

# Genetic effects of stearoyl-coenzyme A desaturase (SCD) polymorphism on milk production traits in the Chinese dairy population

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**Abstract** Stearoyl-CoA desaturase (SCD) is a multifunctional complex enzyme important in the cellular biosynthesis of fatty acids. The present study was to investigate the association of the *SCD* gene with milk production traits in dairy cattle. Two single nucleotide polymorphisms (SNPs) (g.6926A>G and g.8646A>G) in introns 3 and 4, and three SNPs (g.10153A>G, g.10213T>C and g.10329C>T) in exon 5 were identified with pooled DNA sequencing and genotyped using matrix-assisted laser desorption/ionization time of flight mass spectrometry assay in 752 Chinese Holstein cows. Polymorphism g.10329C>T was predicted to result in an amino acid replacement from alanine to valine in the SCD protein. With a mixed animal model, the significant associations of the five SNPs with 305-day milk, fat and protein yields and protein percentage were determined. We further demonstrated cows with heterozygous genotypes (A/G or C/T) had highest 305 day milk yield, fat yield, protein yield and lowest protein percentage. Heterozygous cows with genotype AG at the g.6926A>G locus showed the

greatest milk yield ( $P < 0.0001$ ), fat yield ( $P < 0.0001$ ) and protein yield ( $P < 0.0001$ ) among other heterozygous genotypes at any of the loci. Dominance effects of all identified SNPs on milk, fat and protein yields and protein percentage were significant. Moreover, significant allele substitution effects at g.6926A>G locus on milk yield and at g.10213T>C on protein yield were observed. Five-locus haplotypes and strong linkage disequilibrium ( $D' > 0.9$ ) between the five SNPs were also observed. The results suggest that identified polymorphisms could be potential genetic markers to improve the production performance of Chinese Holstein.

**Keywords** Chinese Holstein · Milk production traits · *SCD* · Polymorphisms

## Introduction

In recent years, advances of molecular genetics in the identification of chromosomal regions or loci affecting traits of economic interest have opened appealing perspectives for the improvement of milk production traits in dairy cattle. Now scientists are trying to increase the milk production without hampering the quality of milk through marker-assisted selection and breeding programs. Gene-assisted selection, the use of functional mutations directly responsible of differences in phenotypes, is at present the most powerful and efficient option of marker-assisted selection in dairy cattle breeding programs [1]. Generally mutation or polymorphism of the gene will affect gene expression [2], rate and regulation of gene transcription, or the amino acid sequence of the gene product [3]. The relationships between polymorphisms in several genes including stearoyl-CoA desaturase (SCD) and dairy traits

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have already been tested in the several independent studies [4–7]. As for the bovine *SCD* gene, the published studies on single nucleotide polymorphisms (SNPs) are from Mele et al. [8] and Moioli et al. [9] reported the existence of SNPs in the *SCD* gene with significant influence on the milk fat composition in dairy cattle. It has been hypothesized that diet-independent variations in CLA content of milk fat may be due to differences in mammary SCD activity, associated with either regulation of expression and polymorphism of *SCD*, or differences in downstream factors that would affect interactions between enzymes and substrates (e.g., phosphorylation) [10]. SCD is a key rate-limiting enzyme in the biosynthesis of monounsaturated fatty acids (FAs) by insertion of a *cis*-double bond in the D9 position (between carbons 9 and 10 atoms) of methylene-interrupted fatty acyl-CoA substrates. This oxidative reaction is catalyzed by the SCD and involves cytochrome *b5*, NADP (H)-dependent cytochrome *b5* reductase and molecular oxygen [11–13].

Because of biological significance on the quantitative traits *SCD* is considered as an excellent candidate gene for QTL analysis. QTL located on BTA26 affecting milk, fat and protein yields and milk composition in dairy cattle have been reported in some independent studies [14–18]. Several genes involved in lipid metabolism that are located on BTA26 including *SCD*, *LPF* (gastric lipase), or *GPAM* (glycerol-3-phosphate acyltransferase mitochondrial) have been suggested as possible candidate genes harboring the functional mutation detected by the QTL analysis [19]. The authors also concluded, the microsatellites used for the study were close to these genes seem to exclude the possibility that the position of the *SCD* locus matches the location of any of the QTL affecting milk production traits found on this chromosome.

