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Research article

Association of a non-synonymous SNP of *IL17RA* gene with litter size traits in Large White and Landrace pigs

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Abstract

Interleukin-17 receptor A (IL17RA) is one of the cytokine receptors of the pro-inflammatory interleukin-17 (IL17) cytokine family. The *IL17* and *IL17RA* genes are involved in inflammatory and immune responses as well as reproductive process of mammals. The purposes of this study were to examine polymorphisms in the porcine *IL17RA* gene and to assess its effects on litter size traits in Large White and Landrace pigs. Three non-synonymous single nucleotide polymorphisms (SNPs) in the porcine *IL17RA* gene were verified. The porcine *IL17RA* c.785C>T (p.Ala262Val) was found to be segregating in the Large White and Landrace pigs. No polymorphisms in the coding region of the porcine *IL17RA* gene at the two non-synonymous SNPs loci of c.997G>A (p.Val333Ile) and c.1962T>G (p.Asp654Glu) were found. The porcine *IL17RA* c.785C>T polymorphism was significantly associated with the total number born (TNB) and the number born alive (NBA) in Large White pigs (P<0.05). Moreover, the porcine *IL17RA* c.785C>T was significantly associated with the TNB, NBA, total birth weight (TBW), and total weaning weight of piglets at 21 days (TWW) in Landrace pigs (P<0.05). These results supported the importance of the porcine *IL17RA* gene in the litter size traits of pigs. Thus, the porcine *IL17RA* could be used as a potential candidate gene for improving litter size traits in pig breeding.

Keywords: *IL17RA*, Litter size, Non-synonymous SNP, Pig

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INTRODUCTION

Litter size trait is one of the major economic traits and plays a significant part in the reproductive performance and the profitableness of the pig production industry (Marantidis et al., 2013; Zhang et al., 2015). Embryonic mortality during the implantation period is a major factor in determining the litter size in pigs (Spötter and Distl, 2006; Lin et al., 2009; Chen et al., 2015). Currently, the cytokine genes are considered to play a key function in the communication process between fetal trophoblasts and maternal decidual immune cells at the feto-maternal interface in the uterus which leads to the acceptance of embryo implantation, embryo expansion, and pregnancy success (Saito, 2001; Bowen et al., 2002; Paria et al., 2002; Schäfer-Somi, 2003; Paulesu et al., 2010). Thus, the cytokines are essential for the embryonic development process and the maintenance of pregnancy in animals and humans (Simón et al., 1994; Chaouat et al., 2007; Ostanin et al., 2007; Paulesu et al., 2010). It has been reported that numerous cytokine genes involved in embryo implantation that affect the relevant litter size traits in pigs such as *LIF* (Spötter et al., 2005; Norseeda et al., 2021a), *EPOR* (Vallet et al., 2005), *OPN* (Kumchoo and Mekchay, 2015), *IL4* and *IL4R* (Norseeda et al., 2021b), and *IL6* genes (Yang et al., 2011; Norseeda et al., 2021c). Currently, several studies demonstrated that the IL17 cytokines and its receptors play a crucial role in implantation and maintenance of pregnancy in mice and humans (Saito et al., 2011; Klein, 2016; Wang et al., 2019). The biological function of IL17 and its role in immune response have been described in pigs (Levast et al., 2010; Stepanova et al., 2012; Xiang et al., 2020). However, there has been no report on the role of IL17 cytokines and its receptors in reproductive function in pigs.

Interleukin-17 receptor A (IL17RA) is the common receptor of the pro-inflammatory interleukin-17 (IL17) cytokine family (Krstic et al., 2015). The IL17 family is composed of six ligand members (IL17A to IL17F) and five receptors (IL17RA to IL17RE) (Ely et al., 2009; Gaffen, 2009). IL17 is mainly produced by T helper 17 (Th17) cells and plays key roles in inflammatory and immune responses (Jin and Dong, 2013; Ge et al., 2014; Krstic et al., 2015). Moreover, IL17 and IL17R are implicated in various reproductive processes including implantation, placentation, and maintenance of pregnancy (Pongcharoen et al., 2006; Pongcharoen and Supalap, 2009; Saito et al., 2011). Genetic polymorphisms and abnormal expression of *IL17* and/or *IL17R* are associated with the pregnancy disorders of preeclampsia, recurrent implantation failure, and recurrent pregnancy loss in women (Najafi et al., 2014; Zidan et al., 2015; Wang et al., 2019; Chen et al., 2020).

