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Effects of *DGAT1* and *GHR* on milk yield and milk composition in the Chinese dairy population

D. Sun*, J. Jia*,†, Y. Ma*,‡, Y. Zhang*, Y. Wang*, Y. Yu* and Y. Zhang*

*Key Laboratory of Animal Genetics and Breeding of Ministry of Agriculture, Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, Beijing 100193, China. †Affiliated High School of Inner-Mongolian Normal University, Hohhot 010020, China. ‡Center for Forensic Science, Tianjin Police Station, Tianjin 300384, China

Summary

Previous studies have demonstrated that the p.Lys232Ala substitution in the *acylCoA: diacylglycerol acyltransferase* (*DGAT1*) gene and the p.Phe279Tyr mutation in the *growth hormone receptor* (*GHR*) gene are the causative quantitative trait loci underlying milk yield and composition on BTA14 and BTA20 respectively. To examine their applications in the genetic improvement of Chinese dairy cattle productivity, we herein investigated the effects of the *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr mutations on milk, fat and protein yield, as well as fat and protein percentage in the milk of 1222 Holstein cows. Genotyping was performed using PCR-RFLP for *DGAT1* or primer-introduced restriction analysis (PCR-PIRA) for *GHR*. With a mixed animal model, the significant associations of the *DGAT1* p.Lys232Ala substitution with 305-day milk, fat and protein yield were identified ($P = 0.0001$). The *DGAT1* allele that encode lysine at position 232 was associated with increased 305-day milk fat yield, but with decreased 305-day milk and protein yield, whereas the *GHR* p.Phe279Tyr mutation was found to be significantly associated with protein percentage ($P = 0.0014$). The allele substitution effect of p.279Phe by p.279Tyr may lead to a significant increase in protein percentage. Our findings indicate that *DGAT1* p.232Ala and *GHR* p.279Phe could be used to increase milk yield and protein yield of Chinese Holstein cows.

Keywords dairy cattle, *DGAT1* p.Lys232Ala, *GHR* p.Phe279Tyr, milk production traits.

Both theoretical and simulation studies agree that application of gene-assisted selection has the potential to increase the rate of genetic gain by pre-selecting young candidate bulls prior to progeny testing in dairy cattle (Khatkar *et al.* 2004). This kind of selection is based on the identification of genes that may affect the traits of interest. In the last two decades, extensive quantitative trait locus (QTL) mapping has been implemented to detect QTL with major effect on the milk production traits of dairy cows. However, only a few proportions of positional candidate SNPs have been confirmed to be true QTL. The AA to GC dinucleotide substitution alters the amino acid sequence from a lysine to an alanine (p.Lys232Ala, formerly called K232A) in the exon8 of the gene encoding acyl CoA: diacylglycerol acyltransferase 1 (*DGAT1*). This is the key enzyme to catalyze the last step in triglyceride synthesis (Cases *et al.* 1998), and has been proven to be the causative mutation underlying an increase in milk fat percentage (Grisart *et al.* 2004). Many studies also confirmed the effects of *DGAT1* p.Lys232Ala in the Holstein population of the Netherlands, New Zealand, and Germany (Spelman *et al.* 2002; Thaller *et al.* 2003; Bennewitz *et al.* 2004; Schennink *et al.* 2007). In addition, another identified QTL affecting milk yield and composition is in the *growth hormone receptor* (*GHR*) gene (Arranz *et al.* 1998; Blott *et al.* 2003). The T/A substitution, which changes the amino acid sequence of the transmembrane domain from a phenylalanine to a tyrosine (p.Phe279Tyr, formerly called F279Y) in the bovine *GHR*, was identified by many investigators to be the quantitative trait nucleotide with a large effect on milk yield and composition (Blott *et al.* 2003; Viitala *et al.* 2006). These aforementioned findings indicated that *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr can be used as practical genetic markers for selective breeding of dairy cattle.

