Effects of the K232A substitution at DGAT1 gene on some economic traits in 3 chinese dairy cattle*

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[**Abstract**] PCR-single-strand conformation polymorphism (PCR-SSCP) assay was used to diagnose the K232A substitution in the DGAT1 exon 8. Animals from three dairy cattle populations (212 Sanhe cattle, 234 Chinese Holsteins and 200 Chinese Simmentals) were used to estimate allele frequencies and gene substitution effects on milk production traits. Another four populations (47 Luxi, 23 Jinnan, 37 Qinchuan and 39 Nanyang cattle) without production records were used to estimate allele frequencies only. The allele frequencies for the lysine-encoding variant (allele K) were 0.17, 0.33 and 0.04 in Sanhe, Holstein, Simmental cows respectively; and 0.72, 0.39, 0.46 and 0.83 respectively in the other four populations. The average milk fat percentage in Sanhe cattle was associated (P = 0.017) with the K232A substitution in DGAT1 gene, individuals with genotypes KK and KA had 0.80% and 0.41% higher average milk fat percentage than those with genotype AA.

[**Key words**] DGAT1 gene; dairy cattle; K232A substitution
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Acyl CoA: diacylglycerol acyltransferase 1 (DGAT1, EC 2.3.1.20), a microsomal enzyme that catalyzes the final step of triglyceride synthesis, is a functional gene for milk production traits, especially for milk fat. Researcher reported mice lacking both copies of DGAT1 were completely devoid of milk secretion[1].

Studies on dairy cattle showed that a quantitative trait loci (QTL) with major influence on milk production was located in the centromeric end of chromosome 14[2-4], where the DGAT1 gene was mapped[5]. Grisart et al[6] and Winter et al[5] reported a GC-TO-AA exchange in DGAT1 gene resulted in a lysine-to-alanine exchange at position 232 of DGAT1 (K232A), and this exchange had association with variation at a quantitative trait locus on BTA 14 for milk fat percentage, individuals with allele K had higher milk fat percentage, and the frenquency of allele K were lower than allele A in most experimental populations.

The present study was aimed at detecting the frequencies distribution of this two alleles and their effect on milk production traits in three Chiense dairy cattle.

1 Materials and methods

1.1 Animals

Experimental animals were three Chinese dairy cattle (212 Sanhe cattle from Xie-er Ta LA Sanhe breeding farm in Mongolia, 117 Chinese Holsteins from Cao Tan dairy farm in Shaanxi and 100 Chinese Simmentals from Gao Lin Tun Simmental breeding farm in Mongolia).

Milk samples were collected for measuring milk fat, milk protein, milk sugar and somatic cell count in 2th, 5th and 8th month of a lactation peri-

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od from Xie-er Ta LA Sanhe breeding farm and Gao Lin Tun Simmental breeding farm. The data for milk product traits in Chinese Holsteins were offered by Cao Tan dairy farm. SCC (Somatic cell count) was converted to SCS (somatic cell score) before Statistic analysis, SCS = lb (SCC/100 000) + 3.

Blood from each cattle was sampled by venipuncture, and genomic DNA was extracted for later genotyping. Blood samples from another four populations (47 Luxi cattle from Juancheng in Shandong province, 23 Jinnan cattle from Shanxi province, 37 Qinchuan cattle from Shaanxi province and 39 Nanyang cattle from Henan province) were collected by my colleagues.

1.2 Primer design

Primers were designed using PRIMER3 program to amplify the 201 bp fragment franking 10 412 bp to 10 612 bp of bovine DGAT1 gene (gi: 21425304), left primer: 5′-CTC GTA GCT TTG GCA GGT AAG-3′, right primer: 5′-AAG TTG AGC TCG TAG CAC AGG-3′.