The *SCD* gene has been cloned and characterized in a number of mammalian species including rodents, ovine, porcine, bovine and human [20–23]. Bovine *SCD* has been localized to chromosome 26q21 [20]. Northern blot analysis provides evidence for the existence of a single *SCD* transcript of approximately 5 kb with an unusually long 3'UTR in bovine mammary gland and adipose tissues [20, 24]. The bovine *SCD* is approximately 17 kb, comprises six exons and five introns (GenBank accession no. AY241932 and AY241933) and contains an open reading frame of 1,080 nucleotides coding for a protein of 359 amino acids and the 3'UTR has 3,884 nucleotides [25]. According to the biosynthesis function and QTL effects on milk fat, it is reasonable to hypothesize that *SCD* gene may also affect the production traits such as milk yield, fat yield, and protein yields that represent the main breeding goals in current selection schemes of dairy cattle. In the present study, the *SCD* gene was chosen as a positional candidate gene for milk production traits, based upon its biological function

and genomic location. We screened its full-length coding region for SNPs and evaluated the genetic effects of polymorphisms on milk production traits in Chinese Holstein.

## Materials and methods

### Experimental animal and phenotypic data

A total of 752 Chinese Holstein cows were chosen from Beijing Dairy Cattle Center and Beijing Sanyuan Lvhe Dairy Farming Center in the Beijing region, China, including 14 sire families with 30–153 daughters from each sire. Performance data for five milk production traits (i.e. milk yield, fat yield, protein yield, fat percentage and protein percentage over 305 days) were collected from the Dairy Data Processing Center, Dairy Association of China. Genomic DNA was extracted from whole blood samples of the cows using a TIANamp Genomic DNA kit (TianGen, Beijing, China) according to the manufacturer's instructions and frozen semen of the 14 sires by a standard phenol–chloroform method.

### Detection and genotyping of the polymorphisms

A DNA pool (50 ng/μl/bull) was constructed from the 14 sires. Six pair of primers (Table 1) were designed to amplify all exons (exons 1–6) and their partial flanking intronic sequences based on the reference sequence of the bovine *SCD* gene (GenBank accession no. AY241932) with Primer3 web Program (v.0.4.0) in order to identify SNPs. PCR amplifications for pooled DNA from 14 sires were performed in a final reaction volume of 25 μl consisting of 50 ng genomic DNA, 0.5 μl of each primer, 2.5 μl 10× PCR buffer, 2.5 mM each of dNTP, and 1 U of

**Table 1** PCR primers used for sequencing the *SCD* gene

Exons	Sequence	Size of PCR product (bp)
Exon 1	L-GTTGGCAACGAATAAAAGAGG R-CGCGGTGATCTCAACTCTTC	384
Exon 2	L-GGACCGGGTCTATGCCTATC R-CCATCCAGCCTCTCAGGAC	552
Exon 3	L-GTTCCCTGGGACTCCTAAGC R-CCGGAACCTAACCACAAGGA	499
Exon 4	L-GGCAACTCCATGACTTCTCC R-CATGACCGTCCTAGGTCAAC	594
Exon 5	L-CCCATTGCTCTTGTCTGT R-CGTGGTCTTGCTGTGGACT	400
Exon 6	L-GCCTCTGAGGGGATCTATTTC R-AGGCAGAGTTGTTGGCTTTC	543

*Taq* DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 5 min at 94 °C for initial denaturing followed by 34 cycles at 94 °C for 30 s; 56 °C for 30 s; 72 °C for 30 s; a final extension at 72 °C for 7 min for all the primers. Then, 40 µl of each PCR product from the pooled DNA was sequenced using the ABI3730XL (Applied Biosystems, Foster City, CA). Both forward and reverse primer sequences were then aligned using the ClustalW program to determine the presence of genetic polymorphism. Matrix-assisted laser desorption/ionization time of flight mass spectrometry (MALDI-TOF MS, Squenom MassARRAY®, Bioyong Technologieies Inc. HK) assay was used for individual genotyping of 752 Chinese Holstein cows.