However, the effect of any identified polymorphism may differ across different populations or breed because of specific genetic backgrounds. The Chinese Holstein originated from

Address for correspondence

Y. Zhang, Department of Animal Genetics and Breeding, College of Animal Science and Technology, China Agricultural University, 2 Yuanmingyuan West Road, Beijing 100193, China.
E-mail: changy@cau.edu.cn

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cross-breeding between Chinese Yellow cattle and European Holsteins over the past 100 years. Continuous import of foreign Holstein bulls, semen and embryos, mainly from USA and a few from Canada and Europe have been implemented, which were directly used in AI or via crosses with Chinese Holstein cows through planned mating to generate breeding bulls. The current selection direction of breeding programmes for the Chinese Holstein is aimed at higher milk yield while retaining no decrease in fat and protein percentage. Before the aforementioned polymorphisms are used for the genetic improvement of Chinese Holstein cattle productivity, their effects should be clearly examined. Thus, we investigated the genetic effects of *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr on milk yield and composition traits in Chinese Holstein cows. Further, we also investigated the 16 sires of genotyped cows to determine the potential impact of bulls on milk production of the Chinese Holstein population.

A total of 1222 cows were selected from 14 Holstein cattle farms in Beijing, including 16 sire families with 30–132 daughters from each sire. Phenotypic data for five milk production traits (i.e. milk yield, fat yield, protein yield, fat percentage and protein percentage over 305 days) at first lactation were obtained from the Dairy Data Processing Center of China. Genomic DNA was extracted from whole blood samples of cows and frozen semen of 16 bulls by a standard phenol–chloroform method. Genotyping was performed using PCR-RFLP. Based on the *DGAT1* sequence (AJ318490), the primers were designed to amplify a 201-bp fragment enclosing the p.Lys232Ala mutation: forward, 5′-CTCGTAGCTTTGGCAGGTAAG-3′; reverse, 5′-AAGTTGAGC TCGTAGCACAGG-3′. As for the *GHR* p.Phe279Tyr locus, an online program PIRA-PCR (http://cedar.genetics.soton.ac.uk/public_html/primer2.html) was used to design a pair of primers according to the *GHR* sequence (AM161140) (5′-AATACTT GGGCTAGCAGTGACAATAT-3′ and 5′-ACTGGGTTGATGAAA CACTTCACTC-3′). The expected PCR product was 175 bp, in which a single-base mismatch 4-bp upstream of the p.Phe279Tyr substitution site close to the 3′-end of forward primer, was created so that an SSpI recognition site (AATATT) was introduced into the PCR product. The PCR products of *DGAT1* and *GHR* were digested by the restriction enzymes Cfr1 and SSpI (New England Biolabs), respectively, and then electrophoresed on a 4% agarose gel.

Pedigree information of the genotyped animals was traced back for three generations. As a result, the total number of animals included in the analysis reached 3204. The kinship matrix (A-matrix) was calculated using Fortran95 code. All variance–covariance matrices have been estimated by restricted maximum likelihood using the EM algorithm, as applied in the REMLF90 programs (Misztal 1999). Finally, the effects of the *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr on first lactation milk yield and composition traits were estimated using the mixed procedure of SAS 8.02 software. With the following animal model, each trait was analysed separately and each polymorphism was also fitted separately: $y = \mu + \text{hys} + b \times M + G + \alpha + e$, where y was the phenotypic value of cows; μ was the general mean; hys was a herd-year-season effect; b was a regression coefficient; M was the co-variable describing the effect of age at first calving; G was a fixed effect corresponding to the genotype of polymorphisms; α was a random polygenic component account for all known pedigree relationships ('animal model'; Lynch & Walsh 1997); and e_i was a random residual. Bonferroni correction was performed for multiple t-testing through dividing the significance level by the number of tests. The additive (a) and dominance (d) and allele substitution (α) effects were estimated according to the equation of Falconer & Mackay (1996), i.e. $a = (\text{AA} - \text{BB})/2$, $d = \text{AB} - (\text{AA} + \text{BB})/2$ and $\alpha = a + d(q - p)$, where AA and BB represent the two homozygous genotypes, AB is heterozygous genotype, p is the allele frequency of *DGAT1* p.Lys232 or *GHR* p.Phe279, and q is that of *DGAT1* p.232Ala or p.*GHR* 279Tyr.