IL17RA is widely expressed in various cell types (e.g. hematopoietic, fibroblastoid, epithelial, endometrial, and conceptus cells) and forms a complex with IL17RC to act as a receptor for IL17A and IL17F cytokines (Iwakura et al., 2011; Wang et al., 2014; Klein, 2016). The *IL17RA* gene has been mapped on the *Sus scrofa* chromosome 5 (SSC5) at position 69.4 Mb. The coding sequence of the porcine *IL17RA* gene is 2777 bp length (ENSSSCT00000033369.3, Ensembl data base, https://asia.ensembl.org). It contains 15 exons and 14 introns and encodes with a peptide of 845 amino acids. The porcine *IL17RA* gene is located within the QTL regions for the total litter weight (67.2 Mb), the total number born (67.8 to 79.0 Mb), the teat number (68.8 to 69.7 Mb), the total number born alive (87.3 Mb), and the number of stillborn (88.8 to 88.9 Mb) (Cassady et al., 2001; Ding et al., 2009; Schneider et al., 2012; Bergfelder-Drüing et al., 2015;

He et al., 2017; Rohrer and Nonneman, 2017; Zhang et al., 2019). The porcine *IL17RA* polymorphisms have been characterized with 630 variant alleles (consisting of 122 upstream, 8 non-synonymous, 15 synonymous, 356 intronic, and 129 downstream variants) and exhibited in the Ensembl database (ENSSSCT00000033369.3, https://www.ensembl.org/Sus_scrofa/Info/Index, online access 23.10.2020). The association of the polymorphisms of the *IL17RA* gene with the pregnancy disorder of preeclampsia has been investigated in humans (Wang et al., 2015; Chen et al., 2020). The downregulated *IL17RA* expressed is related to preeclampsia (Ma et al., 2019). Moreover, the *IL17RA* gene is expressed in endometrial and conceptus tissues during early pregnancy (Klein, 2016).

All this information indicates that the *IL17RA* gene is involved in the immune response and related to the reproductive process of embryonic implantation and maintenance of pregnancy. Thus, the *IL17RA* gene can be regarded as a positional and functional candidate gene for litter size in pigs. Currently, there is no published report on the association of the porcine *IL17RA* polymorphisms (especially non-synonymous SNPs or missense mutation) with litter size traits in pigs. Therefore, in this present study, we have verified the non-synonymous polymorphisms in the coding sequence of the porcine *IL17RA* gene, while its effect on litter size traits has also been assessed in Large White and Landrace pig populations.

MATERIALS and METHODS

Animals and DNA extraction

All of the 437 pigs used in this study were raised under commercial conditions of the Betagro Hybrid International Company, Thailand. Blood samples were taken from 178 Large White and 259 Landrace sows. The litter size traits were assessed in 1369 litters of all pigs. The reproductive traits of total number born (TNB), number born alive (NBA), the number of piglets weaned alive (NWA), total birth weight (TBW), and total weaning weight (TWW) of piglets at 21 days were recorded. Moreover, blood samples of Chinese indigenous pigs (Meishan, n=10) were obtained from Department of Animal and Aquatic Sciences, Faculty of Agriculture, Chiang Mai University and were used to evaluate the genotype and allele frequencies of the porcine *IL17RA* polymorphism. The blood samples were used for DNA isolation by the Chelex procedure (Walsh et al., 2013) and stored at 4°C until being analyzed. The experimental methods were approved by the Animal Ethics Committee of Chiang Mai University, Thailand (2562/AG-0001).

Verification of porcine *IL17RA* polymorphisms and genotyping

To verify the SNPs of the porcine *IL17RA* gene, the non-synonymous SNPs of the porcine *IL17RA* gene in the Ensembl database (ENSSSCT00000033369.3, https://asia.ensembl.org) were selected and examined for polymorphism in various pig breeds by *in silico* analysis. The nucleotide sequences of the porcine *IL17RA* gene of 12 pig breeds were retrieved from the Ensembl database, consisting of Large White (ENSSSCT00025073732), Landrace (ENSSSCT00045011988), Berkshire (ENSSSCT00065110244), Hampshire (ENSSSCT00035071005), Pietrain (ENSSSCT00055035992), USMARC (ENSSSCG00070016171), Bamei (ENSSSCT00050011314), Wuzhishan (ENSSSCT00005022403), Jinhua (ENSSSCT00060017210), Rongchang (ENSSSCT 00030063115), Meishan (ENSSSCT00040102728), and Tibetan (ENSSSCT00015103275). The porcine