### Statistical analysis

With tracing back of pedigree information for three generations, the total number of animals included in the analysis was 2,212. For the association studies, the traits of interest were analyzed using several statistical programs e.g. MATLAB (<http://www.mathworks.com>) version 7.11.0.584 (R2010b) for the kinship matrix (A-matrix), POPGENE (<http://www.ualberta.ca/~fyeh/>) version 1.32 for Hardy–Weinberg equilibrium, Haploview (<http://www.broadinstitute.org/haploview/haploview>) version 4.2 for linkage disequilibrium and haplotypes analysis. The mixed procedure of SAS 9.1.0 software (SAS OnlineDoc® 9.1.0. Cary, NC: SAS Institute Inc. USA) with the following animal model [26] was used to estimate the effects of *SCD* polymorphic genotypes on the milk production traits. Each trait was analysed separately and each polymorphism was also fitted separately in the model

$$Y = \mu + h_{ys} + L + G + \alpha_i + e,$$

where  $Y$  is the phenotypic data of each trait of cows,  $\mu$  is the general mean,  $h_{ys}$  is the herd-year-season effect,  $L$  is the fixed effect of lactation,  $G$  is the fixed effect of polymorphism genotypes,  $\alpha_i$  is the residual polygenic effect,  $e$  is the random residual. The difference between the effects of the polymorphism genotypes were compared using multiple  $t$  test with Bonferroni correction in which the significance level of the multiple tests was equal to the significant level of single test divided by number of tests. Here there are three levels of genotype for each trait, thus three  $t$  tests needed to be performed, so the Bonferroni corrected significance levels of  $0.05/3 = 0.0167$  and  $0.01/3 = 0.0033$  were used.

The equation of Falconer and Mackay [27] was fitted to estimate the additive (a), dominance (d) and allele substitution ( $\alpha$ ) effects, i.e.  $a = (AA - BB)/2$ ,  $d = AB - (AA + BB)/2$  and  $\alpha = a + d(q - p)$ , where AA or BB indicates the two homozygous genotypes, AB indicates

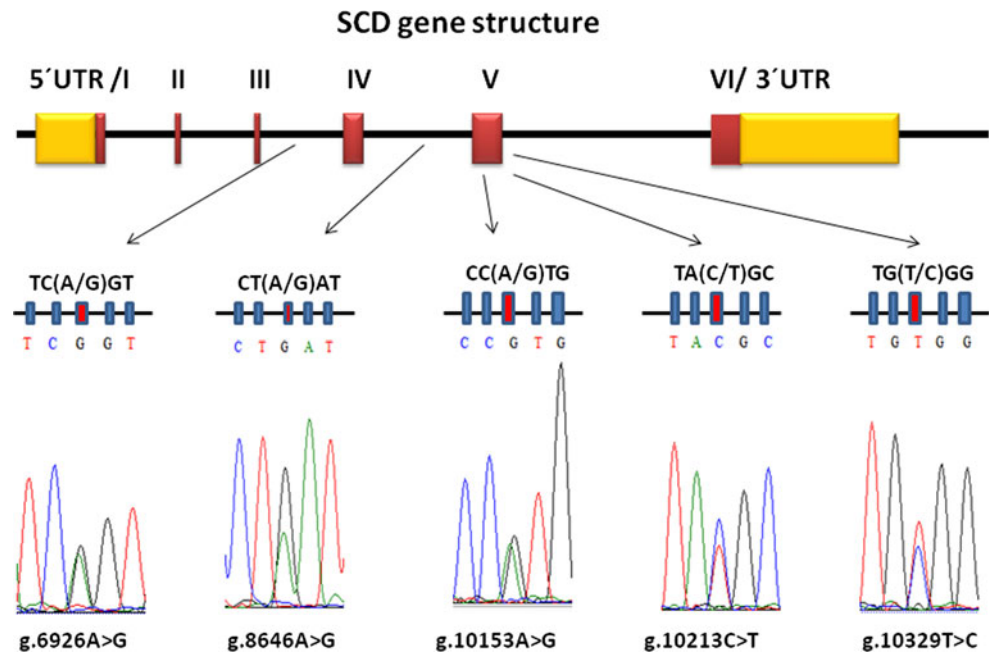
heterozygous genotype,  $p$  and  $q$  are the allele frequencies of corresponding loci.

