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Polymorphism and Sequencing of DGAT1 Gene in Iranian Holstein Bulls

Research Article

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ABSTRACT

Quantitative traits locus for milk production traits has been described on centromeric end of bovine chromosome 14. Reports name the acyl coA: diacylglycerol acyltransferase (DGAT1) gene as a potential candidate gene with dinucleotide substitution (AA to GC) in exon VIII which causes the change of lysine to alanine in amino acid (K232A).The aim of the present study was to estimate the frequency of DGAT1 K232A polymorphism in Iranian Holstein bulls as a potential quantitative trait locus (QTL) for marker assisted selection. Sample of 103 Holstein bulls from the Animal Breeding Center of Iran were genotyped for DGAT1 polymorphism (A and K allele). The PCR-RFLP technique was used to study the DGAT1 gene polymorphism. Frequency of KK, KA and AA genotypes were 0.59, 0.41 and zero respectively. The allele frequencies of the DGAT1 gene were 0.7961 and 0.2039 for K and A allele, respectively. The K allele was sequenced and registered in NCBI gene bank with EU075528 accession number.

KEY WORDS DGAT1, gene, Holstein, PCR-RFLP.

INTRODUCTION

Many studies in dairy cattle have shown that a quantitative traits locus (QTL) with major effect on milk production traits is located in the centromeric end of bovine chromosome 14 (Coppieters *et al*. 1998; Heyen *et al*. 1999; Boichard *et al*. 2003; Looft *et al*. 2001). This QTL had been fine mapped to a 3 cM region (Riquet *et al*. 1999; Farniar *et al*. 2002). It was shown that the QTL variation is most likely caused by a missense mutation (AA to GC) in the candidate gene DGAT1, changing lysine to alanine (K232A) in the enzyme diacylglycerol acyltransferase (DGAT) (Grisart *et al*. 2001; Winter *et al*. 2002). The gene content of chromosomal region flanking DGAT1 was determined by Winter *et al*. (2004). In cattle the lysine variant (K allele) increased fat yield, percent of fat and protein but the alanine variant (A allele) increased milk and protein yield (Grisart *et al*. 2001; Winter *et al*. 2002; Spelman *et al*. 2002).

The single nucleotide polymorphisms (SNPs) underlying lysine to alanine (K232A) substitution can be diagnosed by a PCR-RFLP assay (Kaupe *et al*. 2004). Selection could be based on genetic marker, so before integrating such molecular information in breeding schemes, a rigorous characterization of the alternative variation with respect to improving of genetic gain is necessary. Allele K of DGAT1 gene increases fat yield and fat and protein percentage; whereas, allele A of this gene increases milk and protein

yields (Kaupe *et al*. 2007; Thaller *et al*. 2003). Therefore, the objective of the present study was to estimate the frequency of DGAT1 polymorphism in Iranian Holstein bulls and sequence DGAT1 gene, as a potential marker for marker assisted selection (MAS) in breeding program.

MATERIALS AND METHODS

Samples

Total of 103 samples refer to Iranian proved Holstein bulls were used to genotype for DGAT1 gene.

The molecular experiment was performed in molecular biology laboratory in Avicenna Research Center of Mashhad. Sample preparation of frozen semen to extract genomic DNA was done as follows: one mL TE buffer was added to 0.5 mL frozen semen and centrifuged at 1000 rpm for 10 min then the supernatant was discarded. The formed pellet was vortexed in one ml of TE buffer for 30 s and the suspension was centrifuged as described (Winter *et al*. 2002).

Sperm pellet was diluted with 200 mL PBS buffer and DNA extraction was performed as follows: The genomic DNA of samples were extracted using DIAtom Kit (Biokom Russia) according to the procedure recommended by the manufacturer.

The quality and quantity of DNA were evaluated using spectrophotometer (Bio Aquarius, Cecil, UK) and electrophoresis techniques.

PCR-RFLP analysis

The 411 bp fragment of DGAT1 gene was amplified with standard PCR (Thermo cycler, Biometra, Germany). The total volume of reaction was 25 µL that contained standard Buffer (2.5µL), one unit of Taq polymerase (0.2 µL), 200 µM of each dNTP (0.5 µL), 10-20 pM primer mixture (4 µL) and 50-100 ng DNA (5 µL), 0.5 µL DMSO (was added to each reaction vessels as PCR enhancer), and 12.3 µL dH_2O. The sequences of forward and reverse primers were 5′-GCACCATCCTCTTCCTCAAG-3′ and 5′-GGAAGCGCTTTCGGATG-3′, respectively. The thermal program of PCR included 1 cycle at 94 ºC for 6 min, followed by 35 cycles of 60 s at 94 ºC, 60 s at 60 ºC and 60 s at 72 ºC and followed by 1 cycle of 7 min at 72 ºC .

The PCR product was separated by electrophoresis in 2% agarose gel and visualized with IMAGO gel documentation (B&L system, Germany). The PCR product was digested with RFLP method.