1.3 Genotyping and haplotype identification and sequencing

The PCR-SSCP assay was carried out with a vertical gel to genotype all the individuals. Two microliters of the PCR product was diluted with 8 microliters of a solution containing 95∶100 (*V*∶*V*) formamide, 20 mmol/L EDTA, 0.5 g/L bromophenol blue and 0.5 g/L xylene cyanol. The mixture was then denatured at 98 ℃ for 5 min, cooled in ice for 5 min and loaded on a nondenaturing 120 g/L acrylamide: bis-acrylamide = 29∶1 (*m*∶*m*) gel. Electrophoresis was performed in 1×Tris borate (pH 8.3)-EDTA buffer at 8 volts/cm for 10 h at 4 ℃. DNA was detected by silver staining.

DNA fragments that displayed a modified electrophoretic pattern were selected for sequencing. The amplified PCR products were concentrated and purified by Nucleotrap® Gel Extraction Kit, and were ligated into pGEM®-T easy vectors by T_4 DNA ligase. DNA sequences were determined in Shanghai Sangon Biological Engineering Technology & Service Co. Ltd.

1.4 Statistical Analysis

The differences of the genotypes were evaluated by the general linear model of SAS (SAS Inst. Inc., Cary, NC 2001) with adjustment for the number of lactations, the linear model was $Y_{ij} = \mu + G_i + L_j + E_{ij}$, where Y_{ij}, μ, G_i, L_j and E_{ij} were the record value, average value, genotypic effect, number of lactations effect and random residual effect respectively. For 305-d mature equivalent milk yield, the model was $Y_i = \mu + G_i + E_i$, where G_i was of genotypic effect.

The observed numbers of each genotype were compared with the expected frequencies (Hardy-Weinberg equilibrium) by the χ^2 test.

2 Result and analysis

2.1 PCR product and polymorphism detection

A 201 bp PCR product (Figure 1) was obtained with the designed primers and three genotypes (Figure 2) were detected using SSCP assay, the three genotypes were destined KK, KA and AA. Aligning (Figure 3) the sequences of alleles K and A showed there was an *ApA* to *GpC* dinucleotide substitution at positions 22 and 23 bp of the PCR product, which had been detected by Winter[5] with PCR-RFLP assay. In 120 g/L acrylamide: bis-acrylamide = 29∶1 (*m*∶*m*) gel, genotypes KK and AA had three bands and genotype KA had four bands. One strand of the PCR product must have two conformations, showing no conformation polymorphisms between genotypes KK and AA, the other strand had one conformation, but showing polymorphisms between the two homozygotes.

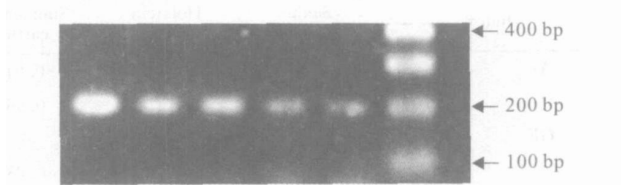

Fig. 1 Detection of PCR product of the designed primers using Agarose gel electrophoresis

