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Research Article

Characterization of single nucleotide polymorphism in diacylglycerol acyltransferase (DGAT1) gene loci of Iranian Holstein Cattle

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Abstract

Genetic improvements of beef and dairy cattle can bring significant advances in satisfying the global food demand expected to be doubled by 2050. Most of the traits in livestock are controlled by multiple genes so called as genetically complex traits. One of the bovine quantitative trait loci (QTL) that affects milk yield and composition was identified to be located on centromeric end of bovine chromosome 14. Diacylglycerol acyl transferase-1 (DGAT1) gene plays an important role in the synthesis and secretion of an enzyme that is able to catalyze the last step of triglyceride biosynthesis. A total of 100 Iranian Holstein dairy cattle blood samples were collected from Mashhad (Iran) dairy farms. Genomic DNA was extracted in a GuSCN-Silica Gel method. The considered fragment of 411 bps was amplified in a standard PCR reaction. The amplified fragments were digested in a PCR-RFLP method making use of Cfr1 restriction enzyme and subsequently were sequenced. Using PopGen analyzing software, genotype frequencies of KK, KA and AA genotypes were estimated to be 0.31, 0.41 and 0.28, respectively. The allele frequencies of K and A alleles were estimated as 0.515 and 0.485, respectively.

Keywords: DGAT1 gene, milk production trait, dairy milk, Holstein cattle

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Introduction

Many advances in molecular genetic techniques have been achieved during last two decades. These achievements especially in the field of DNA markers have had a great effect on gene mapping, making the identification of some multigenic trait genes possible (Hosseinpour, 2007). One of the examples of multigenic traits is milk production trait which is highly significant in today's livestock industry. Economically after protein content, fat is considered as the second important content of the milk. Cattle milk fat involves almost 98 percent triglycerides with relatively high ratio of (nearly 50%) short chain fatty acids (Lehner & Kuksis, 1996; Coleman et al., 2000). A combination of short chain fatty acids with a chain length of 4-6 carbon atoms specifies the ruminants milk fat features. During the synthesis of triglycerides in the endoplasmic reticulum membrane, the attachment of fatty acids to Sn1 and Sn2 positions (stereo specific numbering) are

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catalyzed by several effective enzymes. Among these enzymes, DGAT1 enzyme acts as a catalyzer for the fatty acids that are attached at Sn3 position. The interesting point is that this position is the only one in which short chain fatty acids with 4 to 6 carbon atoms are found (Andersson, 2001). DGAT1 enzyme catalyzes the last step of triglyceride biosynthesis and it may have an inhibitory effect, too (Mayorek et al., 1989). This enzyme is encoded by DGAT1 gene. To date two DGAT encoding genes namely DGAT1 and DGAT2 have been identified (Andersson, 2001; Winter et al., 2003). The coding sequence of DGAT gene in both human and dairy Holstein cattle is 1470 bps in length including 17 exons separated by 16 introns. This gene is located on centromeric end of the bovine chromosome 14 covering 3 cm of this chromosome and gives rise to a protein product of 489 amino acid length (Winter et al., 2002; Farnir et al., 2002). The gene has two alleles called as A and K. Cattles with KK and AA genotypes have, respectively, lysine and alanine in their amino acid chains, while heterozygote cattle with KA genotype produce DGAT1 proteins that include lysine or alanine in their amino acid chains (Winter et al., 2003; Hosseinpoor, 2007). AA → GC mutation in the exon 8 has substituted lysine by alanine at amino acid position 232 in the amino acid chain of this enzyme. Lysine- encoding wild type allele is represented with K letter while the mutant one is shown by a letter. Having mutation followed by substitution of alanine in the place of lysine in DGAT enzyme amino acid chain, the kinetic rate of enzymatic reaction will be reduced resulting in a decrease in fat content, fat yield and protein content (Winter et al., 2002; Farnir et al., 2002). The aim of this study was to investigate DGAT1 gene allele frequency, genotype frequency and single nucleotide polymorphism in a population of Iranian Holstein cattle. The results can be subsequently used in genetically breeding programs.

