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# Association of porcine *STIM1* gene with litter size traits*

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**In commercial pig production, litter size has always been one of the most important production traits. Stromal interaction molecule 1 (STIM1) is a transmembrane-spanning $Ca^{2+}$-binding protein, which is essential for oocyte maturation and fertilization, as well as proper embryo development. The objectives of this study were to identify mutations in the porcine *STIM1* gene and investigate the association between genetic variations and litter size traits in pigs. A novel 1bp - deletion/insertion in intron 10 (g.199893-delT) and a single nucleotide polymorphism (SNP) in exon 10 (g.1969037T>C) were found by sequencing. Association results showed that these two mutations significantly affected litter size traits in both the DIV line and Large White pigs (P<0.05). In all parities sows with the *TT* genotype of g.1969037T>C had significantly more piglets than those of the *CC* genotype (P<0.01), and the number of piglets decreased in the following order of genotypes TT>CT>CC. The sows with the *NN* genotype of g.199893-delT had significantly more piglets than those of the *MM* genotype (P<0.01), and the number of piglets decreased in the following order of genotypes NN>MN>MM. Here we provide evidence that the mutations in the porcine STIM1 gene affected litter size of sows, and that this gene might be a potential important candidate gene related to litter size in pigs.**

**KEY WORDS: pig / litter size / *STIM1* gene / association analysis**

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Stromal interaction molecule 1 (STIM1) is a type 1a single-pass transmembrane protein containing several conserved domains, including a sterile-alpha motif (SAM), a coiled coil region and an EF-hand domain [Manji *et al*. 2000, Williams *et al*. 2002]. Because of the EF-hand and the presence of a single transmembrane domain, STIM1 was suggested to be an ideal candidate as the $Ca^{2+}$ store-sensing component for the $Ca^{2+}$ release-activated $Ca^{2+}$ entry pathway. Additional research confirmed that STIM1 was localized on the endoplasmic reticulum with the EF-hand as the $Ca^{2+}$-sensing domain oriented in the store's lumen. Store depletion triggers a rapid redistribution of STIM1 near the plasma membrane, where it activates CRAC channels and triggers the influx of $Ca^{2+}$ [Liou *et al*. 2005, Zhang *et al*. 2005].

For the appropriate maturation and fertilization of mammalian oocytes, the influx of extracellular calcium through plasma membrane $Ca^{2+}$ channels is required [Koh *et al*. 2009, Gómez-Fernández *et al*. 2009]. STIM1 is expressed in porcine oocytes and plays an important role during oocyte maturation, fertilization and embryonic development by mediating calcium signal transduction. Research showed that STIM1 is essential for $Ca^{2+}$ entry after artificial depletion of the intracellular stores in oocytes [Koh *et al*. 2009]. Mammalian oocytes reach their full capacity to generate $Ca^{2+}$ oscillation during the final stages of maturation [Ajduk *et al*. 2008], and STIM1 expression is important during this process. STIM1 is also required for maintaining the long-lasting $Ca^{2+}$ oscillation at fertilization, and essential for normal embryonic development by mediating store-operated $Ca^{2+}$ entry [Lee *et al*. 2012].

The objectives of the present study were to identify mutations of the porcine *STIM1* gene and analyze their effects on litter size traits in sows.

## Material and methods

### Samples and DNA extraction

A total of 305 blood samples of sows were collected in this study, including 24 Meishan sows from the Jiangsu province, 174 Large White sows raised on the farm of the Hubei Institute of Animal Husbandry and Veterinary, and 107 sows of the DIV line (the 4$^{th}$ Dam line of Chinese lean-type new lines) from the farm owned by the Huazhong Agricultural Universtiy. During the consecutive years (2005-2010), total numbers of born piglets (TNB) and numbers of piglets born alive (NBA) were recorded in 726 litters of Large White and DIV line pigs.

