

Effects of Acyl-CoA:diacylglycerol acyl transferase 1 (DGAT1) gene on milk production traits in crossbred Holstein dairy cattle

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Abstract The objective of this study was to analyze and investigate the genotype frequency and the association between Acyl-CoA:diacylglycerol acyltransferase 1 gene, DGAT1 gene, and milk yield (MY), milk composition, protein yield (PY), fat yield (FY), solid not fat yield (SNF), total solid (TS), the content of fat, protein, solid not fat, and total solid, (%Fat,% Prot,%SNF,%TS) in two herds of crossbred Holstein dairy cattle in Thailand. Two hundred and twenty-seven crossbred Holstein cows were used and their blood samples were taken for the study. PCR–RFLP was used to identify the allele and genotype of DGAT1 gene. A general linear model and the least square method were used to estimate the least square mean and additive, and the dominant effect of the gene on the traits and the least significant differences were used to compare the mean of each trait between genotypes. Two alleles (K, A) and three genotypes (AA, KA, KK) were detected, the highest allele and genotype frequencies were A and AA, respectively. The least mean squares of each genotype were compared and significant differences between genotype were detected. Genotype KK has the greatest effect on all milk composition content traits, while genotype AA has the greatest effect on yield traits. Highly significant additive gene effect was detected. From the results, it can be concluded that the DGAT1 gene can be used as a gene marker for assisted selection in milk composition traits.

Keywords Acyl-CoA:diacylglycerol acyl transferase 1 gene · Crossbred Holstein cattle · Milk composition · Milk production

Introduction

Crossbred Holstein cows that are a cross between Holstein and native cattle are the major strain raised in tropical and subtropical zones, since they can withstand high temperature and a humid climate (Milazzotto et al. 2008; Tadesse and Dessie 2003; Freitas et al. 1998). Furthermore, there is a strong possibility that this strain may become the main breed in the dairy cattle industry in the future, as the crisis in climate change that we are confronting now will continue for a long time. Conditions of high temperatures and humidity are uncomfortable for purebred Holstein cattle and will affect their performance. Crossbred Holstein cattle, however, in general, still have a low performance, which is a problem. Genetic improvement for this strain particularly in the trait of milk production has become an important objective for animal breeders. Gene marker aids for selection can reduce the time and increase the accuracy of selection (Meuwissen and Van Arendonk 1992). Many previous studies investigated the potential of the gene marker in many loci and found some loci have high potential. Acyl-CoA:diacylglycerol acyl transferase 1 (DGAT1) gene is one of the candidate genes that has a very high potential to become a gene marker.

The DGAT1 gene is located on bovine chromosome 14. It encodes Acyl-CoA:diacylglycerol acyl transferase 1 enzyme that has a role in the synthesis of triglyceride with the catalyzation of the reaction of diacylglycerol and fatty acid (Winter et al. 2002; Grisart et al. 2002). Based on the study of both Winter et al. (2002) and Grisart et al. (2002), it was found that a nonconservative substitution of lysine with alanine (K232A) at positions 10,433 and 10,434 in exon8 showed an association between milk production and composition traits. This finding led to many studies that aimed to investigate this association in various breeds and

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population, and the results of the association have been found to depend on the breeds or population. The studies of Signorelli et al. (2009), Näslund et al. (2008), Nowacka-Wozuk et al. (2008), Gautier et al. (2007), Kuehn et al. (2007), Sanders et al. (2006), Thaller et al. (2003), Weller et al. (2003), Grisart et al. (2002), and Spelman et al. (2002), for example, found a negative significant effect of K allele on milk yield and protein yield, but a positive effect on the content of fat. These results contrast with the study of Schennink et al. (2007) and Sun et al. (2009). While the studies of Cardoso et al. (2011) and Streit et al. (2011) found that the K allele of the DGAT1 gene showed a significant association with milk production. Although the results from many studies are inconclusive, it is clear that the DGAT1 gene has a strong association with milk production and milk composition traits. So, there is a possibility that the strong effect and association of this gene with milk production and milk composition traits may be detected in this study. As mentioned, the objective of this study was to analyze the allele and genotype frequency, and investigate the association of the DGAT1 gene with milk production and milk composition traits in crossbred Holstein cattle.

