Effects of KPNA7 gene polymorphisms on reproductive traits in France Large White pigs

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ABSTRACT

KPNA7 is an essential nuclear protein for early embryogenesis and normal fertility. The aim of our study was to determine the associations between single nucleotide polymorphisms (SNPs) in the KPNA7 gene and the reproductive traits in France Large White pigs. Six new SNPs were identified by sequencing. Sows with CT genotype of rs81308652 had a significantly higher total number born than sows with CC and TT genotype in first parity. Litters from sows with TT genotype of rs81308652 had a significantly lower number of weak births than litters from sows with CC and CT genotype in multi-parity. Individuals with GG genotype of rs327848277 showed higher litter weight at birth (LWB) and number of healthy births than individuals with genotype GT and TT in first parity, and individuals with genotype GG had lower LWB compared to other genotypes in multi-parity. However, we obtained no statistically significant results for association between the SNPs in other four loci and reproductive traits in both primiparous and multiparous pigs. In conclusion, our results show that there are significant association of identified SNPs located in KPNA7 gene with pig reproductive traits, and provided a theoretical basis for genetic improvement of pig reproductive traits.

Introduction

Reproductive performance of sows is an important factor in the economics of pig production. Reproductive traits, including total number born (TNB), number of weak births (NWB), number of healthy births (NHB), litter weight at birth (LWB), number of stillborn (NSB) and number born alive (NBA) play important roles in pig breeding (Ye et al. 2018; Sato et al. 2016; Ogawa et al. 2019). Identification of polymorphisms or linked markers is necessary for understanding the genetic basis of reproductive traits and the application of marker-assisted selection in breeding programmes (Wang et al. 2018). Analysis of selected genes provides more accurate information about the genetic structure of a population or pig breeds (Mucha et al. 2013; Hamilton et al. 2000; Mikawa et al. 2011). Therefore, many studies were carried out to identify the effects of polymorphisms of some genes, e.g. leukaemia inhibitory factor (LIF) gene, epidermal growth factor (EGF) gene, estrogen receptor (ESR) gene, leptin (LEP) gene and prolactin receptor (PRLR) gene or retinol-binding protein 4 (RBP4) gene, a-Lactalbumin gene on reproductive traits (Mucha et al. 2013; Terman 2005; Miller et al. 2000). These polymorphisms can contribute to a better understanding of the genetic basis of trait differences among individuals.

Karyopherins, including KPNA1, KPNA2, KPNA3, KPNA4, KPNA5, KPNA6 and KPNA7, regulate the transport of nuclear proteins into the nucleus (Cabot and Prather 2003; Tejomurtula et al. 2009; Kelley et al. 2010). KPNA7 was first reported in cattle and was shown to be exclusively expressed in ovarian tissues, oocytes and cleavage stage embryos (Tejomurtula et al. 2009). Knockdown of KPNA7 in bovine early embryos results in a decreased proportion of embryos developing to the blastocyst stage (Tejomurtula et al. 2009). These results suggest that KPNA7 may have an important role in the transport of essential nuclear proteins required for early embryogenesis. In mice, KPNA7 is required for normal fertility and fecundity, mutation of KPNA7 gene causes reproductive reduction and sex imbalance by inducing preferential foetal lethality in females (Hu et al. 2010). In pigs, KPNA7 gene localized in chromosome 3 is expressed in germinal vesicle (GV) oocytes and cleavage stage embryos, and is required for cleavage development (Wang et al. 2012). Genome-wide association studies (GWAS) have identified QTL region on SSC3 for TNB included KPNA7 gene in Landrace and Yorkshire pigs (Guo et al. 2016). Hence, the KPNA7 gene may be a functional candidate gene for reproductive. However, there are few reports on the association of KPNA7 gene polymorphisms with reproductive traits in pigs.
French White pigs. Therefore, it is necessary to explore the individual and population differences at the molecular level. In this study, to identify potential genetic markers within the KPNA7 gene for economic traits of pigs, we screened the polymorphisms in exon, intron, 5′ regulatory region and in the 5′UTR region of KPNA7 in Large White pigs. We further analysed the correlations with production and reproductive traits (LWB, TNB, NHB and NWB). Our findings provided insights into the genetic variants of porcine KPNA7 gene and identified SNPs marker for the economic traits of pigs. GWAS have identified QTL region on SSC3 for TNB included KPNA7 gene in Yorkshire (Guo et al. 2016).

