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Short Communication

Association of *ATP1A1* gene polymorphism with heat tolerance traits in dairy cattle

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ABSTRACT. Heat stress produces oxidative stress and affects the alternation of plasma K^+ and Na^+. Since Na^+,K^+-ATPase is sensitive to oxidative stress and critical for maintaining the homeostasis of these two ions, we examined the genetic polymorphism of the *ATP1A1* gene in 160 Holstein cows using polymerase chain reaction low ionic strength single-strand conformation polymorphism and DNA sequencing methods. *G* to *A* at position -14103 in exon 14 and *C* to *T* at position -14242 in intron 14 of the bovine *ATP1A1* gene were identified, but the former single nucleotide polymorphism was silent with respect to the amino acid sequence of the protein. However, we found significant correlations between *ATP1A1* gene polymorphism and the coefficient of heat tolerance ($P < 0.01$) and with respiratory rate ($P < 0.01$). Genotype *AC* was the most favorable genotype for heat tolerance. This polymorphism site has potential as a genetic marker for heat tolerance traits in dairy cattle breeding.

Key words: Dairy cattle; *ATP1A1* gene; SNP; Heat stress; Na^+,K^+-ATPase

INTRODUCTION

Cows show heat shock response from May to September because of high temperature in southern China. There are many forms of evidence indicating that the optimum temperature of lactating cows is -5 ~20ºC (Valtorta and Gallardo, 2004). Cows suffer heat shock when the temperature is above 20ºC, and milk yields decline significantly. Previous studies have concentrated on the alleviation of heat stress through physical monitoring of the environment (Armstrong, 1994; Smith et al., 2006). However, little is known about the molecular mechanism of heat resistance in dairy cows.

In summer, high temperatures can produce moderate heat stress, resulting in oxidative stress in dairy cows (Bernabucci et al., 2002), and weak negative effects of heat stress on some plasma markers of oxidative status were revealed in mid-lactating cows. Other studies showed that heat stress influenced the level of plasma Na^+ and K^+ in Holstein cows (El-Nouty et al., 1980; Srikandakumar and Johnson, 2004). All these findings seem to indicate that variable ion concentrations and oxidative stress occurring after heat stress are interrelated events. In order to better understand the molecular mechanism of heat shock response, we selected the Na^+,K^+-ATPase gene as a candidate for heat shock response because it is especially sensitive to oxidative stress (Morel et al., 1998) and is responsible for establishing the electrochemical gradient of Na^+ and K^+ across the plasma membrane, where the ion gradients formed by the enzyme are necessary for Na^+-coupled transport.

The bovine Na^+,K^+-ATPase protein complex consists of α and β subunits, in multiple isoforms (Herrera et al., 1987); distinct γ subunits are now recognized as members of the FXYD family of proteins and are associated with the complex (Sweadner et al., 2000). The *ATP1A1* gene encodes the α1 isoform, the major isoform of the α subunit, which is the only one present in red blood cells. The bovine *ATP1A1* gene has a coding sequence of 3065 nucleotides scattered over 22 exons. As we know, sequence variations can be used for gene mapping, definition of population structure, and performance of functional studies (Dybus and Grzesiak, 2006). Hawken et al. (2004) reported one single nucleotide polymorphism (SNP) in the bovine *ATP1A1* gene.

In this study, we first report the identification of two novel SNPs of the bovine *ATP1A1* gene. Moreover, the effects of the polymorphism site on anti-heat stress traits are evaluated.

MATERIAL AND METHODS

Animals and DNA isolation

A total of 160 Chinese Holstein cows were randomly selected from the Xigang farm of Nanjing city, Jiangsu Province, China, which is situated at latitude 32º02' N and longitude 118º50' E at an altitude of 40.5 m in southern China. The study was performed at 32.5ºC, the mean daily temperature in August.

Blood samples were collected with the anticoagulant acid citrate dextrose and stored at -80ºC. DNA was extracted from 1 mL frozen/thawed blood and diluted with distilled water to a final concentration of 50 ng/μL.

Measurement of physiological parameters

Two physiological parameters, namely heat tolerance coefficient (HTC) and respiratory rate (RR), were measured. HTC was determined according to the Iberia heat tolerance test

(Rhoad, 1944) with the following equation: HTC = 100^{-10} (ART - 38.3), where ART = average rectal temperature before and after 3 h sun exposure for 3 consecutive days, and 38.3ºC is the average normal rectal temperature of cows. The index of HTC was calculated for each cow to assess its heat adaptability. Values of RR at 32.5ºC were also collected in the summer.