Materials and methods

Animals

All cows (227 cows) that have records of individual milk yield and milk composition in two farms, one from the medium privacy dairy cattle farm and the other from

Suranaree University of Technology, were used to genotype. Grading up to increase the level of Holsteins with imported semen has been used for breeding programs in both farms. North America is the main source of semen that has been used in both farms and that is also used on most farms in Thailand.

Genotypes and phenotypes

The genotype of the DGAT1 gene was studied in 227 Crossbred Holstein cows that were crossed between Holstein and native cattle. The percentage level of Holstein cattle varied from 75% to 99.22%. Genomic DNA was extracted from white blood cells using DNA Mini Kit Protocol-Blood (Geneaid). The protocol of Kuehn et al. (2007), Lacorte et al. (2006), and Winter et al. (2002) was used to study the polymorphism of this gene.

Individual milk yield (MY kg/day/cow), the content of fat (%Fat), milk protein (%Prot), solid not fat (%SNF) and total solid (%TS) and the quantity of fat (FY), protein (PY), solid not fat (SNFY), and total solid (TSY) per day from 2008 to 2009, with about 2,250 records were used to analyze the association of this gene with those traits. The average and standard deviation of the traits in each genotype are shown in Table 1.

Statistical analysis

Gene frequency and the Hardy–Weinberg equilibrium test were analyzed by GENEPOP version 3.4 (Raymond and Rousset 2003). The general linear model (GLM) that is shown below and the least square method were used to

Table 1 Frequency of DGAT1 allele and genotype, *P*-value of Hardy–Weinberg test and mean±standard deviation (SD) of traits

Item	Total number	Allele		Genotype		
		K	A	KK	KA	AA
Frequency	227	0.36	0.64	0.115	0.480	0.405
<i>P</i> -value of Hardy–Weinberg equilibrium test	0.57					
Average±SD	No. of data					
MY (kg/day)	2,257			9.99±3.41	10.54±3.95	11.09±4.25
PY (g/day)	2,255			305.2±103.3	314.8±11.8	316.4±115.7
FY (g/day)	2,218			397.0±136.9	387.2±135.1	384.3±139.2
SNF (g/day)	2,218			846.6±283.7	876.4±322.2	907.0±338.9
TS (g/day)	2,257			1,254.4±419.5	1,261.0±444.2	1,290.0±467.4
%Prot	2,255			3.07±0.39	3.03±0.37	2.90±0.34
%Fat	2,218			4.02±0.70	3.75±0.68	3.54±0.61
%SNF	2,218			8.49±0.51	8.35±0.48	8.21±0.49
%TS	2,257			12.62±1.12	12.14±0.99	11.77±0.95

MY milk yield, PY protein yield, FY fat yield, SNF solid not fat yield, TS total solid, %Prot% of protein, %SNF% of solid not fat, %TS% of total solid

Table 2 Least square mean (LSM) of genotype of DGAT1 (Acyl-CoA:diacylglycerol acyltransferase1) gene on milk yield and milk composition that include MY, PY, FY, SNF, TS, %Prot, %SNF, and %TS

