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Functional haplotypes of ARID4A affect promoter activity and semen quality of bulls

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ABSTRACT

The AT-rich interaction domain 4 A (ARID4A) has an important role in regulating Sertoli cell function and male fertility. Its molecular mechanisms, however, remain largely unknown. In this study, two single nucleotide polymorphisms (SNPs) (g.53 G > T, ss 1966531596, and g.826 G > A, rs 210809648) were identified in the promoter region of ARID4A in 215 Chinese Holstein bulls using polymerase chain reaction (PCR)-restriction fragment length polymorphism and created restriction site-PCR. Results revealed that bulls with g.53 G > T-GG and g.826 G > A-G G genotype exhibited higher sperm deformity rate than those with g.53 G > T-TT and g.826 G > A-AA genotype ($P < 0.01$). Furthermore, three haplotypes (H1 (GG), H3 (TG), H4 (TA)) and six haplotype combinations (H1H1, H1H3, H1H4, H3H3, H3H4, H4H4) were obtained. The bulls with H4H4 exhibited lower sperm deformity rate than those with H1H1 and H1H3 ($P < 0.05$). In addition, results of bioinformatics analysis revealed that ARID4A has two promoters and that two SNPs of ARID4A are located in transcription factor binding sites. Compared with g.53 G > T-G and g.826 G > A-G allele, there was a greater fluorescence intensity in g.53 G > T-T and g.826 G > A-A allele by transient transfection in MLTC-1 cells and the luciferase report assay. qRT-PCR indicated the ARID4A expression was greater in bull spermatozoa with H4H4 haplotype combination than those with H1H1 haplotype combination ($P < 0.05$). Results of the present study indicate that g.53 G > T and g.826 G > A are functional mutations that are involved in regulation of ARID4A gene expression by affecting promoter activity and thus semen quality of Chinese Holstein bulls.

1. Introduction

The selective breeding of oxen is very important for the genetic improvement of the dairy herd. Currently, there are various techniques and protocols used to evaluate conventional semen quality variables, including sperm concentration, motility, progressive motility, and deformity rate, which cannot be easily selected directly because of low heritability (Mathevon et al., 1998). The quality of these indicators also affects the conception rate, insemination index, and calving interval as well as several other reproductive variables (Büyükleblebici et al., 2014). Bull fertility, therefore, is a key factor for economic benefits in dairy farming. Bull fertility

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traits are influenced by many factors, including genetics, environment, nutrition, and feeding management. Relying solely on nutrition and feeding management, however, to improve bull semen fertility traits is difficult. Thus, with the continuous development of molecular genetic techniques, the candidate gene approach in genetics and breeding has been widely popularized in recent decades (Fontanesi et al., 2014). The candidate gene approach can provide theoretical guidance for marker-assisted selection by screening the polymorphic loci of candidate genes, analyzing the correlation of polymorphic loci with semen quality, and authenticating molecular markers related to semen quality. Furthermore, results of genome-wide association analysis suggested that single nucleotide polymorphisms (SNPs) and genomic defects are associated with bull fertility (Blaschek et al., 2011). Several research teams have identified SNPs through a genome-wide association study closely related to semen quality and found that these SNPs can affect sperm motility, density, deformity rate, and embryonic development after fertilization (Cochran et al., 2013; Fortes et al., 2013; Hering et al., 2014). Natural mechanisms, particularly genetic variations, therefore, have attracted considerable attention from scientific researchers studying molecular breeding of cattle.