IL17RA nucleotide sequences of these 12 pig breeds were aligned using the multiple sequence alignment program of CLUSTALW (https://www.genome.jp/tools-bin/clustalw) to find out the non-synonymous SNPs. The variant SNPs were selected, based on a high frequency of polymorphisms and the restriction enzyme available, to examine the polymorphisms of the porcine *IL17RA* gene in pig populations. Thus, the variant SNPs were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The specific primers of the porcine *IL17RA* gene were designed based on relevant nucleotide sequence data of the GenBank (Accession number: NC_010447.5), as shown in Table 1. A mismatched primer was designed in order to generate a recognition site of the restriction enzyme for genotyping (Table 1). The PCR amplification was performed in a reaction volume of 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 each primer (Table 1), and 0.2 U *Taq* DNA polymerase (Fermentas). The PCR conditions were started with a denaturation step at 94°C for 3 min, which followed by 35 cycles consisting 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 PCR products were digested by specific restriction enzymes, as shown in Table 1. The digested PCR products were then separated on 6% polyacrylamide electrophoresis gel and visualized using ethidium bromide staining.

Table 1 Primer sequences and restriction enzymes used for genotyping of the porcine *IL17RA* gene.

SNP position	SNP ID	Location	Nucleotide sequence	Product size (bp)	Tm (°C)	Restriction enzyme
c.785C>T	rs322216152	Exon 9	F*: 5′-CTGAAGGCGTCACAGGACG-3′	101	58	*Tai*I
			R: 5′-CCACGCCCTACCTGCACAT-3′			
c.997G>A	rs330277854	Exon 12	F: 5′-AAAGAGGGGCTGGACCGTG-3′	154	60	*Bts*CI
			R: 5′-ATGACGGAACCCACCAGCA-3′			
c.1962T>G	rs1111750270	Exon 14	F: 5′-TGCTTCTCTCGGAGGAAGG-3′	123	60	*Mbo*I
			R: 5′-TGGGAGGGACCGTCTCCAT-3′			

*Mismatched base is underlined to create a recognition site of the restriction enzyme *Tai*I for genotyping.

Statistical analysis

The genotype and allele frequencies of the porcine *IL17RA* polymorphism were estimated. The chi-square test was used to examine differences in genotype frequencies between pig breeds and to determine the Hardy-Weinberg equilibrium (HWE) within the pig populations. The association between the porcine *IL17RA* polymorphism and the litter size traits were analyzed 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 *IL17RA* genotypes (k = 1, 2, 3), and e_{ijkl} is the residual error. Moreover, additive effect of the porcine *IL17RA* polymorphism was estimated as half difference between the estimated effects of homozygous genotypes and the dominance effect was analyzed 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 *t*-test on significant deviations from zero.

RESULTS

Polymorphisms of porcine *IL17RA* gene

Eight non-synonymous SNPs of the porcine *IL17RA* gene (ENSSSCT00000033369.3), leading to non-conservation amino acid exchange, exist in the Ensembl database (https://asia.ensembl.org), consisting of c.785C>T (p.Ala262Val, rs322216152), c.923A>G (p.Lys308Arg, rs342471585), c.997G>A (p.Val333Ile, rs330277854), c.1962T>G (p.Asp654Glu, rs1111750270), c.2045C>T (p.Thr682Met, rs696060511), c.2082C>A (p.Ser694Arg, rs332799787), c.2135C>T (p.Ala712Val, rs691493622), and c.2435C>A (p.Ala812Asp, rs699434149). Based on the *in silico* analysis results, six non-synonymous SNP loci (c.785C>T, c.923A>G, c.997G>A, c.1962T>G, c.2082C>A, and c.2435C>A) of the porcine *IL17RA* gene were found to be polymorphic among 12 pig breeds (Figure 1). From these, three non-synonymous SNPs (c.785C>T, c.997G>A, and c.1962T>G) showed a high frequency of polymorphism among 12 pig breeds (42%, 42%, and 58%, respectively). Thus, these three SNPs were selected to verify the polymorphisms of the *IL17RA* gene in our pig populations. The porcine *IL17RA* c.785C>T was found to be segregating in the Large White and Landrace pigs. This polymorphic site was detected with the restriction enzyme *Tai*I. Two specific alleles revealed a 101-bp fragment for allele C and two fragments of 84 and 17-bp for allele T, as shown in Figure 2. However, no polymorphisms at the porcine *IL17RA* c.785C>T locus was detected in Meishan pigs. Moreover, no polymorphisms at the porcine *IL17RA* c.997G>A and *IL17RA* c.1962T>G loci were observed in these three pig populations.