1-5. The same PCR products; M. The 100 bp marker

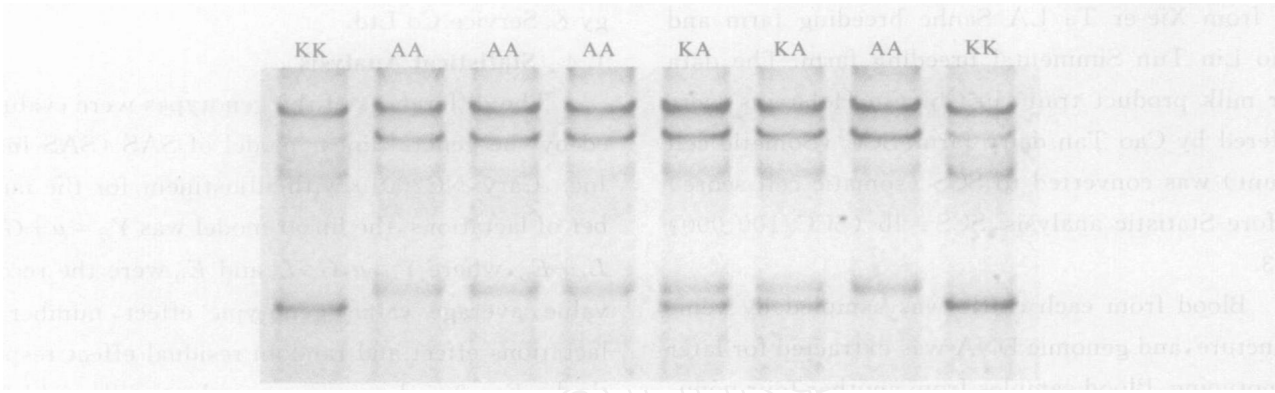

Fig. 2 Detection of SSCP using acrylamide gel electrophoresis

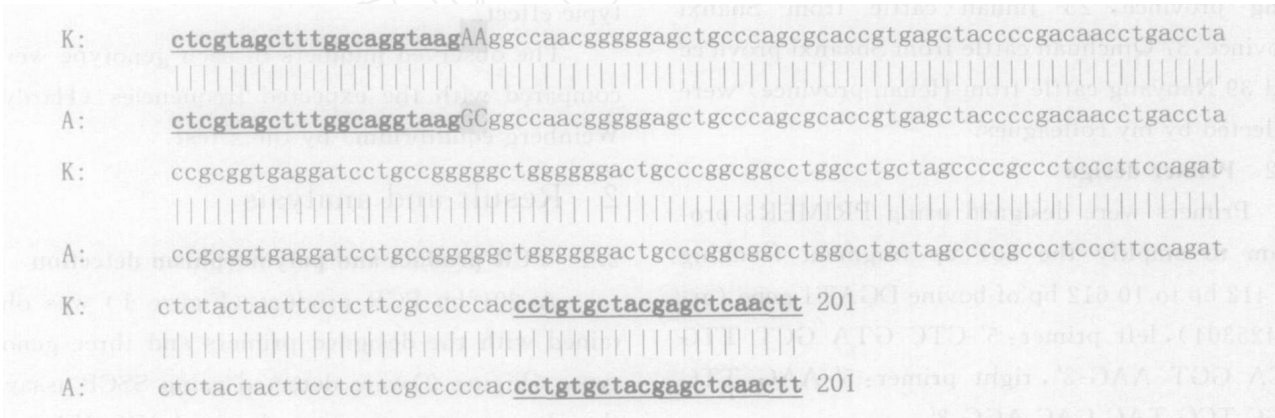

Fig. 3 Sequence alignment of allele K and A at DGAT1 gene exon 8

The sequences with underline were the left primer and the complementary sequence of right primer respectively; the capitalized bases were the mutation site

The allele and genotype frequencies were shown in table 1, the three genotypes distributed in the experimental populations except for the Simmental dairy cattle, in which no individual with genotype KK was found. Only in the two Luxi and Nanyang cattle populations, were the frequencies of genotype KK higher than genotypes AA and KA. The distribution frequencies of alleles K and A were different in all the populations. Allele K was most frequent in Luxi and Nanyang cattle, with frequencies being 0.75, and 0.83 respectively, whereas the frequencies of allele A were higher or much higher than allele K in the other populations. Allele K was very rare in Simmental dairy cattle with frequency being 0.04, and was more frequent in Chinese breeds than in imported breeds. Only Simmental dairy cattle was in Hardy-Weinberg equilibration ($P > 0.05$) at this locus.

Table 1 Allele and genotype frequencies in the three dairy cattle populations and another four populations without production records

Index		Sanhe cattle	Holstein cattle	Simmental cattle	Luxi cattle	Jinnan cattle	Qinchuan cattle	Nanyang cattle
AF	K	0.17	0.33	0.04	0.72	0.39	0.46	0.83
	A	0.83	0.67	0.96	0.28	0.61	0.54	0.17
GF	KK	0.04	0.09	0	0.57	0.04	0.24	0.64
	KA	0.27	0.48	0.08	0.30	0.70	0.44	0.28
	AA	0.69	0.43	0.92	0.13	0.26	0.32	0.08
χ^2-test		6.84*	27.55**	0.15	30.55**	38.55**	8.51*	6.99*

Note: AF. Allele frequency; GF. Genotype frequency; *. means the population was not in Hardy-Weinberg equilibration markedly ($P < 0.05$); **. means the population was not in Hardy-Weinberg equilibration siginificantly ($P < 0.01$).

