 A>G; in primer4, -1175 T>G; in primer5, -1590 C>T, -1665 C>T and -1690 G>A. All these polymorphisms are numbered relative to the adenine of the translation start codon. The sequencing results and SNPs found are shown in Figure 1. The nature and the distribution of PPARGC1A promoter variations are shown in Table 2.

The SNPs -259 T>A, -1175 T>G, -1590 C>T and -1665 C>T occurred only in the Yak population. Conversely, the SNP -915 A>G occurred only in cattle breeds (JX, NY and QC), while -301_-298insCTTT and SNP -1690 G>A occurred both in Yak and cattle populations. The genetic differences of PPARGC1A promoter in the present study give support to the notion that the yak is an independent genus, Poephagus. This notion was also supported by the reports from Zhang (Zhang et al., 2010; Zhang et al., 2011). The discrepancy in different populations was probably caused by the following two possible reasons. First, the yak was an ancient animal species with many wild bovine characteristics, which did not undergo much artificial selective breeding. Meanwhile, the Jiaxian, Nanyang and Qinchuan cattle, three of the most representative indigenous bovine (Bos taurus) breeds in China, are draft and beef dual-purpose cattle breeds which have been selected for beef production in the past decades. Second, yaks must

Figure 1. Sequencing results and polymorphic sites found in the promoter region of the bovine PPARGC1A gene in Chinese breeds. Adenine of the start codon ATG is counted as +1 (GenBank accession No. AC_000163).
Polymorphisms in promoter region numbered relative to the translation start site; Adenine of the start codon ATG is counted as +1 (GenBank accession No. AC_000163).

QC = Qingchuan cattle; NY = Nanyang cattle; JX = Jiaxian cattle.

Table 2. The SNPs identified in the promoter region of the bovine PPARGCIA gene in Chinese breeds and allele frequencies of the SNPs

| Polymorphism site | JX (n = 148) | NY (n = 278) | QC (n = 328) | Yak (n = 57) |
|------------------|--------------|--------------|--------------|--------------|
| -259 T>A        | 0            | 0            | 0            | 1            |
| -301_-298insCTTT | 0.30         | 0.25         | 0.22         | 0.95         |
| -915 A>G        | 0.33         | 0.40         | 0.26         | 0            |
| -1175 T>G       | 0            | 0            | 0            | 1            |
| -1590 C>T       | 0            | 0            | 0            | 1            |
| -1665 C>T       | 0            | 0            | 0            | 1            |
| -1690 G>A       | 0.51         | 0.41         | 0.53         | 1            |

Polygons in promoter region numbered relative to the translation start site; Adenine of the start codon ATG is counted as +1 (GenBank accession No. AC_000163).

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