Veterinary Integrative Sciences 2021; 19(2): 237-246.

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

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

Worrarak Norseeda[1, 2, 3], Guisheng Liu[4, 5], Tawatchai Teltathum[6], Korawan Sringarm[1, 7], Watcharapong Naraballobh[1], Trisadee Khamlor[1], Patcharin Krutmuang[8], and Supamit Mekchay[1, 2, 7, 9*]

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

Article history; *received manuscript: 19 March 2021, revised manuscript: 6 April 2021, accepted manuscript: 19 April 2021, published online: 21 April 2021*
Academic editor; *Korakot Nganvongpanit*

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; Onteru 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×$(NH_4)_2SO_4$ buffer, 1.5 mM $MgCl_2$, 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.

Table 1 Primer sequences and restriction enzymes used for SNPs genotyping of the porcine *IL-6* gene.

SNP position	Location	Primer sequence	Product size (bp)	Tm (°C)	Restriction enzyme
IL-6 g.91506415A>G	Exon 1	F: 5'-TTTCCCTGGTTGTGATTCCT-3' R: 5'-GGGATTTCCTTCACTTACTT-3'	295	58	*Hpy*188I
IL-6 g.91507983A>G	Intron 2	F: 5'-GCCCATTCTCCACTTGTTTG-3' R: 5'-TGCCTGCTTGGTCTACATGT-3'	359	60	*Hpy*188I
IL-6 g.91508173C>T	Intron 3	F: 5'-GCCCATTCTCCACTTGTTTG-3' R: 5'-TGCCTGCTTGGTCTACATGT-3'	359	60	*Msp*I
IL-6 g.91508716C>T	Intron 4	F: 5'-CTTCCCACCATCTTTCCTCT-3' R: 5'-TTGCACAGTCGGGTTGTCTA-3'	232	58	*Hin*fI

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: $Y_{ijkl} = \mu + YS_i + P_j + G_k + e_{ijkl}$ where Y_{ijkl} is the observations of the phenotype values, μ is the overall mean for each trait, YS_i is the fixed effect of year-season (i = 1-8), P_j is the fixed effect of parities (j = 1 and ≥ 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 *Msp*I. 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.

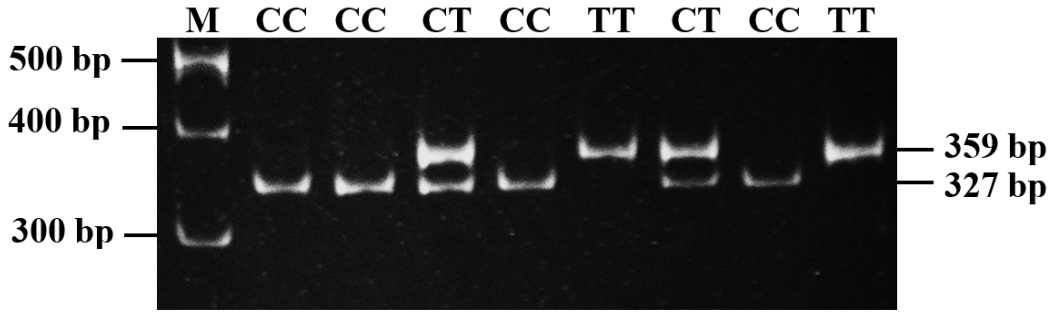

Figure 1 Genotyping SNPs of porcine *IL-6* g.91508173C>T locus with *Msp*I. The molecular marker of 100 bp DNA ladder (M) and the genotypes of the porcine *IL-6* marker are indicated at the top of each line. A 359-bp fragment for allele T and two fragments of 327 and 32-bp for allele C. Notably, the 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 (χ^2) 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-value[1]
		CC	CT	TT	C	T	(χ^2)
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

[1]The P-value is considered a significant level of the chi-square (χ^2) test for Hardy-Weinberg equilibrium of the porcine *IL-6* g.91508173C>T locus in different pig breeds.

Table 3 Association of the porcine *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*	72	58	6		
	TNB	10.95±0.35	11.25±0.36	11.07±1.05	-0.06 ±0.69	0.23±0.77
	NBA	9.56±0.36	9.66±0.37	9.13±1.01	0.22±0.70	0.31±0.77
	NWA	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*	172	145	14		
(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 *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*	98	106	18		
	TNB	10.27±0.28	10.40±0.26	9.71±0.64	0.28±0.34	0.40±0.42
	NBA	8.69±0.30	9.20±0.28	8.46±0.69	0.11±0.36	0.62±0.44
	NWA	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*	245	270	42		
(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^{a}	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.

