Pakistan Veterinary Journal

ISSN: 0253-8318 (PRINT), 2074-7764 (ONLINE)
Accessible at: www.pvj.com.pk

RESEARCH ARTICLE

Single Nucleotide Polymorphism (SNP) in *PPARGC1A* Gene Associates Milk Production Traits in Chinese Holstein Cattle

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ARTICLE HISTORY

Received: January 11, 2012
Revised: February 14, 2012
Accepted: April 06, 2012

Key words:
Chinese Holstein
Single nucleotide polymorphism
PPARGC1A gene

ABSTRACT

This study was designed to find out possible associations between single nucleotide polymorphism (SNP) of *PPARGC1A* gene and milk production traits. In the current study, one SNP at g.85330C>T position was identified in 9th intron of *PPARGC1A* gene through pooled DNA sequencing. The identified SNP was genotyped using *MALDI-TOF MS* technique in 752 individuals from the Chinese Holstein cattle breed. The frequency of C allele at position g.85330C>T was more frequent in our population (0.69), followed by the T allele (0.31). From the association results, significant differences were found between *PPARGC1A* genotypes in the protein concentration and protein yield. Heterozygous cows with CT genotype at the g.85330C>T locus showed the highest protein yield, with more than 4.18 kg compared with homozygous cows with TT genotype; homozygous CC cows were found at intermediate position. In case of protein percentage, homozygous cows with TT genotype were significantly greater than heterozygous CT cows, with a difference of about 0.012%; CC cows were found in lowest position. The allele substitution results demonstrated that g.85330C>T-C allele decreased protein yield (1.23kg) and protein percentage (0.016%). This result clearly indicated that g.85330C>T-T allele increased protein percentage and protein yield.

To Cite This Article: Alim MA, Y Fan, Y Xie, X Wu, D Sun, Y Zhang, S Zhang, Y Zhang, Q Zhang and L Liu, 2012. Single nucleotide polymorphism (SNP) in *PPARGC1A* gene associates milk production traits in Chinese Holstein cattle. Pak Vet J, 32(4): 609-612.

INTRODUCTION

In recent years, advances in molecular technology especially in field of genetics have lead to the identification of genes or markers affecting milk performance traits. There is increasing demand to detect the actual genes controlling economically important traits in dairy cattle and the positional candidate gene approach is mostly used for this process. Identification of genes that are in QTL position can provide not only the more accurate markers for marker assisted selection in livestock population, but also identifies most critical biochemical pathways for further investigation and endogenous or exogenous use.

PPARGC1A has critical and dynamic function in the regulation of energy homeostasis programs and it has the ability to coordinate the metabolic process in liver, muscle, and fat tissue of humans and mice. Bovine *PPARGC1A* gene essentially consists of 13 exons and the levels of expression of it is different in different tissues. Because of its potential function, it is reasonable to hypothesize that the metabolic processes in dairy cattle especially during lactation might be coordinated by *PPARGC1A* (Weikard *et al.,* 2005). Considering the biological function of *PPARGC1A* gene, it is localized on bovine chromosome 6 which linked to a QTL for milk fat synthesis and it is hypothesized that variation in one or more position in the *PPARGC1A* gene could be associated with the QTL effects for other milk production traits (Weikard *et al.,* 2005). QTL for milk production traits on bovine chromosome 6 (BTA6) has already been reported by several independent studies in different cattle populations (Georges *et al.,* 1995; Kuhn *et al.,* 1999; Velmala *et al.,* 1999; Ashwell *et al.,* 2001; Ron *et al.,* 2001; Boichard *et al.,* 2003; Khatkar *et al.,* 2004; Olsen *et al.,* 2005).

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Meta-analysis of QTL was studied by Khatkar *et al.* (2004), who claimed a QTL affecting milk, fat, protein yield and protein concentration located around 50 cM, near microsatellite BM143. Weikard *et al.* (2011) carried out a QTL study on the German Fleckvieh population and reported that two variants in the *PPARGC1A* gene (RW023, RW070) were detected in QTL region 2, affecting milk yield traits. The new SNPs were detected by comparative re-sequencing of the targeted genomic QTL region 2 (Eberlein *et al.,* 2009) comprising the *PPARGC1A* gene. Using haplotype sharing based LD mapping and single marker regression mapping analyses, a QTL for milk production traits has been detected at BTA 6. This region was near to marker BMS470 which has been fine mapped to a marker interval surrounded by the markers BMS483 and MNB209. The QTL allele embedded in the haplotype consisting of alleles 3 of BMS483 and allele 4 of MNB209 has significant effect on milk yield, fat yield and protein yield (Mei *et al.,* 2009). *PPARGC1A* is located within a linkage region with QTL for milk production and being an important biosynthesis of milk fat, thus it represents a plausible candidate for milk production. Several SNPs have been identified within the *PPARGC1A* region, one of which has already been localized at position g.1892T>C in 9th intronic sequences of this gene (GenBank: AY321517) that may underlie the variability of milk production traits (Weikard *et al.,* 2005; Khatib *et al.,* 2007).