Material and methods

**Animals analysed and trait measurements**

All animal procedures were performed according to protocols approved by the Animal Care and Use Committee of Huazhong Agricultural University, Hubei province, P. R. China. All traits were measured by the national standard of swine performance testing of PR China (NY/T822-2004). A total of 384 France Large White sows were enrolled to investigate the distribution of allele and genotype frequency of SNPs. All traits were measured and recorded according to the principles and methods of swine testing (Xiong and Deng 1999). The reproductive traits included, TNB, LWB (kg), NWB, and NHB. Piglets with a LWB greater than 1 kg were recorded as a NHB, and less than 1 kg were recorded as a NWB.

**SNPs detection and genotyping**

Total genomic DNA was isolated from serum of the 384 France Large White sows. DNA collected from the sows was used to estimate the genotype frequency of KPNA7 gene. We designed 26 pairs of primers to detect the SNPs in the full length of KPNA7 DNA by sequencing. The sequencing region covered the most of the full length of KPNA7 gene. Finally, we found that only five PCR products given in Table 1 had SNP sites. The PCR reaction volume was 25.0 μl, containing 100–200 ng template DNA, 1.0 μmol of each primer, and 12.5 μl premix Taq (LA Taq Version 2.0 plus dye) (TakaRa, Japan). The PCR reaction conditions were: initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing for 30 s (annealing temperature in Table 1), and extension at 72°C for 1 min, with final extension at 72°C for 10 min. The amplification products were purified, and then placed in the ABI3730 sequencer for detecting SNPs. Then these sequences were aligned using Clustalw (http://www.ebi.ac.uk/Tools/msa/clustalw2/) to detect the SNPs. The genotyping ratio was 98%, all the SNP sites were successfully genotyped. The corresponding variation ID of SNPs in the SNP database (http://www.ncbi.nlm.nih.gov/SNP) was rs334590006, rs321312903, rs339249916, rs81308652, rs327848277 and rs336259402.

**Statistical analysis**

The association of SNP genotypes with reproductive traits in France Large White pigs was conducted with the mixed linear model procedure of SAS version 8.0 (SAS Institute, Cary, NC, 2000). The data are presented as mean ± S.D., and statistical significance level was set at p < 0.05.

The model of association analysis in 384 Large White pigs was as following:

\[ Y_{ijkt} = u + G_i + P_j + Y_k + S_t + e_{ijkt} \]

Where \( Y_{ijkt} \) is the observed values of a given trait; \( \mu \) is the overall mean; \( G_i \) is the effect of \( i \)th genotype (\( i = 1–3 \)); \( P_j \) is the effect of \( j \)th Parity (\( j = 1, 2, 3, 4 \)); \( Y_k \) is the effect of \( k \)th year; \( S_t \) is the effect of \( f \)th season; \( e_{ijkt} \) is the random error.

