

Effect of *DGAT1*, *leptin* and *TG* gene polymorphisms on some milk production traits in different dairy cattle breeds in Hungary

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Abstract

The objective of this study was to estimate the effect of acylCoA-diacylglycerol-acyltransferase 1 (*DGAT1*), *leptin* and thyroglobulin (*TG*) loci on the milk fat, milk protein and milk yield in Holstein Friesian, Jersey and Hungarian Simmental cows. *Leptin* and *DGAT1* genotypes were determined by qPCR assay, while *TG* genotypes were identified using the polymerase chain reaction-restriction fragment length polymorphism technique (PCR-RFLP). *DGAT1* GC/GC cows had the highest 305-day milk yield values. Difference between AA/AA and GC/GC genotypes was significant ($P<0.05$). *Leptin* CC animals produced significantly higher 305-day milk protein percent values ($P<0.05$) than other genotypes in Hungarian Simmental breed. At *TG* locus TT cows showed the highest 305-day milk fat percent values, although differences between genotypes proved to be significant ($P<0.05$) only in Jersey breed.

Keywords: dairy cattle, *DGAT1*, *leptin*, *TG*, qPCR, PCR-RFLP, milk yield, milk fat, milk protein

Introduction

DGAT1 is a microsomal enzyme that catalyses the final step of triglyceride synthesis. A lysine/alanine (K232A) polymorphism in *DGAT1* has been shown to affect milk fat content (Grisart *et al.* 2002). The lysine allele of *DGAT1* gene has a positive effect on milk fat content in different cattle breeds (Winter *et al.* 2002, Strzałkowska *et al.* 2005). Spelman *et al.* (2002) investigated the effect of the *DGAT1* polymorphism in different dairy breeds and significant differences were demonstrated for milk yield, as well as milk fat and protein content. Highly significant differences were observed for milk fat, milk protein and milk yield in Holstein-Friesian cows (Thaller *et al.* 2003, Bennewitz *et al.* 2004). Variable numbers of tandem repeat (VNTR) polymorphisms in the promoter region of the *DGAT1* gene also seem to affect milk production traits (Kühn *et al.* 2004). Gautier *et al.* (2007) concluded that VNTR polymorphism in Normande and French Holstein breeds was responsible only for a small fraction of the variance of the QTL, while the K232A polymorphism had a large effect on milk production and milk composition. Sanders *et al.* (2006) reported about a specific allele of the *DGAT1* promoter VNTR, which showed significant effects on the lactose content and milk energy content, compared with other alleles.

Leptin is the hormone product of the obese gene synthesised and secreted predominantly by adipocytes and its expression is regulated by body fatness and energy balance. This protein is supposed to be involved in the regulation of body weight by transmission of a lipostatic signal from adipocytes to the *leptin* receptor in the hypothalamus resulting in appetite suppression and increased thermogenesis (Zhang *et al.* 1994, Ji *et al.* 1998). The *leptin* gene has been mapped to bovine chromosome 4 (Stone *et al.* 1996). Polymorphisms in the *leptin* gene have been associated with milk yield in dairy cattle (Liefers *et al.* 2002). Animals with TT genotype of the R25C single nucleotide polymorphism in the bovine *leptin* gene showed increased milk production and milk protein yield compared with CC and CT genotypes, respectively (Buchanan *et al.* 2003). TT animals of the UASMS2 polymorphism in the bovine *leptin* promoter region could be associated with the highest marbling score, serum *leptin* concentration and backfat thickness (Nkrumah *et al.* 2005).

TG is a glycoprotein precursor of hormones that influence lipid metabolism and is synthesised in the thyroid gland. The effect of a C/T single nucleotide polymorphism (SNP) in the 5'-untranslated region of *TG* gene has been deduced to affect intramuscular fat content in cattle (Barendse 1999). *TG* and *DGAT1* genes physically mapped to the centromeric region of the bovine chromosome 14 (Coppieters *et al.* 1998, Winter *et al.* 2002) and are separated by about 25cM (Moore *et al.* 2003, Thaller *et al.* 2003). Many studies mentioned in this chromosomal region a QTL effect on milk fat yield and milk fat percentage in dairy cattle (Khatkar *et al.* 2004). Based on previous QTL studies in different cattle breeds and on the impact of this gene on fat metabolism, Khatib *et al.* (2007) investigated the effect of the *TG* gene on milk production traits. Analysis of 29 sire families showed no significant association between *TG* variants and milk production traits.