The 5 µL of amplified DNA was mixed with 2 µL 10X buffer, 13 µL dH_2O and 5 U of *AcoI* restriction enzyme (product of Sibenzyme) and digested over 6 hours at 37 ºC. The digested fragment was loaded in 3% agarose gel and visualized with IMAGO gel documentation.

Sequencing of DNA

The fragment of 411 bp related to KK genotype was sequenced with Sanger method (ABI machine, USA) machine (Sanger *et al*. 1977). Chromatogram of sequence was analyzed with chromas software (version 2.13). The sequence aligned with registered sequence in Gene Bank by BLAST 2 Sequences software of NCBI. The sequence was also aligned with sequence of DGAT1 gene of *Bos taurus*, *Bos indicus* and water buffalo in gene bank by MultAlin software.

Statistical Analysis

The Hardy Weinberg equilibrium for allele and genotype frequencies were analyzed with Chi square test using PoPGen software, version 1.31 (Yeh and Yong, 1999).

RESULTS AND DISCUSSION

The DNA samples' concentrations were between 61 and 504 ng/µL and DNA concentration average in the final solution was 80 ng/µL. The fragment of 411 bp of DGAT1 gene was amplified with PCR (Figure 1).

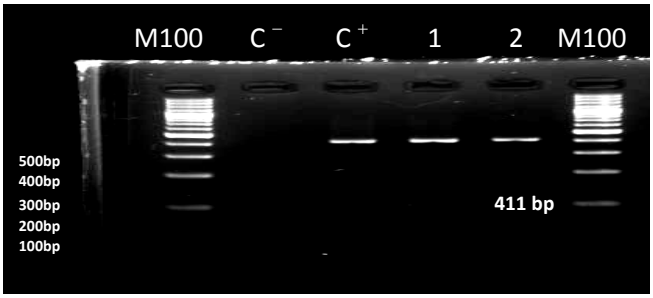

Figure 1 Loading PCR product, negative (C^-) (no PCR amplicon) and positive (C^+) (contained PCR amplicon) control, molecular marker is M100

The gene fragment was digested with *AcoI* enzyme. Two fragments with 208 and 203 bp would have been visualized on agarose gel if the genotype were AA which was not detected in this experiment (Figure 2). Two fragments of 208 and 203 bp were appeared in the agarose gel as one band because two fragments length were almost equal. The genotype would be KK if the gene fragment (411 bp) remained intact after digestion as appeared in gel (Figure 2). Gene fragment could produce three fragments of 208, 203 and 411 bp after enzymatic digestion if genotype be KA (Figure 2).

Gene and genotypic frequency

Number of sire with KK and KA genotype in this study were 61 and 42, respectively. Genotype AA was not observed. The genotypic frequency of KK, KA and AA were

0.592, 0.408 and zero, respectively. Frequency of allele K and A were 0.7961 and 0.2039, respectively.

Grisart *et al*. (2004) and Winter *et al*. (2002) introduced DGAT1 gene. Estimation of allelic and genotype frequencies of gene DGAT1 were studied intensively (Kaupe *et al*. 2007; Chandra *et al*. 2005; Citek *et al*. 2004).

Allelic frequency of DGAT1 was estimated between zero and one (Lacorte *et al*. 2006; Kaupe *et al*. 2004) and observed heterozigosity among different breeds were between 0.0 and 0.499 (Lacorte *et al*. 2006; Chandra *et al*. 2005; Kaupe *et al*. 2004). In this study, frequency of allele K is four times greater than allele A (0.7961 and 0.2039). It is reported that the frequency of allele K is greater than allele A in Holstein breed (Spelman *et al*. 2002; Thaller *et al*. 2003; Chandra *et al*. 2005; Kaupe *et al*. 2007). However, Hori-Oshima *et al*. (2003) and Lacorte *et al*. (2006) reported that allele K frequency is less than allele A frequency in Holstein breed. In this study 61 and 42 sires have genotype KK and KA, respectively and we did not observe genotype AA. So probably, the selection criteria were considered in such a way that reduced AA genotype in the population.

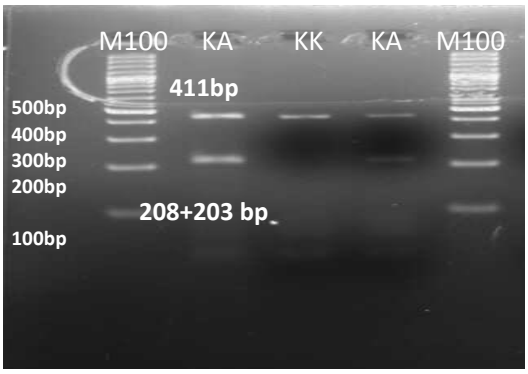

Figure 2 Genotype KK and KA in 3% agarose gel, molecular marker is M100

So probably, the selection criteria were considered in such a way that reduced AA genotype in the population.

The Hardy Weinberg equilibrium was investigated with chi square test (Table 1). The value of chi square in this study was 6.76, greater than the critical value of 6.63 which means that the population under study isn't in Hardy Weinberg equilibrium ($P<0.01$). Observed and expected homozygosis and heterozygosity and average heterozygosity of DGAT1 gene in this study are shown in Table 2. Studies of polymorphism of DGAT1 in *Bos taurus* and *Bos indicus* breeds, showed that allele K was a wild allele and the allele A substitution probably occurred after the divergence of *Bos taurus* and *Bos indicus* (Kaupe *et al*. 2004).