Item	LSM of genotype (SE)		
	AA	KA	KK
MY (kg/day)	11.6(0.18) ^a	11.3(0.15) ^b	10.5(0.25) ^c
PY (g/day)	334.9(5.2) ^a	330.8(4.5) ^{ab}	316.8(7.2) ^b
FY (g/day)	399.4(6.3)	409.7(15.5)	413.2(8.7)
SNF (g/day)	957.7(15.2) ^a	929.1(13.0) ^b	882.5(20.8) ^c
TS (g/day)	1,353.3(20.8)	1,334.3(17.8)	1,306.3(28.3)
%Prot	2.90(0.02) ^c	2.97(0.01) ^b	3.04(0.02) ^a
%Fat	3.5(0.03) ^c	3.7(0.03) ^b	4.0(0.04) ^a
%SNF	8.2(0.02) ^c	8.3(0.02) ^b	8.4(0.03) ^a
%TS	11.68(0.05) ^c	11.98(0.04) ^b	12.53(0.06) ^a

MY milk yield, PY protein yield, FY fat yield, SNF solid not fat yield, TS total solid, %Prot% of protein, %SNF% of solid not fat, %TS% of total solid

^{a,b,c} mean significantly different at P -value < 0.05

estimate the least square mean of each genotype of the traits. Herd–year–season, day in milk, and level of Holstein genetic were used in the model as covariate variables, while lactation (8 levels), and the pattern of genotype (3 levels) were used as fixed effects in the model. The relationship between genotype and the traits was considered significant when $P < 0.05$. The least significant difference was used to compare the effect of each genotype. SPSS for windows (Release 10) (SPSS, Inc., Chicago, IL) was used for the statistical analysis.

$$y = X_1\beta_1 + X_2\beta_2 + \varepsilon$$

where y is the data of MY kg/day/cow, %Fat, %Prot, %SNF, %TS, FY, PY, SNFY, and TSY while X_1 and X_2 are incident matrix of fixed factor that include herd–year–season, day in milk, lactation, and the level of Holstein cattle, incident matrix of the DGAT1 genotypes, respectively, β_1 and β_2 are

the effects of fixed factors in X_1 and the effects of each genotype of DGAT1, respectively. ε is the random effect of residual.

The additive and dominant effects were estimated using the same method as in Falconer and Mackay (1996), namely, additive effect = $1/2(AA - KK)$ and dominant effect = $KA - 1/2(AA + KK)$.

Results and discussion

Allele and genotype frequency and Hardy–Weinberg equilibrium

The allele and genotype frequencies of this locus are shown in Table 1. Two alleles (K, A) and three genotypes (KK, KA, AA) were found. Allele A and genotype KA were the most frequent while allele K and genotype KK were the rarest found in the sample of crossbred Holsteins. This situation was consistent with previous studies of different breeds: Dutch Holsteins (Schennink et al. 2007), Ayrshires (Spelman et al. 2002), Swedish Holsteins (Näslund et al. 2008), UK Holsteins (Banos et al. 2008), Israeli Holsteins (Weller et al. 2003), Fleckvich and German Holsteins (Thaller et al. 2003), Montbe'liarde, Normande and French Holsteins (Gautier et al. 2007), German Holsteins (Winter et al. 2002; Kaupe et al. 2004, 2007), Polish Holsteins (Nowacka-Woszek et al. 2008; Pareek et al. 2005; Strzalkowska et al. 2005), and Chinese Holsteins (Sun et al. 2009). However, some previous studies showed different results, Jerseys (Spelman et al. 2002) and German Angeln (Sanders et al. 2006), for example. From this information, it can be assumed that the different breeding goals of each country resulted in different breeding programs that affected the frequencies of some loci related to particular traits. The nonsignificance of the Hardy–Weinberg equilibrium (HWE) was found ($P = 0.57$). The results could imply that the selection for improving

Table 3 Additive and dominant effects of DGAT1 (Acyl-CoA: diacylglycerol acyltransferase1) gene on milk yield and milk composition that include MY, PY, FY, SNF, TS, %Prot, %SNF, and %TS meaning milk yield, protein yield, fat yield, solid not fat yield, total solid, % of protein, % of solid not fat, % of total solid, respectively

