Research article

Effect of porcine IL-6 polymorphism on litter size traits in commercial pig breeds

Worrarak Norseeda1,2,3, Guisheng Liu4,5, Tawatchai Teltatham6, Korawan Sringarm1,7, Watcharapong Naraballoh1, Trisadee Khamlor1, Patcharin Krutmuang6, and Supamit Mekchay1,2,7,9*

1Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
2Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand
3Graduate School, Chiang Mai University, Chiang Mai 50200, Thailand
4Institute of Animal Science and Veterinary Medicine, Hubei Academy of Agricultural Sciences, Wuhan 430064, China
5Hubei Key Lab for Animal Embryo Engineering and Molecular Breeding, Wuhan 430064, China
6Mae Hong Son Livestock Research and Breeding Center, Mae Hong Son 58000, Thailand
7Cluster of Research and Development of Pharmaceutical and Natural Products Innovation for Human or Animal, Chiang Mai University, Chiang Mai 50200, Thailand
8Department of Entomology and Plant Pathology, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand
9Innovative Agriculture Research Center, Faculty of Agriculture, Chiang Mai University, Chiang Mai 50200, Thailand

Abstract

This study aimed to verify the polymorphisms in the porcine IL-6 gene and to elucidate its effects on litter size traits in Large White and Landrace sows. Four single nucleotide polymorphisms (SNPs) of the porcine IL-6 gene (g.91506415A>G, g.91507983A>G, g.91508173C>T, and g.91508716C>T) were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. There was no polymorphism observed on the three SNPs (g.91506415A>G, g.91507983A>G, and g.91508716C>T) of the porcine IL-6 gene. The porcine IL-6 g.91508173C>T polymorphism was found to be segregating in Large White and Landrace sows. The porcine IL-6 g.91508173C>T polymorphism was significantly associated with the total number born (TNB) and the number of piglets weaned alive (NWA) traits in Large White sows (P<0.05). Moreover, the porcine IL-6 g.91508173C>T polymorphism was significantly associated with the TNB, number born alive (NBA), and NWA traits in Landrace sows (P<0.05). These results indicated that the porcine IL-6 g.91508173C>T polymorphism was associated with litter size traits. These findings confirmed the importance of the IL-6 gene as a candidate gene for litter size traits in pigs.

Keywords: IL-6, Litter size, Pig, Polymorphisms

Corresponding author: Supamit Mekchay, Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University, Chiang Mai, 50200, Thailand. Tel: +66 53944092. E-mail: supamitmekchay@gmail.com.

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INTRODUCTION

Litter size traits are the most important traits for reproduction and have direct effects on the economic advantages in pig production (Martínez-Giner et al., 2013). Embryo mortality during the pregnancy period is one of the main factors that directly affect litter size in pigs (Spötter and Distl, 2006). Moreover, implantation is a critical process for establishing pregnancy due to the most embryonic loss occurring during the peri-implantation period (Lin et al., 2009). Several studies demonstrated that many cytokine genes play an important role in the embryo implantation process and associated with litter size traits in pigs (Lin et al., 2009; Yang et al., 2011; Kumchoo and Mekchay, 2015; Norseeda et al., 2021).

Interleukin 6 (IL-6) is a multifunctional pleiotropic cytokine which involved in several biological functions such as cellular signal transduction (Culig and Puhr, 2012), inflammatory (Vanden Berghe et al., 2000), immune system (Horn et al., 2000), and reproductive system (Syed et al., 2002). The IL-6 protein is secreted by trophoblasts and endometrial stomal cells (Guzeloglu-Kayisli et al., 2009). Moreover, the IL-6 gene is widely expressed in the female reproductive tracts and mediates blastocyst implantation and placental development in several mammal species, including humans (Sherwin et al., 2002), mice (Robertson et al., 2010), sheep (Song et al., 2009), and pigs (Modrić et al., 2000; Blitek et al., 2012; Yoo et al., 2017). The expression levels of the IL-6 gene are the highest in the endometrial epithelium cells at the blastocyst implantation stage (Laird et al., 2000). The IL-6 deficient mice have reduced fertility and decreased in viable implantation sites (Guzeloglu-Kayisli et al., 2009). Therefore, the IL-6 gene is assumed that it is connected with embryo implantation (Yang et al., 2011).

