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The Polymorphisms of β_2 -Adrenergic Receptor Gene on two Cattle Breeds in China

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ABSTRACT

The beta-adrenergic receptors (β -AR) are a class of metabotropic G protein-coupled receptors that responsible for the muscle growth, fat deposition or lactation characteristics of domestic animals. The aim of this article was to scan nucleotide mutations in partial coding region of β_2 -AR gene on cattle. In this study, the polymorphisms of β_2 -AR gene on cattle were detected by methods of Polymerase Chain Reaction-Restriction Fragment Length Polymorphism (PCR-RFLP) and DNA sequencing in two cattle breeds in China, Xinjiang Brown cattle and Chinese Holstein cows. The results revealed that there were two genotypes and homozygote variant was not found in the two breeds. The genotypic frequencies at the Sma I locus were significantly different between the two breeds. The wild-type allele was ascendancy in the two breeds but its allele frequency was distinctly lower in the Chinese Holstein cows than that in the Xinjiang Brown cattle. DNA alignment results showed that three single nucleotide polymorphisms (SNPs), C11A, G53A and C129T were found in 5-coding region of β_2 -AR gene. The C11A mutation resulted in disappearance of Sma I cleavage site. In addition, the C11A and G53A mutations caused two amino acid residues replacement at the 4th and 18th of β_2 -AR. However, the C129T mutation was a synonymous one. In conclusion, the heterozygous frequency in Chinese Holstein cows was obviously higher than that in the Xinjiang Brown cattle. The Sma I locus would be a potential genetic marker for lactation performance of cattle.

Key words: Dairy cattle, β_2 -adrenergic receptor gene, polymorphism, PCR-RFLP

INTRODUCTION

The β -adrenergic receptors (β -ARs, or adrenoceptors) are a class of metabotropic G protein-coupled receptors that are targets of the catecholamines, especially norepinephrine and epinephrine (Perez, 2006). The β -ARs have been subdivided into at least three distinct groups: β_1 , β_2 and β_3 , classically identified in cardiac, airway smooth muscle and adipose tissue, respectively (Johnson, 1998). β -ARs are present on the surface of almost every type of mammalian cell but the distribution of subtypes and proportion of each varies between tissues in a given species (Mersmann, 1998). The use of exogenous β -AR agonists to promote muscle growth and limit fat deposition has been extensively evaluated (Liang and Mills, 2001; Mills *et al.*, 2003; Kim *et al.*, 2010). In general, the most profound effects of exogenous β -AR agonists are observed in cattle and sheep (Mersmann, 1998; Beckett *et al.*, 2009).

Bruckmaier *et al.* (1991) reported that isoproterenol (β -AR agonists) enhanced milk flow without affecting milk ejection. Blum *et al.* (1989) found that using phentolamine (α -AR antagonists) or aleudrin benefited lactating a lot. Over the past decade, researches indicated that stimulation of β -ARs in the bovine mammary gland affected milking characteristics such as milk yield and peak flow rate (Inderwies *et al.*, 2003a). Milk yield and peak flow rate decreased during α -AR stimulation and during the oxytocin receptor blockade and increased during β -adrenergic stimulation (Inderwies *et al.*, 2003b). So if nucleotide mutation take place in β -AR gene, the lactation performance of dairy cattle would be influenced. However, there is little information about polymorphisms of β -AR gene in cattle at present.

In this study, two breeds of cattle (Xinjiang Brown cattle and Chinese Holstein cows) which have obviously differences in lactation performance were chosen as the samples. PCR-RFLP (Polymerase Chain Reaction-Restriction Fragment Length Polymorphism) and DNA sequencing methods were used to scan single nucleotide polymorphisms (SNPs) in the parts of the encoding region of β_2 -AR gene, in order to identify potential genetic markers for lactation performance.