The genomic DNA was extracted from peripheral leucocytes by the standard procedure [Sambrook *et al*. 1989], and the concentrations of DNA samples were measured by both spectrophotometry (at 260 nm and 280 nm absorbance) and electrophoresis (in 1% agarose gel that was stained with ethidium bromide for visualization under UV light). All samples were stored in the refrigerator at -20°C.

**Identification of porcine *STIM1* polymorphisms**

Two fragments were amplified by PCR (polymerase chain reaction), and the PCR primers showed in Table 1 were designed using Primer Premier 5.0 based on the Sus scrofa *STIM1* gene sequence (GenBank acc. No. NC_010451.3). The amplification region of *STIM1* $P_1$ primers covered exon 10, and the amplification region of *STIM1* $P_2$ primers included a partial intron10 and exon 11 of the *STIM1* gene. PCR products were recycled and purified using the DNA recycling and purification kit provided by the Sangon Biological Engineering Technology Company (Shanghai, China).

**Table 1.** Primers designed for porcine *STIM1* gene

| Primer Name | Primer Sequences (5'-3') | Tm (°C) | Product size (bp) |
|---|---|---|---|
| *STIM1* $P_{1}$-L | CCGTGCCGTCATCCTCTT | 60 | 387 |
| *STIM1* $P_{1}$-R | TCGGGCTTTCGCAACCAG | | |
| *STIM1* $P_{2}$-L | GCCTTGCCATCCCCTTGA | 60 | 691 |
| *STIM1* $P_{2}$-R | AGAGACCGCCGATACCCC | | |

Purified PCR products were sequenced for gene mutations according to the methods described by Lee *et al*. [1992] and Yuan *et al.* [2007a]. A pooled DNA sample from 15 different pigs, which were selected randomly from each breed of Meishan, Large White and DIV line populations (5 per each population), was used in sequencing in an ABI 377 automated sequencer (GMI, Inc., Ramsey, MN, USA) to identify the mutation. The sequencer software (dnastar v5.0) was used to assemble the sequences and identify polymorphisms.

**Genotyping**

All samples (305 sows) were genotyped by direct sequencing of the purified PCR products, which were amplified by primers *STIM1* $P_1$ or *STIM1* $P_2$ (Tab. 1).

**Data Analysis**

Associations with gene variants and litter size traits were tested using the general linear model (GLM) procedure (SAS Inst. Inc., Cary, NC). The following unitrait linear model was employed:

$$y_{ijk} = \mu + P_i + S_j + G_k + e_{ijk},$$

where:

$y_{ijk}$ – observation of the trait;

$\mu$ – overall mean;

$P_i$ – effect of i-th parity (i=1,2,3,4,5,6; parity ≥7);

$S_j$ – effect of j-th season;

$G_k$ – the effect of k-th genotype (k=13);

$e_{ijk}$ – random residual.

Significance was established at $P<0.05$, and a highly significant difference was considered at $P<0.01$. Fixed effects were only fitted if they made a significant contribution to the overall variance component ($P<0.05$). The sire effect was not significant in the experimental populations.

## Results and discussion

Two mutations of the porcine *STIM1* gene, a single nucleotide polymorphism (SNP) in exon 10 (g.1969037T>C, rs81411857) and a 1bp-deletion/insertion in intron10 (g.199893-delT), were found by sequencing. Three genotypes at g.1969037T>C were identified after genotyping in each population (Fig. 1). Moreover, three genotypes at g.199893-delT were also detected after sequencing (Fig. 2).