Item	Additive effect		Dominant effect	
	Estimate (SE)	P -value	Estimate (SE)	P -value
MY (kg/day)	0.6 (0.1)	0.0001	0.2 (0.2)	0.27
PY (g/day)	9.0 (3.9)	0.02	4.9(5.0)	0.33
FY (g/day)	−6.9 (4.7)	0.14	3.4 (6.1)	0.58
SNF (g/day)	37.6 (11.3)	0.0009	9.0 (14.6)	0.53
TS (g/day)	23.8 (15.4)	0.12	4.5 (19.8)	0.81
%Prot	−0.1 (0.01)	0.0001	−0.01 (0.02)	0.64
%Fat	−0.3 (0.02)	0.0001	−0.04 (0.03)	0.20
%SNF	−0.12 (0.02)	0.0001	−0.05 (0.02)	0.0086
%TS	−0.42 (0.04)	0.0001	−0.12 (0.05)	0.007

the trait had not been carried out in the herds in accordance with the real situation in dairy cattle production in Thailand. At the same time, there is also the possibility that the gene frequency in the sample reach to the HWE since the imported semen.

Effect of DGAT1 genotype on milk production and milk composition

From this study, it is clear that this locus should be a suitable gene marker in crossbred Holstein cattle. However, a different genotype should be used for yield traits and milk composition content traits. When the least square mean of the traits was considered (Table 2), genotype KK gives the most effect on milk composition content traits, while genotype AA has the most effect on milk yield and milk composition yield traits. This result was in accordance with the studies of Cardoso et al. (2011), Streit et al. (2011), Barbosa et al. (2010), Banos et al. (2008), Bennewitz et al. (2004), Gautier et al. (2007), Grisart et al. (2002), Kuehn et al. (2007), Spelman et al. (2002), Thaller et al. (2003), and Weller et al. (2003) that found that this locus, particularly the KK genotype, has the effect of increasing fat content. These results suggest that the effect of the DGAT1 gene does not only affect milk fat content but also affects the milk composition, particularly, milk protein. The significant effect of the DGAT1 gene on milk protein was found in some previous studies: Schennink et al. (2007), Spelman et al. (2002), Smaragdov (2011), etc. Therefore, it can be postulated that the DGAT1 gene has a multiple pleiotropic effect gene and the function of this gene on milk protein synthesis should be demonstrated. Moreover, the interaction effects between this locus and the other loci, casein gene family, for example, which were confirmed for the function of milk protein synthesis, should be studied further.

Additive and dominant gene effects

The additive and dominant effects of the DGAT1 gene on the traits are shown in Table 3. Highly significant effects of the additive gene were detected in most traits except the traits of FY and TS, while only %SNF and %TS traits were detected with a significant dominant gene effect. In the case of the additive effect, genotype KK has more effects on the traits than the genotype AA according to the study of Spelman et al. (2002) and Sun et al. (2009), which is also consistent with the findings of Winter et al. (2002) and Grisart et al. (2002) that found the strongest effects of genotype KK on milk composition traits. In theory, half of the additive gene effects will be inherited from the parents by their offspring. The animals that have high additive effects can be selected to be parent stock in their herds. These results show that genotype KK can be used as a gene marker aid for dam selection, particularly, when the

breeding goal is milk composition improvement. However, the study of Peterson et al. (2003), which investigated the coordination of the group of genes involved in lipid synthesis pathways with a diet-induced milk fat depression, showed that the diets involve a coordinated effect on mRNA for mammary lipid synthesis pathways. In addition, the study of Hayes et al. (2003), Lillehammer et al. (2009) and the review of Hammami et al. (2009) also found a significant effect of the interaction between the environment and gene expression. Therefore, before KK genotype can be used in breeding programs, the interaction effect between this gene and feed management and other environments should be studied.

Conclusion

The DGAT1 gene has the potential to be used as a gene marker if the aim of genetic improvement is milk composition. With the strongest effect of genotype KK on milk composition traits, this genotype can be used as a gene marker aid for dam selection. However, the interaction effects between this gene and feed management and other environments should be studied.

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