

Association between DGAT1 Genotype and Breeding Value of Milk Production Traits in Iranian Holstein Bulls

Research Article

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ABSTRACT

The aim of this study was to test the genotypes effect of DGAT1 gene on breeding value in Iranian Holstein milk production traits. For this purpose, 103 semen samples of registered bulls were collected and then genotyped for partial region of DGAT1 gene. The studied traits were milk yield (MY), fat yield (FY), protein yield (PY), fat percentage (FP) and protein percentage (PP). First lactation records of 43044 dairy cattle from 2000 to 2007 were used. The PCR-RFLP method was used for genotyping DGAT1 gene. The Hardy Weinberg equilibrium for allele and genotype frequencies was analyzed with Chi-square test using PoP-Gen software. Genetic parameters of studied traits were estimated by REML method under animal model with DFREML software. The breeding value of individual was predicted by BLUP method. Different genotype effect of 97 of 103 proven bulls on breeding value of milk production traits were investigated by GLM procedure of SAS (9.1) software. The mean of breeding value of proven bulls with different genotype were compare with Duncan test. The observed genotype frequency results showed KK (0.592), KA (0.408) and AA (zero) and the allele frequency obtained was K (0.7961) and A (0.2039). The KK genotype was confirmed by sequencing method and the K allele, with long of 411 bp, was registered in gene bank of NCBI with accession number EU077528. Estimated heritability of studied traits were 0.35 (0.02), 0.33 (0.02), 0.31 (0.017), 0.28 (0.02) and 0.27 (0.016) for MY, FY, PY, FP and PP, respectively. The average breeding values of MY, FY, PY, FP and PP were estimated as: 180.2 (28.8) kg, 3.7 (1.26) kg, 2.3 (1.06) kg, -0.036 (0.014) % and -0.03 (0.01) %, respectively. The average breeding value of MY for KA and KK genotypes were 288.8 and 109.6 kg, respectively and the difference was significant ($P < 0.01$). The average breeding values of FY in bulls for KK and KA genotypes were 5.6 kg and 0.91 kg, respectively ($P > 0.05$). The average breeding values of PY for KA and KK genotypes were 0.025 and 5.5 kg, respectively ($P < 0.05$). Differences between means of breeding values for FP and PP were significant ($P < 0.05$), these values for genotype KK and KA were -0.009% and -0.067% for fat and -0.016% and -0.059% for protein, respectively.

KEY WORDS breeding value, DGAT1 gene, Holstein bulls, milk production traits.

INTRODUCTION

Milk consumption per capita in Iran has been reported 73 kg while the average figure of the world is between 300 kg to 350 kg. Holstein cattle are the dominant industrial breed

for providing the majority of dairy products in the country. The population of this breed has been established with the importation of registered heifers from Europe, USA and Canada during 1970s and the first years of 1980s. Each year approximately 80 young bulls were entering into the prog-

eny-testing program by the animal breeding center in the country of which 12-20 bulls were selected as proven sires (Dadpasand *et al.* 2008). Boichard *et al.* (2003) reported that selection of animals in some situation based on genetic markers compare to phenotypic markers is preferable, for example for sex linked traits, type and reproduction traits and some of diseases or selection of bulls. The fore candidate gene approach could be proper platform to test for association of polymorphism within those genes and breeding value of production traits. A quantitative trait locus with major effect was founded on centromic end of chromosome 14 (Coppieters *et al.* 1998; Heyen *et al.* 1999). This QTL was fine mapped with 3 CM length (Farnir *et al.* 2002). In this region diacylglycerol O-acyltransferase 1 (DGAT1) gene was founded (Grisart *et al.* 2001; Winter *et al.* 2002). The function of DGAT1 gene is produced by releasing the diacylglycerol acyltransferase enzyme that catalyzes the final stage of triacylglycerol synthesis. The 98 percent of fat in milk is made of triacylglycerol. DGAT1 gene has large effects on milk production traits, this gene is determined as a major gene (Grisart *et al.* 2004; Winter *et al.* 2004). The nucleotides AA mutate to GC in exon 8 of gene, this mutation causes changes in lysine to alanine into enzymes on amino acid number 232. Wild type allele, which encodes the lysine, and mutant allele are shown with the letter K and the letter A, respectively. Replacement of alanine instead of lysine in the enzyme diacylglycerol acyltransferase causes decreases in enzyme maximum velocity and, therefore, the amount of fat, fat and protein percentage in milk are decreased (Winter *et al.* 2002). Based on previous literature works, DGAT1 gene has a significant effect on milk production traits, so the aim of the present study was to test the association of bulls for DGAT1 genotype with estimate breeding value of milk production traits.