Materials and Methods

In order to do DNA extraction, a total of 100 Holstein dairy cattle blood samples were collected from Mashhad – Iran dairy farms. Tail vein blood samples of 3ml were collected in EDTA containing vacuum tubes. GuSCN- silica Gel-based DNA extraction was carried out using DIAtom DNA prep Kit (BioKom, Moscow). DNA quantification was performed using UV-spectrophotometer. To qualify DNA, 5 µl of extracted DNA was subjected to 1% agarose gel electrophoresis and finally visualized in a gel documentation unit. To amplify a fragment of 411 bp within exon 8 of bovin DGAT1 gene, PCR Universal kit (IsoGene–Moscow) containing master mix PCR diluents and mineral oil was utilized. Standard PCR reactions were performed in a thermo cycler machine (Biometra T3000 Thermo cycler) under the following conditions: initial denaturation at 94°C for 1 min, followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 60°C for 1 min, extension at 72°C for 1 min and final extension at 72°C for 7 mins. PCR was carried out in the final volume of 25 µl including 1U of Taq polymerase, 2.5µl of standard buffer, 0.5µl of 200µM dNTPs, 3µl of 20 pM primers, 0.5µl of DMSO, 13.3 µl of dH_2O and 5µl of 100ng DNA samples. To quantify the amplified fragments, 2µl of PCR products were loaded into a 2% agarose gel and images were recorded using a gel documentation unit. In the next step, the amplified fragments were digested in a PCR-RELP method making use of CfrI restriction enzyme in a mixture of 3µl of amplified DNA sample and 2 units of restriction enzyme and subsequently incubated at 37°C for duration of 6 hours. Carrying out restriction digestion, the fragments were visualized on 3% agarose gel. Sequencing of the desirable fragments was performed in Melligen Company (France) in a ABi 3700 system. Our sequence was compared to the registered sequences available in NCBI and Gen Bank databases. The obtained chromatogram was analyzed using chromas software (version 2.13). DGAT1 DNA sequence of 411 bp in Iranian Holstein Cattles was compared with those of European and Indian cattle making use of Multialin software (Version 5.4.1). PopGene software (version 3.2) was utilized to analyze the allele frequencies, heterozygosity index and chi square test and to check whether the population is in Hardy–Weinberg equilibrium or not.

Results

Quantifying DNA fragments on agarose gel showed neither smeared DNA bands, nor DNA breakage. Quantitative spectrophotometric measurements showed DNA concentration ranging from 61 to 504 ng/ µl with the average of 80ng/µl in the final sample solution. Gel electrophoresis of PCR products confirmed the amplification of 411bps fragment of DGAT1 (Fig. 1). Recognizing the restriction site within the amplified sequence, CfrI restriction enzyme cleaves it into 2 fragments of 203 and 208 bp. During restriction digestion of PCR products, cattle with KA genotype give rise to DNA fragments of 411, 208 and 203 bp on 3% agarose gel electrophoresis. These fragments are observed as two bands on the gel due to the proximity of 203 and 208 bp fragments (Fig. 2). In case of cattle with KK genotype, no restriction digestion is executed on amplified fragment resulting in only one fragment of 411 bps on the gel. In cattle with AA genotype, the restriction site is located on both chromosome strands resulting in 2 fragments of 203 and 208 bp observed as one band due to proximity of these two fragments.