Polymorphism detection

Primers were designed by the Oligo 6.12 software based on the bovine *ATP1A1* gene DNA sequence (GenBank accession No. NC_007301.3). The primers of P14 were used to amplify a 330-bp fragment of the *ATP1A1* gene involving exon 14 and parts of intron 14 (forward: 5'-TGAGCAACCAACGCAACACT-3'; reverse: 5'-TGGAACTGCAATCACTGAGGTC-3'). The 20 µL polymerase chain reaction (PCR) amplification mixture contained 50 ng genomic DNA, 10 pM of each primer, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, and 0.50 U Taq DNA polymerase (TaKaRa). The cycling protocol was 5 min at 95°C and 34 cycles of 94°C for 30 s, annealing at 58°C for 30 s, and 72°C for 1 min, with a final extension at 72°C for 8 min. PCR products were detected by electrophoresis on a 1.5% agarose gel stained with ethidium bromide. Two microliters of the PCR product was added to 10 µL low ionic strength (LIS) loading solution (10% sucrose, 0.01% bromophenol blue, and 0.01% xylenecyanol FF). The mixture was incubated at 98°C for 5 min, cooled on ice, and then loaded onto a 12% polyacrylamide gel (acrylamide/bisacrylamide, 29:1, v/v). The gel was stained with 0.1% silver nitrate. The patterns of DNA bands were observed and photographed with the Biorad Gel Doc 2000. The bands representing different LIS-SSCP (single-strand conformation polymorphism) patterns were purified with the Agarose Gel DNA Purification Kit (TaKaRa), subcloned and sequenced in both directions.

Statistical analysis

Allele and genotype frequencies and their accordance with the Hardy-Weinberg law were determined by the POPGENE 1.31 software (Yeh et al., 1999).

The association between the polymorphism of the *ATP1A1* gene and heat tolerance traits was analyzed with the GLM procedure of SPSS 13.0. The following model was used: $Y_{ij} = \mu + G_i + e_{ij}$, where: Y_{ij} is the phenotypic value; μ is the overall mean; G_i is the fixed effect of *i*th genotype, and e_{ij} is the random error. Considering that all experimental dairy cows were descendants of 18 unrelated proven bulls, we did not observe any preferential sire in the population. So the sire effect was not included in the statistical model for association analysis. As the preliminary statistical analyses indicated that the age, the number of fetuses and the lactation month of dairy cows did not have a significant influence on variability of traits in the populations analyzed, so other effects were not taken in this model. Significance level of differences among genotype groups was determined at $P < 0.01$.

RESULTS

The results of polymorphisms

PCR-LIS-SSCP assay of the PCR products showed that the segments amplified with primer P14 had polymorphisms. The size of the PCR product was about 330 bp (Figure 1A).

The banding patterns could be divided into six genotypes: *AA*, *AB*, *BB*, *AC*, *BC*, and *CC* (Figure 1B). The frequencies of different genotypes were 33.61, 20.49, 3.28, 23.77, 12.29, and 6.56%, and the frequencies of alleles *A*, *B* and *C* were: 0.56, 0.20 and 0.24, respectively. The χ^2 test showed no significant difference at this mutation site ($P > 0.05$). This polymorphism site was in Hardy-Weinberg equilibrium.