MATERIALS AND METHODS

Blood sample source: Ninety eight all unrelated animals (Chinese Holstein cow, n = 42; Xinjiang Brown cattle, n = 56) were collected and used in this research in December of 2009 to July of 2010. The Chinese Holstein cows were from Zhongzhou cattle farm of Yili county of Xinjiang in China, the Xinjiang Brown cattle were from Tacheng Brown Cattle Breeding Centre of Xinjiang in China. Blood samples were drawn from the jugular vein into vacuum blood collection tubes containing ACD (anticoagulant citrate dextrose). The samples were stored at -20°C for latter analyze.

DNA extraction: Genomic DNA was extracted with the use of phenol/chloroform (Sambrook and Russell, 2001) and detected with 0.8% agarose gel electrophoresis (0.5 × Tris-borate-EDTA buffers).

Primer design: According to the DNA sequence of bovine β_2 -AR (GeneBank accession number: NM_174231), one pair of PCR primers was designed with Oligo 6.0 software package (Qiong *et al.*, 2011). Primer sequences used for PCR were as follows:

- **Forward:** 5'-TGC GCT CAC CTG CCA GC-3'
- **Reverse:** 5'-TGC CAG GCC CAT GAC CAG GTC AG-3'

The primers were synthesized by Invitrogen by Life Technologies (Shanghai) and used to amplify 281 bp PCR products, containing partial 5-UTR (26 bp) and coding region (255 bp) of bovine β_2 -AR gene.

PCR amplification: Amplification of the DNA was performed by the PCR with the following conditions in a final volume of 20 μ L: 100 ng genomic DNA, 5 pmol of each primer, 0.2 mM of each dNTP, 4 μ L colorless GoTaq reaction buffer, 0.2 μ L GoTaq™ polymerase (5 IU μ L⁻¹, Promega, Shanghai), nuclease-free water was added to achieve the final volume (Dhanapal *et al.*, 2010). Samples were amplified in a thermocycler (Mycycler, Bio-Rad, USA) using the following programs: 94°C for 5 min followed by 10 cycles of 94°C for 1 min, 70°C for 35 sec, 72°C for 45 sec, then another 25 cycles of 94°C for 45 sec, annealing 63°C for 30 seconds, 72°C for 40 seconds and a final extension at 72°C for 7 min. PCR products were visualized on 1% (w/v) agarose gels prior to RFLP analysis.

Deletion mutation polymorphism in the β_2 -AR gene: Restriction enzymatic digestion was performed with the following conditions in a final volume of 20 μ L: 5 μ L PCR products of β_2 -AR gene, 2 μ L 0.1% BSA, 2 μ L 10 \times T buffer, 0.5 μ L Sma I (10 IU μ L⁻¹, TaKaRa, Japan), deionized water was added to achieve the final volume. All reagents mixed gently and incubated at 30°C for 16 h and then 65 °C for 20 min to inactivate the endonucleases. Afterwards, 2 μ L 10 \times loading buffer was added to terminate the reaction. Aliquots (10 μ L) of the mixture were loaded onto 10% polyacrylamide gel and electrophoresed at a constant voltage (200 V) for 2 h (Alashawkany *et al.*, 2008). At the end of electrophoresis, the gels were stained with ethidium bromide (200 ng mL⁻¹) for 15 min and the gel images were captured with a GelDoc XR gel-documentation system (Bio-Rad, USA).

DNA sequencing: The PCR amplicons from different patterns in the two breeds purified with a DNA Fragment Quick Purification Kit (DingGuo, Beijing, China) and cloned in *Escherichia coli* DH5 α by using pUCm-T vector (Bio Basic Inc, Canada). The recombinant plasmids DNA were isolated and sequenced by using ABI PRISM 377 automated sequencer (Perkin Elmer-Applied Biosystems Division, USA) with primers M13 universal forward primer (5'-GTT GTA AAA CGA CCG CCA GT-3') and M13 reverse primer (5'-CAG GAA ACA GCT ATG ACC-3') by Invitrogen by Life Technologies (Shanghai). These sequences were then compared to those available in GenBank by using Basic Local Alignment Search Tool (BLAST) at <http://blast.ncbi.nlm.nih.gov/Blast.cgi> (Fang *et al.*, 2010).

