Polymorphism of the pig pre-implantation protein 3 (*prei3*) gene and its association with litter size traits

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Abstract

The pre-implantation protein 3 (*prei3*), which might play a role in pre-implantation embryogenesis, is one of the promising candidate genes for litter size traits in pigs. In this study, a single nucleotide polymorphism (SNP: T802G) in intron 6 of the pig *prei3* gene was detected and a genotyping assay for this SNP was developed. An association study for this SNP with litter size was performed in two independent populations. One population consisted of crossbred sows derived from Landrace, Large White, Chinese Tongcheng and/or Chinese Meishan (Line DIV). The other population constituted of crossbred animals derived from Chinese Qingping and Duroc (QD). Statistical analysis demonstrated that, in first parity, 2.65 more piglets were born and 3.82 more piglets were born alive in sows in Line DIV with genotype TT than with genotype GG. For second and subsequent litters, in both the DIV and QD lines there were significant differences in the number of piglets born alive between TG and GG sows, with the TG sows producing more piglets born alive than the GG sows. These results suggest that the *prei3* SNP is significantly associated with litter size in the two populations studied, and could be useful in selection for increasing litter size in pigs. Further investigations on more pig populations with large sample sizes are needed to confirm this.

Keywords: Polymorphism, *prei3*, candidate gene approach, reproductive traits, swine

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Introduction

Since litter size is extremely important to pig production, it would be advantageous for pig producers to be able to select replacement gilts that have the potential to have larger litters than their peers (Campbell *et al*., 2003). However, improvement in litter size traits in pigs through selective breeding has proven to be difficult due to the low heritability for litter size. The candidate gene approach, employed in identifying the polymorphisms in genes likely to cause variation in a trait based on physiological and biochemical evidence could accelerate the improvement of porcine reproductive traits. With the development of candidate gene and comparative mapping approaches, many major genes affecting several traits in pigs have been successfully identified and associations between reproductive performance and polymorphisms at several candidate gene loci have been reported (Kirkpatrick, 2002).

Prenatal survival represents one of the important limits to litter size in pig. In most animals early embryonic development is dependent on the contribution of both maternal and embryonic genomes (Paynton *et al*., 1988). The maternal mRNAs are degraded at the two-cell stage and are replaced by zygotic transcripts following zygotic gene activation (ZGA) such that their amounts increase by the eight-cell and/or blastocyst stages. Studies in mice revealed that the *prei3* gene evinced relatively high levels of expression through the one-cell stage followed by a sharp decrease at the two-cell stage, and a subsequent rise at the eight-cell and/or blastocyst stages (Temeles *et al*., 1994). The expression of *prei3* during oocyte maturation and pre-implantation following ZGA suggests that this gene might play a role in pre-implantation embryogenesis. QTL studies identified several significant QTLs affecting ovulation rate and number of *corpora lutea* within a region on porcine chromosome 15 (Rathje *et al*., 1997; Rohrer *et al*., 1999; Wilkie *et al*., 1999). Comparative mapping data showed that porcine chromosome 15 is orthologous to human chromosome 2 where the human *prei3* gene has been located (http://www.toulouse.inra.fr/lgc/pig/compare /SSCHTML/SSC3S.HTM). Hence, the *prei3* gene might be a positional and functional candidate gene for

reproduction traits. The objective of the present study was to examine *prei3* as a candidate gene for litter size. To achieve this target, we identified mutations in the *prei3* gene sequence, estimated the allele and genotype frequencies of *prei3* gene polymorphism of pigs in different pig breeds, and examined the association between the polymorphism and the reproductive performance of sows in two independent populations.

