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Single nucleotide polymorphism of *CACNA2D1* gene and its association with milk somatic cell score in cattle

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Abstract The objective of the present study was to identify polymorphisms of the *CACNA2D1* gene, and to analyze associations between these polymorphisms and mastitis in several cattle breeds. Through PCR–RFLP methods and DNA sequencing, an allelic variant corresponding to the A→G mutations and Aspartic (Asp) to Glycine (Gly) amino acid replacement at positions 526745 in the exon 25 of bovine *CACNA2D1* gene could be detected. Two alleles, A and G, and three genotypes, AA, AG and GG were defined. Genetic character in the studied populations indicated that the A526745G loci of *CACNA2D1* gene was moderate polymorphism and fitted with Hardy–Weinberg equilibrium ($P > 0.05$). The effects of *CACNA2D1* polymorphisms on somatic cell score (SCS) were analyzed and significant association was found between A526745G and SCS. The mean of genotype GG was significantly lower than those of genotype AG and AA ($P = 0.0469$). Information provided in this research could be useful in further studies to determine the role of *CACNA2D1* gene in the mastitis resistance.

Keywords Cattle · *CACNA2D1* gene · Somatic cell count · Somatic cell score · Mastitis

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Introduction

Mastitis is one of the most frequently occurring diseases of dairy cattle, which causes huge economic losses to the dairy industry worldwide [1–6]. Mastitis, an inflammatory response of the mammary gland caused usually by bacteria, is probably the most costly of the infectious, endemic diseases to affect dairy cows and other dairy species. Its impact is on animal production, animal welfare and the quality of the milk produced. For many years, breeding goals for dairy cattle had focused mainly on increasing the productivity and had ignored health traits such as disease resistance. Higher yielding cows tend to have higher health costs. Dairy cattle were less resistant to mastitis than dual-purpose breeds [7, 8]. Holstein cows were more easier have mastitis than Sanhe and Simmental cows because the Chinese Sanhe cattle and Chinses Simmenta cattle were dual-purpose breed for meat and milk in China while Holstein was almost for milk in long time. Molecular markers can play an important role in cattle genetic improvement through conventional breeding strategies. Although, it is difficult to make appreciable genetic progress by traditional breeding methods because the heritability of the trait is low [9–11] and there is unfavorable genetic correlation with production traits [11, 12]. In addition, the trait is difficult to record objectively, resistance to mastitis is still a candidate for MAS and many studies have reported quantitative trait loci (QTLs) affecting this trait. At present, several studies have identified several QTLs for Somatic cell score (SCS) and clinical mastitis (CM). It is concluded that many of the genes affecting SCS also affect CM. SCS is easier to record and used as an indicator trait for CM as the genetic correlation is around 0.7 [11–13].Since it is difficult to measure the mastitis phenotype using a direct index, milk SCS has been

most widely used as an indirect way to evaluate mastitis. Milk SCS is a $\log_2$ score of the milk somatic cell count (SCC) and has positive correlation with clinical mastitis [14, 15]. The study of associations of candidate genes is a step for the knowledge of the genetic basis of productive traits, and compared to other genomic approaches is potentially more easily and efficiently implemented in breeding program and the candidate gene may be selected on the basis of a known relationship between physiological or biochemical processes and production traits, and can be tested as QTL [16, 17]. Using this method, some important candidate genes for the production traits such as *CACNA2D1*, *MC4R*, *SLC27A4*, *MyoG*, *CSRP3*, *HGD*, and *FTO* genes have been identified in cattle, pig and other livestocks [18–24]. Calcium channel, voltage-dependent, alpha-2/delta subunit 1 (*CACNA2D1*) gene encodes a member of the alpha-2/delta subunit family, proteins are accessory molecules associated with voltage-gated calcium channels, and increase the density at the plasma membrane of calcium channels activated by high voltage. In addition, several mutations in this gene result in neurological disorders and so on [25]. Cattle *CACNA2D1* gene has been mapped to BTA 4q18 [26]. It is located within the genomic region of SCS QTL [27, 28] and nearby the QTL of SCC [29, 30] (http://www.animalgenome.org/cgi-bin/QTLdb/BT/draw_chromap?optqtl=&chromos=4&orderqtl=QTL_symbol&scale=4&density=10&submit=GO). Therefore, the *CACNA2D1* gene is considered to be one of the potential candidate gene influencing SCS and mastitis. Through PCR–RFLP and DNA sequencing methods, the A526745G allelic variant corresponding to the A→G mutations (aspartic (Asp) to glycine (Gly) amino acid) replacement of bovine *CACNA2D1* gene could be detected and the A526745G was significantly associated with carcass weight, dressing percentage, meat percentage and backfat thickness [16]. In this study,*CACNA2D1* the aim of the current study was to investigate the A526745G SNPs of *CACNA2D1* gene, and assess their possible associations between SNPs, combined genotypes of SNPs, and breeds with mastitis (Via milk SCS) in cattle.