The porcine IL-6 gene has been mapped on the Sus scrofa chromosome 9 (SSC9) at position 91.5 Mb. It is composed of five exons and four introns and encoded for a peptide of 241 amino acids (ENSSSCG000000 20970; Ensembl Sscrofa 11.1; https://asia.ensembl.org/Sus_scrofa/Info/ Index). In addition, the porcine IL-6 gene is closely located within the QTL regions for piglet mortality (70.3 to 102.3 Mb), the number of mummified pigs (84.3 to 86.6 Mb), total number born (84.6 to 96.3 Mb), and total number born alive (95.5 Mb) (Uimari et al., 2011; Onterus et al., 2012; He et al., 2017; Zhang et al., 2019). The polymorphisms of the porcine IL-6 gene have been characterized (Burk et al., 1997; Daniłowicz et al., 2008) and reported in the Ensembl database (https://asia.ensembl.org/Sus_scrofa/Info/Index). Furthermore, the association of an SNP in the 5’-regulatory region of the porcine IL-6 gene with litter size traits has been reported in commercial Landrace pigs (Yang et al., 2011). Therefore, the IL-6 gene can be regarded as a functional and positional candidate gene for the determination of the litter size traits of pigs. However, the effect of the porcine IL-6 polymorphism on litter size traits has been limited. This study aimed to verify the polymorphisms in the porcine IL-6 gene and to elucidate the association of the porcine IL-6 polymorphism with litter size in Large White and Landrace sows.
MATERIALS and METHODS

Animals and DNA extraction

Blood samples were taken from 136 sows of the Large White breed and 222 sows of the Landrace breed. These sows were acquired from a commercial nucleus herd. All sows were reared under commercial conditions of the Betagro Hybrid International Company, Thailand. The litter size traits of sows were assessed in 467 and 779 litters for Large White and Landrace breeds, respectively. Litter size traits were recorded that consisted of total number born (TNB), number born alive (NBA), number of piglets weaned alive (NWA), mean birth weight of the piglets (MBW), and mean weight of piglets at weaning (21 days, MWW). Genomic DNA was extracted from blood samples using the Chelex method and kept at 4°C until analysis. The experimental procedures were approved by the Animal Ethics Committee of Chiang Mai University, Thailand (2562/AG-0001).

Verification of porcine IL-6 polymorphisms and genotyping

Four SNPs (g.91506415A>G, g.91507983A>G, g.91508173C>T, and g.91508716C>T) of the porcine IL-6 gene were selected based on the restriction enzymes available in the Ensembl database (ENSSSCG00000020970; http://asia.ensembl.org/index.html). These SNPs were used to verify the SNPs in the Large White and Landrace sows. The specific primers of the porcine IL-6 gene were designed based on relevant nucleotide sequence information (GenBank accession number: NC_010451.4), as shown in Table 1. The PCR amplification was performed in a final reaction volume 20 μL consisting of 50 ng of a genomic DNA sample, 1×(NH₄)₂SO₄ buffer, 1.5 mM MgCl₂, 0.2 mM dNTPs, 0.4 μM for each primer (Table 1), and 0.2 U Taq DNA polymerase (Fermentas). The PCR conditions were as follows: 94°C for 3 min at the initial denaturing stage, followed by 35 cycles of 94°C for 30 sec, 58 to 60°C for 30 sec, 72°C for 30 sec, and then 5 min at 72°C to complete the reaction. The single nucleotide polymorphisms of the porcine IL-6 gene were genotyped by PCR-RFLP method with restriction enzymes (Table 1). The digested products were separated by electrophoreses on 6% polyacrylamide gels in 1×TBE buffer and visualized by ethidium bromide staining.