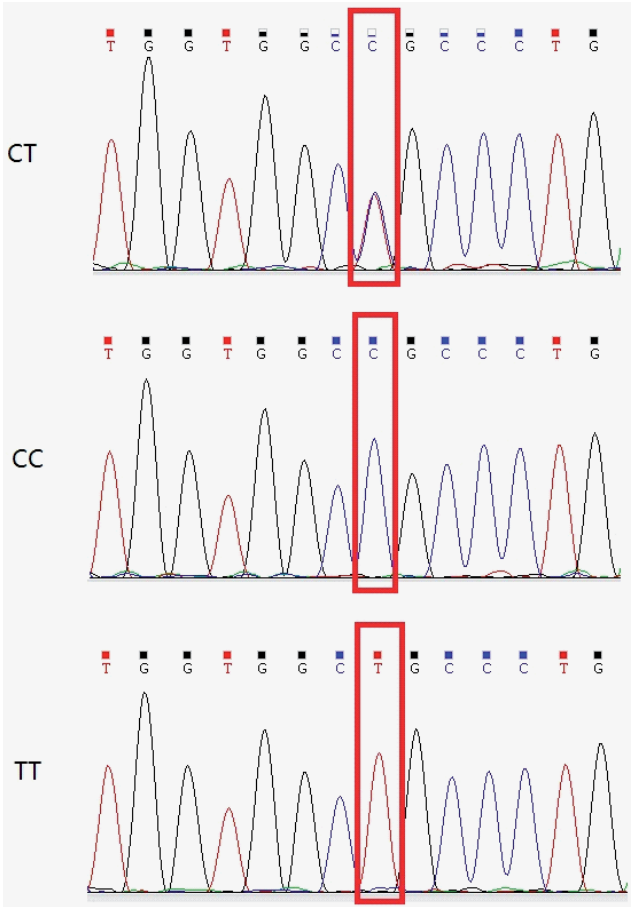


Fig. 1. Sequencing results of three genotypes of g.1969037T>C at exon 10 of the *STIM1* gene.

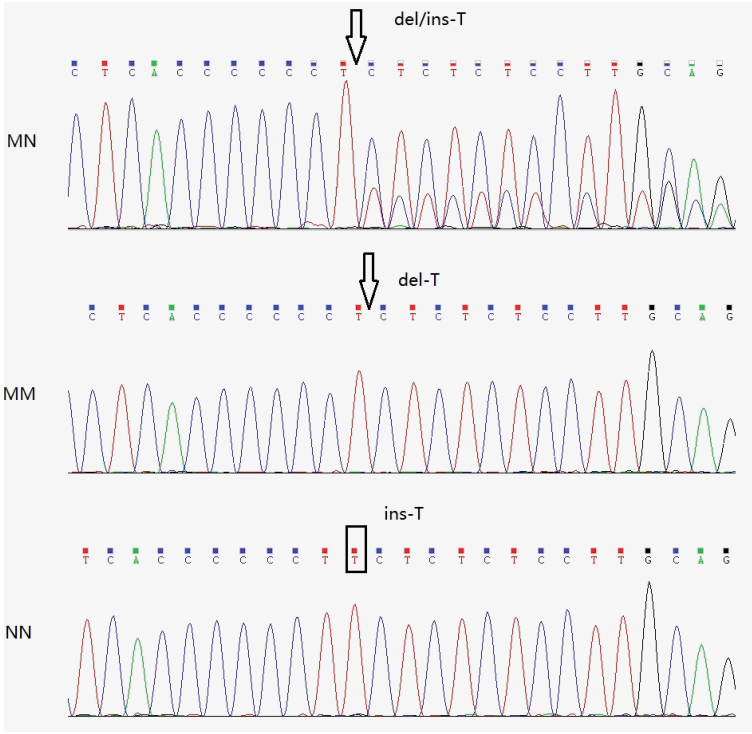


Fig. 2. Sequencing results of three genotypes of g.199893-delT at intron 10 of the STIM1 gene.

The allele and genotype frequencies of the two mutations are shown in Tables 2 and 3. For SNP g.1969037T>C, the frequency of allele *T* was much higher than that of allele *C* in the Meishan pigs, while the frequency of allele *T* was much lower than that of allele *C* in the DIV line and Large White pigs. For the other mutation g.199893-delT locus, the frequency of allele *N* was higher than that of *M* in the Meishan pigs, but the frequency of allele *M* was much higher than that of *N* in the DIV line and particularly in the Large White pigs.