**Results and discussion**

To explore the single nucleotide polymorphisms in the KPNA7 gene, sequencing method was used to scanning the full length of KPNA7 gene in the French Large White pigs. Six new SNPs in KPNA7 gene were found including, rs334590006 located in exon 1, rs321312903 in exon 3, rs339249916 in intron 1, rs81308652 in intron 3, rs327848277 in the 5′ regulatory region, and rs336259402 in the 5′UTR. The haplotypes of six SNPs were inferred in France Larger White. The two SNPs rs334590006 and rs321312903 were in strong linkage disequilibrium (\( D = 0.96 \)) (Figure 1) and the haplotype frequency of GC, AT, GT and AC was 59.0%, 39.1%, 11.0% and 0.0%, respectively. The two SNPs rs334590006 and rs336259402 were in strong linkage disequilibrium (\( D = 0.91 \)) (Figure 1) and the haplotype frequency of AG, GA, GG and AA was 38.9%, 31.0%, 29.1% and 10.0%, respectively. The two SNPs rs321312903 and rs336259402 were also in strong linkage disequilibrium (\( D = 0.93 \)) (Figure 1) and the haplotype frequency of GT, AC, GC and AT was 39.3%, 31.2%, 28.6% and 0%, respectively. The results suggested that the allele G at rs334590006 of the KPNA7 was mostly linked with allele C at rs321312903 and allele A at rs336259402. While, the allele A at rs334590006 of the KPNA7 was mostly linked with allele T at rs321312903 and allele G at rs336259402. The two SNPs rs81308652 and rs336259402 were in strong linkage disequilibrium (\( D = 0.95 \)) (Figure 1) and the haplotype frequency of GC, AT, GT and AC was 45.8%, 31.4%, 22.2% and 0%, respectively. The two SNPs rs81308652 and rs327848277 were in linkage disequilibrium (\( D = 0.85 \)) (Figure 1) and the haplotype frequency of TC, GT, TT and GC was 44.0%, 32.7%, 20.9% and 2.40%, respectively. The results suggested that the allele C at rs81308652 of the KPNA7 was mostly linked with allele G at rs336259402 and the allele T at rs327848277 in the France Larger White population studied. The genotype frequency and alleles of KPNA7

| Primer | Sequence of Primer (5′–3′) | Annealing (°C) | Length (bp) |
|--------|---------------------------|----------------|-------------|
| KPNA7-F1 | TAGCTTCCCCACCCACCAC | 57°C | 585 |
| KPNA7-R1 | CCGGCTCTAGGACCGCT | 55°C | 863 |
| KPNA7-F2 | GATGCGCCGATGCCGAC | 53°C | 483 |
| KPNA7-R2 | GACTAAAGGGCCGGGCTG | 53°C | 365 |
| KPNA7-F3 | CTCCAACCGACGACGACCT | 52°C | 671 |
| KPNA7-R3 | TGGACATGCGCGGCACTAC | 52°C | 671 |
Haplotype and linkage disequilibrium analysis

The association of the genotypes in the KPNA7 with the reproductive traits was analysed in the French Large White pig population. For the rs81308652 polymorphism, only in the first parity, the TNB of sows with the CT genotype differ significantly from that of sows of the CC and TT genotype, by 1.56 and 2.07 piglets, respectively (Table 3), which indicated that heterozygous genotype promoted the TNB-related gene expression. In multiparous, no significant difference was observed in TNB between sows with different genotypes of rs81308652 polymorphisms. Sows with the CC genotype have the highest values of NWB in multiparous, and sows with the CT genotype were characterised by higher values of NWB compared to sows with the TT genotype in multiparous (P < 0.05) (Table 3). No statistically significant differences (P > 0.05) were noted when comparing sows for the genotypes of the analyzed genes in terms of LWB and NHB (Table 3). We analyzed 384 pigs and only 10 CT genotype pigs were found. These sows born the highest number of pigs but the pigs may be the weakest because their LWB is the lowest in multiparous. Therefore, the population needs to be further expanded and the results were verified in other populations. Schneider has reported to using GWAS to located QTL for TNB and LWB on SSC1, SSC2, SSC3, SSC4, SSC13, SSC14 and SSC15 (Schneider et al. 2012).

Introns play important roles in the regulation of gene expression. There are important regulatory elements in the intron of porcine MyHC (Myosin heavy chain) gene, which can regulate transcription and increase gene expression (Chang et al. 2000). Previous study showed that intron mutation caused the change of gene expression. For example, IGF2 is a major candidate gene for meat quality traits (Clark et al. 2014), and its intron 2, 3 and 8 are related to gene expression regulation (Jungerius et al. 2004). The rs81308652 polymorphism is localized in intron 3, which may be an important regulatory region binding transcription factors and regulate the expression of TNB-related genes.