Materials and Methods

Two hundred and eighty-two individuals were randomly selected from seven herds as an experimental sample to genotype the *prei3* polymorphism, and include 52 Chinese Meishan pigs, 24 Tongcheng pigs, 30 Erhualian pigs, 7 Hezuo pigs, 21 Huainan pigs, 57 Bamei pigs and 91 Large White pigs. Two populations with different genetic backgrounds were studied to evaluate associations between *prei3* polymorphism and litter size traits. One population (Line DIV) included 146 sows belonging to a synthetic line of Landrace, Large White, Tongcheng and/or Meishan origin. These animals were reared on the experimental pig station of the Huazhong Agricultural University. During consecutive years (2003 - 2005), both the number of piglets born and the number of piglets born alive were recorded in 253 litters of sows that farrowed. The other population included 73 Qingping × Duroc (QD) sows kept at the Qingping research farm in the Hubei Province of China, and collection of phenotypic data from these sows was similar to that done on Line DIV. Genomic DNA was extracted from blood, using methods described by Xiong *et al*. (1999).

Total RNA was isolated from kidney samples, previously frozen in liquid nitrogen immediately after slaughter, using a Trizol Reagent (Invitrogen, Carlsbad, CA, USA). Three micrograms of RNA were reverse transcribed with oligo-dT primer and M-MLV reverse transcriptase (Promega, Madison, Wisconsin, USA). Several primer pairs (Table 1) were designed on porcine ESTs sequences and the exon/intron organization of the human *prei3* gene (GenBank: NT_086634) (http://www.ncbi.nlm.nih.gov/IEB/Research/Ostell/Spidey) and used to amplify from cDNA or total genomic DNA. PCR amplification (25 μL final volume) was performed using 1 μL kidney cDNA or genomic DNA, 0.2 mM dNTPs, 1.5 mM $MgCl_2$, 0.5 μM of each PCR primer, 1 U Taq DNA polymerase and reaction buffer (Promega, Madison, Wisconsin, USA). The cycling conditions included an initial incubation at 94 °C for 4 min followed by 33 cycles comprising 45 sec at 94 °C, 40 sec at 50 °C, 90 °C sec at 72 °C and a final extension at 72 °C for 8 min. The purified PCR products were cloned into the pMD18-T vector (TaKaRa, Tokyo, Japan) and sequenced commercially (Sangon, Shanghai, China).

Table 1 Primer pairs used in the analysis of the porcine *prei3* gene

Primers	Primer sequence (5' - 3')	Size (bp)	Primer location
Pair 1	F: 5'-ATCCAACAGAACATAAGAGC-3'	697	Exon 4
	R: 5'-AAAGGCAACACTCAGCAT-3'		Exon 8
Pair 2	F: 5'-CTT GAGCTA AATGGACTTG-3'	302	Exon 4
	R: 5'-CTCTTTTGGAGTTTTGTGA-3'		Exon 5
Pair 3	F: 5'-GTGCTGCATGTCTTCTGA-3'	1297	Exon 6
	R: 5'-CTACGGATGATTCCTTTAT-3'		Exon 7
Pair 4	F: 5'-AATCATCCGTAGCAA AAC-3'	499	Exon 7
	R: 5'-AAAGGCAACACTCAGCAT-3'		Exon 8
SNP	F: 5'-GTTTTAGTAAGTTTTGATTGGTCCG-3'	294	Intron 6
	R: 5'-AAACCCCTGTTCCTCATTCTTG-3'		Intron 6
G3PDH	F: 5'-ACCACAGTCCATGCCATCAC-3'	480	
	R: 5'-TCCACCACCCTGTTGCTGTA-3'		