Statistical analysis: Based on the genotypes of Sma I locus in the two cattle breeds, the genotypic frequencies and allelic frequencies were calculated and Hardy-Weinberg equilibriums were detected by using PopGen32 software package (Jun *et al.*, 2010). The distribution of these genotypes among all cattle populations was analyzed using χ^2 -test which were performed by PASW Statistics 18.0 (Norusis, 2009). Population genetic indexes, such as He (gene heterozygosity), Ho (gene homozygosity), Ne (effective allele numbers) and PIC (Polymorphism Information Content) were calculated according to Nei and Roychoudhury (1974), Nei and Li (1979), respectively.

RESULTS

PCR amplification: All of the PCR amplification products were detected by 1% agarose and obtained the expected fragments with the clear and specific bands. The agarose electrophoresis profiles of β_2 -AR gene obtained from the primers are shown in Fig. 1. The result showed that the amplification products had a good specificity and it could be used for enzymatic digestion.

RFLP typing: In this study, polymorphisms of β_2 -AR gene were scanned by PCR-RFLP in the two breeds. The results revealed that there were 2 genotypes (named as genotype CC and CA) and 2 alleles (namely C and A) at Sma I locus (Fig. 2). The restriction fragment lengths included 243 and 38 bp for CC genotype and 281, 243 and 38 bp for CA genotype. Genotypic and allele frequencies of β_2 -AR gene in the two breeds were shown in Table 1. The results showed that both of CC and CA genotype existed in the two breeds and the frequency of CC genotype was higher than that of CA. in both of the two breeds. The frequency of CC genotype in Chinese Holstein cows was 0.5714 which was obviously lower than that in the Xinjiang Brown cattle. Frequencies of allele C in the analyzed populations were 0.9018 and 0.7857 for Xinjiang Brown cattle and Chinese Holstein cow. The genotypic frequencies at the Sma I locus were significantly different

Table 1: Genotype and allele frequencies of β_2 -AR gene in different breeds

Breeds	N	Genotypic frequency		Allele frequency		χ^2 (HWE)	p-value
		CC	CA	C	A		
Xinjiang Brown cattle	56	0.8036	0.1964	0.9018	0.0982	0.664	>0.05
Chinese Holstein cows	42	0.5714	0.4286	0.7857	0.2143	3.124	>0.05

χ^2 (HWE): Hardy-Weinberg equilibrium χ^2 value

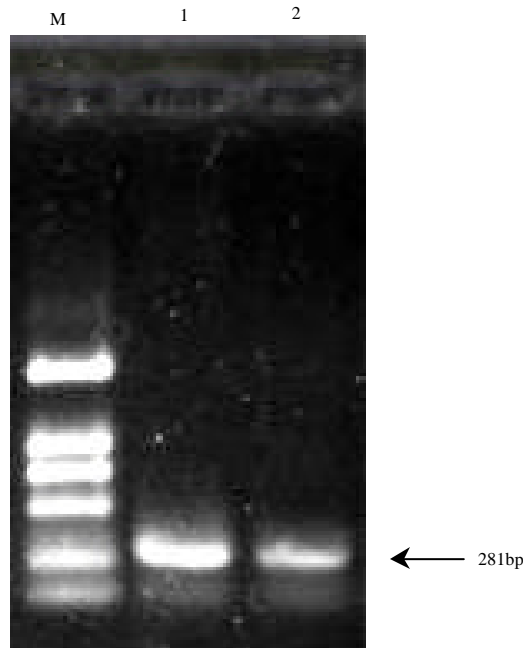


Fig. 1: The atlas of β_2 -AR gene PCR products. Line 1 and 2: PCR products; line M: DNA marker

