September 2016 Vol. 23 No. 3 31-38
Journal of Northeast Agricultural University (English Edition)

Identification of Novel Polymorphisms in Porcine *Ring Finger Protein* 4 and *Matrix Metalloproteinase* 9 Genes and Association Analysis with Litter Size Traits

Niu Bu-yue[1, 2], Lan Xiao-ming[1], Xiong Yuan-zhu[2], and Wang Xi-biao[1*]

[1] College of Animal Sciences and Technology, Northeast Agricultural University, Harbin 150030, China

[2] Key Laboratory of Swine Genetics and Breeding of Ministry of Agriculture & Key Laboratory of Agricultural Animal Genetics, Breeding, and Reproduction of Ministry of Education, Huazhong Agricultural University, Wuhan 430070, China

Abstract: Reproduction trait plays an important role in pig production. Identification of molecular markers that are linked to litter size may contribute to the genetic development of porcine reproduction traits. In this study, porcine *ring finger protein* 4 (*pRNF*4) and *Matrix metalloproteinase* 9 (*pMMP*-9) were selected as candidate genes on the basis of their physiological roles in reproduction. Two single-nucleotide polymorphisms (416C>T in *pRNF*4 and -1257G>A in *pMMP*-9) that could be detected by PCR restriction fragment length polymorphism (PCR-RFLP) were discovered and tested for statistical associations with litter size traits in three populations. For 416C>T, TT genotype was associated with a significantly higher (p<0.05) number of live births than those recorded for CC sows and the additive effect was significant (p<0.05) in Qingping and Min Pigs populations in later parities. For -1257G>A, inconsistent results were found in three populations. The results suggested that T allele in *pRNF*4 gene might confer a high prolificacy in breeding and further studies were needed to confirm the results.

Key words: pig, litter size, *pRNF*4, *pMMP*-9, polymorphism

CLC number: S813.3 **Document code:** A **Article ID:** 1006-8104(2016)-03-0031-08

Introduction

Improvement in litter size has become of great interest in pig industry as good fecundity is directly related to a sow's productive life. However, improvements using concepts of the quantitative genetics make genetic progress of litter size slow, due to its low heritability. Marker assisted selection (MAS) allows to dissect litter size in its component traits and using molecular genetic markers for the components of litter size traits promise more progress and advantages in optimum balancing of different physiological mechanisms influencing litter size (Distl, 2007). Therefore, identifying genetic variations of potential candidate genes and examining their associations with litter size are important in MAS approach.

Litter size is defined as the number of piglets born (TNB) or numbers of piglets born alive (NBA) and the main components of litter size are ovulation rate (OR, numbers of ovulated eggs) and uterus capacity. A great quantity of researches and studies corroborate that follicle-stimulating hormone (FSH) and luteinizing hormone (LH) performe a critical role in follicular

Received 7 September 2015
Supported by the National Natural Science Foundation of China (31301935); Ph. D. Programs Foundation of Ministry of Education of China (20112325120013)
Niu Bu-yue (1980-), female, Ph. D, engaged in the research of animal breeding and genetics. E-mail: niubuyue@163.com
* Corresponding author. E-mail: wxibiao@yahoo.com.cn

development, maturation and ovulation. Expression of the follicle-stimulating hormone *β* subunit (FSH*β*) or luteinizing hormone *β* subunit (LH*β*) is limiting for the synthesis of overall FSH or LH. Previous research reported that *FSHβ* gene was associated with litter size traits (Li *et al*., 1998). The expression of *LHβ* gene is regulated by many transfactors, such as steroidogenic factor-1(SF-1), SP-1 and Egr-1. The ring finger protein 4 (RNF4) is a nuclear receptor coregulator which can serve as a coactivator for steroid receptor-dependent and independent promoters. Overexpression of *RNF*4 gene can enhance the transcription of steroid receptors, including the glucocorticoid, progesterone and estrogen receptors (Moilanen *et al*., 1998; Saville *et al*., 2002). Especially, RNF4 can stimulate transcription of rat LH*β* through mediate interactions between the distal and proximal gonadotropin-releasing hormone (GnRH) response regions of LH*β* promoter (Curtin *et al*., 2004). The previous study showed that porcine RNF4 (pRNF4) was expressed highly in ovary and testis (Niu *et al*., 2009), which suggested that *pRNF*4 gene might play a role in the ovulation by regulating the expression of the porcine *LHβ* gene.

Matrix metalloproteinase 9 (MMP-9; gelatinase B) is an important member of MMPs family which participates in ovulation, implantation and embryonic development through degradation uterine stromal extracellular matrix (ECM; Lee *et al.*, 2005; Daimon and Wada, 2005; Bai *et al.*, 2005; Chen *et al.*, 2007). Gene knock-out experiments demonstrate that MMP-9-deficient mice can develop normally and are fertile (Vu *et al.*, 1998; Itoh *et al.*, 1999), but the litters of these mice are smaller and the percentage of infertile breeding pairs increases (Dubois *et al*., 2000). In swine, previous studies showed that porcine MMP-9 (pMMP-9) may play roles in reproduction as well. Porcine follicular fluid contains pMMP-9 activity that is highly increased after administration of human chorionic gonadotrophin (hCG; Driancourt *et al*., 1999). In addition, pMMP-9 is present in porcine corpora lutea at a high level during the early luteal phase, luteolysis and pregnancy (Pitzel *et al*., 2000; Ribeiro *et al*., 2006; 2007).

Hence, in this study *pRNF*4 and *pMMP*-9 were selected as candidate genes on the basis of their physiological roles in reproduction, the novel mutations in these genes were identified, and the association between litter size and genetic variants in these genes were examined in three populations with diverse genetic background.

Materials and Methods

Experimental populations

The association analysis was done in three populations with different genetic backgrounds. One population included 54 purebred Qingping sows kept at the Qingping Research Farm in Hubei Province of China. Another population included 48 purebred Min Pigs sows kept at the Min Pigs Research Farm in Heilongjiang Province of China. The third population included 210 sows from a synthetic line (Line DIV) of Landrace, Large White, Tongcheng or Meishan origin that were raised in the experimental pig station of Huazhong Agricultural University. During 2001-2006, the total numbers of piglets born (*TNB*) and the numbers of piglets born alive (*NBA*) were recorded for 223 litters in Qingping sows, 228 litters in Min Pigs sows and 611 litters in Line DIV sows. Genomic DNA was prepared from blood samples using the standard phenol/chloroform extraction procedure (Xiong, 1999).

Isolation of 3'-untranslation region of *pRNF*4 gene

In an attempt to isolate 3'-UTR of *pRNF*4, a 590 bp partial cDNA fragment of *pRNF*4 (GenBank: DQ208407) which encompassed the complete CDS and 14 bp of 3'-UTR, was compared to all the available sequences in EST database using BLAST algorithm(http://blast.ncbi.nlm.nih.gov/Blast.cgi). Two highly conserved ESTs (GenBank: BI181237; BP157988) together with the sequence of *pRNF*4