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Short communication

Variants and haplotypes within *MEF2C* gene influence stature of chinese native cattle including body dimensions and weight

Xiu-Kai Cao [a,1], Zhao-Yang Zhan [a,1], Yong-Zhen Huang [a], Xian-Yong Lan [a], Chu-Zhao Lei [a], Xing-Lei Qi [b], Hong Chen [a,*]

[a] College of Animal Science and Technology, Northwest A&F University, Shaanxi Key Laboratory of Molecular Biology for Agriculture, Yangling, Shaanxi 712100, China
[b] Bureau of Animal Husbandry of Biyang County, Biyang, Henan 463700, China

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ABSTRACT

Myocyte enhancer factor 2C (*MEF2C*) gene was annotated for its potential phenotypic effects on bovine stature including body dimensions and body weight. The aim of this study was the analysis of *MEF2C* as a candidate for bovine stature. Four SNPs including two novel SNPs were identified and the polymorphisms were genotyped by (forced) PCR-RFLP. Using a population of 805 cows from two Chinese native breeds, we report confirmation of this effect, demonstrating strong association of different components of bovine stature with *MEF2C* genotypes. Six combined haplotypes were constructed as well and diplotype H1H6/H3H5 (CTGGTCAA) affected bovine stature negatively, including body weight, hucklebone widt, and withers height ($P < 0.05$). Our results suggest that the four variants within bovine *MEF2C* can be used as candidate SNP markers for marker-assisted selection in beef cattle breeding programmes.

Introduction

Myocyte enhancer factor 2C (*MEF2C*) gene annotated for its potential phenotypic effects on body shape and growth traits by GWAS is one promising candidate for bovine stature without confirmation to date(Cole et al., 2011; Sorbolini, 2014). It is located at position ~90.5 Mb on BTA 7 the region that involved in a high density of QTLs for various traits of economic importance, especially body weight and carcass weight for which QTLs peaks are closely linked to our target—*MEF2C* (Fig. S1). The gene pertains to *MEF2* family of MADS (MCM1, agamous, deficiens, and serum response factor)-box transcription factors (Martin et al., 1993). The MADS box of MEF2 is responsible for DNA binding, dimerization, and interaction with basic helix–loop–helix (bHLH) transcription factors that control development and differentiation of many cell types, including muscle, neural, and hematopoietic cells (Wilson-Rawls et al., 1999). As an essential member of MEF2 family, MEF2C has been the focus of intense interest given its indispensable role in muscle (Han et al., 1997; Wang et al., 2001; Wimmers et al., 2006; Panda et al., 2014), bone (Arnold et al., 2007; Ackert-Bicknell et al., 2010), and even neuro (Li et al., 2008; Rashid et al., 2014) development. Interestingly, evidence indicates unexpected commonalities in the mechanisms governing muscle and bone development with respect to their regulation by MEF2C (Arnold et al., 2007). Together, these findings of MEF2C functional properties add considerable weight to its probability of being a genetic regulator for bovine stature. At present the role of *MEF2C* in regulating stature, however, is only beginning to be realized, as well as that in regulating reproductivity (Onteru et al., 2010; 2012) and meat quality (Zhao et al., 2011; Sevane et al., 2013). Hence the objective of this article is to ascertain the effects of *MEF2C* on bovin stature via preliminary association analysis, which possibly provided some useful information for cattle breeding and genetics.

Materials and methods

Animals and data collection

A total of 805 cows were used in this study, including Qingchuan (QC, $n=382$) and Jiaxian (JX, $n=423$) reserved in Shaanxi and Henan, respectively, which were the main beef cattle breeds in China because of their superior growth and meat traits (Cao et al., 2013). The animals of each breed were selected to be unrelated for at least three generations, with the aim of having distant lineage within each breed. After weaning at 6 months of age, these animals were fed *ad libitum* on concentrated diet and straw to adult

* Corresponding author at: No.22 Xinong Road, College of Animal Science and Technology, Northwest A&F University, Yangling, Shaanxi 712100, China.
E-mail address: chenhong1212@263.net (H. Chen).
[1] The authors contributed equally to this work.

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stage. Stature records of these cows ($n=805$) were collected following the method of Gilbert et al. for association analysis, including body length (BL), body weight (BW), chest girth (CHEGRT), hucklebone width (HUCWDT), rump length (RUMLGT), withers height (WHT)(Gilbert et al., 1993).

DNA pool sequencing

Genomic DNA were extracted from 1 mL 2% heparin-treated whole blood samples procured from jugular according to standard procedures, followed by dilution to 50 ng/L on the basis of spectrophotometrical results (Sambrook et al., 2001). Fifty DNA samples were randomly selected from each breed to construct two DNA pools. Eleven pairs of primers (Table S1) were designed for PCR amplification using touchdown protocol based on the sequence of *Bos taurus MEF2C* gene (GenBank Accession No. AC_000164.1) (Don et al., 1991). PCR products were commercially sequenced in both directions.