| SNP position | Location | Primer sequence | Product size (bp) | Tm (°C) | Restriction enzyme |
|--------------|----------|----------------|------------------|--------|-------------------|
| IL-6 g.91506415A>G | Exon 1 | F: 5'-TTTCCCTGGTTGATTCCCT-3' R: 5'-GGGATTTCCCTCACTTT-3' | 295 | 58 | Hpy188I |
| IL-6 g.91507983A>G | Intron 2 | F: 5'-GCCCATTCCTCGATTTGGT-3' R: 5'-TGCCGCTTGCTAGCTATGT-3' | 359 | 60 | Hpy188I |
| IL-6 g.91508173C>T | Intron 3 | F: 5'-GCCCATTCCTCGATTTGGT-3' R: 5'-TGCCGCTTGCTAGCTATGT-3' | 359 | 60 | MspI |
| IL-6 g.91508716C>T | Intron 4 | F: 5'-CTTCCACACATTTTCCTC-3' R: 5'-TTGCAACAGTCGGTTGCTA-3' | 232 | 58 | Hinfl |
Statistical analyses

The allele and genotype frequencies of the porcine IL-6 polymorphism were estimated. Hardy-Weinberg equilibrium (HWE) was analyzed with the chi-square test. Association analyses of the porcine IL-6 polymorphism with the litter size traits were performed with a statistical model as expressed below: \( Y_{ijkl} = \mu + Y_{S_i} + P_j + G_k + e_{ijkl} \) where \( Y_{ijkl} \) is the observations of the phenotype values, \( \mu \) is the overall mean for each trait, \( Y_{S_i} \) is the fixed effect of year-season \((i = 1-8)\), \( P_j \) is the fixed effect of parities \((j = 1 \text{ and } \geq 2)\), \( G_k \) is the fixed effect of the IL-6 genotypes \((k = 1, 2, 3)\), and \( e_{ijkl} \) is the residual error. Moreover, additive effect of the porcine IL-6 polymorphism was calculated as half difference between the estimated effects of homozygous genotypes and the dominance effect was estimated as the deviation of the heterozygous genotype effect from the mean effect of the homozygous genotypes (Muñoz et al., 2007). The estimated effects were calculated using a t-test on significant deviations from zero.

RESULTS

Polymorphisms of the porcine IL-6 gene

The results in this study showed a polymorphic site of the porcine IL-6 g.91508173C>T (rs1109532035) locus was located in intron 3. This polymorphic site was detected with the restriction enzyme MspI. Two specific alleles revealed a 359-bp fragment for allele T and two fragments of 327 and 32-bp for allele C (Figure 1). However, no polymorphisms of the three SNPs (g.91506415A>G, g.91507983A>G, and g.91508716C>T) of the porcine IL-6 gene were observed in this study.

![Figure 1](image.png)

**Figure 1** Genotyping SNPs of porcine IL-6 g.91508173C>T locus with MspI. The molecular marker of 100 bp DNA ladder (M) and the genotypes of the porcine IL-6 marker are indicated at the top of each line. A 359-bp fragment for allele T and two fragments of 327 and 32-bp for allele C. Notably, the 32-bp fragment is not shown in the gel.
Genotype and allele frequencies

The genotype and allele frequencies of the porcine IL-6 gene are shown in Table 2. At the porcine IL-6 g.91508173C>T locus, three genotypes were found to be segregating in these Large White and Landrace sows. The IL-6 g.91508173C allele was the major allele in these sows. However, the three SNPs of the porcine IL-6 gene at g.91506415, g.91507983, and g.91508716 loci were fixed as g.91506415A, g.91507983A, and g.91508716C (data not shown). The chi-square (χ²) test showed that the genotype distribution of the porcine IL-6 g.91508173C>T locus in Large White and Landrace sows were in agreement with the HWE specifications (P>0.05).

Associations of porcine IL-6 polymorphism with litter size traits

Associations of the porcine IL-6 g.91508173C>T polymorphism with litter size traits in Large White and Landrace sows are shown in Tables 3 and 4, respectively. No significant association of the porcine IL-6 g.91508173C>T polymorphism with litter size traits was found in the first parity of Large White and Landrace sows. However, the porcine IL-6 g.91508173C>T polymorphism was significantly associated with TNB and NWA traits in later parities of Large White sows. Moreover, this polymorphic site was significantly associated with TNB, NBA, and NWA traits in later parities of Landrace sows. The sows with the CC genotype had higher TNB, NBA, and NWA values than those of the sows with the CT and TT genotypes. In addition, the significant additive effect of TNB trait was observed in later parities of Landrace sows. Therefore, the porcine IL-6 g.91508173C allele seems to be a favorable allele for litter size traits in the Large White and Landrace sows.

