Influence of the Bovine Acyl-CoA: Diacylglycerol Acyltransferase1 (DGAT1) K232A on Milk Production and Somatic Cell Score Holstein Cows

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ABSTRACT

This experiment was aimed to study the association between the DGAT1 K232A polymorphism and milk production traits and somatic cell score (SCS) in Iranian Holstein dairy cows. The records of 408 animals from five dairy herds were randomly identified and then genomic DNA was extracted from blood using the modified-salting method described by Miller. RFLP-PCR was performed to obtain all the polymorphisms and two alleles, K and A were observed with frequency of 0.37 and 0.63. Genotypic frequencies of AA, KA and KK were 0.3578, 0.5515 and 0.0907, respectively. The relationship between DGAT1 K232A and milk traits and somatic cell score in the first lactation was studied. The results showed significant difference ($p \leq 0.05$) between the genotypes on milk production, fat percent but not for protein percent and SCS. According to this research, the DGAT1 K232A polymorphism can be considered for increasing milk performance traits in Holstein dairy cows in the proximal region of bovine chromosome 14.

Key words: polymorphism, DGAT1, milk, RFLP, SCS.Overview

INTRODUCTION

In animal breeding taking advantage of the knowledge from molecular genetics and important development analytical tools has resulted in identification of causal genes underlying QTL (Quantitative Trait Loci) for various economic traits (Jones et al.). Di-acyl glycerol acyl transferase 1 (DGAT1) was introduced as a suggested gene for milk production traits and it has been mapped in the centromeric end of bovine chromosome 14 (BTA14), within a region that contains QTL influencing milk yield and composition (Farnir et al. 2002). This gene is the encoder of enzyme Di-acyl glycerol transferase 1 which catalyzes the last step toward of triglyceride synthesis (Grisart et al. 2002, Naslud et al. 2008). Milk fat is primarily composed of triglyceride because more than 95 percent

milk fat consists of triacylglycerol and therefore, DGAT1 enzyme could be considered as a key enzyme in milk triacylglycerol synthesis in the mammary glands (Jensen 2002, Smith et al.2000). The considered polymorphism results AA→GC nucleotide substitution in DGAT1 gene exon 8 in Bos Taurus for changing Lysine to Alanine at position 232 of the encoding protein. The allele encodings Lysine (K) 232 variant proved to be more efficient considering to milk fat synthesis. Allele K has its impact on the milk fat synthesis in this way that it is capable to produce triglycerides faster (Vmax) than allele A (Grisart et al. 2002, Grisart et al.2004). The K allele is a wild type and the A allele substitution probably happened after the mutation of Bos Taurus and Bos Indicus (Kaupe et al. 2004). Many studies showed a significant association between polymorphism of this gene and milk production traits (Ripoli et al.2006, Smith et al. 2000).This polymorphism has been associated with increased fat yield, fat and protein percent also reduction in milk and protein yield (Jensen 2002, Kaupe et al. 2004, Winter et al. 2002). Recently, allelic and genotypic frequencies of DGAT1 K232A gene polymorphism in Iranian Holstein dairy cows were reported (Asadollahpour et al. 2013). In dairy cattle breeding schemes, the animals not only improve for milk production and functional traits, the traits related to animal health are considered. As a result, better animal survival in the herd and increase the production of high quality were achieved (Meredith et al. 2012). Somatic Cell Score (SCS) has been studied as an indicator of susceptibility to mastitis in dairy cows. Many reports have showed genotypic and phenotypic relationship between SCS and milk production. The results proved the existence of unfavorable correlation between high milk production and increased SCS (Jamrozik et al. 2010, Meredith et al. 2012). Since a poor relationship between DGAT1 K232A polymorphism and SCS has been reported, the objective of this experiment was to study the association between DGAT1 K232A polymorphism and SCS and milk production traits in Iranian Holstein population.

MATERIAL AND METHODS

The records of 408 animals from five dairy herds were randomly identified and then genomic DNA was extracted from blood using the modified-salting method described by Miller (Miller et al. 1988). RFLP-PCR was performed to obtain all the polymorphisms and two alleles were observed (K and A). Characteristics of the studied population, the extraction of genomic DNA, genotyping and the polymorphism across the herds was described earlier by Asadollahpour et al. (2013). In current study, the relationship between DGAT1 K232A gene with milk production, milk compositions (fat and protein) and SCS were investigated.