Three kinds of band patterns corresponding to genotypes KK (homozygous lysine), KA and AA (homozygous alanine), 201 and 201 bp/178 bp/23 and 178 bp/23 bp, were observed for the *DGAT1* p.Lys232Ala. As for the *GHR* p.Phe279Tyr locus, genotype FF (homozygous phenylalanine) yielded two bands of 151 and 24 bp, FY yielded 175, 151 and 24 bp, and YY (homozygous tyrosine) yielded one band of 175 bp. Direct sequencing of PCR products showed that both mutations found in this study were consistent with those described previously (Blott *et al.* 2003; Grisart *et al.* 2004). Genotypic and allelic frequencies for the two polymorphisms are presented in Table 1. With chi-squared tests, both *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr were found to deviate significantly from Hardy–Weinberg equilibrium ($P < 0.01$). Such deviations may be because of the different allele frequency distributions at *DGAT1* p.Lys232Ala and/or *GHR* p.Phe279Tyr between imported Holstein bulls and Chinese Holstein cows.

The estimated effects of the *DGAT*1 p.Lys232Ala and *GHR* p.Phe279Tyr on milk production traits are shown in Table 2. Associations of *DGAT*1 p.Lys232Ala with 305-day milk, fat and protein yield were revealed to be significant ($P = 0.0001$); such associations remained significant even after Bonferroni correction for multiple testing. *DGAT1* 232Lys was associated with higher fat yield, but with lower 305-day milk and protein yield. Further, all the additive effects as well as allelic substitution effects at the *DGAT1* p.Lys232Ala locus on the three yield traits were significant ($P < 0.01$). On the other hand, *GHR* p.Phe279Tyr was found to be significantly associated with milk yield ($P = 0.0098$), fat percentage ($P = 0.0178$), and protein percentage ($P = 0.0014$). However, this polymorphism remained significantly associated only with protein percentage after adjusting for multiple testing. Replacement of phenylalanine by tyrosine at the *GHR* locus appeared to lead to a significant decrease in protein percentage. Signif-

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Table 1 Genotypic and allelic frequencies in the *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr polymorphisms.

		Variant		Genotypic frequency			Allelic frequency	
Locus	Polymorphism	0	+	00	0+	++	0	+
DGAT1	p.Lys232Ala	Lysine	Alanine	0.14	0.61	0.25	0.45	0.55
GHR	P.Phe279Tyr	phenylalanine	Tyrosine	0.38	0.51	0.11	0.64	0.36

Table 2 Effects of the *DGAT1* p.Lys232Ala and *GHR* p.Phe279Tyr on milk production traits.

Traits	00[1] (LSM ± SE)	0+[1] (LSM ± SE)	++[1] (LSM ± SE)	Additive effect	Dominance effect	Allele substitution effect
DGAT1 (substitution of alanine for lysine)						
Milk yield (kg)	8060.13 ± 98.87^{A}	8114.91 ± 59.66^{B}	8521.70 ± 78.60^{C}	230.79**	176.01	248.39**
Fat yield (kg)	339.87 ± 4.55^{A}	319.34 ± 2.76^{B}	314.48 ± 3.61^{C}	−12.70**	7.84	−11.91**
Protein yield (kg)	259.43 ± 2.87^{A}	259.35 ± 1.74^{A}	268.76 ± 2.28^{B}	4.67**	4.75	5.14**
Fat percentage (%)	4.24 ± 0.004	3.95 ± 0.002	3.69 ± 0.003	−0.275	0.015	−0.274
Protein percentage (%)	3.23 ± 0.001	3.21 ± 0.001	3.16 ± 0.001	−0.035	−0.015	−0.037
GHR (substitution of tyrosine for phenylalanine)						
Milk yield (kg)	8053.06 ± 72.98^{a}	8281.40 ± 64.27^{b}	8081.73 ± 119.68^{ab}	14.34	−214.01**	74.26
Fat yield (kg)	317.66 ± 3.28	319.74 ± 2.89	313.42 ± 5.38	−2.12	−4.20	−0.94
Protein yield (kg)	258.96 ± 2.11	262.88 ± 1.86	255.77 ± 3.47	−1.60	−5.52*	−0.05
Fat percentage (%)	3.97 ± 0.030^{a}	3.88 ± 0.025^{b}	3.91 ± 0.046^{ab}	−0.030	0.060	−0.047*
Protein percentage (%)	3.23 ± 0.011^{Aa}	3.19 ± 0.010^{Bb}	3.18 ± 0.018^{b}	−0.025**	0.015	−0.029**