Following amplification of genomic DNA with primer pairs 2, 3 and 4 (Table 1), partial sequences of porcine *prei3* gene from 3 Meishan, 2 Large White and 2 Duroc pigs were sequenced and compared for variation. To detect the SNP T802G in intron 6 of *prei3* gene, a primer mutagenesis assay was established. PCR was performed to amplify a 294-bp fragment using primer SNP-R as reverse primer and the forward mutated primer SNP-F adjacent to the mutation site, exchanging the 3'-end based nucleotides AC with CC, creating an *MspI* recognition site (CCGG). PCR amplification (20 μL final volume) was performed using 50 ng genomic DNA, 100 μM dNTP, 0.3 μM of each primer, 1.5 μM $MgCl_2$ and 0.5 U Taq DNA polymerase with the reaction buffer supplied by the manufacturer. The thermal cycling profile was: 94 °C for 4 min; 33 cycles at 94 °C for 30 s, 53 °C for 30 s and 72 °C for 1 min. For the PCR-RFLP assays, 8.4 μL of the PCR product was digested with 6 units *MspI* (TaKaRa, Tokyo, Japan) for 3 h at 37 °C, separated by an 8% polyacrylamide gel electrophoresis in 1 × TBE buffer and stained with silver.

The relationship between *prei3* gene genotypes and reproductive traits was evaluated with the general linear model (GLM) procedure of SAS (1999). The phenotypic data for TNB and NBA were analyzed in fixed effect models that included the effects of parity, month, year, boar, line and the phenotype for the SNP of the *prei3* gene. In addition, the data were analyzed separately for first and later parities. Both additive and dominance effects were estimated using the REG procedure of SAS (1999), where the additive effect was estimated as -1, 0 and 1 for TT, TG and GG, respectively, and the dominance effect represented as 1, -1 and 1 for TT, TG and GG, respectively (Liu., 1998).

Total RNA from 12 tissues (heart, liver, spleen, lung, kidney, small intestine, stomach, muscle, fat, uterus, ovary and testis) of adult Large White pigs was extracted and reverse transcription reactions were performed as described above. The expression pattern of *prei3* was analyzed by RT-PCR amplification using the gene-specific primer pair 1 (Table 1). The glyceraldehyde-3-phosphate dehydrogenase (G3PDH) (Table 1) as internal control was employed. The PCR profile of *prei3* and G3PDH amplification was: initial denaturation at 94 °C for 4 min, 28 cycles at 94 °C for 45 s, 50 °C for 40 s, 72 °C for 45 s, and a final extension of 72 °C for 8 min.

Results and Discussion

Porcine ESTs (GenBank: AJ655305, CB479769, BX924510) homologous to the *prei3* gene were identified with BLAST searches using the human cDNA (GenBank: NM_015387) sequences, and were used to design primers. Primer pair 1 yielded a consensus sequence of 697bp spanning from putative exon 4 to 8 (GenBank: AY884042) by RT-PCR. RT-PCR analysis of 12 tissues of adult pigs indicated that the porcine *prei3* gene was widely expressed in all tissues examined, except the small intestine (Figure 1).