**Table 2.** Allele and genotype frequencies of g.1969037T>C in the three populations

| Breed | Genotype frequency | | | Allele frequency | |
|---|---|---|---|---|---|
| | CC | CT | TT | C | T |
| Meishan(n=24) | 0.083(n=2) | 0.375(n=9) | 0.542(n=13) | 0.271 | 0.729 |
| DIV line(n=107) | 0.505(n=54) | 0.318(n=34) | 0.178(n=19) | 0.664 | 0.336 |
| Large White(n=174) | 0.701(n=122) | 0.178(n=31) | 0.121(n=21) | 0.790 | 0.210 |

**Table 3.** Allele and genotype frequencies of g.199893-delT in the three populations

| Breed | Genotype frequency | | | Allele frequency | |
|---|---|---|---|---|---|
| | MM | MN | NN | M | N |
| Meishan(n=24) | 0.125(n=3) | 0.625(n=15) | 0.250(n=6) | 0.437 | 0.563 |
| DIV line(n=107) | 0.542(n=58) | 0.112 (n=12) | 0.346(n=37) | 0.598 | 0.402 |
| Large White(n=174) | 0.759(n=132) | 0.103(n=18) | 0.138(n=24) | 0.810 | 0.190 |

The association analysis was performed in the DIV line and Large White pigs. Statistical analysis demonstrated that g.1969037T>C was significantly associated with TNB and NBA in the DIV line pigs for both the first parity and all parities (P<0.05) (Tab. 4). The sows with the *TT* genotype had significantly more piglets of both TNB and NBA in the first parity than those of the *CC* genotype (P<0.05). Furthermore, the

**Table 4.** Association analysis between genotypes of g.1969037T>C and litter size traits in DIV line and Large White

| Breed | Genotype | First parity | | | | | All parities | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | TNB | | NBA | | n | TNB | | NBA | |
| | | | mean | SD | mean | SD | | mean | SD | mean | SD |
| DIV line | CC | 27 | $10.15^{b}$ | 0.43 | $8.26^{b}$ | 0.46 | 207 | $10.56^{B}$ | 0.20 | $9.63^{B}$ | 0.22 |
| | CT | 19 | $11.79^{a}$ | 0.59 | $10.21^{a}$ | 0.60 | 144 | $11.26^{Ab}$ | 0.17 | $10.65^{Ab}$ | 0.17 |
| | TT | 8 | $12.00^{a}$ | 0.34 | $11.00^{a}$ | 0.38 | 86 | $12.33^{Aa}$ | 0.22 | $11.52^{Aa}$ | 0.24 |
| Large White | CC | 57 | 10.11 | 0.35 | $9.07^{b}$ | 0.38 | 205 | $10.00^{B}$ | 0.18 | $8.84^{C}$ | 0.20 |
| | CT | 14 | 11.29 | 0.68 | $10.07^{ab}$ | 0.62 | 53 | $11.60^{A}$ | 0.31 | $10.66^{B}$ | 0.31 |
| | TT | 11 | 11.82 | 0.38 | $11.18^{a}$ | 0.50 | 31 | $12.90^{A}$ | 0.28 | $12.45^{A}$ | 0.32 |

TNB – total number of born piglets; NBA – number of piglets born alive, n – number of individuals.
$^{aA...}$Within columns means bearing different superscripts differ significantly at: small letters P<0.05; capitals – P<0.01.

**Table 5.** Association analysis between genotypes of g.199893-delT and litter size traits in DIV line and Large White pigs