The polymorphism of rs327848277 was significantly associated with LWB and NHB in primiparous sows, and significantly correlated with NWB in multiparous sows. In the first parities, the LWB and NHB of sows with the GG genotype were significantly higher than that of TT and GT genotype (P < 0.05) (Table 3). And the homozygote of polymorphism rs327848277 was more favourable for LWB and NHB-related gene expression. And in multiparous, NWB of sows with the GG genotype was significantly lower than that of TT and GT genotype (P < 0.05) (Table 3). The results suggested that allele G of polymorphism rs327848277 was favourable for the number of healthy biglets. The results also showed NWB of sows with the homozygous genotype was significantly lower than that of heterozygous genotype. Our results suggested that the homozygous genotype inhibited the expression of NWB-related genes. SNPs in 5' the regulatory region play an important role in regulation of gene expression. Epigenetic regulation regulates the expression of KPNA7 (Wang et al. 2019). In this study, the mechanism of SNP regulating KPNA7 gene expression may be that SNP affects DNA methylation. The SNPs in the 5' regulatory region can be linked to some regulatory elements bound (Sun et al. 2018). The mutations located in the 5' regulatory region affect the binding capacity of transcription factor 7-like 2 (TCF7L2) and regulated the

Figure 1. Haplotype and linkage disequilibrium analysis

gene were presented in Table 2. All of the three genotypes were detected for the six candidate SNPs. As the result of genotyping showed that, in the rs81308652 (C/T), CT genotype was the least frequent (0.03). Compared to the frequency of C allele, the frequency of T allele (0.54) was higher. For the rs321312903 (C/T), TT genotype was least frequency (0.17). The frequency of C allele is higher than that of T allele, suggesting C was the dominant allele in the France Large White pigs. And the allele T at rs327848277 was the dominant allele compared to the G allele. For both of the rs334590006 and rs336259402, we found higher frequency of GG genotype compared to the frequency of AA genotype (Table 2). We found that except for rs321312903, the other five novel SNPs were not in Hardy–Weinberg equilibrium in the French Large White pig population. Deviation from these proportions can be caused by many factors, including purifying selection, copy number variation, inbreeding or population substructure (Chen et al. 2017; Graffelman et al. 2017). Hardy–Weinberg equilibrium was also affected by the presence of full sibs and sample size (Sánchez-Montes et al. 2017). In our study, the sample size is limited, the population needs to be further expanded and the results need to be further verified in other populations.

Table 2. Genotype and allele frequencies of KPNA7 gene in pig populations

| SNPs            | Genotype frequency | Allele frequency |
|-----------------|--------------------|------------------|
| rs81308652      | CC 166 (0.44)      | C 0.455          |
|                 | CT 10 (0.03)       | T 0.545          |
|                 | TT 209 (0.53)      |                  |
| rs321312903     | CC 142 (0.37)      | C 0.60           |
|                 | CT 177 (0.46)      | G 0.40           |
|                 | TT 65 (0.17)       | T 0.60           |
| rs327848277     | GG 94 (0.25)       | G 0.355          |
|                 | GT 81 (0.21)       | T 0.645          |
|                 | TT 209 (0.54)      |                  |
| rs334590006     | AA 67 (0.17)       | A 0.36           |
|                 | AG 144 (0.38)      | G 0.64           |
|                 | GG 173 (0.45)      |                  |
| rs336259402     | AA 54 (0.14)       | A 0.32           |
|                 | AG 140 (0.36)      | G 0.68           |
|                 | GG 190 (0.50)      |                  |
| rs339249916     | AA 128 (0.34)      | A 0.515          |
|                 | AT 134 (0.35)      | T 0.485          |
|                 | TT 118 (0.31)      |                  |
transcription activities of promoter fragments of PPARD gene (Zhang et al. 2015). The rs327848277 polymorphism is localized in 5′ regulatory region sequence, which can be an important regulatory region binding transcription factors and affecting KPN7 gene expression.

The rs321312903, rs334590006, rs336259402, rs339249916 polymorphisms were not significantly associated with the reproductive traits in both primiparous and multiparous pigs. In conclusion, KPN7 genes can be used as candidate genes for the reproductive performance of the French Large White pigs. Further work will be necessary to confirm the effects of these functional SNPs within porcine KPN7 gene in more pig populations or different pig breeds.

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Disclosure statement
No potential conflict of interest was reported by the author(s).

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