Effect of leukemia inhibitory factor polymorphism on litter size traits in Thai commercial pig breeds

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Abstract

Leukemia inhibitory factor (LIF) is a crucial candidate gene that impacts on implantation process. In this study, the effects of the porcine LIF polymorphism on litter size traits were elucidated in Thai commercial pig populations. Genotyping of three single nucleotide polymorphisms (SNPs) of the porcine LIF gene was detected in coding and 3’-untranslated regions. The porcine LIF c.*24C>T was segregating in Large White, Landrace, and Large White × Landrace (LW × LR) crossbred sows. No polymorphisms at two non-synonymous SNPs loci (LIF c.28C>A and LIF c.161A>G) were found in this study. The porcine LIF c.*24C>T was significantly associated with the total number born (TNB), the number born alive (NBA), and the number of piglets weaned alive (NWA) traits in Large White and Landrace sows. Moreover, the porcine LIF c.*24C>T was associated with the NBA and NWA traits in LW × LR crossbred sows. The favorable LIF c.*24C allele was positively correlated with the litter size traits. These findings indicated that the polymorphism of the porcine LIF gene was associated with litter size traits and confirms the significance of porcine LIF as a candidate gene for litter size traits in pig breeding. Thus, the porcine LIF gene could be used for improving prolific traits in these Thai commercial pig populations.

Keywords: LIF, Pig, Litter size, SNPs

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INTRODUCTION

Litter size is the main determinant influencing the productivity and economics of pig production (Buske et al., 2006; Nielsen et al., 2013). Embryonic mortality during the implantation period is a critical factor in determining litter size in pigs (Spötter and Distl, 2006; Lin et al., 2009; Chen et al., 2015). Leukemia inhibitory factor (LIF) is a pleiotropic cytokine of the interleukin 6 family that is produced by the endometrium and blastocyst (Suman et al., 2013; Salleh and Giribabu, 2014) and regulates several cellular functions (Haines et al., 2000; Mathieu et al., 2012). LIF is related to the implantation, the survival of cells, and blastocyst development in humans (Dunglison et al., 1996; Nachtigall et al., 1996; Devjak et al., 2016), mice (Stewart et al., 1992; Stewart, 1994; Cheng et al., 2001; Robb et al., 2002; Fouladi-Nashta et al., 2005; Kobayashi et al., 2014), and pigs (Blitek et al., 2012; Kim et al., 2015; Yoo et al., 2019). LIF is contributed to providing the uterine environment for blastocyst implantation and growth of embryos (Rodríguez et al., 2007). Expression of LIF gene in endometrium increases during implantation (Salleh and Giribabu, 2014). The expression of LIF gene in the uterine endometrium during implantation was up-regulated and reached its highest levels in the porcine endometrial surface epithelial cells on day 24 of pregnancy (Chen et al., 2015). Additionally, the expression profiling of the LIF gene in the endometrium of humans and pigs was regulated in a stage-specific manner during pregnancy (Chen et al., 2015; Kim et al., 2015; Devjak et al., 2016; Yoo et al., 2019).

The LIF gene is mapped on the Sus scrofa chromosome 14 (47,221,560-47,238,749 bp, ENSSSCG000000040961; Ensembl Sscrofa 11.1). It consists of 4 exons and 3 introns and encodes for 202 amino acids of the peptide sequence. Moreover, the porcine LIF gene is located closely with the QTL regions for total number born alive (37.5-38.5 Mb), total number born (41.8-42.0 Mb), and total litter weight (56.4-56.6 Mb) (Onteru et al., 2012; Schneider et al., 2012). Additionally, significant associations of the porcine LIF polymorphism with litter traits have been reported in several pig breeds (Spötter et al., 2005; Lin et al., 2009; Spötter et al., 2009; Mucha et al., 2013; Leonova et al., 2015; Ding et al., 2020). All these shreds of evidence indicated that the LIF function was critical for embryonic implantation and embryonic survival. Thus, the porcine LIF gene has reasonable reliability to be used as a candidate gene for the litter size traits of pigs. However, the effects of the porcine LIF gene on prolific traits have not been reported in Thai commercial pig populations. This study aimed to elucidate the effects of porcine LIF polymorphisms on the litter size traits in Thai commercial pig populations.