The objective of this study was to estimate the effect of *DGAT1*, *leptin* and *TG* loci on some milk production traits in different dairy cattle breeds in Hungary. Considering that previous results for the possible use of the mentioned polymorphisms in selection to improve milk production traits are rather contradictory, it has been decided to carry out studies in different Hungarian dairy cattle populations with the aim to provide additional data to this particular subject.

Material and methods

1 236 blood samples were collected from Holstein Friesian (n=415), Jersey (n=340) and Hungarian Simmental (n=481) cows. Genomic DNA was isolated from whole blood (Zsolnai *et al.* 2003). Blood samples were stored at -20 °C until DNA extraction. Genotyping of the *DGAT1* and *leptin* polymorphisms was performed using a TaqMan allelic discrimination method in a Rotor-Gene RG 3000 Real-Time PCR system (Corbett Research UK Ltd, Cambridge, UK). Primers and labeled oligonucleotide probes were designed based on the *leptin* sequence (UASMS2, C/T substitution at position 528 according to GenBank acc. no. AB070368): forward, 5'-AGG TGC CCA GGG ACT CA-3'; reverse, 5'-CAA CAA AGG CCG TGT GAC A-3'; FAM probe, 5'-CAA GCT CTA GAG CCT GTG T-3'; HEX probe, 5'-AAG CTC TAG AGC CTA TGT-3'. PCR cycling conditions were: 95 °C for 10 min, followed by 40 cycles of 95 °C for 7 s, 55 °C for 7 s and 72 °C for 15 s. For the *DGAT1* sequence (SNPs are located in position 10 433 and 10 434 of the sequence under GenBank acc. no. AJ318490) primers and labeled oligonucleotide probes were designed as

follows: forward, 5'-CGC TTG CTC GTA GCT TTG G-3'; reverse, 5'-CGC GGT AGG TCA GGT TGT C-3'; FAM probe, 5'-TTG GCC GCC TTA C-3'; HEX probe, 5'-CGT TGG CCT TCT TAC-3'. In this case PCR conditions were: 95 °C for 10 min, followed by 15 cycles of 94 °C for 20 s, 62 °C for 30 s and 72 °C for 30 s and 35 cycles of 94 °C for 20 s, 38 °C for 20 s and 72 °C for 20 s. *TG* polymorphism was detected using the PCR-RFLP method described by Barendse (1999). Digested PCR products were separated in 4% Meta-Phor agarose gel (Rockland, ME, USA) in 1× TBE buffer and stained with ethidium bromide. As for each polymorphism about 5% of samples were genotyped in duplicate and repeatability was in all cases perfect. Milk production data of the genotyped cows were collected throughout three consecutive lactations and statistical analyses have been carried out to find association between genotypes and milk production traits.

Statistical analysis

Dataset was analysed with SPSS 15.0 for Windows (SPSS Inc., Chicago, IL, USA). Multivariate analysis of variance (general linear model, GLM) was applied to determine differences in milk production traits in case of all polymorphisms in all studied breeds. *DGAT1*, *TG* and *leptin* genotypes, birth year, number of lactations and calving season were included as fixed effects in mathematical models and 305-day-milk yield, 305-day-milk fat percentage and 305-day-milk protein percentage were considered as dependent variables.

So the formula of general linear model (GLM) was as follows in Hungarian Simmental breed:

$$y_{ijklmn} = \mu + Lep_i + TG_j + DGAT_k + birth\ year_i + calving\ no_m + calving\ season_n + Lep_i \times DGAT_k + Lep_i \times TG_j + e_{ijklmn} \quad (1)$$

can be written in Holstein-Friesian breed as follows:

$$y_{ijklmn} = \mu + Lep_i + TG_j + DGAT_k + birth\ year_i + calving\ no_m + calving\ season_n + Lep_i \times DGAT_k + DGAT_k \times TG_j + e_{ijklmn} \quad (2)$$