Materials and methods

Animals and measure of Mastitis

A total of 240 unrelated animals including 73 Holstein (Caotan Dairy Farm, Xi'an, Shaanxi Province),78 Sanhe (Xiertala Breeding Farm, Hailar, Inner Mongolia Autonomous Region), and 89 Simmental(Gaolintun Breeding Farm, Tongliao, Inner Mongolia Autonomous Region)were used in this study. The average age was 3.50 ± 1.20, the parity was first or second and the stage of lactation was 3–6 month. All individuals were self-help feeding and milked by milking machine.

Genomic DNA was extracted from whole blood by the standard phenol/chloroform/isoamyl alcohol extraction protocol [31] then dissolved in TE buffer (10 mmol/l Tris–HCl and 1 mmol/l EDTA, pH 8.0), and kept at −20°C. The milk samples with antiseptic were collected. Traits of interest are somatic cell count (SCC) on test day, which was measured in Beijing Dairy Cattle Centre, China, then converted into the SCS using formula: SCS = log2 (SCC/100000) + 3 [32], and was rectified to eliminate the effect of lactation days and period of sampling on SCS. Our experimental protocols and animals care met the requirements of the institution animal care and use committee (IACUC) and performed according to authorization granted by the Chinese Ministry of Agriculture.

Primer design and PCR amplification

One pair of primers (forward:5′-GTTTCCACTACCTATGATTGC-3′and reverse:5′-ACTGAACCAAGATTTGACCAC-3′) was designed to amplify a 249 bp product of the *CACNA2D1* exon 25 and its intron region [16]. Polymerase chain reaction (PCR) amplifications were performed in 20 μl reaction mixture containing 50 ng mixed DNA template, 10 pM of each primer, 0.20 mM dNTP, 2.5 mM $MgCl_2$ and 0.5 U Taq DNA polymerase (TaKaRa, Dalian, China). The PCR protocol was 95°C for 5 min followed by 32 cycles of 94°C for 30 s, 54°C annealing for 30 s, and 72°C for 30 s, and a final extension at 72°C for 10 min. The PCR products were separated on 1.5% agarose gel (Promega) including 0.5 μg/ml of ethidium bromide, photographed under UV light.

PCR–RFLP and genotype determination

According to the YUAN and XU reported paper [16], genotyping of the SNP polymorphism at position 526745 of the *CACNA2D1* gene was carried out using PCR restriction fragment length polymorphism (PCR–RFLP). Aliquots of 5 μl PCR products were digested with 2 U *HaeIII* (MBI, Fermentas) at 37°C for 10 h following the supplier's manual. The digested products were detected by electrophoresis in 2.5% agarose gel stained with ethidium bromide. To verify the genotypes and SNP, PCR amplified products of the different genotypes were purified using a Wizard Prep PCR purification kit (Shanghai Bioasia Biotechnology, P. R. China) and sequenced by an ABI 3730 sequencer (Bioasia Biotechnology Co., Ltd. Shanghai, China).

Statistical analyses

Genetic characters of cattle *CACNA2D1* gene among three populations were analyzed by Popgene32 [33]. The Hardy–Weinberg equilibrium of the mutation was determined by χ^2 test. Analysis of associations between the genotypes of SNP and SCS that reflects mastitis traits was carried out with GLM procedure using SAS software (Statistical Analysis System 9.1, SAS Institute Inc.) [34] by the following formula: $y_{ijklm} = \mu + b_i + f_j + a_k + p_l + g_m + e_{ijklm}$, where y_{ijklm} = lactation average SCS, μ = global mean, b_i = breed-herd-year-season effect, f_j = parity effect, a_k = age effect, p_l = lactation effect, g_m = genotype effect, e_{ijklm} = residual effect.

Results and discussion

Identification and genotyping of SNP

An allelic variant corresponding to the A→G mutations and Aspartic (Asp) to Glycine (Gly) amino acid replacement at positions 526745 in the exon25 of bovine *CACNA2D1* gene could be detected [16]. The PCR product of A526745G was digested with *HaeIII* enzyme. Three possible genotypes were defined by three distinct banding patterns: AA (249 bp fragment), AG (249, 154 and 95 bp fragments), and GG (154 and 95 bp fragments).

Genotype and allelic frequencies

The PCR–RFLP analysis for A526745G showed that all three possible genotypes for the A/G polymorphism were identified in the studied populations and allele *A* was the predominant allele in Holstein and Sanhe populations (Holstein, 0.6027; Sanhe, 0.6410), but allele *G* was the predominant allele in Simmental populations (Simmental, 0.5112). The value of *AA* genotypic frequency was maximum in Holstein and Sanhe populations (Holstein, 0.4247; Sanhe, 0.4487), while *AG* genotypic frequency was maximum in Simmental populations (Simmental, 0.4382). The genotype and allelic frequencies of A526745G polymorphism in different populations were given in Table 1.