MATERIALS AND METHODS

Phenotypic

The studied traits were milk yield (MY), fat yield (FY) and protein yield (PY), fat percentage (FP) and protein percentage (PP). First lactation records of 43044 dairy cattle from 2000 to 2007 were used.

Molecular study

In overall, one hundred and three registered semen sample bulls were collected from Animal Breeding Center of Iran and were used for DNA extraction. The genomic DNA of samples was extracted based on GuSCN-Silica Gel method and standard protocol was used with commercial kit of DI-Atom DNA Prep (product of Biokom co) based on Boom's *et al.* (1990) report. DNA concentration and purity measured at 260 nm long was determined by spectrophotometry (Bio Aquarius, Cecil, UK). The 411 bp fragment in exon 8

of DGAT1 gene was amplified with standard PCR using a Biometra Thermocycler. The sequence of primers was:

F 5'-GCACCATCCTCTTCCTCAAG-3'
R 5'-GGAAGCGCTTTCGGATG-3'

The total volume of reaction was 25 µl that contained one unit of Taq polymerase (Sina Gene), 2 µM of each dNTP, 200 mM MgCl₂, 10-20 pM primer mixed and 50-100 ng DNA and standard buffer. The values of material based on density were as follow: 2.5 µL standard buffer, 0.5 µL dNTPs, 3 µL primer mixed, 0.2 U Taq polymerase, 0.5 µL DMSO, 5 µL DNA and 13.3 µL dH₂O. The thermal program of PCR reaction was as follows for 35 cycles: initial denaturation at 94 °C for 6 min, denaturation at 94 °C for 60 s, annealing of primers at the temperature at 60 °C for 60 s, elongation at 72 °C for 60 s and final elongation at 72 °C for 7 min.

The PCR products were separated by electrophoresis in 2% agarose gel and then visualized with IMAGO gel documentation. The PCR product was digested with RFLP method.

The 5 µL of amplified DNA mixed with 2 µL 10X buffer, 13 µL dH₂O and 5 U of *AcoI* (product of Sibenzyme) and digested over 6 hours at 37 °C. The digested fragment was loaded in 3% agarose gel and then it was visualized with gel documentation. The fragment of 411 bp related to KK genotype was sequenced with Sanger method (ABI machine, USA) machine (Sanger *et al.* 1977). Chromatogram of sequences was analyzed with chromas software (version 2.13).

Statistical analysis

The Hardy Weinberg equilibrium for allele and genotype frequencies was analyzed with Chi-square test using PoP-Gen software (version 1.31).

Phenotypic and genotypic parameters of five traits were estimated by REML method under animal model with DFREML software (Meyer, 2000). The breeding value of individuals was predicted by BLUP method. Different genotypes effects of 97 proven bulls on breeding value of milk production traits were investigated by GLM procedure of SAS (9.1) software. The mean of breeding values of proven bulls with different genotypes were compare with Duncan test.