### Results and discussion

Through sequence analysis of the pooled DNA samples, five SNPs in *SCD* gene were discovered in this study, of which, two (g.6926A>G and g.8646A>G) were found in introns 3 and 4 respectively and another three (g.10153A>G, g.10213T>C and g.10329C>T) in exon 5 (Fig. 1). The genotypic and allelic frequencies and Hardy–Weinberg equilibrium test ( $\chi^2$ ) are summarized in Table 2.  $\chi^2$  Test showed that all genotypic frequencies in the population were in Hardy–Weinberg equilibrium ( $P > 0.05$ ) (Table 2), indicating that selection pressure (mainly on production traits) did not have a large influence on the genotypic frequencies of these sites. Five-locus haplotypes are observed as shown in Table 3. The two haplotypes [AAATC] and [GGGCT] were detected in this study and the frequencies were 66 and 34 % respectively. The linkage disequilibrium between the five SNPs was estimated, and the results (Fig. 2) showed the five SNPs were in linkage disequilibrium ( $D' > 0.9$ ).

In Table 4, association analysis demonstrated that cows with genotype AG at the g.6926A>G locus showed the greatest 305-day milk yield ( $P < 0.0001$ ), fat yield ( $P < 0.0001$ ) and protein yield ( $P < 0.0001$ ) among genotypes with 354, 12 and 7 kg more milk, fat and protein yield respectively compared with GG cows. This SNP had no effect on fat percentage. Moreover, the protein percentage of AA or GG cows was significantly greater than that of AG cows, with a difference of about 0.029 and 0.045 kg, respectively. Cows with heterozygous genotype AG at locus g.8646A>G and g.10153A>G had highest milk ( $P < 0.0001$ ), fat ( $P < 0.05$ ) and protein ( $P < 0.05$ ) yields than the ones with homozygous AA and GG, whereas genotype GG (at both locus) showed highest protein percentage ( $P < 0.0001$ ). The SNPs g.10213T>C and g.10329C>T have significant effects on milk, fat and protein yields as well. Cows with CT genotypes at g.10213T>C and g.10329C>T locus had highest milk yield ( $P < 0.001$ ), fat yield ( $P < 0.05$ ) and protein yield ( $P < 0.0001$ ;  $P < 0.05$ ) whereas lowest protein percentage ( $P < 0.0001$ ;  $P < 0.05$ ) than in those with TT or CC respectively. However, no significant association with fat percentage was observed. We further demonstrated that dominance effects of all identified SNPs on milk yield ( $P < 0.001$ ), fat yield ( $P < 0.001$ ), protein yield ( $P < 0.001$ ), and protein percentage ( $P < 0.001$ ) were significant (Table 5). Additive effects were not significant for all of the traits whereas the significant allele substitution effects at g.6926A>G locus on milk yield ( $P < 0.05$ )

**Fig. 1** *SCD* gene structure and location of SNPs (red boxes are exons and yellow box are 3'UTR). Exons are indicated as rectangles labeled by exon numbers in roman numerals. (Color figure online)



**Table 2** Genotypic and allelic frequencies and Hardy–Weinberg equilibrium test of five SNPs of *SCD* gene in Chinese Holstein