MATERIALS and METHODS

Animals and DNA extraction

Blood samples were taken from a total of 610 Thai commercial pigs (132 Large White, 143 Landrace, and 335 Large White × Landrace crossbred sows). All pigs were obtained from the Betagro Hybrid International Company, Thailand and were reared under commercial conditions. The litter size traits of sows were assessed in 443, 429, and 1669 litters for Large White, Landrace,
and Large White × Landrace pig breeds, respectively. The reproductive traits of the sows were recorded in terms 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 (Walsh et al., 2013) and stored at 4°C until being analyzed.

**Single nucleotide polymorphism of the porcine LIF gene and genotyping**

To verify the polymorphisms of the porcine LIF gene, three polymorphic sites on porcine LIF were selected based on the restriction enzymes available in the Ensembl database (ENSSSCT00000044908.2; http://asia.ensembl.org/index.html) and a previous study (Spötter et al., 2005), consisting of two non-synonymous SNPs (c.28C>A, rs710637571 and c.161A>G, rs706594954) and a 3’-untranslated region (3’-UTR) SNP (c.*24C>T, rs322167972) corresponding to g.6988C>T as ascribed by a previous study (Spötter et al., 2005). The specific primers of the porcine LIF were designed based on the nucleotide sequence in the GenBank (Accession No. NC_010456.5), as shown in Table 1. These primers were used to genotype the Thai commercial pig breeds of Large White, Landrace, and LW × LR crossbred. The polymerase chain reaction (PCR) was carried out in a reaction volume of 20 μL consisting of 50 ng of a genomic DNA sample, 1×(NH₄)₂SO₄ buffer, 0.2 mM dNTPs, 1.5 mM MgCl₂, 0.4 μM each primer, and 0.2 U Taq DNA polymerase (Fermentas). The PCR conditions were used as the initial denaturing stage at 95 °C for 3 min, followed by 32 cycles of denaturation at 95 °C for 30 sec, annealing at 58-60 °C for 30 sec, elongation at 72 °C for 45 sec, and then 5 min at 72 °C to complete the reaction. The PCR products of the porcine LIF gene were digested with restriction enzymes (Table 1). The digested products were electrophoresed on 6% polyacrylamide gels and stained with ethidium bromide for visualization.

| SNP position | Location | Primer sequence | Product size (bp) | Tm (°C) | Restriction enzyme |
|--------------|----------|-----------------|------------------|---------|------------------|
| c.28C>A      | Exon 3   | F: 5´- ACACTGGGTGTCCTCAAAGGA -3´ R: 5´- TGACAGGGATGTGGAAGG -3´ | 173     | 60      | BseSI           |
| c.161A>G     | Exon 3   | F: 5´- ATGAACCAGATCAAGAACCAG -3´ R: 5´- TCTGCACTAGTGAGCCGA -3´ | 205     | 60      | AluI            |
| c.*24C>T     | 3’-UTR   | F: 5´- TCCTGGGGAAGTATAAGCAG -3´ R: 5´- GCCTTCTCTCTGTTCTG -3´ | 262     | 58      | DraIII          |
Statistical analyses

Allele and genotype frequencies of the porcine LIF polymorphism were estimated. Hardy-Weinberg equilibrium (HWE) was examined with the chi-square test. Effects of the porcine LIF polymorphism on 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, $\mu$ 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 $\geq 2$), $G_k$ is the fixed effect of the LIF genotypes ($k = 1, 2, 3$), and $e_{ijkl}$ is the residual error. Moreover, additive effect of the porcine LIF polymorphism was calculated as half difference between the two homozygous genotypes and the dominance effect was estimated as the deviation of the heterozygous genotype effect from the mean effect of the two homozygous genotypes (Falconer and Mackay, 1996; Muñoz et al., 2007). The effects were estimated by $t$-tests on significant deviations from zero.