Finally GLM equation can be described in Jersey breed as follows:

$$y_{ijklmno} = \mu + Lep_i + TG_j + DGAT_k + birth\ year_i + calving\ no_m + calving\ season_n + farm_o + e_{ijklmn} \quad (3)$$

where y is the phenotypic record of the studied traits (e.g. milk%), μ is the general mean, *Lep* is the *leptin* hormone genotype (CC, TC, TT), *TG* refers to the *TG* polymorphism (CC, TC, TT), *DGAT* represents the effect of *DGAT1* genotypes (AA/AA, AA/GC, GC/GC), *birth year* means the year of birth, *calving no* stands for the number of completed lactations, *calving season* refers to the season in which the given cow calved, *farm_o* represents the farm effect and e is the residual error. Dominance effects were estimated as the deviation of mean values of the studied traits in heterozygotes from the mean of homozygotes, using the least square means. Additive effect was calculated as the half of the difference between the two homozygotes. Significant level of these factors was detected by the method of the least square difference (LSD). Variance in tables below represents the proportion of variation in the dependent variable explained by the regression model itself.

Results and discussion

With regard to herd genetic structure analyses, differences between the observed and expected genotype frequency values of *DGAT1* genotypes were significant only in the Jersey breed (Table 1). The calculated χ^2 values for the *leptin* and *TG* genotypes indicated Hardy-Weinberg equilibrium in the population.

Table 1
Frequencies of *DGAT1* genotypes in the studied breeds

Breed	No.	AA/AA*	AA/GC	GC/GC**	χ^2	<i>P</i>
Holstein-Friesian	415	18 (20)	148 (142)	249 (253)	0.517	0.772
%	100	4.3 (4.8)	35.7 (34.3)	60.0 (60.8)		
Jersey	340	233 (217)	86 (109)	21 (14)	9.533	0.009
%	100	68.5 (64.0)	25.3 (32.0)	6.2 (4.0)		
Hungarian Simmental	481	8 (5)	79 (86)	394 (390)	2.411	0.300
%	100	1.7 (1.0)	16.4 (18.0)	81.9 (81.0)		

The expected values are presented in brackets (df=2), *coding for lysine, **coding for alanine

Referring to *leptin* genotypes, only the Jersey breed fit the Hardy-Weinberg equilibrium, in case of Holstein-Friesian and Hungarian Simmental breeds the calculated χ^2 values did not indicate Hardy-Weinberg equilibrium in the population (Table 2).

Table 2
Frequencies of *leptin* genotypes in the studied breeds

Breed	No.	CC	TC	TT	χ^2	<i>P</i>
Holstein-Friesian	415	291 (300)	121 (106)	3 (9)	6.393	0.041
%	100	70.1 (72.3)	29.2 (25.5)	0.7 (2.3)		
Jersey	341	255 (258)	84 (77)	2 (6)	3.338	0.188
%	100	74.8 (75.7)	24.6 (22.6)	0.6 (1.7)		
Hungarian Simmental	485	257 (273)	212 (182)	16 (30)	12.416	0.002
%	100	53.0 (56.3)	43.7 (37.5)	3.3 (6.3)		

The expected values are presented in brackets (df=2).

Concerning *TG* genotypes there was a good agreement in all breeds between the observed and expected frequency values (Table 3). The calculated least square means (LSM) and standard errors for milk production traits of the three breeds included in this study are presented in Table 4, 5 and 6.

Table 3
Frequencies of *TG* genotypes in the studied breeds

Breed	No.	CC	TC	TT	χ^2	<i>P</i>
Holstein-Friesian	415	309 (310)	100 (97)	6 (8)	0.596	0.742
%	100	74.5 (74.8)	24.1 (23.4)	1.4 (1.8)		
Jersey	283	170 (172)	99 (97)	14 (14)	0.064	0.968
%	100	60.1 (60.8)	35 (34.3)	4.9 (4.9)		
Hungarian Simmental		234 (233)	171 (173)	33 (32)	0.059	0.971
%	100	53.5 (53.3)	39.0 (39.4)	7.5 (7.3)		

The expected values are presented in brackets (df=2).