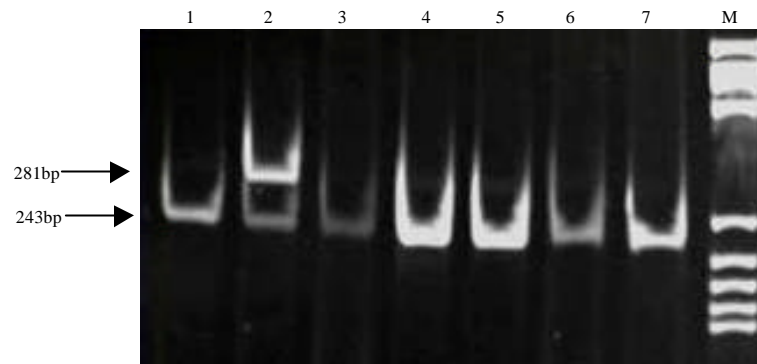


Fig. 2: The PCR-RFLP PAGE patterns of β_2 -AR-Sma I locus. Line 1 and 3 to 7: CC genotype, line 2: CA genotype. Line M: pBR322/BsuR I DNA marker

between Xinjiang Brown cattle and Chinese Holstein cows based on a χ^2 -test ($\chi^2 = 6.208$, $df = 1$, $p = 0.013$). The C allele was ascendency in the two breeds but its allele frequency was distinctly

lower in the Chinese Holstein cows than that in the Xinjiang brown cattle. The χ^2 -test showed that the genotype distributions of Xinjiang Brown cattle and Chinese Holstein cow breeds were in consistent with Hardy-Weinberg equilibrium ($p > 0.05$).

The population genetic parameters (namely, homozygosity, heterozygosity, effective allele numbers (Ne) and PIC (Polymorphism Information Content)) were demonstrated in Table 2. Value of homozygosity estimate varied from 0.663 to 0.823 and Ne ranged from 1.215 to 1.508. PIC values varied from 0.161 to 0.280.

DNA sequencing results: According to the sequencing and sequence of bovine β_2 -AR gene (GeneBank accession No. NM_174231), CC genotype was indentified as wild-type and CA genotype as heterozygote. Alignment results showed that three SNPs, C11A, G53A and C129T were found in coding region at β_2 -AR gene (Fig. 3). The C11A mutation resulted in disappearance of Sma I cleavage sites (CCCGGG→CACGGG). In addition, amino acid sequence analysis showed that the C11A and G53A mutations led to the fourth and eighteenth amino acid residues changed from the proline to histidine and arginine changed to histidine of β_2 -AR. However, the C129T mutation was a synonymous one.

Table 2: Genetic index in two cattle breeds in China

Breeds	Type	Ho	He	Ne	PIC
Xinjiang Brown cattle	Dual-purpose cattle	0.823	0.177	1.215	0.161
Chinese Holstein cows	Dairy cattle	0.663	0.337	1.508	0.280

Ho: Gene homozygosity; He: Gene heterozygosity; Ne: Effective allele number; PIC: Polymorphic information content