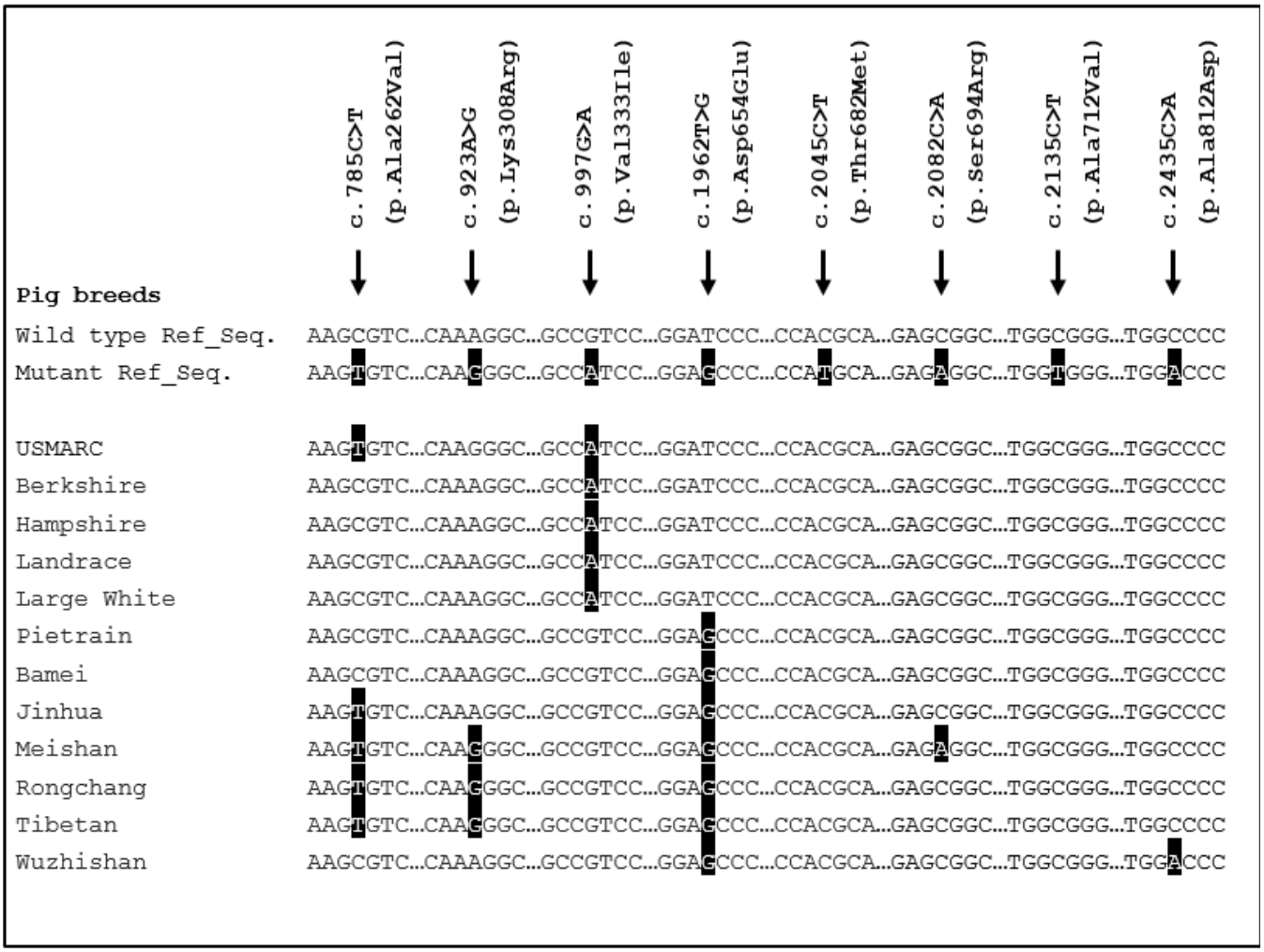

Figure 1 Alignment of nucleotide sequences of the porcine *IL17RA* gene among 12 pig breeds, consisting of USMARC (ENSSSCG00070016171), Berkshire (ENSSSCT00065110244), Hampshire (ENSSSCT00035071005), Landrace (ENSSSCT00045011988), Large White (ENSSSCT00025073732), Pietrain (ENSSSCT00055035992), Bamei (ENSSSCT00050011314), Jinhua (ENSSSCT00060017210), Meishan (ENSSSCT00040102728), Rongchang (ENSSSCT00030063115), Tibetan (ENSSSCT00015103275), and Wuzhishan (ENSSSCT00005022403). The wild type and mutant reference sequences (Ref_Seq.) are ENSSSCT00000033369.3. The non-synonymous polymorphic sites of the porcine *IL17RA* gene are indicated by arrows.