ACKNOWLEDGEMENTS

This research is partially supported by Ching Mai University and the National Science and Technology Development Agency (NSTDA), NSTDA University Industry Research Collaboration (NUI-RC) project (NUI-RC_CPMO01-55-006) and Betagro group (CPM-54-016) as well as the Center of Excellence on Agricultural Biotechnology, Science and Technology Postgraduate Education and Research Development Office, Office of Higher Education Commission, Ministry of Higher Education, Science, Research and Innovation (AG-BIO/PERDO-CHE). We would like to thank Julius Rajula, Ph.D. for the English language editing of this manuscript. Moreover, we wish to thank all anonymous reviewers for their valuable suggestions.

AUTHOR CONTRIBUTIONS

Worrarak Norseeda; Methodology, investigation, data curation, writing - original draft.
Guisheng Liu; Conceptualization, methodology, writing - review and editing.
Tawatchai Teltathum; 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.

REFERENCES

Blitek, A., Morawska, E., Ziecik, A.J., 2012. Regulation of expression and role of leukemia inhibitory factor and interleukin-6 in the uterus of early pregnant pigs. Theriogenology 78, 951-964.

Bradding, P., Feather, I.H., Wilson, S., Bardin, P.G., Heusser, C.H., Holgate, S.T., Howarth, P.H., 1993. Immunolocalization of cytokines in the nasal mucosa of normal and perennial rhinitic subjects. The mast cell as a source of IL-4, IL-5, and IL-6 in human allergic mucosal inflammation. J. Immunol. 151, 3853-3865.

Burk, N.E., Vincent, A.L., Rothschild, M.F., 1997. Linkage mapping of porcine interleukin 6 (IL6). J. Anim. Sci. 75, 3367.

Chaouat, G., Dubanchet, S., Ledée, N., 2007. Cytokines: Important for implantation?. J. Assist. Reprod. Genet. 24, 491-505.

Culig, Z., Puhr, M., 2012. Interleukin-6: a multifunctional targetable cytokine in human prostate cancer. Mol. Cell. Endocrinol. 360, 52-58.

Daniłowicz, E., Akouchekian, M., Drogemuller, C., Haase, B., Leeb, T., Kuiper, H., Distl, O., Iras, F.C., 2008. Molecular characterization and SNP development for the porcine IL6 and IL10 genes. Anim. Biotechnol. 19, 159-165.

Franczak, A., Wojciechowicz, B., Zmijewska, A., Kolakowska, J., Kotwica, G., 2013. The effect of interleukin 1β and interleukin 6 on estradiol-17β secretion in the endometrium of pig during early pregnancy and the estrous cycle. Theriogenology 80, 90-98.

Guzeloglu-Kayisli, O., Kayisli, U.A., Taylor, H.S., 2009. The role of growth factors and cytokines during implantation: endocrine and paracrine interactions. Semin. Reprod. Med. 27, 62-79.

He, L.C., Li, P.H., Ma, X., Sui, S.P., Gao, S., Kim, S.W., Gu, Y.Q., Huang, Y., Ding, N.S., Huang, R.H., 2017. Identification of new single nucleotide polymorphisms affecting total number born and candidate genes related to ovulation rate in Chinese Erhualian pigs. Anim. Genet. 48, 48-54.

Horn, F., Henze, C., Heidrich, K., 2000. Interleukin-6 signal transduction and lymphocyte function. Immunobiology 202, 151-167.

Kumchoo, T., Mekchay, S., 2015. Association of non-synonymous SNPs of *OPN* gene with litter size traits in pigs. Arch. Anim. Breed. 58, 317-323.

Kwon, S.G., Hwang, J.H., Park, D.H., Kim, T.W., Kang, D.G., Kang, K.H., Kim, I.S., Park, H.C., Na, C.S., Ha, J., Kim, C.W., 2016. Identification of differentially expressed genes associated with litter size in Berkshire pig placenta. PLoS One 11, e0153311.

Laird, S.M., Tuckerman, E.M., Cork, B.A., Li, T.C., 2000. Expression of nuclear factor kappa B in human endometrium; role in the control of interleukin 6 and leukaemia inhibitory factor production. Mol. Hum. Reprod. 6, 34-40.

Lin, H.C., Liu, G.F., Wang, A.G., Kong, L.J., Wang, X.F., Fu, J.L., 2009. Effect of polymorphism in the leukemia inhibitory factor gene on litter size in Large White pigs. Mol. Biol. Rep. 36, 1833-1838.

Martínez-Giner, M., Noguera, J.L., Balcells, I., Fernández-Rodríguez, A., Pena, R.N., 2013. Selection of internal control genes for real-time quantitative PCR in ovary and uterus of sows across pregnancy. PLoS One 8, e66023.