2.2 Associations between the K232A substitution in the DGAT1 gene exon 8 with some economic traits

For all the analyzed traits, only average milk fat content had association (P = 0.017) with the K232A substitution in Sanhe cattle (Table 2), individuals bearing genotypes KK and KA had 0.80% and 0.41% higher average milk fat content than those bearing genotype AA, but this result did not emerge in Holstein dairy cattle (Table 3). There were no significant associations (P > 0.05) between milk production traits with genotype KA and AA in Simmental cows (Table 4).

Table 2　Effect of the K232A substitution in the DGAT1 exon 8 on some milk traits in Sanhe cows

Index	Milk yield/kg	Milk fat content/(g·kg^{-1})	Milk protein content/(g·kg^{-1})	Milk sugar content/(g·kg^{-1})	Dry matter content/(g·kg^{-1})	SCS
KK	3 096.00±210.24	42.8±2.7 a	34.7±1.2	43.8±1.7	118.9±5.2	4.38±0.67
KA	3 106.39±124.84	38.9±1.0 a	33.5±0.6	44.5±0.6	121.2±2.0	3.60±0.25
AA	3 205.53±103.25	34.8±0.7 b	34.4±0.4	43.6±0.4	119.2±1.3	4.05±0.17
P value	0.89	0.017	0.70	0.70	0.76	0.44

Note: The different superscripts in a same line mean significant difference (P < 0.05). SCS is Somatic cell score

Table 3　Effect of the K232A substitution in the DGAT1 exon 8 on milk traits in Chinese Holstein

Index	Milk yield/kg	Milk fat content/(g·kg^{-1})	Milk protein content/(g·kg^{-1})	SCS
KK	6 829.83±724.75	37.5±3.0	30.8±1.9	5.62±0.62
KA	8 051.56±452.52	37.1±1.5	29.6±0.8	5.57±0.29
AA	8 586.09±513.01	37.1±1.7	29.0±0.9	5.18±0.34
P value	0.30	0.746	0.173	0.85

Table 4　Effect of the K232A substitution in the DGAT1 exon 8 on milk traits in Chinese Simmental

Index	Milk fat content/(g·kg^{-1})	Milk protein content/(g·kg^{-1})	Milk sugar content/(g·kg^{-1})	Dry matter content/(g·kg^{-1})	SCS
AA	43.0±3.9	37.1±0.4	45.4±0.2	132.7±1.2	3.88±0.19
KA	45.7±7.2	38.9±0.9	44.9±0.4	136.7±1.6	4.34±0.39
P value	0.27	0.119	0.437	0.178	0.57

3 Discussions

3.1 The method of detecting the AA→GC substitution at 10 433- 10 434 bp of DGAT1 gene

The SSCPs detected in the present study resulted from the AA→GC substitutions at nucleotide positions 10 433- 104 34 bp of the DGAT1 gene, which could also be detected by PCR-RFLP assay with CfrI[5], Cleavage by CfrI was diagnostic for the allele encoding alanine (GC), but to detect the 10 433- 10 434 bp substitutions in a large population, PCR-PFLP assay would be much more expensive than PCR-SSCP assay.

3.2 Allele frequencies in different populations and their associations with selection

Weller et al[7] found the distribution frequencies of the allele K (substitution of a lysine residue with alanine) had decreased from 15% to 5% from 1981 until 1990, and since had increased to 10% in Israeli Holstein population. Grisart et al[8] confirmed the allele K had undergone a selective sweep. Because the allele K had association with low milk production, the long-term selection aimed at high milk volume resulted in the decline of distribution of allele K, and this could explain why allele K was much more frequent in Chinese local breeds, but selection may not be the only reason for the decline, because the frequencies of allele K were as high as 54.8%; 35% and 69% respectively in German Holstein[9], Holand Holstein and New Zealand Holstein, and 33% in Chinese Holstein. Furthermore, allele K was more frequent in Chinese Holstein than in Chinese Simmental, but the milk fat percentage was lower in the former breed in present study.