RESULTS AND DISCUSSION

Gene and genotypic frequencies

Numbers of bulls with KK and KA genotypes in this study were 61 and 42, respectively. AA genotype was not observed. The genotypic frequencies 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. Some researchers have reported that the frequency of K allele is greater than A allele in Holstein breed (Thaller *et al.* 2003; Kaupe *et al.* 2007). However, some studies reported that K allele frequency is less than A allele frequency in Holstein breed. Hardy Weinberg equilibrium was investigated by using Chi-square test. The value of Chi-square was 6.76 that compares with Chi-square of table (6.63) was higher, therefore, the population under study was not found to be in Hardy Weinberg equilibrium ($P < 0.01$). The fragment of 411 bp of KK genotype was sequenced from forward and reverse side. The K allele and the fragment 411 bp were registered in gene bank of NCBI with accession number EU077528.

Genetic parameters

The estimated heritabilities were 0.35 (0.02), 0.33 (0.02), 0.31 (0.017), 0.28 (0.02) and 0.27 (0.016) for MY, FY, PY, FP and PP, respectively (Table 1). The estimated breeding values of proven bulls are shown in Table 1. Minimum and maximum breeding values of MY were -265 and 1287 (kg), the mean of BV was 180.2 (28.8). These values for bulls with KK and KA genotypes were 109.5 and 288.2, respectively. The maximum and minimum of BV for FY in bulls were -27.5 and 27.3 (kg), respectively. The mean of BV was 3.7 (1.26).

The estimated BV of bulls with KK and KA genotypes were 5.6 and 0.9 (kg), respectively. The mean of BV for PY was 2.3 (1.06) and this value for KK and KA genotypes were 0.025 and 5.46, respectively. The mean of BV for FP and PP were -0.036 (0.014) and -0.028 (0.009), respectively.

Estimated BV of bulls with KK and KA genotypes for FP were -0.009 and -0.06 and for PP were -0.01586 and -0.05971, respectively. The means of breeding value for KK and KA genotypes of proven bulls were comparing and the result of that are shown in Table 2.

Milk yield

The mean of breeding value for KA genotype (288.23) was significant ($P < 0.01$) different to KK genotype (109.57). The K allele has low effect on MY and A allele has high effect on traits. So, the breeding value of bulls with KK genotype was lower than in those with KA genotype. The positive effect of allele A was reported by other researchers (Kühn *et al.* 2004; Kaupe *et al.* 2007). Citek *et al.* (2004) reported that differences between breeding values of AA (811 kg) and KK (256 kg) were significant ($P < 0.001$). The allele substitution effect of DGAT1 gene was investigated in some reports. The K allele substitution for MY in Jersey, Holstein and Ayrshires breeds ($P < 0.005$) were -110, -130 and -220, respectively (Spelman *et al.* 2002). These effects

were -242 to -180 for Fleckvieh and -260 for Germany Holstein (Thaller *et al.* 2003).

Fat yield

The result shows that the differences between the means of breeding values of KA and KK genotypes were not significant ($P > 0.05$). These results indicated the high effect of K allele on FY and the negative effect of A allele on these traits, that is consistent with other reports (Kühn *et al.* 2004; Winter *et al.* 2002). The average substitution effect of K allele for breeding value of Holstein dairy cattle for FY was 7.46 kg and the half of this effect was 5.23 kg for daughter yield deviation (DYD) (Grisart *et al.* 2004). Spelman *et al.* (2002) reported that this effect for allele K was 5.76 kg and 3.3 kg for both Holstein and Jersey breeds. Thaller *et al.* (2003) estimated this value for FY as 705 to 14.8 kg for Fleckvieh and from 7.6 to 10.7 for Germany Holstein cows.

Protein yield

The average value of inbreeding for KA (5.46) was higher and statistically significant ($P < 0.05$) compare to KK genotype (0.025). The results of this study showed a significant effect of allele A on PY that it was consistent with other studies (Grisart *et al.* 2004; Kühn *et al.* 2004; Kaupe *et al.* 2007). Citek *et al.* (2004) reported that the breeding value of PY (25 kg) for AA genotype in Holstein bulls was higher ($P < 0.001$) than for KK genotype (10.9 kg). Kaupe *et al.* (2004) reported an average value of -2.64 kg for the allele K substitution effect on breeding value of PY and -2.28 kg for DYD of bulls.