Generally any polymorphism in the gene region will affect expression pattern of gene (Zan *et al.,* 2007), rate and regulation of gene transcription or the amino acid sequence of the gene product (Nott *et al.,* 2003). The actual goal of our experiment was rather to draw attention on Chinese Holstein Cattle showing how single nucleotide polymorphisms (SNPs) responsible for milk production traits at genomic level and exploitable in selective breeding programs. The objective of the study was to find out possible associations between single nucleotide polymorphism of the *PPARGC1A* gene and milk production performance of Chinese Holstein Cattle breed.

MATERIALS AND METHODS

Experimental animals and phenotype data: For genomic DNA extraction, semen samples were collected from the 14 sire bulls and blood samples were collected from the 752 Chinese Holstein cows from Beijing Dairy Cattle Center and Beijing Sanyuan Lvhe Dairy farming Center, including 14 sire families with 49–150 daughters from each sire. Frozen semen samples of bulls and whole blood samples of cows were used for genomic DNA extraction by standard phenol–chloroform method for semen and a commercial kit [TIANamp Kit; Tiangen Biotech Co., Ltd., China] for blood. The commercial kit was used according to the instructions of manufacturer. Performance data were collected from the Dairy Data Processing Center of China, Dairy Association of China (DAC) for 5 milk production traits such as milk yield, fat yield, fat concentration, protein yield, and protein concentration over 305 days.

Detection and Genotyping of the Polymorphisms: Exon 9 and partial sequence region of intron 9 was used to design the primer for selective amplification of *PPARGC1A* fragment using reference GenBank sequence (Accession No. NC_007304.4) and Primer3 web Program (v .0.4.0). A DNA pool (50ng/µL/bull) was constructed from the aforementioned 14 sires. Polymerase chain reaction (PCR) was performed to amplify 411 bp DNA fragment of *PPARGC1A* gene for detection of genotypes and it was set by adding left primer (5′- GCC GGT TTA TGT TAA GAC AG -3′) and right primer (5′- GGT ATT CTT CCC TCT TGA GC -3′). PCR were run with a programmable thermal cycler (Bio-Rad, DNA Engin, Dyad and Tetad 2, Peltier Thermal Circlers, Mexico) in a final reaction volume of 25 µl using reagents from Invitrogen (Invitrogen Life technologies, Beijing, China). The PCR reaction mixture consisted of 1 µl of each primer, 12.5 µl primix, 8.5 µl ddH_2O and 2 µl containing 50 ng genomic DNA. The PCR protocol was 5 min at 94ºC for denaturing followed by 34 cycles at 94ºC for 30 s; 56ºC for 30 s; 72ºC for 30 s; 72ºC for 7 min for the final extension and incubated 2 min at 20ºC. PCR product (40 µl of each) was sent to the company (TSINGKE, China) for both the forward and reverse sequencing. BioEdit Sequence Alignment Editor (version 7.0.9.0) was used for processing the chromatographs generated from sequencing. Using the ClustalW multiple sequence alignment programs both forward and reverse primer sequences were then aligned to detect the presence of genetic polymorphism. Matrix-assisted laser desorption/ ionization time of flight mass spectrometry (*MALDI-TOF MS*) technique (Squenom MassARRAY®, Bioyong Technologies Inc. HK) was used for individual genotyping.

Statistical Analysis: Because of tracing back pedigree information for three generations, the total number of animals included in this experiment reached 2212. For the association studies, the traits of interest were analyzed using several statistical programs e.g. MATLAB version 7.11.0.584 for the kinship matrix (A-matrix), POPGENE version 1.32 for Hardy–Weinberg equilibrium. The mixed procedure of SAS 9.1.0 software with the following animal model (Lynch and Walsh, 1997) was used to check the final effects of genotypes on the milk performance traits:

$Y=\mu+hsy+L+G+\alpha+e,$

where, Y = performance data of each trait of cows;
μ=overall mean;
hys=herd- season - year effect;
L=fixed effect of lactation;
G=fixed effect of polymorphisms corresponding to the genotype;
α=random polygenic effect for all known pedigree relationships;
e=random residual.