RESULTS

Polymorphisms of the porcine LIF gene

The porcine LIF c.28C>A, c.161A>G, and c.*24C>T loci were genotyped in Thai commercial pig breeds. The polymorphic site of the porcine c.*24C>T locus was detected with the restriction enzyme DraIII. Two specific alleles revealed a 262-bp fragment for allele C and two fragments of 190 and 72-bp for allele T. Genotype patterns for the porcine LIF c.*24C>T polymorphism in Thai commercial pig populations were indicated in Figure 1. However, no polymorphisms of the porcine LIF c.28C>A (p.Pro10Thr) and c.161A>G (p.Gln54Arg) loci were found in this study.

![Figure 1](image)

**Figure 1** Genotyping SNP of porcine LIF c.*24C>T locus with DraIII. The molecular marker of 100 bp DNA ladder (M) and the genotypes of porcine LIF c.*24C>T SNP are indicated at the top of each line. A 262-bp fragment for allele C and two fragments of 190 and 72-bp for allele T. Notably, the 72-bp fragment is not shown in the gel.
Genotype and allele frequencies

The genotype and allele frequencies of the porcine LIF c.*24C>T polymorphism of Thai commercial pig populations were indicated in Table 2. Three genotypes were segregating in Large White, Landrace, and LW × LR crossbred sows. The CC and CT genotypes had higher frequencies than the TT genotype and the C allele had higher frequencies than the T allele in these pig populations. However, the porcine LIF c.28C>A and c.161A>G SNPs were fixed as c.28C and c.161A (data not shown). The chi-square test revealed that the genotype distributions of the porcine LIF c.*24C>T locus in Large White and Landrace sows agreed with the HWE specifications. In contrast, the genotype distributions of the porcine LIF c.*24C>T locus in LW × LR crossbred sows deviated from the HWE specifications (P<0.05).

Table 2 Genotype and allele frequencies of the porcine LIF c.*24C>T.

| Breeds                | n    | Genotype frequencies | Allele frequencies | Chi-square test* |
|-----------------------|------|----------------------|--------------------|-------------------|
|                       |      | CC   | CT  | TT  | C   | T   | (χ²) |
| Large White (LW)      | 132  | 0.54 | 0.37| 0.09| 0.72| 0.28| 0.69 |
| Landrace (LR)         | 143  | 0.42 | 0.43| 0.15| 0.63| 0.37| 0.96 |
| LW × LR crossbred     | 335  | 0.37 | 0.41| 0.22| 0.58| 0.42| 9.43*|

1 A significant level of the chi-square (χ²) test for Hardy-Weinberg equilibrium of porcine LIF c.*24C>T locus in different pig breeds, *P<0.05.

Effects of the porcine LIF gene polymorphism on litter size traits

Effects of the porcine LIF c.*24C>T polymorphism on litter size traits in Thai commercial pig populations were indicated in Tables 3, 4, and 5. There was no significant association of the porcine LIF c.*24C>T polymorphism with any litter size traits in the first parity of these three Thai commercial pig populations. However, the porcine LIF c.*24C>T polymorphism was significantly associated with TNB, NBA, and NWA traits in later parities of Large White and Landrace sows. Moreover, the porcine LIF c.*24C>T polymorphism was significantly associated with NBA and NWA traits in later parities of LW × LR crossbred sows. Notably, the sows with the CC genotype had higher TNB, NBA, and NWA values compared to those of the sows with the CT and TT genotypes. Thus, the porcine LIF c.*24C allele seems to be a favorable allele for litter size traits in these Thai commercial pig populations. The significant additive effects of NBA and NWA traits were observed in later parities of Large White sows. Moreover, the significant dominance effects of NBA trait were detected in later parities of Large White sows. The significant additive effects of TBA, NBA, and NWA traits were observed in later parities of Landrace sows, however the later parities of LW × LR crossbred sows only showed additive effects of NWA trait.
### Table 3: Association of the porcine LIF c.*24C>T with litter size traits in Large White sows.