Polymorphisms	Genotypic frequency (n = animal genotyped)			Allelic frequency		Hardy–Weinberg equilibrium $\chi^2$ test, <i>P</i>
	AA	AG	GG	A	G	
g.6926A>G	AA (n = 287)	AG (n = 328)	GG (n = 74)	A	G	>0.05
	0.43	0.45	0.12	0.65	0.35	
g.8646G>A	AA (n = 295)	AG (n = 322)	GG (n = 72)	A	G	>0.05
	0.44	0.45	0.11	0.66	0.34	
g.10153G>A	AA (n = 288)	AG (n = 329)	GG (n = 72)	A	G	>0.05
	0.43	0.45	0.12	0.66	0.34	
g.10213T>C	CC (n = 72)	TC (n = 328)	TT (n = 289)	C	T	>0.05
	0.12	0.45	0.43	0.34	0.66	
g.10329C>T	CC (n = 288)	CT (n = 330)	TT (n = 70)	C	T	>0.05
	0.43	0.45	0.12	0.66	0.34	

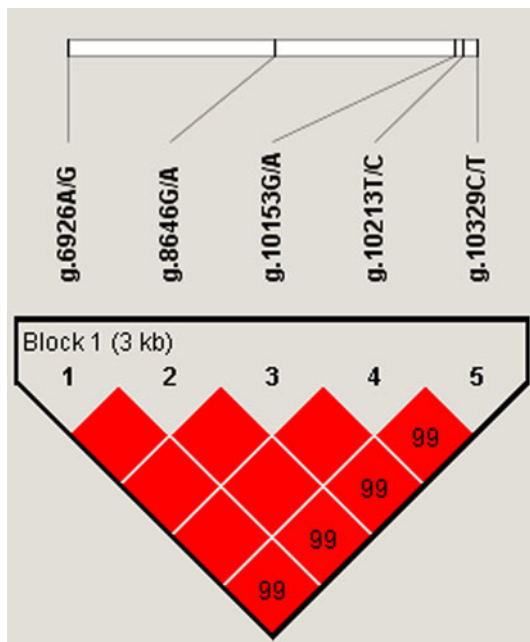
**Table 3** Haplotype distribution of polymorphisms in *SCD* gene

Haplotypes	SNPs					Frequency
	g.6926A>G	g.8646G>A	g.10153G>A	g.10213T>C	g.10329C>T	
1	A	A	A	T	C	0.66
2	G	G	G	C	T	0.34

and at g.10213T>C locus on protein yield ( $P < 0.05$ ) were observed. As a whole, for all five loci, heterozygous genotypes led to much better performance than the homozygous genotypes for milk yield fat yield and protein yield, while the homozygous genotypes had slightly higher protein percentage and fat percentage, and differences between the two homozygous genotypes were little.

*SCD* is a multifunctional complex enzyme in the cellular biosynthesis of FAs. The *SCD* has been suggested as a

candidate gene affecting milk FA profile [8, 9, 19], the FA profile of carcass fat [25, 28] and a fundamental aspect of milk nutritional quality [29, 30]. In our study, to evaluate the effect of SNPs, 752 cows were genotyped by MALDI-TOF array. The average genotyping success rate was 91.6%. In contrast to Taniguchi et al. [25], in the current study a strong linkage disequilibrium ( $D' > 0.9$ ) was found among the SNPs; only polymorphism g.10329C>T was predicted to result in an amino acid replacement from



**Fig. 2** Pairwise linkage disequilibrium in bovine *SCD* gene. Note: ‘99’ indicates  $D' = 0.99$

alanine into valine in the *SCD* protein. The C allele was identified as the ancestral allele. As a result, similar significant associations on milk production traits were