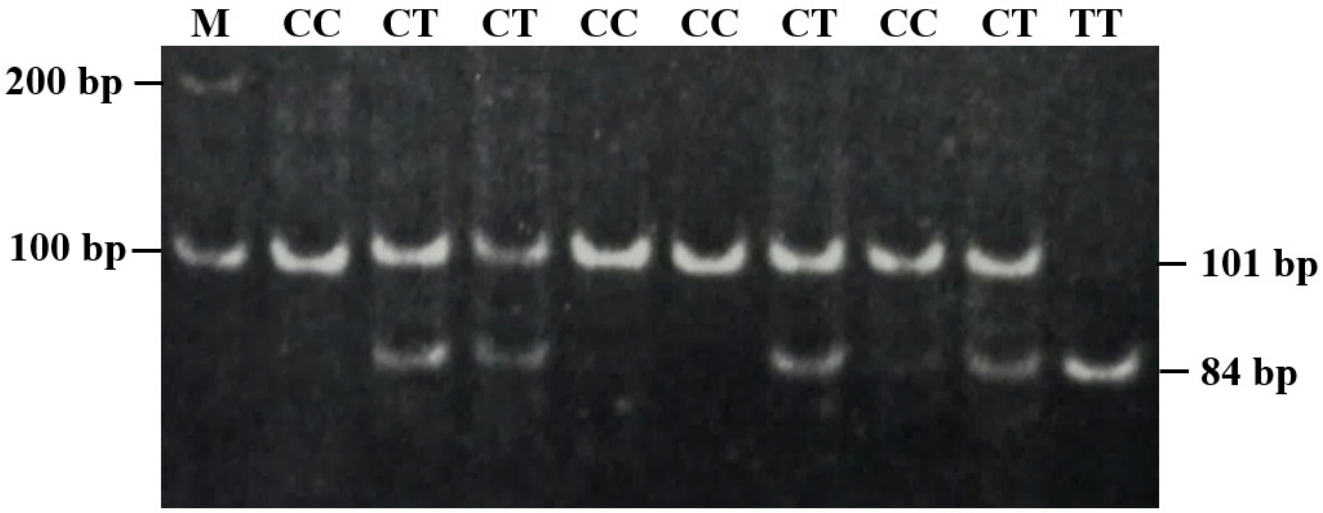

Figure 2 Genotyping of the porcine *IL17RA* gene at c.785C>T locus with *Tai*I. The molecular marker of 100 bp DNA ladder (M) and the genotypes of *IL17RA* c.785C>T are indicated at the top of each line. A 101-bp fragment for allele C and two fragments of 84 and 17-bp for allele T. Notably, the 17-bp fragment is not shown in the gel.

Genotype and allele frequencies

The genotype and allele frequencies of the porcine *IL17RA* c.785C>T locus are shown in Table 2. At the porcine *IL17RA* c.785C>T locus, three genotypes (CC, CT, and TT) were observed in Landrace pigs, whereas two genotypes (CC and CT) were observed in Large White pigs. The CC genotype had higher frequencies than the CT and TT genotypes, as well as the C allele, which had higher frequencies than the T allele in these pig populations. In addition, the porcine *IL17RA* c.785C>T locus was fixed as c.785T in Meishan pigs. The chi-square test showed that the difference in genotype frequency distribution between Large White and Landrace pigs was statistically significant (χ^2 = 53.78, P<0.001). Moreover, the genotype distributions of the porcine *IL17RA* c.785C>T locus within these Large White and Landrace populations deviated from the HWE specifications (P<0.05). Furthermore, the two SNPs at the porcine *IL17RA* c.997G>A and *IL17RA* c.1962T>G loci were fixed as c.997A and c.1962T in Large White and Landrace populations and as c.997G and c.1962G in Meishan pigs.

Table 2 Genotype and allele frequencies of the porcine *IL17RA* c.785C>T gene.