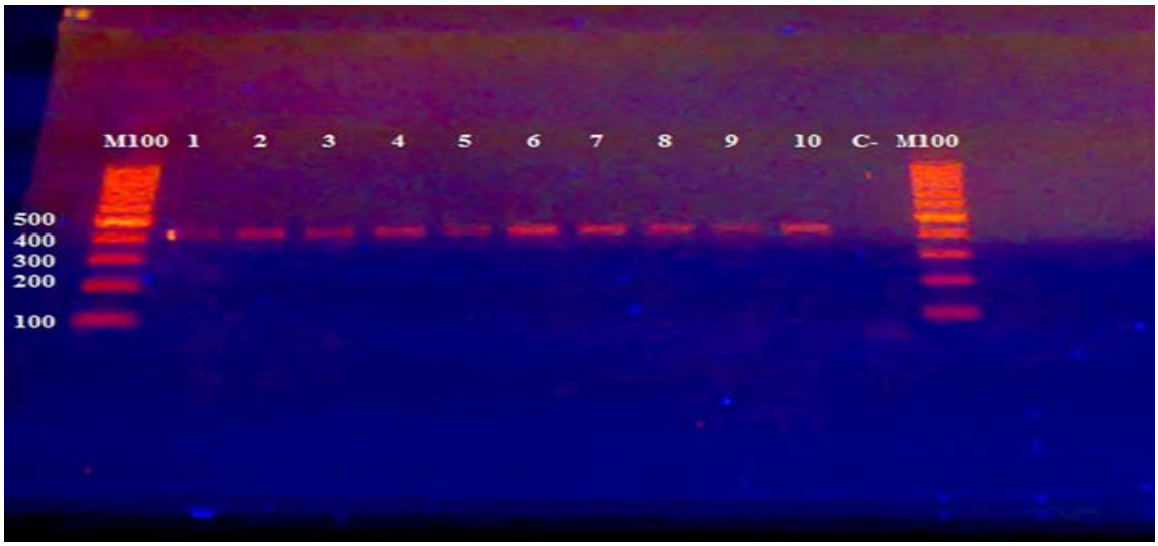

Fig. 1: Gel electrophoresis of the amplified 411bps fragment of DGAT1 gene.