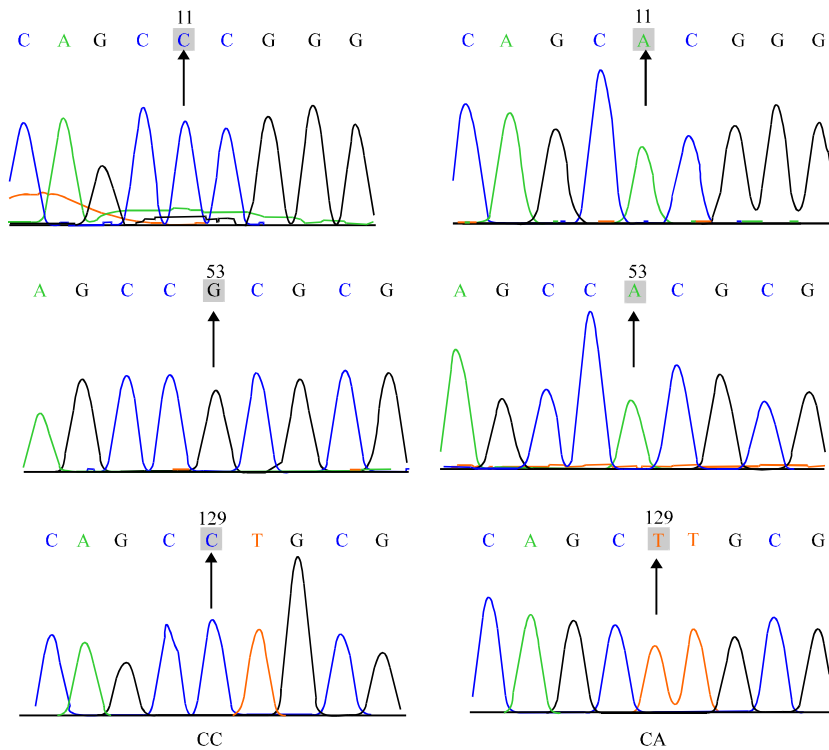


Fig. 3: Sequencing maps from different genotype of β_2 -AR gene

DISCUSSION

β_2 -AR prevails on the surface of cardiac muscle cell, adipose cell, nervous system, ren tissue and mammary gland (Bray and Boerwinkle, 2000; Inderwies *et al.*, 2003c). It is involved in the process of physiological and metabolism regulation, including heart rate, blood pressure, remaining of renal water and milk yields (Stoffel and Meyer, 1993; Bray and Boerwinkle, 2000). Roets *et al.* (1986) reported that there were some relevance between milk yields of cows and α_2 -AR and β_2 -AR which were found both in mammary papilla organs and blood cells. So nucleotide mutation of β_2 -AR may influence the lactation performance of cattle. However, there have been no reports about whether nucleotide mutation of β_2 -AR gene may generate some influence on the quantity of β_2 -AR or on the lactation performance of cattle.

In this study, PCR-RFLP was adopted to analyze polymorphism of β_2 -AR gene in two cattle breeds in China and the results showed that there were two genotypes, namely CC and CA. DNA sequence showed that there were three mutation sites. The C11A mutation resulted in the disappearing of the recognition site of restriction enzyme Sma I. Amino acid sequence showed that proline was changed into histidine at the forth amino acid residue. Due to the fact that proline is an imino acid while histidine is a basic amino acid, meanwhile, this site is very conservative in cattle, swine and sheep (Carron *et al.*, 2005; Chen *et al.*, 2002; Zhang *et al.*, 2010), so the three-dimensional structure and function of β_2 -AR would be affected by the C11A mutation. This mutation whether affect milk yield needs more further study.

Xinjiang Brown cattle are an improved breed with some Brown Swiss, Alatau and Kesiteluomu blood. They are dual-purpose, medium-sized cattle (Suttie and Reynolds, 2003). Its milk yields situate between 1600-3000 kg (305-day basis) (Zheng, 1984). However, Chinese Holstein cows (named Chinese Black and White before the year of 1997) bred with abroad Holstein cows and indigenous yellow cattle and its milk yields situate between 6500-7500 kg (305-day basis). In the present study, the CA genotypic frequency in Chinese Holstein cows was obviously higher than that in the Xinjiang Brown cattle. There seems to be certain relationship between CA genotype and lactation performance. To prove this presumption, more researches still need.

In this research, the population genetic parameters were calculated. PIC values varied from 0.161 to 0.280. According to the classification of PIC (high polymorphism if PIC value>0.5, median polymorphism if 0.25<PIC value<0.5 and low polymorphism if PIC value<0.25) (Botstein *et al.*, 1980). Among the loci of the two populations showed low polymorphism.

CONCLUSION

In conclusion, three SNPs existed in the 5'-coding region of β_2 -AR gene on two cattle breeds in China. The heterozygous frequency in Chinese Holstein cows was obviously higher than that in the Xinjiang Brown cattle. The Sma I locus would be a potential genetic marker for lactation performance of cattle.

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