Breeds	*n*	Genotype frequencies			Allele frequencies		*P*-value[1]
		CC	CT	TT	C	T	(χ^2)
Large White	178	0.66	0.34	0.00	0.83	0.17	0.026*
Landrace	259	0.31	0.67	0.02	0.64	0.36	0.001**
Meishan	10	0.00	0.00	1.00	0.00	1.00	-

[1]A significant level of the chi-square (χ^2) test for Hardy-Weinberg equilibrium of porcine *IL17RA* c.785C>T locus in different pig breeds, *P<0.05, **P<0.01.

Associations of porcine *IL17RA* polymorphism with litter size traits

Associations of the porcine *IL17RA* c.785C>T with litter size traits in Large White and Landrace pigs are shown in Tables 3 and 4, respectively. No significant association of porcine *IL17RA* c.785C>T with any litter size traits was found in the first parity of Large White and Landrace pig populations. However, a significant association of the porcine *IL17RA* c.785C>T with litter size traits was found in the later parities of both pig populations. The porcine *IL17RA* c.785C>T was significantly associated with TNB and NBA traits in Large White pigs and TNB, NBA, TBW, and TWW traits in Landrace pigs. In addition, no significant additive and dominance effects on the litter size traits were observed in the Large White and Landrace breeds. Notably, the sows with the TT and CT genotypes had higher litter size values than those of the sows with the CC genotype. Hence, the porcine *IL17RA* c.785T allele seems to be a favorable allele for litter size traits in these pig populations.

Table 3 Association of the porcine *IL17RA* c.785C>T with litter size traits in Large White pigs.

Parity	Traits[1]	Genotypes (means±SE)[2]			Additive	Dominance
		CC	CT	TT		
First parity	*n*	118	60	0		
	TNB	11.11±0.29	11.24±0.29	-	-	-
	NBA	9.33±0.29	9.92±0.28	-	-	-
	NWA	8.40±0.28	9.24±0.27	-	-	-
	TBW	12.90±0.41	15.04±0.40	-	-	-
	TWW	56.00±1.84	61.35±1.76	-	-	-
Later parities	*n*	257	135	0		
(2^{nd} – 8^{th} parities)	TNB	11.06±0.38^{a}	12.05±0.35^{b}	-	-	-
	NBA	9.78±0.38^{a}	10.60±0.34^{b}	-	-	-
	NWA	8.86±0.37	9.63±0.32	-	-	-
	TBW	13.01±0.53	15.56±0.49	-	-	-
	TWW	57.82±2.37	63.91±2.12	-	-	-

[1]*n*: number of investigated litters, TNB: total number born, NBA: number born alive, NWA: number of piglets weaned alive, TBW: total birth weight, and TWW: total weaning weight of piglets at 21 days. TBW and TWW 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 *IL17RA* c.785C>T with litter size traits in Landrace pigs.

Parity	Traits[1]	Genotypes (means±SE)[2]			Additive	Dominance
		CC	CT	TT		
First parity	*n*	81	172	6		
	TNB	9.60±0.44	9.59±0.37	9.70±1.15	-0.05±0.57	-0.06±0.61
	NBA	8.53±0.48	8.35±0.41	8.82±1.25	-0.15±0.61	-0.33±0.66
	NWA	8.12±0.47	7.59±0.41	8.01±1.21	0.06±0.59	-0.48±0.64
	TBW	12.94±0.65	13.37±0.56	12.88±1.68	0.03±0.84	0.46±0.89
	TWW	52.78±2.89	51.53±2.46	52.15±7.32	0.31±3.62	-0.93±3.87
Later parities	*n*	176	347	17		
(2^{nd} – 8^{th} parities)	TNB	9.72±0.42^{a}	10.33±0.37^{b}	10.16±0.87^{ab}	-0.22±0.42	0.38±0.45
	NBA	8.84±0.46^{a}	9.48±0.42^{b}	9.76±0.90^{ab}	-0.46±0.43	0.18±0.46
	NWA	8.19±0.44	8.86±0.41	8.98±0.85	-0.39±0.40	0.27±0.43
	TBW	13.99±0.62^{a}	15.21±0.56^{b}	15.12±1.24^{ab}	-0.56±0.59	0.65±0.64
	TWW	54.11±2.68^{a}	57.90±2.47^{b}	57.97±5.17^{ab}	-1.93±2.44	1.86±2.65

[1]*n*: number of investigated litters, TNB: total number born, NBA: number born alive, NWA: number of piglets weaned alive, TBW: total birth weight, and TWW: total weaning weight of piglets at 21 days. TBW and TWW 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 ($^{a, b}$ $P<0.05$).