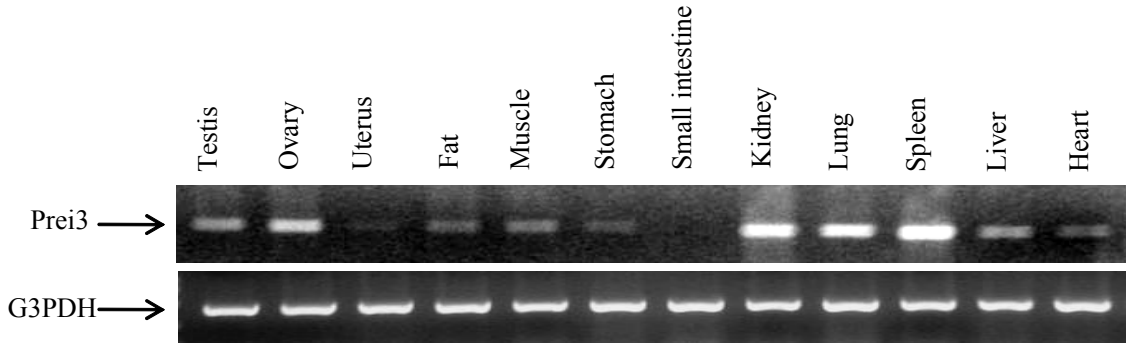

Figure 1 Expression of porcine *prei3* in different tissues

The 302-bp, 1297-bp and 499-bp genomic fragments, encompass complete intron 4 (GenBank: AY887127), 6 (GenBank: DQ191745) and 7 (GenBank: DQ180739), were amplified from total porcine genomic DNA using primer pairs 2, 3 and 4, respectively. A SNP (T802G) in intron 6 was discovered by comparative sequencing of animals from Meishan, Large White and Duroc breeds and a PCR-RFLP assay for this SNP was developed. For the PCR-RFLP assay, the 294-bp PCR product with primer SNP was

digested into two fragments of 183-bp and 111-bp. The 183-bp fragments (T allele) were alternatively split into a 24-bp and a 159-bp fragment (G allele) due to an additional *MspI* restriction site (Figure 2).

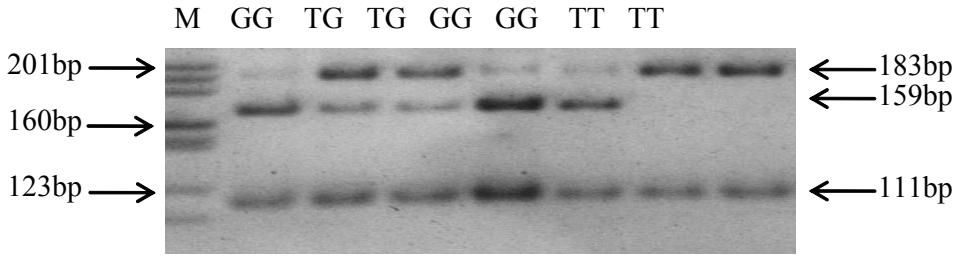

Figure 2 The three different PCR-RFLP (*Msp*I) genotypes of porcine *prei3*. The genotypes (TT, TG, GG) are shown at the top. M: *pBR*322 DNA/*Msp*I marker

The genotype and allele frequencies of the polymorphism obtained from the seven pig breeds are shown in Table 2. This polymorphism was segregated into Large White, Bamei, Huainan and Tongcheng pigs. The Chi square (χ^2) test showed there were highly significant ($P < 0.01$) differences in genotype frequencies between indigenous Chinese breeds and the Large White breed (Table 3).

Table 2 Genotype and allele frequencies of SNP : T802G in different pig breeds

Breeds	N	Genotype			Allele frequency	
		TT	TG	GG	T	G
Meishan	52	0.00 (0)	0.00 (0)	1.00 (52)	0.00	1.00
Erhualian	30	0.00 (0)	0.00 (0)	1.00 (30)	0.00	1.00
Hezuo	7	0.00 (0)	0.00 (0)	1.00 (7)	0.00	1.00
Tongcheng	24	0.00 (0)	0.08 (2)	0.92 (22)	0.04	0.96
Huainana	21	0.05 (1)	0.38 (8)	0.57 (12)	0.24	0.76
Bamei	57	0.05 (3)	0.46 (26)	0.49 (28)	0.28	0.72
Large White	91	0.91 (83)	0.08 (7)	0.01 (1)	0.95	0.05
Total	282					

N - number of pigs

In order to investigate the possible functional or positional role of this mutation in the pig *prei3* gene, association studies were performed in the two different populations (Line DIV, QD). For the first parity, sows in Line DIV with TT genotype had outperformed GG sows by 2.65 piglets born and 3.82 piglets born alive ($P < 0.05$). However, the difference between *prei3* genotypes and reproductive traits in the first parity of QD sows was not significant (data not given). In the second and subsequent litters, TG animals in Line DIV population had more piglets born alive (+1.2 piglets) than sows with the GG genotype ($P < 0.05$). Meanwhile, as indicated in Table 3, QD sows with the TG genotype had an additional 0.72 piglets born alive compared to the GG animals ($P < 0.05$) in later parities. The TG animals seemed to have more NBA in later parities. Both additive and dominance effects of the genotypes are also shown in Table 3. For TNB in the first parity, an additive effect of -1.26 ± 0.53 piglets/litter ($P < 0.05$) was detected in Line DIV population. In

total, there was a trend for animals carrying the T allele to increase litter size in both Lines DIV and QD animals and the differences in litter size between the genotypes can be explained by an advantageous effect of a T allele over a G allele. Thus, increasing the frequency of the favourable allele T could be beneficial in Line DIV and QD animals to accelerate the genetic improvement of reproductive traits.