Multiple t-test was performed through dividing the significance level by the number of tests for Bonferroni correction. The equation of Falconer and Mackay (1996), was fitted to estimate the additive (a), dominance (d) and allele substitution (α) effects. The equation is-

$a = (AA - BB)/2,$
$d = AB -(AA + BB)/2$
$\alpha = a + d(q - p)$

where AA or BB= homozygous genotypes,
AB= heterozygous genotype,
p or q = allele frequency of corresponding locus.

RESULTS AND DISCUSSION

In our study, a single nucleotide polymorphism (SNP) at position g.85330C>T in 9th intronic sequences of

PPARGC1A gene (Fig. 1) compared with GenBank reference sequence (GenBank: NC_007304) in Chinese Holstein cattle was detected by sequence analysis of the pooled DNA samples. The identified SNP was genotyped by *MALDI-TOF MS* technique and its effects on 5 milk performance traits such as 305 days milk yield, fat yield, protein yield, fat percentage and protein percentage were estimated.

It was found that TT genotype was less frequent at position g.85330C>T in our population and it was only 9% whereas frequency of CC genotype was found as 49% and the C allele was more frequent in the population (0.69), followed by the T allele (0.31). The results of Chi-square test (χ^2) showed that all genotypic frequencies in our population were not in Hardy-Weinberg equilibrium (P>0.05). These values indicated that selection pressure was not too powerful on this site in the population. In current study, significant associations on protein yield and protein percentage were observed only. These associations were found significant even after Bonferroni correction. Association results demonstrated that heterozygous cows with CT genotype at the g.85330C>T locus showed the highest protein yield (Table 1), with more than 4.18 kg compared with homozygous cows with TT genotype; homozygous CC cows were found at intermediate position. There was no effect of g.85330C>T locus on milk yield, fat yield or fat concentration, whereas protein percentage in cows with TT genotype was significantly greater than that of cows with CT genotype, with a difference of about 0.012%; CC cows were found at lowest position (Table 1). Significant allele substitution effects (P<0.001) on protein yield and protein percentage were also observed (Table 1). The allele substitution result demonstrated that g.85330C>T-C allele decreased protein yield (1.23kg) and protein percentage (0.016%). These results clearly indicated that g.85330C>T-T allele increased protein yield and protein concentration (Table 1).

The results of the present experiment are consistent with previous results of different studies that had shown significant association of *PPARGC1A* genotypes with milk performance traits (Weikard *et al.,* 2005; Khatib *et al.,* 2007; Komisarek and Dorynek 2009; Schennink *et al.,* 2009; Kowalewska-Luczak *et al.,* 2010). In case of g.85330C>T polymorphism (they named it c.1892T>C), the more frequent genotype was the CC (0.49) in our population. Similar result was reported by Weikard *et al.* (2005) in German Holstein population (0.68); Komisarek and Dorynek (2009) in Polish Holstein-Friesian population (0.53) and Schennink *et al.* (2009) in Dutch Holstein–Friesian population (0.56), while Khatib *et al.* (2007) and Kowalewska-Luczak *et al.* (2010) reported that CT genotype was most frequent in University of Wisconsin resource population (0.65) and Jersey population (0.72), respectively. However, the lowest frequency (0.09) was observed in TT genotype in the population analyzed. Our findings are in agreement with previous reports by Weikard *et al.* (2005), Khatib *et al.* (2007), Komisarek and Dorynek (2009) and Schennink *et al.* (2009), who claimed that TT had the lowest frequency and it was 0.02, 0.02, 0.06 and 0.08 respectively. Our results disagree with literature data demonstrated by Kowalewska-Luczak *et al.* (2010), who reported that CC allele had the lowest frequency (0.01).

Because of possible involvement in fat metabolism and known QTL effects of *PPARAGC1A* gene on milk production, it was chosen for association test with milk performance traits (Weikard *et al.,* 2005). A significant association between g.85330C>T and milk fat traits and milk yield was reported by Weikard *et al.* (2005) in 434 bulls from the German Holstein population whereas Khatib *et al.* (2007) did not find any association with SNP g.85330C>T and milk production traits in University of Wisconsin resource population. The results of Kowalewska-Luczak *et al.* (2010) were similar to the result of Khatib *et al.* (2007), who claimed that no significant associations were observed in milk performance traits between g.85330C>T genotypes. Kowalewska-Luczak *et al.* (2010) reported that cows with genotype CC had a higher fat and protein content and a lower milk yield compared with cows but the association result was not statistically significant. However, statistically significant association (P≤0.05; P≤0.001) results were observed between the *PPARGC1A* gene combined genotype of cows (c.1892T>C/c.3359A>C) and milk production traits. Cows with the combined CC/AC genotype showed lowest milk yield and highest protein content than the other cows (Kowalewska-Luczak *et al.,* 2010). These conflicting results of our study compared with other studies could be explained by other several reasons, e.g. the genetic backgrounds of the analyzed population, gene fusion, population size, paternal effect and the different number of samples considered in each study.