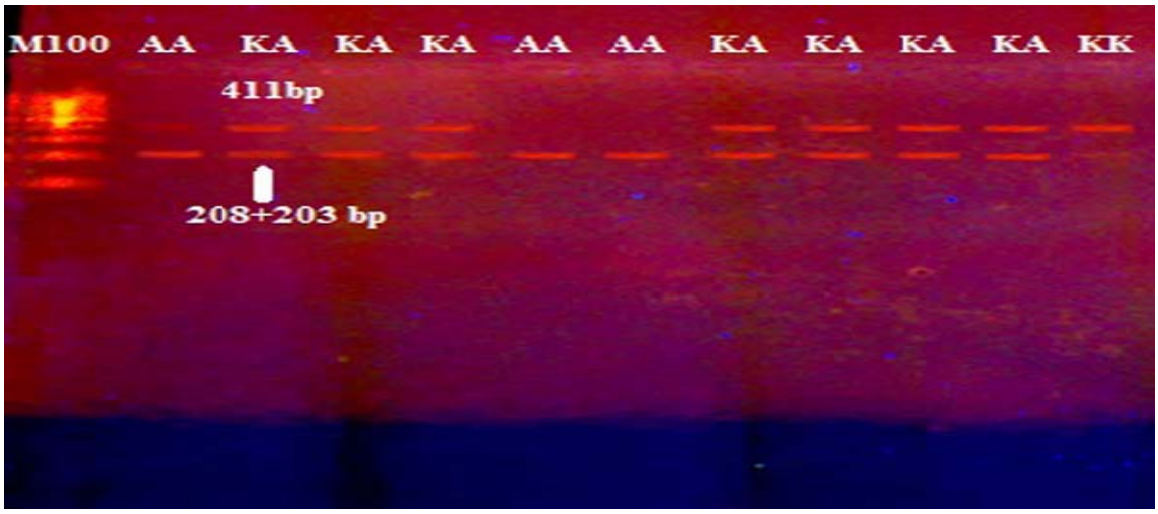

Fig. 2: Gel electrophoresis of the PCR products after CfrI restriction digestion

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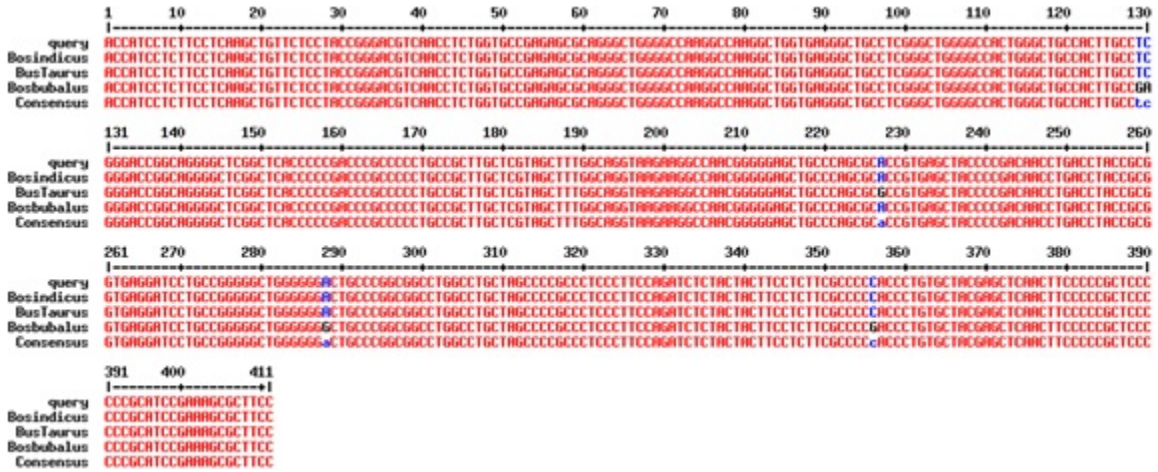

Fig. 3: Alignment of DGAT1 gene among different breed.

Table 1: The observed and expected genotypic and allelic frequencies of DGAT1 gene polymorphism

Genotype	Number of animal	Observed frequency	Expected Frequency	$\frac{(O-E)^2}{E}$	Chi-square test	Alleles
AA	28	0.28	0.235	0.852	X^2 =3.21 Critical Value=3.841	A=0.485 K=0.515
KA	41	0.41	0.499	1.605		
KK	31	0.31	0.265	0.755		

The Genotype frequency within the study population was estimated as follows: KK genotype (31%), KA genotype (41%) and AA genotype (28%). Chi square test was performed to assess whether the population is in Hardy-Weinberg equilibrium or not. The results are represented in Table 1. Taking degrees of freedom of 1 and P value of <0.05, the population was considered to be in Hardy-Weinberg equilibrium.

Comparing DGAT1 DNA sequence of 411 bp in Iranian Holstein Cattles with those of European and Indian cattle and buffleheads showed that the concerned sequence is completely similar in all three Holstein cattle types while it was different from those of buffleheads in four nucleotides (Fig. 3).