Table 2 Genotype and allele frequencies of the porcine IL-6 g.91508173C>T.

| Breeds    | n   | Genotype frequencies | Allele frequencies | P-value¹ (χ²) |
|-----------|-----|----------------------|--------------------|--------------|
|           |     | CC       | CT     | TT     | C      | T      |     |
| Large White | 136 | 0.53     | 0.43   | 0.04   | 0.74   | 0.26   | 0.40 |
| Landrace   | 222 | 0.44     | 0.48   | 0.08   | 0.68   | 0.32   | 0.35 |

¹The P-value is considered a significant level of the chi-square (χ²) test for Hardy-Weinberg equilibrium of the porcine IL-6 g.91508173C>T locus in different pig breeds.
### Table 3 Association of the porcine \textit{IL-6} g.91508173C>T with litter size traits in Large White sows.

| Parity      | Traits\(^1\) | Genotypes (means±SE)\(^2\) | Additive | Dominance |
|-------------|---------------|-----------------------------|----------|-----------|
|             |               | CC                          | CT       | TT        |
| First parity|               |                             |          |           |
|             | \(n\)         |                             |          |           |
| TNB         | 72            | 10.95±0.35                  | 11.25±0.36 | 11.07±1.05 | -0.06±0.69 | 0.23±0.77 |
| NBA         | 58            | 9.56±0.36                   | 9.66±0.37 | 9.13±1.01 | 0.22±0.70 | 0.31±0.77 |
| NWA         | 6             | 8.70±0.34                   | 8.69±0.35 | 8.87±1.30 | -0.08±0.66 | -0.09±0.73 |
| MBW         |               | 1.39±0.03                   | 1.35±0.03 | 1.52±0.12 | -0.06±0.06 | -0.11±0.07 |
| MWW         |               | 6.58±0.04                   | 6.48±0.04 | 6.59±0.15 | -0.01±0.08 | -0.10±0.08 |
| Later parities | \(n\)         |                             |          |           |
|              |               |                             |          |           |
| (2\(^{nd}\) - 8\(^{th}\) parities) | TNB | 12.01±0.35\(^a\)        | 11.24±0.34\(^b\) | 11.51±1.06\(^ab\) | 0.25±0.53 | -0.52±0.60 |
|             | NBA           | 10.66±0.34                  | 10.07±0.33 | 10.24±1.01 | 0.21±0.51 | -0.38±0.57 |
|             | NWA           | 10.12±0.32\(^a\)        | 9.32±0.31\(^b\) | 9.90±0.99\(^ab\) | 0.11±0.50 | -0.69±0.56 |
|             | MBW           | 1.52±0.03                   | 1.51±0.03 | 1.60±0.09 | -0.04±0.05 | -0.05±0.05 |
|             | MWW           | 6.57±0.03                   | 6.62±0.03 | 6.70±0.10 | -0.06±0.05 | -0.01±0.06 |

\(^1\)\(n\): number of investigated litters, TNB: total number born, NBA: number born alive, NWA: number of piglets weaned alive, MBW: mean birth weight of the piglets, MWW: mean weight of piglets at weaning. MBW and MWW traits are presented in kg. 
\(^2\)Means±SE represents the least square means±standard error. Values in each row with differing superscripts are considered significantly different (\(^a\), \(^b\) \(P<0.05\)).