| Breed | Genotype | First parity | | | | | All parities | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | n | TNB | | NBA | | n | TNB | | NBA | |
| | | | mean | SD | mean | SD | | mean | SD | mean | SD |
| DIV line | MM | 23 | 10.52 | 0.54 | 9.39 | 0.51 | 284 | $10.87^{B}$ | 0.16 | $10.20^{b}$ | 0.17 |
| | MN | 8 | 11.25 | 0.92 | 8.25 | 0.82 | 36 | $11.22^{AB}$ | 0.36 | $9.78^{b}$ | 0.38 |
| | NN | 23 | 11.39 | 0.43 | 9.70 | 0.57 | 117 | $11.76^{A}$ | 0.23 | $10.84^{a}$ | 0.24 |
| Large White | MM | 65 | 10.40 | 0.31 | 9.35 | 0.34 | 215 | $10.31^{B}$ | 0.18 | $9.18^{B}$ | 0.19 |
| | MN | 7 | 10.43 | 0.65 | 9.57 | 0.89 | 31 | $11.26^{AB}$ | 0.33 | $10.39^{AB}$ | 0.37 |
| | NN | 10 | 11.50 | 1.01 | 10.60 | 0.79 | 43 | $11.60^{A}$ | 0.43 | $10.88^{A}$ | 0.42 |

TNB – total number of born piglets; NBA – number of piglets born alive, n – number of individuals.
$^{aA...}$Within columns means bearing different superscripts differ significantly at: small letters P<0.05; capitals – P<0.01.

TNB and NBA in all parities of the *TT* genotype sows were significantly greater than those of *CT* ($P<0.05$) and *CC* ($P<0.01$) ones. Similar results were found in the Large White pigs. The first parity NBA and all parities TNB and NBA of the *TT* genotype sows were significantly higher than those of the *CC* genotype sows ($P<0.05$ or $P<0.01$).

The results of association analysis between genotypes of g.199893-delT and litter size traits are shown in Table 5. For the litter size of all parities the *NN* genotype sows had significantly more piglets of TNB and NBA ($P<0.05$ or $P<0.01$) than the *MM* genotype in both the DIV line and Large White populations. However, for litter size of the first parity, no significant difference was found either in the experimental Large White or DIV line sows.

In commercial pig production litter size has always been one of the most important production traits. The use of direct selection for some genes associated with reproductive traits has been recommended to achieve further genetic progress for litter size in pig breeding schemes [Rothschild 2004]. Some genes or genetic markers were reported to be associated with litter size in pigs [Yuan *et al*. 2007a; Yuan *et al*. 2007b; Wu *et al*. 2013], which had a relationship with the sex-related hormones or their receptors, and thus affected ovulation rate and uterus capacity.

STIM1, a single transmembrane-spanning $Ca^{2+}$-binding protein, plays an essential role in oocyte fertilization and embryo survival rate. For example, STIM1 is a necessary component of the signalling mechanism that links $Ca^{2+}$ store depletion to the activation of store-operated $Ca^{2+}$ entry in porcine oocytes [Koh *et al*. 2009]. STIM1 suppression in oocytes inhibited the sperm-induced $Ca^{2+}$ oscillations, indicating that STIM1 is essential for the maintenance of the long-lasting $Ca^{2+}$ signal during fertilization [Lee *et al*. 2012]. Furthermore, downregulation of STIM1 before fertilization prevented the onset of repetitive $Ca^{2+}$ oscillations in fertilized oocytes, which eventually resulted in poor embryonic development [Lee *et al*. 2012]. Moreover, the potential involvement of STIM1 in signalling during fertilization was also shown in the mouse [Gómez-Fernández *et al*. 2009]. In addition, knockdown of STIM1 expression causes sterility due to the loss of sheath cell and spermatheca contractile activity required for ovulation in *C. elegans* [Yan *et al*. 2006].

In the present study, we found two mutations in the porcine *STIM1* gene, i.e. g.1969037T>C in exon 10 and g.199893-delT in intron 10. Our results showed that these two mutations significantly affected litter size traits (TNB and NBA) in the DIV line and Large White pigs. Sows with the *g.1969037-TT* and *g.199893-NN* genotypes had more piglets. This indicates that the porcine *STIM1* gene might be a potential important candidate gene related to litter size. These two variations may influence porcine oocyte fertilization and early embryonic development possibly via their effects on the regulations of *STIM1* gene transcriptions and expressions, although they do not result in any changes in the amino acid sequence of STIM1 protein. To provide a better understanding of their mechanisms functional researches need to be conducted.

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