The average substitution effect for K allele were estimated ($P < 0.05$) as -2.45 and -2.48 for Holstein and Jersey breeds. These averages values were also similar to those reported for Ayrshires but there were not significant (Spelman *et al.* 2002). The allele K had a negative effect on PY in Fleckvieh with average values ranging from -3.6 to 0.2 kg and in Germany Holstein ranging from -4.8 to -5.2 kg (Thaller *et al.* 2003).

Fat percentage

The means of breeding values for this trait were -0.00933% and -0.06674% for KK and KA genotypes, respectively and the differences were significant ($P < 0.05$). The result of this study indicated the positive effect of K allele on FP that is consistent with other reports.

The breeding value of FP was estimated as 0.42% for KK genotype and this was considered to be higher ($P < 0.001$) than that obtained for AA genotype (Citek *et al.* 2004). The k allele substitution for breeding value of FP was 0.42% and half of this effect for DYD of bulls reported 0.17% (Grisart *et al.* 2004).

Table 1 The estimated heritability and breeding value of milk production traits

	MY	FY	PY	FP	PP
h ² (SD)	0.35 (0.02)	0.33 (0.02)	0.31 (0.017)	0.28 (0.02)	0.27 (0.016)
Mean BV (SD)	180.2 (28.8)	3.7 (1.26)	2.3 (1.06)	-0.036 (0.014)	-0.028 (0.009)
Max BV	1287	27.3	26.9	0.505	0.19
Min BV	-265	-27.5	-29	-0.31	-0.3

h²: heritability; SD: standard deviation and BV: breeding value.

MY: milk yield; FY: fat yield; PY: protein yield; FP: fat percentage; PP: protein percentage and CV: coefficient of variation.

Table 2 The compare of breeding value means

Genotype	Sample number	Mean of breeding value of studied traits				
		MY	FY	PY	FP	PP
KA	39	288.23 ^a	5.6 ^a	5.46 ^a	-0.00933 ^a	-0.01586 ^a
KK	58	109.56 ^b	0.908 ^a	0.025 ^b	-0.06674 ^b	-0.05971 ^b
SEM	-	42.38	1.73	1.51	0.02	0.012
Pr ≥ F	-	0.0037	0.058	0.012	0.049	0.017

MY: milk yield; FY: fat yield; PY: protein yield; FP: fat percentage and PP: protein percentage.

The means within the same column with at least one common letter, do not have significant difference (P>0.05).

SEM: standard error of the means.

The K allele increased the percentage of fat milk to a level of 0.35% and 0.28% for Fleckvieh and Germany Holstein (Thaller *et al.* 2003).

Protein percentage

The differences between the average breeding values for animals with KK (-0.01586) and KA (-0.05971) genotypes were significant (P<0.05).

These results imply that the K allele has a positive effect on milk PP and the same results were reported by other researchers (Thaller *et al.* 2003; Citek *et al.* 2004). The breeding value for PP in bulls that carry K allele (P<0.05) was 0.02% and for homozygote bulls for A allele was -0.03 (Citek *et al.* 2004).

In Fleckvieh and Holstein breeds the K allele increased between 0.1 and 0.06% the PP of milk (Thaller *et al.* 2003). Grisart *et al.* (2004) reported that the K allele substitution effect for PP (0.08%) and half of this value for daughter yield differences (DYD) was 0.04%.

CONCLUSION

Association study between candidate gene polymorphism and important traits in livestock is a first step to investigate on favorable allele verification for marker assisted selection. Quantitative traits such milk are regulated by many genes and are affected by interactions among them, and thus, a candidate gene associated with a trait in one population may have a different effect, or show no effect at all, in another population due to the negative effects of other genes and epistatic interactions of the candidate gene with other genes in the population. This theory is supported by many association studies, in which a polymorphism was significantly associated with performance traits in one family or breed, but not in another family or breed.

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