### Table 4 Association of the porcine \textit{IL-6} g.91508173C>T with litter size traits in Landrace sows.

| Parity      | Traits\(^1\) | Genotypes (means±SE)\(^2\) | Additive | Dominance |
|-------------|---------------|-----------------------------|----------|-----------|
|             |               | CC                          | CT       | TT        |
| First parity|               |                             |          |           |
|             | \(n\)         |                             |          |           |
| TNB         | 98            | 10.27±0.28                  | 10.40±0.26 | 9.71±0.64 | 0.28±0.34 | 0.40±0.42 |
| NBA         | 106           | 8.69±0.30                   | 9.20±0.28 | 8.46±0.69 | 0.11±0.36 | 0.62±0.44 |
| NWA         | 18            | 7.93±0.29                   | 8.61±0.28 | 7.78±0.68 | 0.08±0.36 | 0.75±0.44 |
| MBW         |               | 1.56±0.02                   | 1.52±0.02 | 1.49±0.05 | 0.04±0.03 | -0.01±0.03 |
| MWW         |               | 6.47±0.04                   | 6.45±0.04 | 6.59±0.10 | 0.06±0.05 | -0.08±0.06 |
| Later parities | \(n\)         |                             |          |           |
|              |               |                             |          |           |
| (2\(^{nd}\) - 8\(^{th}\) parities) | TNB | 11.69±0.24\(^a\)        | 10.63±0.24\(^b\) | 10.47±0.54\(^b\) | 0.61±0.28* | -0.44±0.34 |
|             | NBA           | 10.43±0.25                  | 9.71±0.26\(^b\) | 9.54±0.56\(^ab\) | 0.44±0.29 | -0.28±0.35 |
|             | NWA           | 9.70±0.24\(^a\)       | 9.16±0.25\(^b\) | 8.85±0.53\(^ab\) | 0.43±0.28 | -0.11±0.34 |
|             | MBW           | 1.60±0.02                   | 1.60±0.02 | 1.53±0.05 | 0.04±0.02 | 0.03±0.03 |
|             | MWW           | 6.58±0.02                   | 6.57±0.03 | 6.58±0.06 | -0.01±0.03 | -0.01±0.04 |

\(^1\)\(n\): number of investigated litters, TNB: total number born, NBA: number born alive, NWA: number of piglets weaned alive, MBW: mean birth weight of the piglets, MWW: mean weight of piglets at weaning. MBW and MWW traits are presented in kg. 
\(^2\)Means±SE represents the least square means±standard error. Values in each row with differing superscripts are considered significantly different (\(^a\), \(^b\) \(P<0.05\), *\(P<0.05\)).
DISCUSSION

Litter size is one of the most economically important traits in pig production (Lin et al., 2009). Successful implantation is a critical factor in determining litter size in pigs (Spötter and Distl, 2006). Numerous studies have demonstrated that many cytokines are essential to establish a pregnancy, especially to embryo implantation in mammalian species (Chaouat et al., 2007; Paulesu et al., 2010). IL-6 is a pro-inflammatory cytokine gene and plays a major role in implantation and establishing pregnancy in several species (Blitek et al., 2012; Yoo et al., 2017). Moreover, previous studies have reported the polymorphisms of the porcine IL-6 gene are associated with litter size (Yang et al., 2011) and fatness traits in pigs (Szydlowski et al., 2011).

In this study, the polymorphisms of the porcine IL-6 were verified and elucidated its effects on litter size traits in Large White and Landrace sows. Three genotypes of the porcine IL-6 g.91508173C>T locus were segregated in these Large White and Landrace sows. The IL-6 g.91508173C was the major allele in these sows. Moreover, the genotype distribution of the porcine IL-6 g.91508173C>T locus in Large White and Landrace sows were in agreement with the HWE specifications. The results imply that the porcine IL-6 g.91508173C>T polymorphism in these sows was within homeostasis when accompanied by the effects of artificial selection.

The results in this study showed that the polymorphism of the porcine IL-6 gene had a significant association with TNB and NWA traits in later parities of Large White sows and with TNB, NBA, and NWA traits in later parities of Landrace sows. The positive effect of the favorable porcine IL-6 g.91508173C allele on litter size traits was observed in these two pig populations. Although, no effect of the porcine IL-6 gene on litter size traits in the first parity was observed in this study. This may be due to physiological differences in the reproductive system of gilts (immaturity) and sows (maturity). Obviously, the litter size traits at the first parity of gilts are smaller partly than the later parities of sows. However, the porcine IL-6 g.91508173C>T polymorphism was located in the non-coding sequence of the porcine IL-6 gene. We assume that the porcine IL-6 g.91508173C>T locus may be in linkage of disequilibrium with other causal SNPs of a positive effect on litter size traits in pigs. Similarly, a previous study showed that a polymorphism in the 5’-regulatory region (non-coding sequence) of the IL-6 gene was associated with TNB and NBA traits in Landrace sows (Yang et al., 2011).