Generally the effect of any polymorphism in the specific gene region will differ across different populations or breed because of specific originating backgrounds. Holsteins cattle in China belongs to dairy, which was originated from cross-breeding or up grading between imported Holstein sire (pure-bred bull) from America or Europe and native cow (Chinese Yellow cattle) over the past 100 years (Qiu, 2002).

Conclusion: Considering that the environment is the major cause of phenotypic variation in milk production traits, the dairy farm was chosen in the Beijing region, China where the experiments were conducted. Regular and standard performance testing, i.e. Dairy Herd Improvement system, have been carried out in each farm since 1999. The present study revealed one SNP in 9th intronic sequence of bovine *PPARGC1A* gene which was associated with protein percentage and protein yield in our population. Although intron is not the protein coding sequence but it has a potential regulating function in gene expression by changing RNA splicing and stability and rate and regulation of gene transcription. Although whether the identified polymorphism g.85330C>T affected gene expression remain to be explored. The detected SNP in our study would provide an excellent opportunity for marker-assisted selection (MAS) programs in dairy cattle. The inconsistent association results necessitate further investigation prior to apply in MAS programs.

Acknowledgement: This work was financially supported by the National "948" Project (20011-G2A), Key Development of New Transgenic Breeds Program of China (2009ZX08009-156B) and National Natural Science Foundation of China (31072016). The authors would like to acknowledge the current and former members of the department for their valuable contributions and comments and the Dairy Association of China for providing semen samples and pedigree information of bull.

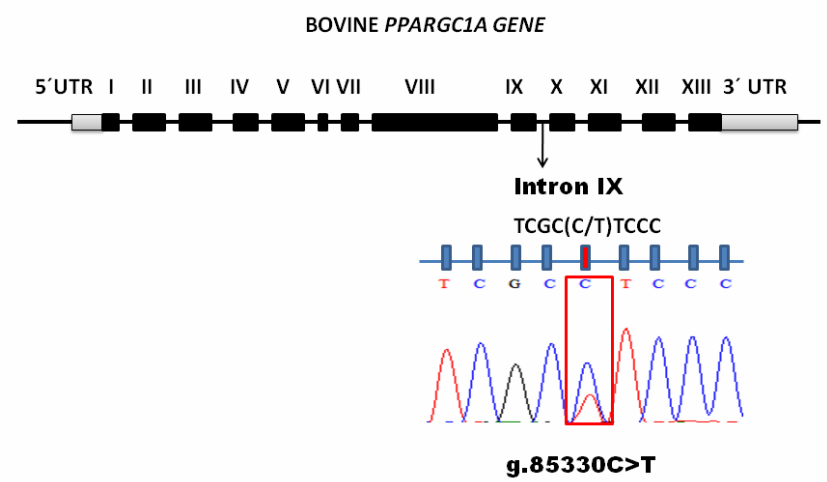

Fig. 1: Detection of polymorphic site in bovine *PPARGC1A* gene. Black mark indicates the coding sequences, gray mark indicates the 5_-UTR and 3_-UTR and black line indicates the introns.

Table 1: Effects of g.85330C>T polymorphism on *PPARGC1A* genotypes in Chinese Holsteins (LSM±SE).

Locus/Variants	Génotypes	Milk yield (kg)	Fat yield (kg)	Fat (%)	Protein yield (kg)	Protein (%)
g.85330C>T	CC	9933.05±80.98	373.77±3.66	3.784±0.033	$307.71±2.66^{A}$	$3.107±0.010^{A}$
	CT	9988.82±83.12	373.43±3.75	3.751±0.034	$311.28±2.72^{B}$	$3.125±0.011^{AB}$
	TT	9817.16±113.85	370.51±4.86	3.783±0.046	$307.10±3.54^{AB}$	$3.137±0.015^{B}$
P-value	-	0.1725	0.6924	0.2687	0.0123	0.0037
Additive (a)	-	57.94	1.63	0.0008	0.30	-0.0149*
Dominant (d)	-	113.71	1.28	-0.0328	3.87*	0.0030
Allele substitution (α)	-	12.74	1.11	0.0139	-1.23*	-0.0161**

Notes: Different superscripts of capital letters in the same column refers significantly different at P<0.01. The single asterisk (*) refers statistical difference at P<0.05 while two asterisks (**) refer statistical difference at P<0.01.

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