| Parity                  | Traits | Genotypes (means±SE) | Additive | Dominance |
|-------------------------|--------|----------------------|----------|-----------|
|                         |        | CC                   | CT                   | TT                   |           |           |
| First parity            |        |                      |                      |                      |           |           |
|                         | $n$    | 71                   | 49                   | 12                   |           |           |
|                         | TNB    | 11.39±0.37           | 10.70±0.44           | 10.27±0.87           | 0.15±0.54 | -0.17±0.74 |
|                         | NBA    | 10.04±0.35           | 9.01±0.42            | 9.18±0.82            | 0.43±0.44 | -0.60±0.59 |
|                         | NWA    | 8.93±0.33            | 8.14±0.40            | 7.50±0.78            | 0.71±0.41 | -0.07±0.56 |
|                         | MBW    | 1.38±0.03            | 1.37±0.04            | 1.41±0.06            | -0.01±0.03 | -0.03±0.05 |
|                         | MWW    | 6.57±0.04            | 6.53±0.04            | 6.47±0.08            | 0.05±0.04 | 0.01±0.06  |
| Later parities          | (2nd – 8th parities) |                      |                      |                      |           |           |
|                         | $n$    | 151                  | 130                  | 30                   |           |           |
|                         | TNB    | 12.26±0.35           | 10.94±0.38           | 10.92±0.69           | 0.67±0.36 | -0.65±0.47 |
|                         | NBA    | 10.83±0.32           | 9.47±0.33            | 9.46±0.60            | 0.68±0.31 | -0.68±0.42 |
|                         | NWA    | 10.11±0.29           | 8.67±0.31            | 8.89±0.56            | 0.61±0.29 | -0.84±0.38 |
|                         | MBW    | 1.50±0.03            | 1.52±0.03            | 1.55±0.07            | -0.02±0.04 | 0.01±0.04  |
|                         | MWW    | 6.60±0.03            | 6.59±0.04            | 6.56±0.05            | 0.02±0.03 | 0.01±0.03  |

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.

2Means±SE represent least square means±standard error. Values in each row with differing superscripts are considered significantly different (A, B $P<0.01$), *$P<0.05$.

### Table 4: Association of the porcine LIF c.*24C>T with litter size traits in Landrace sows.

| Parity                  | Traits | Genotypes (means±SE) | Additive | Dominance |
|-------------------------|--------|----------------------|----------|-----------|
|                         |        | CC                   | CT                   | TT                   |           |           |
| First parity            |        |                      |                      |                      |           |           |
|                         | $n$    | 60                   | 61                   | 22                   |           |           |
|                         | TNB    | 11.21±0.42           | 10.21±0.39           | 11.34±0.65           | -0.07±0.36 | -1.06±0.51 |
|                         | NBA    | 9.74±0.43            | 9.17±0.39            | 9.62±0.65            | 0.06±0.36 | -0.51±0.52 |
|                         | NWA    | 9.12±0.42            | 8.69±0.39            | 8.83±0.53            | 0.15±0.35 | -0.28±0.51 |
|                         | MBW    | 1.50±0.03            | 1.51±0.03            | 1.43±0.05            | 0.03±0.03 | 0.04±0.04  |
|                         | MWW    | 6.46±0.04            | 6.52±0.04            | 6.54±0.06            | -0.04±0.03 | 0.02±0.05  |
| Later parities          | (2nd – 8th parities) |                      |                      |                      |           |           |
|                         | $n$    | 101                  | 143                  | 42                   |           |           |
|                         | TNB    | 11.86±0.40           | 11.07±0.36           | 10.32±0.56           | 0.77±0.31* | -0.02±0.43 |
|                         | NBA    | 10.93±0.40           | 9.77±0.36            | 9.66±0.55            | 0.63±0.29* | -0.53±0.41 |
|                         | NWA    | 10.21±0.38           | 9.17±0.34            | 8.81±0.53            | 0.70±0.28* | -0.34±0.39 |
|                         | MBW    | 1.53±0.03            | 1.57±0.03            | 1.61±0.04            | -0.04±0.02 | -0.01±0.03 |
|                         | MWW    | 6.57±0.04            | 6.56±0.03            | 6.64±0.05            | -0.03±0.03 | -0.04±0.03 |

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.