Modrić, T., Kowalski, A.A., Green, M.L., Simmen, R.C., Simmen, F.A., 2000. Pregnancy-dependent expression of leukaemia inhibitory factor (LIF), LIF receptor-beta and interleukin-6 (IL-6) messenger ribonucleic acids in the porcine female reproductive tract. Placenta 21, 345-353.

Muñoz, G., Ovilo, C., Estellé, J., Silió, L., Fernández, A., Rodriguez, C., 2007. Association with litter size of new polymorphisms on *ESR1* and *ESR2* genes in a Chinese-European pig line. Genet. Sel. Evol. 39, 195-206.

Norseeda, W., Liu, G., Teltathum, T., Sringarm, K., Naraballobh, W., Khamlor, T., Mekchay, S., 2021. Effect of leukemia inhibitory factor polymorphism on litter size traits in Thai commercial pig breeds. Vet. Integr. Sci. 19, 185-196.

Onteru, S.K., Fan, B., Du, Z.Q., Garrick, D.J., Stalder, K.J., Rothschild, M.F., 2012. A whole-genome association study for pig reproductive traits. Anim. Genet. 43, 18-26.

Paulesu, L., Jantra, S., Ietta, F., Brizzi, R., Avanzati, A.M., Bigliardi, E., 2010. Cytokines in vertebrate reproduction. Herpetol. Conserv. Biol. 5, 335-340.

Robertson, S.A., Christiaens, I., Dorian, C.L., Zaragoza, D.B., Care, A.S., Banks, A.M., Olson, D.M., 2010. Interleukin-6 is an essential determinant of on-time parturition in the mouse. Endocrinology 151, 3996-4006.

Sherwin, J.R., Smith, S.K., Wilson, A., Sharkey, A.M., 2002. Soluble gp130 is up-regulated in the implantation window and shows altered secretion in patients with primary unexplained infertility. J. Clin. Endocrinol. Metab. 87, 3953-3960.

Song, G., Satterfield, M.C., Kim, J., Bazer, F.W., Spencer, T.E., 2009. Progesterone and interferon tau regulate leukemia inhibitory factor receptor and IL6ST in the ovine uterus during early pregnancy. Reproduction 137, 553-565.

Spötter, A., Distl, O., 2006. Genetic approaches to the improvement of fertility traits in the pig. Vet. J. 172, 234-247.

Syed, V., Ulinski, G., Mok, S.C., Ho, S.M., 2002. Reproductive hormone-induced, STAT3-mediated interleukin 6 action in normal and malignant human ovarian surface epithelial cells. J. Natl. Cancer Inst. 94, 617-629.

Szydlowski, M., Buszka, A., Maćkowski, M., Lechniak, D., Switonski, M., 2011. Polymorphism of genes encoding cytokines IL6 and TNF is associated with pig fatness. Livest. Sci. 136, 150-156.

Uimari, P., Sironen, A., Sevón-Aimonen, M.L., 2011. Whole-genome SNP association analysis of reproduction traits in the Finnish Landrace pig breed. Genet. Sel. Evol. 43, 42.

Vanden Berghe, W., Vermeulen, L., De Wilde, G., De Bosscher, K., Boone, E., Haegeman, G., 2000. Signal transduction by tumor necrosis factor and gene regulation of the inflammatory cytokine interleukin-6. Biochem. Pharmacol. 60, 1185-1195.

Yang, L., Fu, J., Fu, Y., Wang, A., 2011. Association analysis between a polymorphism in the 5′-regulatory region of the IL-6 gene and litter size in pigs. J. Anim. Sci. Biotech. 2, 187-191.

Yoo, I., Han, J., Kim, M., Jang, H., Sa, S., Choi, S.H., Ka, H., 2017. Expression and regulation of interleukin 6 and its receptor at the maternal-conceptus interface during pregnancy in pigs. Theriogenology 96, 85-91.

Zhang, Q., Huang, R., Ma, X., Jiang, N., Zhou, W., Gao, C., Zhao, M., Niu, P., Zhang, Z., Li, Q., Zhou, J., Li, P., 2019. Association of *rs339939442* in the *AHR* gene with litter size are inconsistent among Chinese indigenous pigs and western commercial pigs. Animals 10, 11.

How to cite this article;

Worrarak Norseeda, Guisheng Liu, Tawatchai Teltathum, Korawan Sringarm, Watcharapong Naraballobh, Trisadee Khamlor, Patcharin Krutmuang and Supamit Mekchay. Effect of porcine *IL-6* polymorphism on litter size traits in commercial pig breeds. Veterinary Integrative Sciences. 2021; 19(2): 237-246.