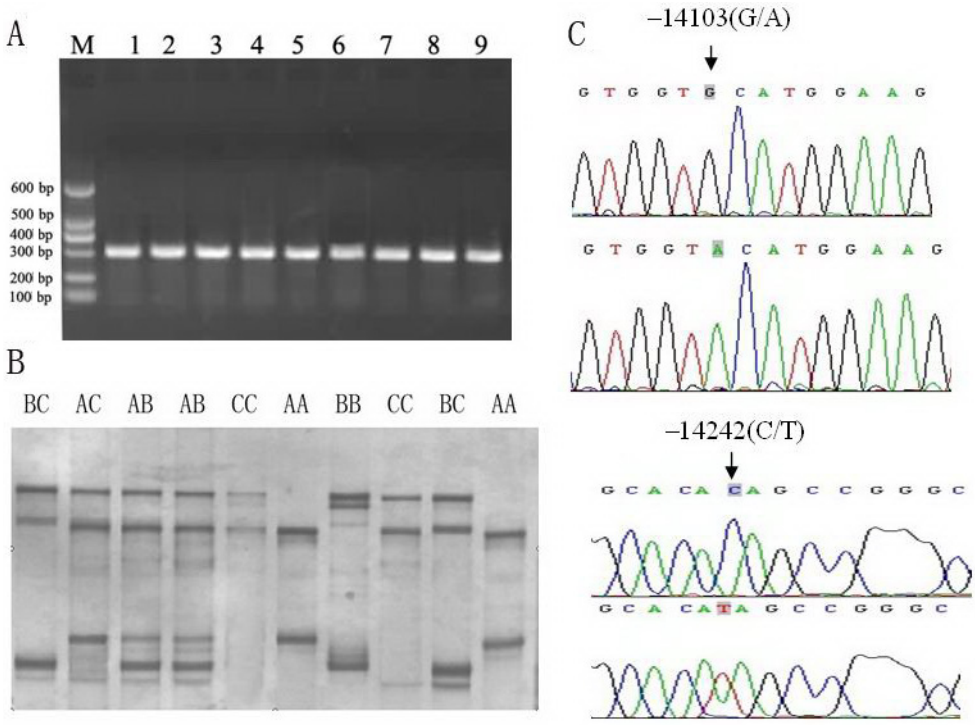

Figure 1. The electrophoresis result of polymerase chain reaction (PCR) products, low ionic strength single-strand conformation polymorphism (LIS-SSCP) patterns and mutation sites. **A.** PCR results of P14 (lanes 1-9). M = Molecular marker. **B.** LIS-SSCP results: corresponding to *AA*, *AB*, *AC*, *BB*, *BC*, and *CC* genotypes, respectively. **C.** Mutation sites: -14103(*G*/*A*) and -14242(*C*/*T*).

After sequencing, two novel SNPs were identified according to accession No. NC_007301.3, *G* to *A* at position -14103 in exon 14 and *C* to *T* at position -14242 in intron 14 of the bovine *ATP1A1* gene (Figure 1C); but the -14103 coding SNP was silent with respect to the amino acid sequence of the protein.

Association between bovine physiological parameters and *ATP1A1* gene polymorphism

The association between the *ATP1A1* polymorphism and physiological parameters was analyzed in the dairy cows (N = 160) (Table 1). We noticed significant relationships be-

tween genotypes and HTC and RR in dairy cows ($P < 0.01$). Cows with genotype *AC* showed the highest HTC (80.22 ± 3.13) and lowest RR (71.76 ± 5.24) among the six genotypes ($P < 0.01$; Table 1). Such results indicate that the *AC* genotype was the most favorable at the *ATP1A1-P14* locus for anti-heat stress trait in the population.

Table 1. Least square means and standard error for physiological parameters of different genotypic heat-stressed cows.

Locus	Genotype	Number	HTC	RR
P14	*AA*	48	$76.30 + 3.32^{AB}$	92.15 ± 3.63^{A}
	AB	36	74.63 ± 1.77^{B}	91.62 ± 3.54^{A}
	AC	38	80.22 ± 3.13^{A}	71.76 ± 5.24^{B}
	BB	6	73.93 ± 4.07^{B}	96.52 ± 6.12^{A}
	BC	23	75.16 ± 2.57^{B}	89.45 ± 5.14^{A}
	CC	9	$76.30 + 3.32^{AB}$	84.32 ± 3.63^{AB}

Different superscript letters indicate significant differences ($P < 0.01$). HTC = heat tolerance coefficient; RR = respiratory rate.

DISCUSSION

Among various candidates in marker-assisted selection of dairy cows, Na^+,K^+-ATPase seems to be promising as a potential candidate for anti-heat stress trait in dairy cows. The literature so far suggests that a specific Na^+,K^+-ATPase could be necessary to fulfill different physiological roles. The genomic organization and evolutionary history of the Na^+,K^+-ATPase family are particularly intriguing given the highly conserved sequences of these genes and their ubiquity in biological systems. We found two novel SNPs and deposited them in the dbSNP GenBank (ss159831431, ss159831431). As described by Maruya et al. (1996), our study indicates that the PCR-LIS-SSCP and DNA sequencing methods are rapid, economical and effective in the detection of novel SNPs of the bovine *ATP1A1* gene.

In human, there is evidence that the polymorphism of human *ATP1A1* is associated with diseases, and for instance, Jannot et al. (2002) suggested that it influences Na^+,K^+-ATPase activity in the case of complete or partial C-peptide deficiency, which proved the high relative risk of developing the neuropathy observed in type 1 diabetic patients. Glorioso et al. (2007) reported that *ATP1A1* may contribute to the known renal and vascular endothelial dysfunction associated with essential hypertension. In this study, the association between the *ATP1A1* polymorphism and physiological parameters in dairy cattles indicates that the polymorphism site has a positive effect on anti-heat stress trait, the *AC* genotype of the *ATP1A1* gene is the most favorable genotype at the P14 locus for heat resistance. It is suggested that the bovine *ATP1A1* gene may play an important role in heat resistance, and the P14 locus within the bovine *ATP1A1* gene could be considered as a DNA marker for bovine anti-heat stress trait in marker-assisted selection. Further studies on larger populations are needed to verify the results and expand their application.

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