observed in several cases. This amino acid replacement considered in this study was in agreement with previous reports on the different cattle populations [8, 9, 19, 25, 28, 31, 32]. Strong LD between the analyzed three SNPs of exon 5 ( $D' > 0.9$ ) were also detected by Milanese et al. [33], with correlation values ( $r^2$ ) between g.10213T>C and g.10329C>T and between g.10153A>G and g.10213T>C (they named the SNPs A702G, T762C and T878C) reported as 0.98 and 0.65, respectively, indicating a higher recombination frequency between the latter pair of SNP. Our results also were in agreement with these results. The study carried out by Milanese et al. [33] on Italian cattle breed reported that in the 11 breeds three-locus five haplotypes had been reconstructed. The most frequent haplotypes, ATC (55.6 %) and GCT (34.8 %), were present in all the breeds studied. These two haplotypes in the exon 5 also reported in the Japanese Black [25] and in the Italian Holstein breeds [34]. However, literature data on six-locus haplotypes frequency were not available for comparison with our results.

Higher frequency of the C allele than the T allele at position g.10329C>T was reported for Italian Holsteins (0.57) [8], Valdostana (0.65), Jerseys (0.94) [9], Grey Alpine (0.89) [34] and Japanese Black cattle (0.59) [25]. The allele frequencies of the present study at this locus were consistent with these studies. The higher C allele

**Table 4** Associations of five SNPs of *SCD* with milk production traits in Chinese Holstein (LSM  $\pm$  SE)

Locus	Genotype	Milk yield (kg)	Fat yield (kg)	Fat (%)	Protein yield (kg)	Protein (%)
g.6926A>G	AA(287)	9831.30 $\pm$ 85.95 <sup>A</sup>	366.98 $\pm$ 3.86 <sup>A</sup>	3.749 $\pm$ 0.036	307.57 $\pm$ 2.81 <sup>aA</sup>	3.129 $\pm$ 0.011 <sup>A</sup>
	AG(328)	10150.00 $\pm$ 85.07 <sup>B</sup>	376.16 $\pm$ 3.84 <sup>B</sup>	3.715 $\pm$ 0.035	315.15 $\pm$ 2.80 <sup>bB</sup>	3.100 $\pm$ 0.011 <sup>B</sup>
	GG(74)	9796.30 $\pm$ 110.39 <sup>A</sup>	364.15 $\pm$ 4.76 <sup>A</sup>	3.734 $\pm$ 0.035	308.46 $\pm$ 3.47 <sup>A</sup>	3.141 $\pm$ 0.015 <sup>A</sup>
<i>P</i> value		<0.0001	<0.0001	0.3646	<0.0001	0.0003
g.8646G>A	AA(295)	9855.62 $\pm$ 85.19 <sup>A</sup>	367.90 $\pm$ 3.84 <sup>A</sup>	3.750 $\pm$ 0.035	308.33 $\pm$ 2.79 <sup>A</sup>	3.129 $\pm$ 0.011 <sup>A</sup>
	GA(322)	10145.00 $\pm$ 85.88 <sup>B</sup>	375.46 $\pm$ 3.87 <sup>B</sup>	3.708 $\pm$ 0.036	314.86 $\pm$ 2.82 <sup>B</sup>	3.098 $\pm$ 0.011 <sup>B</sup>
	GG(72)	9801.36 $\pm$ 111.28 <sup>A</sup>	365.25 $\pm$ 4.80 <sup>A</sup>	3.744 $\pm$ 0.045	308.45 $\pm$ 3.49 <sup>AB</sup>	3.139 $\pm$ 0.015 <sup>A</sup>
<i>P</i> value		<0.0001	0.0013	0.2021	0.0006	0.0002
g.10153G>A	AA(288)	9839.52 $\pm$ 85.94 <sup>A</sup>	367.30 $\pm$ 3.86 <sup>A</sup>	3.749 $\pm$ 0.036	307.82 $\pm$ 2.82 <sup>A</sup>	3.129 $\pm$ 0.011 <sup>A</sup>
	GA(329)	10140.00 $\pm$ 85.09 <sup>B</sup>	375.52 $\pm$ 3.84 <sup>B</sup>	3.712 $\pm$ 0.035	314.90 $\pm$ 2.80 <sup>B</sup>	3.100 $\pm$ 0.011 <sup>B</sup>
	GG(72)	9798.4 $\pm$ 111.23 <sup>A</sup>	365.21 $\pm$ 4.79 <sup>A</sup>	3.745 $\pm$ 0.045	308.42 $\pm$ 3.49 <sup>A</sup>	3.140 $\pm$ 0.015 <sup>A</sup>
<i>P</i> value		<0.0001	0.0006	0.2667	0.0002	0.0005
g.10213T>C	CC(72)	9797.99 $\pm$ 111.23 <sup>A</sup>	365.17 $\pm$ 4.80 <sup>A</sup>	3.745 $\pm$ 0.045	308.4 $\pm$ 3.49 <sup>A</sup>	3.14 $\pm$ 0.015 <sup>A</sup>
	CT(328)	10141 $\pm$ 85.11 <sup>B</sup>	375.43 $\pm$ 3.84 <sup>B</sup>	3.710 $\pm$ 0.035	314.92 $\pm$ 2.80 <sup>AB</sup>	3.1001 $\pm$ 0.011 <sup>B</sup>
	TT(270)	9838.66 $\pm$ 85.94 <sup>A</sup>	367.38 $\pm$ 3.86 <sup>A</sup>	3.751 $\pm$ 0.036	307.79 $\pm$ 2.81 <sup>B</sup>	3.1289 $\pm$ 0.011 <sup>A</sup>
<i>P</i> value		<0.0001	0.0007	0.2302	0.0002	0.0005
g.10329C>T	CC(288)	9736.57 $\pm$ 85.84 <sup>A</sup>	368.46 $\pm$ 3.87 <sup>A</sup>	3.780 $\pm$ 0.036	302.69 $\pm$ 2.81 <sup>A</sup>	3.120 $\pm$ 0.011 <sup>a</sup>
	CT(330)	9980.67 $\pm$ 84.71 <sup>B</sup>	373.89 $\pm$ 3.82 <sup>A</sup>	3.758 $\pm$ 0.035	308.60 $\pm$ 2.78 <sup>B</sup>	3.098 $\pm$ 0.011 <sup>b</sup>
	TT(70)	9621.62 $\pm$ 112.85 <sup>A</sup>	363.64 $\pm$ 4.86 <sup>B</sup>	3.780 $\pm$ 0.046	300.13 $\pm$ 3.54 <sup>A</sup>	3.130 $\pm$ 0.016 <sup>ab</sup>
<i>P</i> value		<0.0001	0.0067	0.1925	0.0003	0.0107