DISCUSSION

In the current study, we verified the variation of the non-synonymous SNPs in the porcine *IL17RA* gene and assessed its effects on litter size traits in Large White and Landrace pig populations. Three non-synonymous SNPs (c.785C>T, c.997G>A, and c.1962T>G) of the porcine *IL17RA* gene revealed a high frequency of polymorphisms (>40%) among various Western and Chinese indigenous pig breeds (Large White, Landrace, Berkshire, Hampshire, Pietrain, USMARC, Bamei, Wuzhishan, Jinhua, Rongchang, Meishan, and Tibetan) based on the *in silico* analysis. In this study, the porcine *IL17RA* c.785C>T polymorphism was found to be segregating in our pig populations. However, the porcine *IL17RA* c.997G>A and *IL17RA* c.1962T>G loci were fixed as *IL17RA* c.997A and *IL17RA* c.1962T in these pig populations. Three genotypes of the porcine *IL17RA* c.785C>T polymorphism were detected in Landrace pigs, meanwhile, only two genotypes (CC and CT) were found in Large White pigs. The porcine *IL17RA* c.785C was the major allele in both pig populations. The difference in genotype frequency distribution between these two pig breeds was statistically significant ($P<0.001$) and these may be caused by the different genetic backgrounds of these two pig breeds. Moreover, the chi-square test revealed that the porcine *IL17RA* c.785C>T polymorphism in two populations was a significant deviation from the HWE specifications. These results suggest that there are effects of selective mating based on some desirable production traits that are related to the porcine *IL17RA* c.785C>T polymorphism locus in Large White pigs. In addition, null alleles may be another reason for this pig population deviating from the HWE. Moreover, excess heterozygosity of the

porcine *IL17RA* c.785C>T loci occurred in the Landrace pig population, that might be due to outcrossing among their parent lines and this could have caused populations to be found deviating from the HWE (Namipashaki et al.,2015; Chen et al., 2017).

Although the association of *IL17A* polymorphism with reproduction traits has been scarcely studied, a previous study has demonstrated that the human *IL17RA* (rs4819554) polymorphism is associated with the pregnancy disorder of preeclampsia in a Chinese Han population (Chen et al., 2020). Conversely, there is no association of the human *IL17RA* polymorphism with preeclampsia in Chinese women (Wang et al., 2015). However, the downregulated *IL17RA* expression is related to preeclampsia (Ma et al., 2019). Furthermore, the *IL17RA* gene is hypermethylated in pregnant women with preeclampsia (Halvatsiotis et al., 2019). There is evidence indicating that the *IL17RA* gene is expressed in endometrial and conceptus tissues during early pregnancy (Klein, 2016). Thus, the *IL17RA* gene is assumed to relate to embryonic implantation and maintenance of pregnancy.

The porcine IL17RA peptide sequence corresponds to the human IL17RA peptide sequence with a 68% amino acid identity. It has conserved structural features and consists of a 35-amino acid signal peptide, a 286-amino acid extracellular, a 26-amino acid transmembrane, and a 498-amino acid cytoplasmic domains (UniProtKB accession: K7GT29; UniProtKB database; https://www.uniprot.org/). The extracellular domain of the porcine IL17RA molecule contains six potential N-linked glycosylation sites at the position of asparagine (N) 53, 58, 71, 228, 245, and 268 and six intra-chain disulfide bonds between cysteine residuals at the positions 47-54, 61-129, 188-199, 248-279, 280-306, and 293-297, which are conserved with the human IL17RA receptor (UniProtKB:Q96F46) (Yao et al., 1997). Moreover, the extracellular domain composes of a fibronectin type-III (FnIII) D1 (amino acids; aa 52-202) and FnIII D2 (aa 203-307) domains. The two FnIII domains are linked together with the IL17RC (heteromeric receptor complex) bind to the IL17A and IL17F molecules (Ely et al., 2009; Liu et al., 2013). These binding complexes trigger the intracellular signaling pathways through the activation of transcription factors of the mitogen-activated protein kinases, nuclear factor-kappa B, and CCAAT enhancer-binding protein (Iwakura et al., 2011; Wang et al., 2014; Okamura et al., 2020).