Moreover, in this study we also found that TT is the preferred genotype but the prolific breeds are all GG. This can be assumed that the effects of the genetic background influenced the results and further investigations in more pig populations are required to confirm the present results.

This polymorphism is located in a non-coding region (intron) of the porcine *prei3* gene and this variation does not directly alter any amino acid residue, and may consequently not involve a substantial change on the biochemical properties of *prei3*. Thus, the probable hypothesis to explain the association results obtained is that this polymorphism indirectly affects litter size traits by being in linkage disequilibrium with QTL or another causative polymorphism affecting reproduction traits.

Table 3 Association between *prei3* genotypes and reproductive traits (piglet)

Traits	*Prei3* Genotype (μ ± s.e.)			Effect (μ ± s.e.)	
	TT	TG	GG	Additive	Dominance
Prei3 effects for second and subsequent litter in QD pigs					
N	24	50	23		
TNB	11.75 ± 0.59	11.66 ± 0.40	10.38 ± 0.62	-0.56 ± 0.43	-0.25 ± 0.29
NBA	10.63^{ab} ± 0.56	11.11^{a} ± 0.39	10.39^{b} ± 0.62	-0.45 ± 0.41	-0.48 ± 0.28
Prei3 effects for first litter in Line DIV pigs					
N	8	27	43		
TNB	11.36^{a} ± 0.96	10.14^{a} ± 0.53	8.71^{b} ± 0.43	-1.26 ± 0.53 *	-0.02 ± 0.37
NBA	10.61^{a} ± 1.43	8.28^{ab} ± 0.81	6.79^{b} ± 0.63	-1.33 ± 0.80	0.12 ± 0.56
Prei3 effects for second and subsequent litter in Line DIV pigs					
N	23	67	85		
TNB	11.84 ± 0.64	11.43 ± 0.39	10.88 ± 0.34	-0.47 ± 0.36	0.03 ± 0.26
NBA	10.55^{ab} ± 0.68	10.74^{a} ± 0.41	9.54^{b} ± 0.36	-0.50 ± 0.39	-0.35 ± 0.28

Least square means of genotypes within the same row with different superscripts are significantly different ($P < 0.05$)
* $P < 0.05$; N - number of litters investigated
TNB - number of piglets born; NBA - number of piglets born alive

Conclusion

A T/G substitution in the sequence of porcine *prei3* gene was detected and three genotypes of *prei3* gene were identified in two independent populations (Lines DIV and QD). Association analyses showed that litter size (TNB, NBA) was greater ($P < 0.05$) in the first parity of sows carrying the TT genotype compared with those of GG genotype. In the later parities, significant differences in NBA ($P < 0.05$) were found also between the TG and GG sows in both Line DIV and QD populations.

This study revealed a significant relationship between *MspI* PCR-RFLP genotype and TNB and/or NBA, indicating that the SNP in *prei3* gene is a potential molecular marker for litter size traits. However, before the polymorphism can be used for marker-assisted selection in pig genetic improvement, further evaluation and confirmation studies in more pig populations with large sample sizes are necessary.

Acknowledgements

This work was supported financially by the Key Project of the Chinese Ministry of Education (104132), Innovation Fund of Huazhong Agricultural University, the National High Technology Development Project (No. 2002AA211041) and the National "973" Project of P.R. China (No. G2000016105).

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