According to Kaupe *et al*. (2004) frequency of allele A in beef cattle is greater than in dairy cattle which have a low to high frequency of allele K.

Study polymorphism of DGAT1 in New Zealand dairy cattle population showed that frequency of allele K in Holstein, Jersey and Air Shire was 0.6, 0.88 and 0.23 respectively (Spelman *et al*. 2002). Results from this and other studies imply that the frequency of gene and genotypic of DGAT1 in different breeds are diverse worldwide (Ripoli *et al*. 2006; Lacorte *et al*. 2006).

Sequencing

Result of comparing the sequence of 411 bp of DGAT1 gene with the same fragment in gene bank, shows that the sequence refers to allele K and the fragment 411 bp registered in gene bank of NCBI with accession number EU077528. In fact sequencing confirmed the results of RFLP techniques. The sequence of DGAT1 gene of *Bos taurus*, *Bos indicus*, water buffalo in gene bank were aligned with sequence of allele K in this study (Figure 3). According to estimated frequency of alleles and Hardy Weinberg equilibrium status of the gene in the population it could be concluded that the population is under selection for milk fat percentage.

Table 1 Chi square test of data

Genotype	Observed	Expected	$(O-E)^2/E$
AA	0	4.28	4.28
KA	42	33.4	2.2
KK	61	65.27	0.28
sum	103	-	6.76

Table 2 Summary of homozigosity and heterozygosity of DGAT1 gene

Gene	Sample size	Observed homozygoty	Observed heterozygoty	Expected homozygoty	Expected heterozigoty	Average heterozigoty
DGAT1	206	0.592	0.408	0.6738	0.3262	0.3264

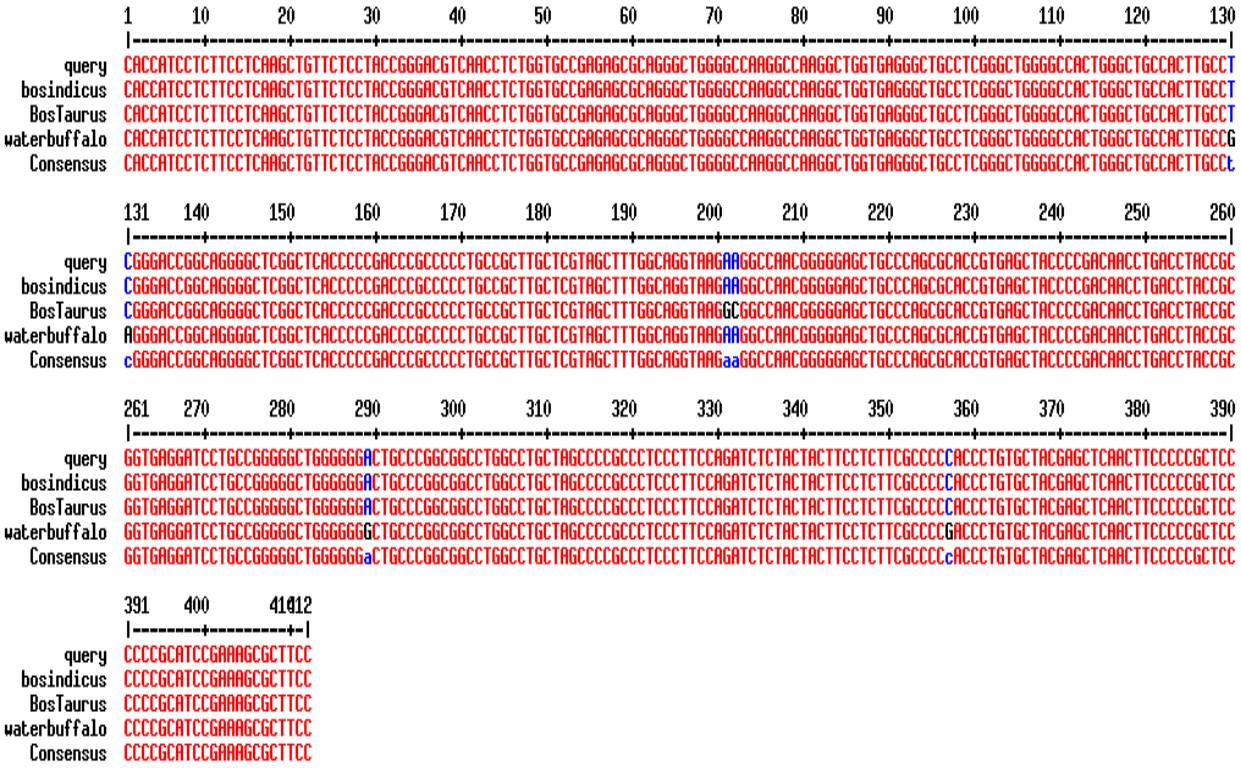

Figure 3 Alignment of DGAT1 gene with different species

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