*Notes:* *P* value refers to the results of association analysis between each SNP and milk production traits. Different letter (small letters:  $P < 0.05$ ; capital letters:  $P < 0.01$ ) superscripts (adjusted value after correction for multiple testing) indicate significant differences among the genotypes

**Table 5** Additive, dominant and allele substitution effects of the five SNPs on milk production traits of SCD in Chinese Holstein

Locus	Genetic effects	Milk yield (kg)	Fat yield (kg)	Fat (%)	Protein yield (kg)	Protein (%)
g.6926A>G	Additive (a)	17.50	1.41	0.008	-0.44	-0.006
	Dominant (d)	335.71**	10.54**	-0.027	7.14**	-0.035**
	Allele substitution ( $\alpha$ )	-86.25*	-1.81	0.016	-2.65	0.005
g.8646G>A	Additive (a)	27.13	1.35	0.003	-0.06	-0.005
	Dominant (d)	316.59**	8.83**	-0.039	6.47**	-0.036**
	Allele substitution ( $\alpha$ )	-75.15	-1.50	0.016	-2.15	0.007
g.10153G>A	Additive (a)	20.56	1.04	0.002	-0.30	-0.006
	Dominant (d)	321.52**	9.20**	-0.036	6.78**	-0.034**
	Allele substitution ( $\alpha$ )	-80.10	-1.81	0.013	-2.42	0.005
g.10213T>C	Additive (a)	-20.34	-1.12	-0.003	0.31	0.006
	Dominant (d)	323.16**	9.10**	-0.037	6.83**	-0.034**
	Allele substitution ( $\alpha$ )	80.05	1.70	-0.014	2.42*	-0.005
g.10329C>T	Additive (a)	57.47	2.41	-0.0005	1.28	-0.005
	Dominant (d)	301.57**	7.84**	-0.041	7.19**	-0.027**
	Allele substitution ( $\alpha$ )	-36.59	-0.04	0.012	-0.96	0.004