Different superscripts of small ($P < 0.05$) and capital ($P < 0.01$) letters in the same row mean significantly different.
*$P < 0.05$; **$P < 0.01$.

icant additive effects of *GHR* on protein percent ($P < 0.01$) and dominance effects on milk ($P < 0.01$) and protein yield ($P < 0.05$) were also observed.

In addition, we also genotyped the 16 Holstein bulls at the *DGAT*1 p.Lys232Ala and *GHR* p.Phe279Tyr polymorphic loci, which constituted the sire families of the cows used in this study. Interestingly, we found that none of the bulls were homozygous for the variant A (alanine) of p.Lys232Ala, and six out of 16 bulls were homozygous for the variant K (lysine). Similarly, at the *GHR* p.Phe279Tyr locus, only two sires were homozygous YY and seven sires were homozygous FF. The skewed distribution of genotypic frequencies in Holstein bulls is most likely because of the selection direction of breeding programmes.

In this study, the allele frequency of *DGAT1* p.232Lys is consistent with some previous studies (0.40) (Schennink *et al.* 2007), but slightly different from others (0.60 and 0.55) (Spelman *et al.* 2002; Grisart *et al.* 2004). We found that the *DGAT1* p.232Lys increased fat yield, but decreased milk yield and protein yield, confirming results of previous studies (Spelman *et al.* 2002; Thaller *et al.* 2003; Grisart *et al.* 2004; Schennink *et al.* 2007). In all these studies, *DGAT*1 p.Lys232Ala was observed to have opposite effects on fat vs. milk and protein. This may be because of the fact that p.232Lys is associated with increased fat percentages. In contrast to previous studies (Thaller *et al.* 2003; Grisart *et al.* 2004; Schennink *et al.* 2007), we found that *DGAT1* p.Lys232Ala does not affect fat percentage. From the results given in Table 2, however, we can see the trend that *DGAT1* p.232Lys increases fat and protein percentage, although such effects do not reach significance. The causes of the above discrepancies between allele frequencies and genetic effects may be the interactions with background genes in the different Holstein populations. On the other hand, estimated effects of *GHR* p.Phe279Tyr polymorphism on milk yield, fat percentage and protein percentage are consistent with previous reports (Blott *et al.* 2003; Viitala *et al.* 2006). However, the allele frequency of *GHR* p.Phe279 greatly differed from that of Finnish Ayrshire dairy cattle (Viitala *et al.* 2006). This is likely because of the specific characteristics of the two breeds. Because the dominant effect is not heritable across generations, *GHR* is not a useful genetic marker for selection of milk yield.

Considering the current breeding objectives for Chinese Holstein and also considering Chinese eating habits, increasing the frequency of the *DGAT1* p.232Ala is desirable because of its association with increased milk and protein yields and lower fat yields. As for *GHR* p.Phe279Tyr, the p.279Phe allele is desirable because of its association with higher protein percentages. It should be possible to improve the efficiency of selective breeding programmes by integrating these alleles into breeding schemes of the Chinese Holstein.

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