Statistical analysis

The effect of DGAT1 allele on milk yield and milk compositions, and SCS in the first lactation was analysed using GLM procedure in SAS (Watson and Neoh 2008). The following statistical model was used:

$$Y_{ijkl} = \mu + G_i + HYS_j + S_k + b_1(X_{ijkl} - \bar{X}) + b_2(W_{ijkl} - \bar{W}) + b_3(Z_{ijkl} - \bar{Z}) + e_{ijkl}$$

Where; Y_{ijkl} : milk traits, μ : overall mean, G_i : fixed effect of the genotypes (AA, AK and KK), HYS_j : fixed effect of the herds (1, 2, 3, 4, and 5), birth year and season, S_k : random effect of sires (1,…,155), b_1 : linear regression coefficient of milk yield, X_{ijkl} : individual milk yield, $\bar{X}$ is mean of milk yield, b_2 : linear regression coefficient of open days, W_{ijkl} : individual open days, $\bar{W}$: mean of open days, b_3 : linear regression coefficient of days in lactation, Z_{ijkl} : individual days in lactation, $\bar{Z}$: mean of days in lactation, and e_{ijkl} is random residual effects. For milk yield, the covariate of days in lactation and open days was ignored and for fat and protein percent and SCS, the covariate effect of milk yield was excluded from the model.
Somatic cell score index as following formula was used, in order to normalize the distribution of somatic cell count (Ali, shook 1980):$SCS = \log_2(SCC/100000) + 3$

RESULTS

In this study, influence of DGAT1 gene on milk production traits, especially on milk and fat yield in different genotypes was observed (Table 1). Functional polymorphism of this gene polymorphisms on milk composition have been reported in dairy cattle by Grisart et al. (2002). Allelic and genotypic frequencies of DGAT1 across the herds by Asadollahpour et al. (2013) indicated that this gene was polymorphic and therefore it could be considered in the animal breeding schemes.

DISCUSSION

Results of DGAT1 K232A polymorphism showed significant difference ($p \leq 0.05$) for milk yield and AA was higher than KA and AA. In opposite, AA was significantly ($p \leq 0.05$) less that KA and AA genotypes for fat percent. In general, differences were observed between the genotypes carrying the K allele (KK and KA) and the genotype that is lacking this allele (AA). It seems to be an allele has been causing difference between the genotypes. Furthermore, regarding the report of Grizaret et al (2004), both DGAT1 alleles were investigated in baculovirus system and it proved that Allele K is capable to produce triglycerides faster (Vmax) than allele A. Therefore, DGAT 1 gene may lead to different amount of milk fat in genotypes KK and KA against genotype AA.

Since the reverse relationship between milk production and fat percent, association of allele K with higher fat percentage and lower milk production is in accordance with previous studies such as Grisart et al. (2004) in Holstein – Friesian cows, Winter et al. (2002) in Bos taurus and Bos indicus breeds, Kaupe et al. (2007) in German Holstein cattle, Ripoli et al. (2006) in European breeds. In current study, no significant difference was found between genotypes in protein percentage, which was indicated by others (Grisart et al. 2002, Grisart et al. 2004, Strzałkowska et al. 2005).

In current study, no significant association ($p \leq 0.05$) was revealed between the K232A DGAT1 polymorphisms and SCS. In other words, there is an inappropriate relationship between milk production level and SCS and we had more SCS in the cows with high milk production. Phenotypic correlations between milk yield and SCS can be studied by several different mechanisms. Amount of SCS may indicate a bacterial infection of the udder that could have an inappropriate effect on milk production. High-producing cows are more tending to mastitis. Naslud et al. (2008) and Kaupe et al. (2007) reported no association in German Holstein cattle and Swedish dairy cattle, respectively. Besides, Vinicius et al. (2010) reported association between DGAT1 allelic effects and SCS in a sample of North American Holstein cattle ($p \leq 0.01$). The correlation between milk production and SCS causes higher SCS in high-producing cows. Hence, it was expected to observe the difference between AA and other genotypes, but it was not significant. This could be due to numerous problems inherent with SCS phenotype and therefore reduced power to identify any relationship. Differences in environmental effects in statistical models, population and genetic level and using DYD or EBV by different reports may be the probable reasons to observe these differences in the present and others studies.

In conclusion, the present study, show a significant association of K232A polymorphism in the DGAT1 gene with fat percentage. In fact the individuals with genotypes KK and KA had higher fat percent and lower milk production than AA. This can be a source that underlies the reported QTL for fat yield and percent in this proximal region of bovine chromosome 14. The genotypes KK and KA for having allele K can be selected in animal breeding plans with goal of increasing fat percent of the Holstein cattle.

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Table1. Least square means and standard error (±SE) for milk production traits and somatic cell score (SCS) in Holstein dairy cows

Traits	Genotypes		
	AA	KA	KK
Milk yield (kg)	9606b(±141)	9296a(±127)	9043a(±242)
Fat percent	3.06b(±0.04)	3.2a(±0.043)	3.3a(±0.08)
Protein percent	2.93a(±0.01)	2.97a(±0.01)	3.00a(±0.03)
SCS	1.87a(±0.09)	1.86a(±0.08)	1.7a(±0.16)

Different superscript letters in each row indicate significant differences at p ≤ 0.05