Review

Associations of *GBP2* gene copy number variations with growth traits and transcriptional expression in Chinese cattle

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A B S T R A C T

Copy number variations (CNVs) recently have been recognized as another important genetic variability followed single nucleotide polymorphisms (SNPs). The guanylate binding protein 2 (*GBP2*) gene plays an important role in cell proliferation. This study was performed to determine the presence of *GBP2* CNV (relative to Angus cattle) in 466 individuals representing six main cattle breeds from China, identify its relationship with growth, and explore the biological effects of gene expression. There were two CNV regions in the *GBP2* gene, for three types, CNV1 loss type (relative to Angus cattle) was more frequent in XN than other breeds, and CNV2 loss type (relative to Angus cattle) was more frequent in XN and CDM than other breeds. Though the *GBP2* gene copy number presented no correlation with the transcriptional expression of JX ($P > .05$), but the transcriptional expression in heart is higher than other tissues, and the copy number in muscles and fat of JX is higher than others breeds. Statistical analysis revealed that the *GBP2* gene CNV1 and CNV2 were significantly associated with growth traits ($P < .05$). In conclusion, this research established the correlations between CNVs of *GBP2* gene and growth traits in different cattle breeds, and our results suggested that the CNVs in *GBP2* gene may be considered markers for the molecular breeding of Chinese beef cattle.

1. Introduction

With the cattle genome assembly release and functional elements annotation (Elsik et al., 2009), the study of genetic variations were used to improve meat productivity and marbling grades in animals breeding (Casas et al., 2005). In previous studies, the detection and genetic effect analysis of single nucleotide polymorphisms (SNPs) and indels has made significant breakthrough. Through high-throughput sequencing and genome-wide association study (GWAS), a large number of SNPs were detected and showed the associated with growth traits in cattle (Pryce et al., 2010).

However, copy number variations (CNVs) of genomic segments, a wider variation degree than SNPs and Indels were come up in 2004 (Iafrate et al., 2004; Sebat et al., 2004). As a kind of the microstructure variation, CNVs ranges from 50 bp to several Mb and comprised large-scale insertions, deletions, copy, inversions and translocations compared with a reference genome (Feuk et al., 2006). There are four kinds of forming mechanism of CNV, consisting of Non-Allelic Homologous Recombination (NAHR), Non-Homologous End-Joining (NHEJ), Fork Stalling and Template Switching (FoSTeS) and L1-mediated Retro transposition (Gu et al., 2008; Hastings et al., 2009). CNVs affected gene function through a variety of mechanisms, including gene dosage effect, gene blocking effect, gene fusion, position effect, stealth allele revealing, functional polymorphism and potential condensed effect, etc. Extensively studies on CNVs have been conducted in humans (Altshuler et al., 2010), the human genome had detected 200,000 CNV regions coverage rate reached 70% (Mac Donald et al., 2014). Many studies found that CNV associated with many complex neurological diseases,

such as SNCA gene copy number increase lead to changes in its dose, cause the PD disease happen (Hannes et al., 2009). In addition, CNV detection and map construction have been completed in chicken (Wang et al., 2010), goat (Fontanesi et al., 2010), cattle (Bae et al., 2010; Fadista et al., 2010). These results demonstrated that CNV regions influence phenotypic traits by changing the gene expression (Seroussi et al., 2010).

Compelling evidence has indicated that CNVs responsible for growth traits by changing the copy numbers of function genes. Our previous study revealed that guanylate binding protein 2 (GBP2) gene was identified existing CNVs region in Chinese cattle genome. GBP2 gene, a member of GBP family, plays an important role in regulating cell proliferation and resistance to pathogen infection. In addition, GBPs protein has a very high GTP enzyme activity, can hydrolysis GTP to GDP and GMP without depend on the exchange factor and enzyme activation protein (Cheng et al., 1991). However, there are no studies being reported on the potential associations of GBP2 copy number variations with cattle growth traits. In this study, we conducted to test the hypothesis by performing quantitative real-time PCR (qPCR) in genomic DNA and mRNA level, which could provide molecular basis for further utilization of the GBP2 CNVs as promising markers for economic traits in cattle breeding programs.