The AT-rich interaction domain (ARID) is an ancient helix-turn-helix motif-based DNA-binding domain (DNA-binding properties of ARID family proteins), and ARID proteins have important roles in various biological processes, such as chromatin remodeling, gene expression, and differentiation and proliferation during cell development as well as cancer (Patsialou et al., 2005; Guo et al., 2013; Cajuso et al., 2008). In mammals, sequence relationships reveal seven distinct subfamilies of ARID-containing proteins in metazoans, namely, ARID1, ARID2, ARID3, ARID4, ARID5, JARID1, and JARID2 (Wilsker et al., 2005). The ARID1 contains two members (ARID1A and ARID1B) that are mutually exclusive subunits of the BAF complex. The specific presence of these subunits can determine whether SWI/SNF functions as a corepressor (ARID1A) or coactivator (ARID1B) of the cell cycle genes (Flores-Alcantar et al., 2011). The ARID2 is also an intrinsic component of the polybromo-associated BAF complex, SWI/SNF subcomplex, and mutations in *ARID2* associated with developmental delay and intellectual disabilities (Shang et al., 2015). The third ARID subfamily, ARID3, contains three members, ARID3A, ARID3B, and ARID3C. ARID3B has an important role in the expression of pro-apoptotic p53-target genes and apoptosis by specifically binding to putative ARID3-binding sites in p53 target genes (Pratama et al., 2015). Thus, ARID proteins have important roles in regulating biological process.

The ARID4A, also known as retinoblastoma (RB)-binding protein 1 (RBBP1 or RBP1), is a homologous member of the ARID-containing gene family, which consists of 15 ARID genes in humans, six in *Drosophila*, and two in yeast (Wilsker et al., 2004). Results from recent studies indicate that ARID4A gene regulates Sertoli cell function and male fertility as transcriptional coactivators for Androgens and androgen receptor (AR) and RB (Wu et al., 2013). The molecular mechanisms involved in the regulation of ARID4A gene expression, however, remain largely unknown. To investigate the functional markers of the ARID4A gene associated with semen quality, the SNPs that affect promoter activity were identified and analyzed. In the present study, there was a focus on the relevance of ARID4A SNPs to semen quality in Chinese Holstein bulls and the possible mechanism in regulating ARID4A gene expression. Thus, significant molecular markers associated with semen quality were identified for theoretical guidance in selective breeding.

2. Materials and methods

2.1. Animals and tissue samples

A total of 215 normal mature Chinese Holstein bulls from Beijing and the Shandong Bull Station were included in the study. Semen samples were collected from 85 bulls from the Beijing Dairy Center and 130 bulls from the Shandong OX Bio-Technology Co., Ltd. and examined. The means and standard errors of sperm quality variable data, including semen volume per ejaculate (mL), sperm motility (%), sperm concentration ($\times 10^8$/mL), post-thaw cryopreserved sperm motility (%), and sperm deformity rate (%) in the 215 Chinese Holstein bulls were detected following the assessment method described in the 2.2 subchapter. The criteria entitled Frozen Bovine Semen standard (GB/T 4143-2008, China) set the semen quality standards for frozen and fresh sperm. For example, fresh semen must be greater than or equal to 65%, fresh sperm concentration must be greater than or equal to 6×10^8, the post-thaw cryopreserved sperm motility must be 35% or greater, the sperm deformity rate must be less than 18%, and so on. Semen samples that met the standard (GB/T 4143-2008, China) were collected by professional employees and applied for SNP screening, genotyping, and association analysis.

The present study was approved by the Bureau of Animal Husbandry and Veterinary and the Dairy Cattle Frozen Semen Quality Supervision Testing Center of the Chinese Ministry of Agriculture. Considering the unavailability of testis in Chinese Holstein bulls, RNA was extracted from semen of Chinese Holstein bulls with different genotypes. The murine Leydig tumor cells (MLTC-1) used for analysis of relative luciferase activity were obtained from the Cell Culture Collection of the Chinese Academy of Sciences in Shanghai, China.

2.2. Semen assessment and cryopreservation

Semen was collected with an artificial vagina, and all ejaculates were examined individually by routine laboratory measurements (ejaculate volume, sperm density, and sperm motility) to ensure that samples present similar characteristics. The ejaculate volume was measured in a semen-collecting vial, and the number of sperm cells was counted with a hemocytometer. The sperm concentration was calculated using a sperm densitometer (Accucell; IMV Biotechnology, L'Aigle, France). The motilities of the fresh and post-thaw cryopreserved sperms were observed on a TV monitor, which was connected to a camera mounted onto a phase-contrast microscope (AndroVision, Minitube, Germany) at 200× magnification. Sperm abnormality refers to abnormal deformation and defect and generally include head, neck, and tail deformities. The percentage of sperm deformities was determined at 400× and 1000×