In this study, the results showed that the porcine IL-6 g.91508173C>T polymorphism was significantly associated with litter size traits. It may hypothesize that this polymorphic site was related to the function of the IL-6 gene in the process of blastocyst implantation (Laird et al., 2000). Endometrial IL-6 mRNA expression was upregulated on day 12 in pregnant gilts compared with non-pregnant pigs. Furthermore, IL-6 protein was higher in pregnant than in cyclic gilts. Thus, the IL-6 gene is an essential constitutive of embryo-uterine interactions during early pregnancy in the pig and may encourage successful conceptus implantation (Blitek et al., 2012).

In addition, the IL-6 affects the endometrial inflammation at pig blastocyst attachment sites (Bradding et al., 1993). This may suggest that IL-6 might relate to the inflammation of the endometrium in close apposition to
the attaching conceptuses. It is probable to be beneficial in the nutrition of the conceptus because the inflammation may result in increased blood flow to implantation sites and capillary permeability at implantation sites (Modrić et al., 2000). Recently, transcriptomic analysis conducted using RNA-sequencing revealed that the expression levels of the IL-6 gene are significantly increased in Berkshire pig placentas with larger litter size group compared to the smaller litter size group (Kwon et al., 2016). This evidence suggests that the induction of IL-6 may play an important role in increasing nutrition supply through the placenta from the sow to the piglet during gestation (Kwon et al., 2016).

Besides, a previous study of the expression and regulation of IL-6 at the maternal-conceptus interface during pregnancy in pigs revealed that IL-6 and its receptors are expressed in the uterine endometrium and conceptus tissues (Yoo et al., 2017). Based on the localization of IL-6 and IL-6R in the uterine endometrium and conceptus tissues, it is possible that the IL-6 acts on endometrial epithelial cells and conceptus tissues in an autocrine and/or paracrine feature to involve the endometrial and conceptus function during pig pregnancy. It has been suggested that the IL-6 induces endometrial estrogen and prostaglandin F2α (PGF2α) production during early pregnancy and attachment and proliferation of trophoblast cells in pigs (Blitek et al., 2012; Franczak et al., 2013; Yoo et al., 2017). Hence, the IL-6 may play an important role in endometrial and placental tissues for pregnancy maintenance (Yoo et al., 2017). From all these pieces of evidence, it has been hypothesized that the porcine IL-6 gene may involve in the reproductive processes of pigs especially the implantation process, and might impact litter size traits. The results in this study demonstrated that the porcine IL-6 gene could be expected to implicate in the litter size traits of pigs.

CONCLUSION

In the current study, we have verified the polymorphisms of the porcine IL-6 gene and elucidated its effects on litter size traits in commercial pig breeds. The porcine IL-6 g.91508173C>T polymorphisms had effects on TNB and NWA traits in Large White sows and on TNB, NBA, and NWA traits in Landrace sows. These findings confirmed the importance of the porcine IL-6 gene in the litter size traits of pigs. Therefore, the porcine IL-6 could be used as a candidate gene for selection of litter size traits in pigs.

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AUTHOR CONTRIBUTIONS

Worrarak Norseeda; Methodology, investigation, data curation, writing - original draft.
Guisheng Liu; Conceptualization, methodology, writing - review and editing.
Tawatchai Teltatham; Methodology, investigation, writing - review and editing.
Korawan Sringarm; Methodology, data curation, writing - review and editing.
Watcharapong Naraballobh; Formal analysis, writing - review and editing.
Trisadee Khamlor; Data curation, formal analysis, writing - review and editing.
Patcharin Krutmuang; Data curation, formal analysis, writing - review and editing.
Supamit Mekchay; Conceptualization, supervision, investigation, formal analysis, writing - original draft, writing-review and editing, project administration.

CONFLICT ON INTEREST

The authors declare there is no conflict of interest.

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