2Means±SE represent least square means±standard error. Values in each row with differing superscripts are considered significantly different ($^a$,$^b$ $P<0.05$, A, B $P<0.01$), *$P<0.05$. 

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### Table 5: Association of the porcine \textit{LIF} c.*24C>T with litter size traits in LW × LR crossbred sows.

| Parity          | Traits | Genotypes (means±SE) | Additive       | Dominance       |
|-----------------|--------|----------------------|----------------|-----------------|
|                 |        | CC       | CT       | TT    |                   |                 |
|                 |        |          |          |       |                   |                 |
| First parity    |        |          |          |       |                   |                 |
|                 |        | 123      | 137      | 75    | 0.18±0.21         | 0.37±0.32       |
|                 | TNB    | 10.35±0.37 | 10.55±0.38 | 10.00±0.44 | 0.13±0.20 | 0.62±0.31 |
|                 | NBA    | 9.01±0.37   | 9.49±0.38   | 8.75±0.43   | 0.12±0.20 | 0.50±0.31 |
|                 | NWA    | 8.41±0.36   | 8.79±0.37   | 8.16±0.43   | -0.02±0.02 | -0.02±0.03 |
|                 | MBW    | 1.52±0.03   | 1.52±0.04   | 1.55±0.04   | -0.01±0.04 | -0.03±0.06 |
|                 | MWW    | 6.24±0.07   | 6.21±0.07   | 6.25±0.09   | -0.01±0.04 | -0.03±0.06 |
| Later parities  |        |          |          |       |                   |                 |
| (2nd – 8th parities) | n  | 487      | 583      | 264    | 0.21±0.14         | 0.19±0.21       |
|                 | TNB    | 11.63±0.35  | 11.61±0.34  | 11.21±0.38  | 0.25±0.13 | 0.20±0.19 |
|                 | NBA    | 10.58±0.35\(^a\) | 10.53±0.34\(^b\) | 10.08±0.37\(^b\) | 0.26±0.12* | 0.09±0.18 |
|                 | NWA    | 9.83±0.34\(^a\) | 9.66±0.33\(^b\) | 9.30±0.36\(^b\) | 0.01±0.01 | -0.01±0.02 |
|                 | MBW    | 1.62±0.03   | 1.61±0.03   | 1.61±0.04   | 0.06±0.02  | -0.03±0.03 |
|                 | MWW    | 6.33±0.07   | 6.23±0.07   | 6.20±0.07   | -0.01±0.04 | -0.03±0.06 |

1: 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 (\(^a\)P<0.05), \(^b\)P<0.05