Notes: \* The estimated effect differs from zero at  $P < 0.05$ , \*\* the estimated effect differs from zero at  $P < 0.01$

frequency in the Chinese Holstein population may have resulted from long term selective breeding for improving the milk production or other associated traits, or from drift. In contrast to Milianesi et al. [33], in the current study A (0.66) and T (0.66) allele were found to be more frequent at position g.10153A>G and g.10213T>C respectively.

A study was carried out by Macciotta et al. [35], who claimed that cows with homozygous genotype CC at the SCD locus (g.10329C>T) producing more milk (about 2 kg/day) and protein (about 0.07 kg/day) compared with TT cows; heterozygous cows were in an intermediate position but our results disagree with their reported literature. The results of the present experiment were consistent with previous results of Conte et al. [34] who had shown that this SNP had been associated with higher SCD (g.10329C>T) activity and fat concentration in milk in Italian Holstein.

In contrast to the results of Taniguchi et al. [25] on FA composition of carcass fat in cattle, Mele et al. [8] found a significant association between SCD genotypes and *cis*-9 C18:1, *cis*-9 C14:1, and total MUFA contents of milk. In particular, the homozygous TT genotype was associated with 9.3, 37.9, and 11.7 % more MUFA, *cis*-9 C14:1 and *cis*-9 C18:1, respectively, when compared with homozygous CC animal whereas Moioli et al. [9] did not find any association in CLA, polyunsaturated FA, and MUFA in either Valdostana or Jerseys breed. Taniguchi et al. [25] also reported that heterozygous Val/Ala (C/T) animals had higher C16:1/C16:0 in intramuscular fat than did homozygous Val (TT) animals. Another study by Conte et al. [33] on Italian brown cattle reported a significant association between SCDI genotypes and milk FA C14:1 *cis*-9 and DI 14 ( $P \leq 0.01$ ). In particular, the CC genotype was

associated with 18.3 and 20.6 % more C14:1 *cis*-9 and DI 14, respectively, compared with the TT genotype.

This seemingly paradoxical result could be explained by several reasons, e.g. the genetic background, linkage disequilibrium, artificial selection for other traits and paternal effect. Generally the effect of polymorphism will differ across different populations or breed because of specific genetic backgrounds. Chinese Holsteins cattle originated from cross-breeding between native cow (Chinese Yellow cattle) and American or European Holsteins (pure-bred bull) over the past 100 years. Continuous import of Holstein sires, semen and embryos which were directly used in AI or via crosses with Chinese Holstein cows through planned mating to increase the milk production and other traits [36]. Another possible reason for the conflicting findings between different studies could be that some of the studies were based on relatively small samples and thus the results might be involved in relatively large sampling errors.

In addition, two SNPs in introns 3 and 4 of SCD gene were detected in this study. Although, intron was not the sequence for coding protein but it played an important regulating effect in gene expression, regulation [2] and transcription and mRNA splicing [3]. Further study is needed to explore whether the detected mutations affect gene expression or not. In conclusion, our findings showed that SCD gene polymorphisms are associated with milk production traits in Chinese Holstein population; however, heterozygous cows yielded more milk, fat and protein. The results suggest that SCD is a candidate gene that influences milk production traits, and provides potentially useful information for in dairy breeding programs. The identified polymorphisms could be potential genetic markers to

improve the production performance of Chinese Holstein. Further studies using long term production data and in vitro biological analysis should be conducted in order to check the effects of such polymorphisms and validate its function on milk production traits.

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