2. Materials and methods

2.1. Animals and growth traits measurements

All experiments were approved by the Review Committee for the Use of Animal Subjects of Northwest A&F University. In order to detect the intergroup distribution of GBP2 gene copy number variations, 466 adult cows (24 months old) representing six main cattle breeds from China were enrolled: Xianan cattle (XN, n = 96, Biyang county, Henan Province, China), Pinan cattle (PN, n = 145, Xinye county, Henan Province, China), Jinjiang cattle (JJ, n = 65, Gaoan country, Jiangxi Province, China), Jian cattle (JA, n = 70, Taihe country, Jiangxi Province, China), Chaidamu cattle (CDM, CDM, n = 60, Dulan country, Qinghai Provence, China), Yak (YAK, n = 30, Tianjun country, Qinghai Provence, China). In addition, for detecting mRNA expression level of GBP2 gene, four Chinese beef cattle breeds were enrolled: Jiaxian cattle, Angus crossbreed F1 generation (AJ, Angus × Jiaxian), Simmental crossbreed F1 generation (SJ, Simmental × Jiaxian), Charlotte's cattle hybrid F1 generation (CJ, Charlotte × Jiaxian). All of the individuals were selected randomly as tested cases. In comparative genomic hybridization (CGH) array, six individuals from three breeds (Nanyang, Qinchuan and Luxi) have been used for genomic CNVs screening, and they were also selected for CNVs validating analysis. Angus cattle were the reference sample in the hybridization array (Supplementary Table 2). Moreover, the individuals from each breed were selected from the same breeding farm. All aforementioned cattle (without genetic relationships) were female and fed ad libitum with corn–corn silage from weaning to slaughter. Blood samples were

obtained, and genomic DNA was isolated from leukocytes with phenol–chloroform extraction. The phenotypic data of growth traits body weight, body height, body length, chest girth, hucklebone width and average daily gain were recorded and collected at the same age in every breed for the further association analysis.

2.2. Sample collected, genomic DNA and total RNA extraction

Blood samples were collected and genomic DNA was extraction according to the procedure described in Sonstegard et al. (2000). The adult JX, AJ, SJ and CJ cattle were selected for tissue sampling. None of the animals exhibited any adverse health conditions. Different tissues, including heart, liver, kidney, skeletal muscles, fat and stomach were obtained from adult (n = 3) JX cattle, in addition, different skeletal muscles, and fat tissues were isolated from adult JX (n = 3), AJ (n = 3), SJ (n = 3), and CJ (n = 3) breeds. These tissues were snap-frozen in liquid nitrogen and stored at −80 °C for subsequent use. Total RNA was isolated using Trizol reagent (Takara, Dalian, China) according to the manufacturer's instructions. The quality of RNA was assessed by visualizing the ribosomal RNA bands via agarose gel electrophoresis and measuring spectrophotometrically. First strand cDNA was synthesized using a cDNA synthesis kit (Takara, Dalian, China) with 1 μg of total RNA as template.

2.3. Primer design and amplification detection

According to our previous CGH analysis results, further validation of the CNVs region including GBP2 gene was performed in this study. The GBP2 gene copy number variation region GBP2-CNV1 is located in the GBP2 gene reference genome sequence NC_007301.6 from 54593301 bp to 54,594,300 bp, a total of 999 bp, and GBP2-CNV2 is located from 54636901 bp to 54638000 bp, a total of 1099 bp. Two distinct primer sets were designed in two separate regions CNV1 and CNV2 of GBP2 gene, meanwhile, the reference primer were designed in gene BTF3 and β-actin gene using Primer v5.0 software (PREMIER Bio soft International, California, USA). The primers information was shown in Table 1 as illustrated, the amplified fragment by primer pair 1 (GBP2-CNV1) was in the CNV1 region, while the primer pair 2 (CBP2-CNV2) was located in the CNV2 region.

2.4. Copy number analysis and expression profiling of GBP2 gene

In this study, we investigated the relative copy numbers and mRNA expression of bovine GBP2 gene. We choose bovine basic transcription factor 3 (BTF3) as internal reference gene, because there are neither CNVs nor segmental duplication in the Database of Genomic Variants of BTF3 (Bickhart et al., 2012) (Table 1). The copy number of GBP2 gene was confirmed based on the assumption that there were two copies of the DNA segment in the calibrator animals. Genomic qPCR experiments were conducted using SYBR® Green in triplicate reactions. A total of 12.5 μl reaction mixtures contained 10 ng of cDNA, 6.25 μlSYBR®

Table 1
The information of primers used in this study.

	Locus	Primer sequence (5′ to 3′)	Amplification length bp
DNA level	GBP2-CNV1	5′-CTCTCAGGCGGTATCACAGTCAATG-3′	147 bp
		5′- TCCTTGGCATCATTAGACTCTGTAT-3′	
	GBP2-CNV2	5′-ATGGGCAGCCTGGACTATC-3′	134 bp
	BTF3	5′-GGTTCTCCTTGGACGGGTG-3′	166 bp
		5′-AACCAGGAGAAACTCGCCAA-3′	
		5′-TTCGGTGAAATGCCCTCTCG-3′	
	GBP2-Dbta	5′-GACAGAGCTGACACGTCGAA-3′	140 bp
mRNA level	β-Actin	5′-GTCATCACCATCGGCAATGAG-3′	84 bp
		5′-AATGCCGCAGGATTCCATG-3′	

Notes: Two distinct primer pairs in the GBP2 CNVs locus for validation analysis.