3.3 The K232A substitution and its association with some economic traits in dairy cattle

Since Grisart first reported the nonconserva-

tive K232A substitution in the DGAT1 gene had major effect on milk fat content and other milk characteristics, with the lysine-encoding allele being associated with higher milk fat content, several studies have reported the similar result[5, 7, 9]. In present study, allele K did have favorable effect on the milk fat percentage in Sanhe cattle, but no significant association with milk yield and milk protein percentage, and the favorable effect on milk fat percentage didn't emerge in Chinese Holstein. Our study didn't test the association of allele K and milk traits in the Chinese Simmental because of lacking individuals with genotype KK.

Spelman et al[10] investigated the effect of the reported K232A substitution in three dairy breeds in New Zealand, and found statistically significant results for milk fat, milk protein, and volume for Jersey and Holstein-Friesian breeds, but only milk volume for Ayrshires, and the size of the fat response were nearly double for Holstein-Friesians comparing to Jerseys. The reason for the unconsistent effect of the K232A substitution in the different breeds was unclear, Bennewitz et al[11] applied three different statistical models to investigate whether the diallelic DGAT1 polymorphism was responsible for all the genetic variation at the centromeric region of this chromosome for milk, fat, and protein yield and fat and protein percentage, and confirmed there must be an additional source of genetic variance on this chromosome for these traits and that was one of the reasons for the unconsistent effect of the K232A substitution.

4 Conclusions

The K232A substitution was popular in all the three Chinese dairy cattle, but the allele frequencies were different. The favorable effect of lysine-encoding allele on milk fat was only found in Sanhe cows.

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Studies on optimizing the signal peptide of AmyX protein from *B. subtilis*

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Abstract: The gene sequence of the signal peptide AmyX was cloned from *B. subtilis* 1A747, and the pYGAmyX expression system was constructed with the signal peptide AmyX, then it was transformed into WB700 (WB700(pYGAmyX)). The other expression system pYGMUT, which was applied to the AmyX mutation gained by overlap PCR, was transformed into WB700(WB700(pYGMUT)) in order to study the effect of Tat pathway in *Bacillus subtilis* by the direct-mutation of the signal peptide AmyX. The result showed the β-galactosidase was secreted into periplasmic successfully, the highest activity level of WB700(pYGAmyX) reached 2 300 U, and that of WB700(pYGMUT) reached 5 200 U. This testified the expression system using the rebuilding signal peptide secreted β-galactosidase by Tat pathway.

Key words: *B. subtilis*; Tat pathway; β-galactosidase; gene of the signal peptide

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牛DGAT1基因K232A取代对3个品种奶牛群体部分经济性状的影响

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[**摘 要**] 利用单链构向多态性方法检测了牛*DGAT1*基因第8外元AA→GC碱基突变, 分析了该突变在三河牛、中国荷斯坦奶牛和中国西门塔尔奶牛中等位基因的分布频率, 以及该突变对这3个品种奶牛群体产奶量、乳脂和乳蛋白含量的影响, 并分析了该突变在鲁西牛、晋南牛、秦川牛和南阳牛中等位基因分布频率。结果表明, 编码赖氨酸的等位基因K在三河牛、中国荷斯坦牛和中国西门塔尔牛中的分布频率分别为0.17, 0.33和0.04, 在鲁西牛、晋南牛、秦川牛和南阳牛4个中国地方品种中的分布频率分别为0.72, 0.39, 0.46和0.83; K232A取代与三河牛的平均乳脂率相关(P = 0.017), KK和KA基因型个体的平均乳脂率分别较AA基因型个体高0.80%和0.41%。

[**关键词**] DGAT1基因; 奶牛; K232A取代

[**中图分类号**] S813.1 [**文献标识码**] A [**文章编号**] 1671-9387(2006)09-0006-05