### DISCUSSION

In the present study, we verified the porcine \textit{LIF} polymorphisms and assessed its effects on litter size traits in Thai commercial pig populations. The porcine \textit{LIF} c.*24C>T polymorphism was segregating in three Thai commercial pig populations and three possible genotypes were observed. The porcine \textit{LIF} c.*24C was the major allele in these pig populations. These results are agreed with previous studies, which showed that the porcine \textit{LIF} c.*24C was a major allele in several pig breeds such as German Landrace (Spötter et al., 2009), Large White (Leonova et al., 2015), and four Chinese pig breeds (Ding et al., 2020). However, the porcine \textit{LIF} c.*24T was the major allele in German synthetic line (Spötter et al., 2005), German Large White (Spötter et al., 2009), Polish Large White and Polish Landrace (Mucha et al., 2013), and Landrace and Duroc (Leonova et al., 2015). These data indicated that the different genetic backgrounds or population structure of pig breeds may have an effect on the porcine \textit{LIF} allele frequencies in those pig populations. The chi-square analysis results revealed that the porcine \textit{LIF} c.*24C>T locus in Large White and Landrace populations met the HWE specifications. These data indicated that the different genetic backgrounds or population structure of pig breeds may have an effect on the porcine \textit{LIF} allele frequencies in those pig populations. The chi-square analysis results revealed that the porcine \textit{LIF} c.*24C>T locus in Large White and Landrace populations met the HWE specifications.
The porcine $LIF$ gene is localized on chromosome 14 closely with the QTL regions for TNB, NBA, and total litter weight (Onteru et al., 2012; Schneider et al., 2012). Moreover, the porcine $LIF$ c.*24C>T polymorphism was found in the 3’-UTR sequence that can be a significant regulatory region binding transcription factors and influencing $LIF$ gene expression (Mucha et al., 2013). In our study, the porcine $LIF$ c.*24C>T had pleiotropic effects on litter size traits in three Thai commercial pig populations. The porcine $LIF$ c.*24C was a favorable allele to increase litter size. This result agreed with previous studies which found the porcine $LIF$ c.*24C to be a favorable allele for litter size traits in several pig breeds (Spöttner et al., 2005; Lin et al., 2009; Leonova et al., 2015; Ding et al., 2020). However, at porcine $LIF$ c.*24C>T locus of the Polish Large White and Polish Landrace pigs with TT genotype had higher litters compared with CC and CT genotypes (Mucha et al., 2013). These contrasting results might be caused by the different genetic background effects of those pig populations. However, in this study the porcine $LIF$ c.*24C>T polymorphism revealed a significant association with the TNB, NBA, and NWA traits in Thai commercial pig breeds. Thus, we hypothesized that this SNP might affect the transcription process (Mucha et al., 2013) and might be in linkage of disequilibrium with causal SNPs that may be located in QTL regions for litter size traits.

The polymorphisms of the $LIF$ gene in mice and humans lead to defect in maternal reproduction attributable to a failure of implantation (Steck et al., 2004; Mikolajczyk et al., 2007; Paiva et al., 2009; Mojarrad et al., 2013). The human $LIF$ polymorphism is implicated in membrane-associated, diffusible, and truncated forms that act as paracrine factors in embryonic implantation (Aghajanova, 2004; Salleh and Giribabu, 2014). In addition, the expression of $LIF$ plays a critical role in blastocyst implantation to the endometrium in humans (Leduc et al., 2012). Uterine expression of $LIF$ in humans and rabbits is suggested to exhibit a key function in embryo implantation (Cullinan et al., 1996; Lei et al., 2004). Defective expression of $LIF$ in mice embryo reaches the blastocyst stage but cannot implant into the uterus (Lee et al., 2005; Moodley et al., 2010; Mojarrad et al., 2013). All these shreds of evidence imply that the expression of $LIF$ in the reproductive organs of various mammal species might play an important role through an autocrine and paracrine between the mother and the conceptus and may contribute to success of embryo implantation process (Raheem, 2018). Moreover, the gene expression profiling in uterine endometrium was studied in pigs (Chen et al., 2015). The results displayed that the $LIF$ gene was expressed in the immune response process during the implantation of pigs. It may indicate that the $LIF$ gene relates to preventing fetal rejection, resulting in a more successful implantation (Chen et al., 2015). These findings suggested that the polymorphism of the $LIF$ gene may contribute to embryonic implantation and related to litter size traits of pigs. These results in this study confirm that the porcine $LIF$ c.*24C>T polymorphism is correlated with TNB, NBA, and NWA traits in Thai commercial pig breeds. This result implies that the porcine $LIF$ gene could be involved in the reproductive traits especially litter size traits.
CONCLUSION

In the current study, we verified the polymorphism of porcine LIF gene and elucidated its effect on litter size traits in Thai commercial pig breeds. The porcine LIF c.*24C>T polymorphism showed significant effects on the litter size traits in Thai commercial pig populations. Obviously, the favorable LIF c.*24C allele was positively related to the litter size traits. Therefore, the porcine LIF can be used as a candidate gene for prolific traits in these Thai commercial pig breeds.

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AUTHORS CONTRIBUTION

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; Data curation, formal analysis, writing - review and editing.
Trisadee Khamlor; 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 OF INTEREST

The authors declare that no conflict of interest.
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