Discussion

So far, cattle have been selected mainly based on their phenotypes and functionality, which is the result of interaction between genetic and environmental factors. With recent advancements, some of the important genes, which are responsible for milk production trait, have been identified. All over the world, several molecular genetic attempts have been carried out to assess the allele frequencies of these genes among different races. Finally, a quantitative trait locus was identified in the centromeric end of bovine chromosome 14 harbouring DGAT1 gene as one of the most important factors affecting milk production trait specially milk protein and fat content (Weiss et al., 1960; Komisarek et al., 2004). This gene has two alleles named as K and A alleles. While K allele increases milk fat yield, fat content and protein content, A allele increases milk production and protein yield in dairy cattle. In some reports allele frequency of K allele is greater than that of A allele (Kaupe et al., 2004; Kaupe et al., 2007; Nowacka et al., 2008) while in others the results is reverse (Lacorte et al., 2006; Gautier et al., 2007; Banos et al., 2008; Schennink et al., 2008; Scotti & Fontanesi, 2010). In cattle with KK genotype lysine is located within amino acid chain of DGAT1 enzyme while in those with AA genotype alanine is located within their amino acid chains. In case of heterozygote cattle with KA genotype either lysine or alanine is placed within amino acid chain of DGTA1 enzyme (Winter et al., 2003; Kuehn et al., 2007; Naslund & Fikse, 2008). The genotype frequency of DGTA1 gene in our study population was: KK genotype (31%), KA genotype (41%) and AA genotype (28%). Estimating DGAT1 gene allele frequency in different population has shown high rates of variation ranging from zero to one. The heterozygosity index within different population has been reported from zero to 0.5 (Kaupe et al., 2004). Investigating DGAT1 polymorphism in a population of 100 Jerzy Dairy cattle using PCR-RFLP method, allele frequency of DGAT1 gene was reported as follows: K allele (0.83) and A allele (0.17) (Komisarek et al., 2004). In a study on DGAT1 K232A polymorphism in Irish Holstein cattle, genotype frequencies of KK, KA and AA genotypes were reported as 0.11%, 0.32% and 0.47%, respectively. According to the results of this study, one copy of K allele is associated with 77 kg decrease in milk production, 4.22 kg increase in fat yield and 0.99 kg decrease in protein yield. Genetic variance of milk production trait, milk fat content and milk protein content was reported as 4.8%, 10.2% and 1%, respectively. No association was found between DGAT1 K232A polymorphism and fertility, survival and stature traits (Berry et al., 2010). In another study on DGAT1 K 232A polymorphism in Swedish dairy Holstein and red cattle, the mean allele frequency of K allele in Swedish red cattle was reported as 0.09 while it was reported as 0.12 among dairy Holstein cattle. The results of this study showed that K allele is associated with an increase in milk fat and protein content and a decrease in milk production compared to A allele (Naslund & Fikse, 2008). Investigating DGAT1 polymorphism in *Bos tarus* and *Bos indicus* cattle showed that K allele is the ancestral wild type allele and replacement of this allele with A allele has been occurred probably after the lineage divergence of European and Indian breeds. Genotyping 1748 *Bos tarus* and *Bos indicus* cattle, from 13 countries of 4 Continents (Europe, Africa, Asia, North and South America) showed the stabilization of A allele in some of the European breeds and K allele in one of the Indian breeds. The frequency of K allele in breeds with no obvious ancestral history (European domesticated cattle in the center of near east and taurine N'Dama cattle in Africa) was in medium range. While the allele frequency of A allele was higher in beef cattle, the allele frequency of K allele was reported from too low to too high in dairy cattle (Kaupe et al., 2007). In another study, DGAT1 polymorphism was investigated in a population of Italian dual purpose cattle. Dairy cattle of different races including Holstein, brown, Simmental, Rendena, Reggina, Valdostana and

Modenese were genotyped in a PCR-RFLP method. While the allele frequency of K allele in Simmental, valdostana and vedena breeds was too low, it was reported as 24.5% among Holstein breeds and 17.2% among Reggiana breeds. K allele was reported neither in brown breeds nor in Modenese breeds. Comparing the results of DGAT1 allele frequency in their population with those of other populations, Scotti et al. (2010) showed a wide range of variations between various populations. Analyzing DGAT1 polymorphism in 14 populations of cattle from Argentina, Bolivia and Uruguay showed a heterozygosity variation ranging from 0.00 to 0.524. While the highest K allele frequency was reported among *Bos Indicus* cattle, European *Bos taurus* breeds with the exception of Jersey breeds showing the lowest frequency of this allele. Their results are in accordance with those of our study showing nearly equal ratio of both alleles [K allele (0.51) and A allele (0.48)].

Conclusion

The results of different studies on DGAT1 polymorphism show a high degree of variation among various populations all over the world. Considering the great effect of DGAT1 gene on milk production trait, the allele frequencies can be manipulated in a genetically breeding program. As DGAT1gene has been selected as the marker of milk production trait in several studies, investigating DGAT1 polymorphism along with those of other genes is highly valuable in today's livestock industry.

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