Table 4

Least square means (LSM) and standard errors, variance, additive effect and dominance for 305-day milk yield, milk fat percent and milk protein percent in Holstein-Friesian cows

Loci	Genotype	LSM±SE		
		305-day milk yield, kg	305-day milk fat, %	305-day milk protein, %
<i>DGAT1</i>	AA/AA	8247.1±563.3 ^a	4.344±0.126 ^a	3.410±0.054 ^a
	AAGC	9079.1±385.0 ^a	3.782±0.086 ^b	3.275±0.037 ^b
	GC/GC	9532.7±335.8 ^b	3.725±0.075 ^c	3.229±0.032 ^c
	Variance, %	2.9	11.5	4.0
	Additive effect	642.8*	0.310*	0.091*
	Dominance	189.2*	-0.253*	-0.045*
<i>Leptin</i>	CC	9213.9±182.9	3.821±0.041	3.301±0.018 ^a
	TC	9262.9±283.2	3.946±0.063	3.258±0.027 ^b
	TT	8783.8±663.3	3.845±0.148	3.287±0.064 ^{a,b}
	Variance, %	0	0	0.9
	Additive effect	215.1	0.012	0.007
	Dominance	264.1	0.113	-0.036*
<i>TG</i>	CC	9179.7±226.1 ^a	3.878±0.050	3.269±0.022
	TC	8964.7±314.1 ^b	3.857±0.070	3.297±0.030
	TT	9367.1±556.8 ^a	3.906±0.124	3.275±0.054
	Variance, %	0.1	0	0
	Additive effect	93.7	0.014	0.003
	Dominance	-308.7*	-0.035	+0.025

Variance: percentage of variance due to the studied loci in the total phenotypic variance, ^{a,b,c}Different letters indicate significant difference between genotypes, *confidence level of the predicted factors ($P<0.05$)

Table 5

Least square means (LSM) and standard errors, variance, additive effect and dominance for 305-day milk yield, milk fat percent and milk protein percent in Jersey cows

Loci	Genotype	LSM±SE		
		305-day milk yield, kg	305-day milk fat, %	305-day milk protein, %
<i>DGAT1</i>	AA/AA	4676.6±221.2 ^a	5.383±0.168 ^a	3.958±0.065 ^a
	AAGC	4848.3±230.9 ^b	5.047±0.176 ^b	3.844±0.068 ^b
	GCGC	4853.2±243.7 ^{a,b}	5.054±0.186 ^c	3.785±0.071 ^b
	Variance, % [#]	0.1	8.2	4.3
	Additive effect	88.3	0.165*	0.087*
	Dominance	83.4*	-0.172*	-0.028*
<i>Leptin</i>	CC	4904.6±181.8	5.040±0.138	3.842±0.053
	TC	4806.4±191.7	5.113±0.146	3.851±0.056
	TT	4667.1±425.8	5.031±0.324	3.894±0.124
	Variance, % [#]	0	0	0
	Additive effect	118.75	0.005	0.026
	Dominance	20.55	0.078	-0.017
<i>TG</i>	CC	4858.9±216.6 ^a	4.987±0.165 ^a	3.850±0.063
	TC	4829.3±216.8 ^{a,b}	5.078±0.165 ^b	3.863±0.063
	TT	4689.9±238.7 ^b	5.118±0.182 ^{a,b}	3.875±0.070
	Variance, % [#]	1.0	0.7	0
	Additive effect	84.5*	0.066	0.013
	Dominance	54.9	0.026*	0.0005

^{a,b}different letters indicate significant difference between genotypes, *confidence level of the predicted factors ($P<0.05$), [#]percentage of variance due to the studied loci in the total phenotypic variance

In all breeds *DGAT1* GC/GC cows had the highest and AA/AA cows the lowest 305-day milk yield values. Contrasting the AA/AA genotype vs. GC/GC, the difference proved to be significant ($P<0.05$). There was a negative correlation between milk yield and milk fat percentage, so in case of 305-day milk fat percent a decreasing tendency could be observed from genotype AA/AA to GC/GC. Differences between genotypes were significant ($P<0.05$). In respect of milk fat content, similar results were obtained by Winter *et al.* (2002) in Holstein-Friesian, Simmental and Braunvieh breeds, by Strzałkowska *et al.* (2005) in Polish Black-and-White (Friesian) cattle, and by Schennink *et al.* (2007) in Dutch Holstein-Friesian cows. Concerning 305-day milk fat percentage, estimated variances within dependent variables caused by *DGAT1* genotypes in the Holstein-Friesian, Jersey and Hungarian Simmental breeds were 11.5, 8.2 and 9.2%, respectively. Considering 305-day milk protein percent, AA/AA animals showed significantly higher values ($P<0.05$) than other genotypes. Effects of the *DGAT1* K232A polymorphism on milk yield, fat and protein percentage are consistent with previous studies made by Grisart *et al.* (2002) and Spelman *et al.* (2002).