In this study, the porcine *IL17RA* c.785C>T polymorphism was significantly associated with the TNB, NBA, TBW, and TWW traits. The porcine *IL17RA* c.785T allele is positively related to litter size traits. Interestingly, this *IL17RA* c.785T allele is also exhibited in the Chinese Meishan pig (Table 2) which is commonly known to be a high prolificacy breed with low embryonic mortality. These findings suggest that the porcine *IL17RA* c.785T allele seems to be a favorable allele for litter size traits. The porcine *IL17RA* c.785C>T polymorphism was a non-synonymous SNP leading to a non-conservation amino acid exchange at position p.Ala262Val of exon 9. Although this variant p.Ala262Val of the porcine *IL17RA* gene has not yet been functionally identified, it is located within the extracellular FnIII D2 of the IL17RA molecule which is involved in binding of the IL17A and IL17F molecules and it is also located close to the N-linked glycosylation sites and disulfide bonds which are involved in signal transduction and post-translation

modification process. Moreover, the porcine *IL17RA* is located (SSC5, 69.4 Mb) within the QTL regions for TNB, NBA, the number of stillborn, and the total litter weight (Cassady et al., 2001; Schneider et al., 2012; Bergfelder-Drüing et al., 2015; He et al., 2017; Zhang et al., 2019). Therefore, it could be expected that the observed amino acid change of the porcine *IL17RA* (p.Ala262Val) might promote the functional explanation of the reproductive process or might be in the close linkage of disequilibrium with the causal SNPs of a positive effect on litter size traits in pigs.

Moreover, the interaction of IL17RA and its ligand molecules may be involved in the regulation of reproductive function in animals. It has been reported that the mice deficient in *IL17RA* and *IL17RC* fail to respond to both IL17A and IL17F (Iwakura et al., 2011), as well as the specific mutations in functional motifs of the *IL17RA* gene, were exhibited to impair cells' response to IL17A molecule (Gaffen, 2009; Krstic et al., 2015). Moreover, previous studies have demonstrated that the human *IL17A* and *IL17F* polymorphisms, coding for ligands of the IL17RA and IL17RC molecules, are associated with recurrent pregnancy loss (Najafi et al., 2014; Zidan et al., 2015). Obviously, the serum circulation of IL17 can induce abortion in mammals. A previous study has exhibited that the exogenous recombinant IL17 induced fetal loss in a regular mouse model, whereas an anti-IL17 neutralizing antibody prevented the fetal loss in an abortion mouse model (Xu et al., 2016). Moreover, higher serum IL17 levels corresponded to the early fetal loss in mice (Li et al., 2013). Similarly, the imbalance expressions of IL17, IL10, and TGF-beta induced chronic endometritis and related to the recurrent implantation failure in women (Wang et al., 2019). However, the IL17 which also expressed in the human placenta may play a key role in angiogenesis, placental development, and/or immunoregulation in the establishment of pregnancy (Pongcharoen et al., 2007). Furthermore, the IL17 and IL17R may have a regulatory role in trophoblast invasion by increasing progesterone secretion (Pongcharoen et al., 2006; Pongcharoen and Supalap, 2009) and these two genes are exhibited in endometrial and conceptus tissues during pre-implantation (Klein, 2016). It is possible that the IL17 acts upon endometrium and contributes to the maintenance of pregnancy by binding to its receptors (Klein, 2016). These pieces of evidence suggest that the *IL17RA* and its ligand may be necessary for the reproductive process, especially embryonic attachment, implantation, and maintenance of pregnancy, in animals and humans. However, further study is needed in order to clarify the role of *IL17RA* and its ligand in implantation and embryo survival. Moreover, the association of porcine *IL17RA* gene including its ligand with litter size traits is required to confirm in various pig populations.

CONCLUSION

The polymorphism of the porcine *IL17RA* gene was verified and its effects on litter size traits were elucidated in commercial pigs. The porcine *IL17RA* c.785C>T had obvious effects on litter size traits in Large White and Landrace pig populations. These results promote the emphasis of the porcine *IL17RA* gene in the litter size traits of pigs. Thus, the porcine *IL17RA* could be used as a potential candidate gene for improving litter size traits in pig breeding.

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AUTHORS' CONTRIBUTIONS

Worrarak Norseeda; Methodology, investigation, data curation, writing - original draft.
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Tawatchai Teltathum; Methodology, investigation, writing - review and editing.
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Watcharapong Naraballobh; Formal analysis, writing - review and editing.
Trisadee Khamlor; Data curation, formal analysis, writing - review and editing.
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