Genetic diversity/character in the cattle

Genetic character in the studied populations indicated that the A526745G loci of the three populations was moderate polymorphism and fitted with Hardy–Weinberg equilibrium ($P > 0.05$). The value of gene homozygosity (Ho), gene heterozygosity (He), effective number of alleles (Ne), polymorphism information contents (PIC), and χ^2 value were shown in Table 2. The value of PIC, He of Simmental was higher than that of the other populations, which implied that the polymorphism and the genetic variation Simmental were higher than that of the other populations.

Association of the *CACNA2D1* gene polymorphisms with SCS

Mastitis is the most prevalent production disease in dairy herds world-wide and disease resistance traits usually have low heritabilities and limited amounts of data, which hamper the potential for genetic improvement by traditional selection methods. Recent advances in molecular biotechnology provided great opportunities to incorporate molecular information into the traditional genetic evaluation models and to improve selection accuracies in livestock populations. Consequently, there has been considerable interest in defining genetic and immunological markers that could be used to select for improved disease resistance [35].The candidate gene approach may provide a more direct understanding of the genetic basis for the expression of quantitative differences between individuals [36], and revealing genomic regions and specific markers that are associated with traits. In the present study, *CACNA2D1* was considered to be a potential candidate gene influencing mastitis, because the cattle *CACNA2D1* gene has been mapped to BTA 4q18 [26] and located within the genomic region of QTL for SCS [27, 28] and nearby SCC [29, 30]. The analysis of variance on SCS was calculated using the model with genetic marker effect. Breed, stage of lactation and genotype of SNP extremely affected the SCS, but the age effect and parity of cattle on SCS was not significant (Table 3). The relationship between genotypes and SCS was evaluated and shown in Table 4. Polymorphism A526745G was significantly associated with SCS. Cows of genotypes AA and AG had higher SCS than those of GG genotype

Table 1 Genotypic and allelic frequencies of A526745G in three cattle breeds

Breeds	Number	Genotype frequencies (number)			Allelic frequencies	
		AA	AG	GG	A	G
Holstein	73	0.4247 (31)	0.3562 (26)	0.2191 (16)	0.6027	0.3973
Sanhe	78	0.4487 (35)	0.3846 (30)	0.1667 (13)	0.6410	0.3590
Simmental	89	0.2697 (24)	0.4382 (39)	0.2921 (26)	0.4888	0.5112

Table 2 Genetic diversity at the *CACNA2D1* locus in bovine populations

Breeds	Ho	He	Ne	PIC	$\chi 2$
Holstein	0.5211	0.4789	1.9190	0.3642	4.7942
Sanhe	0.5398	0.4602	1.8526	0.3543	2.1052
Simmental	0.5003	0.4997	1.9990	0.3749	1.3498

Ho homozygosity, *He* gene heterozygosity, *Ne* effective number of alleles, *PIC* polymorphism information contents

Table 3 Effects of different factors on SCS

Factors	Breeds	Age	Parity	Stage of lactation	Genotype of SNP
F value	29.21**	1.06	2.00	5.84**	3.10*

*$P < 0.05$;**$P < 0.01$

Table 4 Effects of different genotypes on SCS

SNP	Genotype	SCS (LSM ± SE)
A526745G	GG	3.04 ± 0.76^{a}
	AG	3.73 ± 0.78^{b}
	AA	3.75 ± 0.79^{b}
	P-value	0.0469

Least squares means with the different superscripts letters differ significantly ($P < 0.05$)

Table 5 Effects of different breeds on SCS

SNP	Breeds	SCS (LSM ± SE)
A526745G	Holstein	5.05 ± 0.79^{a}
	Sanhe	3.35 ± 0.78^{b}
	Simmental	2.12 ± 0.79^{c}

Least squares means with the different superscripts letters differ significantly ($P < 0.05$)

($P = 0.0469$). Meanwhile, the effect of different breed type on SCS was analyzed and shown in Table 5. The Least squares means value of SCS of Holstein was significantly higher than that of the Sanhe and Simmental. Zhang et al. and Wang et al. [7, 8] had reported the similar result, respectively. Therefore it was concluded that dairy cattle were easier to be infected with latent mastitis than dual-purpose breeds. Results from this study indicate that that the *CACNA2D1* gene has potential effects on SCS and mastitis resistance. There is no similar research about the association analysis of the *CACNA2D1* gene with SCS and mastitis in cattle. Since the detection of association for such a relative small sample size as ours is low, further work will be necessary to study these SNPs in larger population and other breeds to better clarify the role of *CACNA2D1* gene on SCS and mastitis.

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