Table 6

Least square means (LSM) and standard errors, variance, additive effect and dominance for 305-day milk yield, milk fat percent and milk protein percent in Hungarian Simmental cows

Loci	Genotype	LSM±SE		
		305-day milk yield, kg	305-day milk fat, %	305-day milk protein, %
<i>DGAT1</i>	AA/AA	4 417.8±315.3 ^a	4.746±0.102 ^a	3.811±0.046 ^a
	AAGC	4 788.4±194.6 ^b	4.359±0.063 ^b	3.661±0.028 ^b
	GC/GC	5 109.1±122.2 ^b	4.110±0.040 ^c	3.554±0.018 ^c
	Variance, % [#]	0.4	9.2	6.0
	Additive effect	345.7*	0.318*	0.129*
	Dominance	25.0*	-0.069*	-0.022*
<i>Leptin</i>	CC	4 685.1±170.7	4.362±0.055 ^a	3.694±0.025 ^a
	TC	5 164.2±191.0	4.339±0.062 ^b	3.607±0.028 ^b
	TT	4 490.2±340.1	4.398±0.099 ^c	3.681±0.044 ^c
	Variance, % [#]	0	0.1	0.3
	Additive effect	97.5	0.018*	0.007*
	Dominance	576.6	-0.041*	-0.081*
<i>TG</i>	CC	5 001.4±141.4 ^a	4.283±0.046	3.628±0.020 ^a
	TC	4 963.2±148.9 ^b	4.330±0.048	3.643±0.022 ^b
	TT	4 483.6±223.0 ^b	4.474±0.072	3.704±0.032 ^b
	Variance, % [#]	1.0	0	1.1
	Additive effect	255.2*	0.096	0.038*
	Dominance	217.0*	-0.049	-0.023*

^{a,b,c}Different letters indicate significant difference between genotypes, *confidence level of the predicted factors ($P<0.05$), [#]percentage of variance due to the studied loci in the total phenotypic variance

As for *leptin* locus there was no demonstrable effect of the genotype on milk yield. Differences of means could not be proved to be significant in Holstein-Friesian and Jersey breeds, due to the small number of TT cows and the large standard error. Concerning Hungarian Simmental breed, *leptin* CC animals showed significantly higher 305-day milk protein percent values ($P<0.05$) than other genotypes. In this breed *leptin* TT cows realised the highest milk fat percentage values; the difference between genotypes was significant ($P<0.05$). Some previous studies have focused on associations between polymorphisms in

the coding region of the *leptin* gene and milk production traits (Liefers *et al.* 2002, Buchanan *et al.* 2003). However there are no former results concerning the effect of promoter region UASMS2 polymorphism (present study) on milk production traits.

Referring to *TG* locus, TT cows produced in all breeds the highest 305-day milk fat percent values; in case of Jersey and Hungarian Simmental breeds differences between CC and TT genotypes were significant ($P < 0.05$). Former analysis of 29 Holstein sire families showed no significant association between *TG* variants and milk production traits (Khatib *et al.* 2007).

Our previous research in Angus cattle (Anton *et al.* 2011) revealed that *leptin* and *thyroglobulin* TT animals could be associated with the highest intramuscular fat content values, similarly to the milk fat content in the current study.

The present study was carried out to test the possible association of three specific polymorphisms with milk production traits. An eventual marker assisted selection (MAS) approach - such as selecting for AA/AA genotypes at the *DGAT1* locus - might be performed, if higher milk yield is preferred. In this case, simultaneous selection for *leptin* and *thyroglobulin* TT genotypes could increase milk fat and protein percentage as well. However, from public health point of view Schennink *et al.* (2007) recommend to increase alanin variant (GC/GC) of *DGAT1* in the population by selective breeding (because of its association with more unsaturated milk-fat), reducing in this way saturated fatty acid intake of humans.

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