Genotyping by forced PCR-RFLP

Four single nucleotide polymorphisms (SNPs) were screened via the DNA pool sequencing and Blastn analyses, namely, AC_000164.1:g.90688021C > T, g.90707761G > T, g.90707849C > T, and g.90766467A > C, among the specimens.To define genotypes of the variants efficiently and inexpensively, mutations were induced into primers to create expected restriction sites for endonucleases (Table S2, Figure S2). For example, two mismatches (AA) were introduced to forward primer at g. 90688025C and g. 90688026C loci, respectively, which created a *Hin*d III restriction site (AAGCTT) in the PCR products from the g.90688021T individual carrier, whereas PCR products from the g.90688021C bovine carrier blocked this restriction endonuclease site. Aliquots of 10 μL PCR products were digested with endonucleases (MBI Fermentas, Vilnius, Lithuania) following the manuals. All digested products were detected by electrophoresis in a 3.0% agarose gel stained with ethidium bromide (Figure S3).

Prediction of splicing sites

ESEfinder 3.0 online software was used for bioinformatic prediction on change of splicing sites given the non-exonic location of the four associated SNPs (Cartegni et al., 2003; Smith et al., 2006). Matrix library of the software was SRProteins and other parameters were set as default. Sequence of 51 nt with wild/mutant site in center was analyzed. The differences on binding sites of splicing factors were showed in Figure S5.

Statistical analysis

The χ^2 test for Hardy–Weinberg equilibrium (HWE) were computed by PASW Statistics (version 18). Linkage disequilibrium (LD) and haplotypes analyses were performed by the online SHEsis software (Li et al., 2009). For the individual SNP, contrasts between *MEF2C* genotypes for the measured traits were estimated using the approximated *F*-statistic provided by ASReml (Butler et al., 2009). Apart from the genotype effects, fixed effect was birth season where there are four seasons in a year. Age and farm were fitted as random effects. The significance of random effects were tested using log likelihood ratio tests within ASReml. Combined haplotypes were aslo constructed to explore the potential joint action of the four loci on stature. Given the population size of a certain combined haplotype, breed and interaction between pairwise loci were additionally included as random and fixed effects, respectively (Huang et al., 2011). Additive effects were calculated as the mean of the difference between homozygotes, using the least squares means (Falconer et al., 1996). Dominance effects were calculated as the deviation of heterozygotes from the mean of the homozygotes (Short et al., 1997). Allele substitution effects were estimated by using linear regression techniques, regressing phenotypes on number of copies of the mutant alleles (0, 1, and/or 2) of the four loci separately (Rothschild et al., 1996). Notably, multiple tests were not corrected at $P=0.05$ and $P=0.01$.

Results and discussion

Four variants mapped to three introns of bovine *MEF2C* were identified according to the pool sequencing and alignment analysis (Table S2, Figure S2). Unexpectedly, we did not find discernible variants in exons. Among the four loci, g.90707761G > T (dbSNP Accession No. rs379077428) and g.90707849C > T (dbSNP Accession No. rs385763063) were both in intron 3, while the other two novel SNPs, g.90688021C > T (SubSNP, ss1553721894) and g.90766467A > C (SubSNP, ss1537037927), were in intron 4 and 1, respectively. Additional information of the two novel SNPs were listed in Table S3. The χ^2 test by PASW Statistics (version 18) showed all variants in the analyzed breeds were at the Hardy–Weinberg equilibrium except for the g.90688021C > T locus in QC breed ($P<0.05$) (Table S4). Furthermore, slight difference were detected between the two breeds in allelic frequencies, gene homozygosity (*Ho*), effective allele numbers (*Ne*) and polymorphism information content (PIC) of the four loci (Table S4).

We correlated each single SNP with bovine stature in the two populations separately on account of the genetic background, and the results are visualized in Fig. 1 (detailed in Table S5 a, b). All the

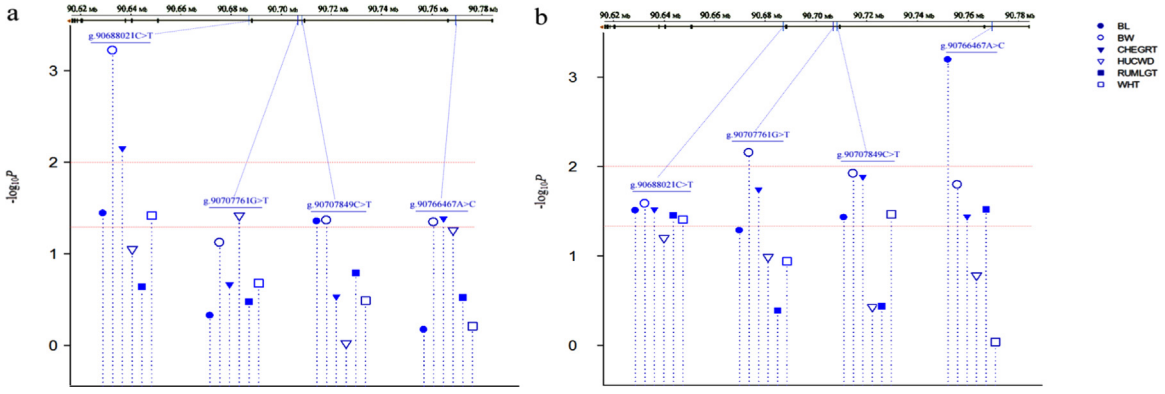

Fig. 1. Bovine *MEF2C* gene structure and association analysis of its four mutations with stature of QC (a) and JX (b) breeds. Significance test *P* values were showed as $\log_{10}$ values (left *y* axis). The two dotted red line meant significance level at $P=0.01$ (upper) and $P=0.05$ (lower).