Association of IL-4 and IL-4R Polymorphisms with Litter Size Traits in Pigs

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Simple Summary: The IL-4 and IL-4R cytokine genes are responsible for immune response in the reproductive system and are related to embryonic implantation and fetal survival during pregnancy in females. However, to date, their effects on litter size traits in pigs have been not elucidated. Therefore, the present study was conducted to verify the porcine IL-4 and IL-4R polymorphisms and assess how they affect litter size traits in commercial pigs. The findings suggested that the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T polymorphisms were associated with the litter size traits. Therefore, the porcine IL-4 and IL-4R genes may be potentially effective genetic markers to improve the litter size traits in pigs.

Abstract: The interleukin-4 (IL-4) and interleukin-4 receptor (IL-4R) are cytokines that are involved in the immune and reproductive systems. This study aimed to verify the polymorphisms in the porcine IL-4 and IL-4R genes and to assess their effects on litter size traits in commercial pigs. Single nucleotide polymorphisms (SNPs) in the porcine IL-4 and IL-4R genes were genotyped by the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. A non-coding SNP of IL-4 g.134993898T > C and a non-synonymous SNP of IL-4R c.1577A > T (amino acid change at position 526, Q526L) were found to be segregating in Landrace sows. The IL-4 polymorphism was significantly associated with the number of piglets weaned alive (NWA) trait. Therefore, the present study was conducted to verify the porcine IL-4 and IL-4R polymorphisms with Litter Size Traits in Pigs. Animals 2021, 11, 1154. https://doi.org/10.3390/ani11041154
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1. Introduction

Litter size traits are among the most important traits for breeding in commercial pig production. Increasing litter size is of great economic interest as a means of enhancing the productivity of the pig industry [1–3]. Major limitations to increasing litter size in pigs are embryonic mortality and fetal losses during the pregnancy period [4,5]. Successful pregnancy depends on coordinated and precisely regulated cellular and molecular processes of the conceptus and the maternal endometrium for the establishment of pregnancy. Communication between the conceptus and the endometrium is mediated by cytokines and cell surface receptors [6], and it is essential for embryo implantation, placental development, and the effective maintenance of pregnancy [7,8]. Several cytokine genes involved in embryo attachment and implantation that affect the relevant litter size traits have also been identified in pigs, e.g., erythropoietin-producing hepatocellular A4 (EphA4) [5], ring finger protein 4 gene (RNF4) [9], leukemia inhibitory factory (LIF) [10], interleukin-6 (IL-6) [11], and osteopontin (OPN) [12]. Currently, numerous studies demonstrate that interleukin-4 (IL-4) and its receptor (IL-4 receptor, IL-4R) play an important role in embryo implantation and pregnancy in pigs [13,14] and humans [15].

The IL-4 is an anti-inflammatory cytokine that is mainly produced by T-helper (Th) 2 cells, natural killer (NK) cells, mast cells, and basophils [16]. It is involved in the regulation of immune system responses [17]. The expression levels of IL-4 mRNAs are increased in the uterine endometrium during implantation in pigs and humans [13–15]. It has been documented that a deficiency of IL-4 cytokine can lead to infertility and various pregnancy disorders in mammals [18]. The IL-4 gene has been mapped on the long arm of the *Sus scrofa* chromosome 2 (SSC2q) and is composed of four exons and three introns that are encoded with a peptide of 133 amino acids (ENSSCG0000075333; Ensembl Sscrofa 11.1; https://asia.ensembl.org/Sus_scrofa/Info/Index, accessed on 2 September 2020). Besides, the porcine IL-4 gene is closely located (SSC2, 134.9 Mb) to the quantitative trait loci (QTL) regions for ovulation rates (128.8–145.1 Mb), total number born (139.9–140.9 Mb), mummified fetuses (133.4–145.1 Mb), numbers of stillborn (128.5–137.1 Mb), the mortality of piglets (138.7–143.7 Mb), gestation length (153.0 Mb), and age at puberty (133.3–157.3 Mb) [19–22].

The IL-4R is an immune gene that encodes the alpha-chain of the IL-4R molecule, a type-I transmembrane protein that can bind IL-4 and IL-13, and mediates its effect through kinases of the Janus kinase (JAK) family, leading to tyrosine phosphorylation of several substrates in cells [28,29]. The IL-4R mRNAs are expressed in the endometrium and conceptus [30,31]. The IL-4R gene has been mapped on *Sus scrofa* chromosome 3 (SSC3). It contains ten exons and nine introns that are encoded with a peptide of 824 amino acids (ENSSCG0000007817; Ensembl Sscrofa 11.1; https://asia.ensembl.org/Sus_scrofa/Info/Index, accessed on 2 September 2020). Moreover, the porcine IL-4R gene is closely located (SSC3, 19.5 Mb) to the QTL regions for total number born (15.3–20.0 Mb), litter birth intervals (11.9–33.7 Mb), and age at puberty (11.1–27.20 Mb) [20,22,32]. The polymorphisms of the porcine IL-4R gene have been characterized and reported in the Ensembl database (https://asia.ensembl.org/Sus_scrofa/Info/Index, accessed on 2 September 2020). Moreover, the polymorphisms of *IL4R* gene are associated with the pregnancy disorders and various cancer types in humans [27,33–36].
All these shreds of evidence suggest that the *IL-4* and *IL-4R* genes are responsible for immune response in the reproductive system of mammals. Moreover, their functions are critical for embryonic implantation and fetal survival during pregnancy, as well as their positions, which are closely located to the QTLs for reproductive traits in pigs. Therefore, the *IL-4* and its receptor (*IL-4R*) genes can be regarded as positional and functional candidate genes for the determination of the reproductive traits of pigs. However, information on the association of the *IL-4* and *IL-4R* genes with litter size traits in pigs has been limited. Thus, the porcine *IL-4* and *IL-4R* SNPs were selected based on their segregation in the pig population to elucidate their association with litter size traits. In the present study, we have verified the polymorphisms in porcine *IL-4* and *IL-4R* genes, while their association with litter size traits was assessed in commercial pigs.

2. Materials and Methods

2.1. Animals and DNA Extraction

Blood samples were taken from a total of 323 sows of the Landrace pig breed. These Landrace breeding stocks were obtained from a commercial nucleus herd that is established for improved growth performance and reproductive traits. All sows were reared under commercial conditions of the Betagro Hybrid International Company, Thailand. These sows were kept in closed houses with an evaporative cooling system and were fed a corn-soybean-based diet containing 16% crude protein and 3388 kcal/kg digestible energy. The reproductive performance traits of the sows were assessed in 1162 litters (1 to 8 parities) and were recorded in terms of litter size. These traits consisted of total number born (TNB), number born alive (NBA), the 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 [37] and kept at 4 °C until analysis.

2.2. Verification of Porcine IL-4 and IL-4R Polymorphisms and Genotyping

To verify the SNPs in the porcine *IL-4* gene, specific primers were designed based on the available nucleotide sequence information (GenBank accession number: NC_010444.3) to cover four exons and three introns of the porcine *IL-4* gene, as shown in Table 1. Ten DNA samples were selected from the Landrace population with the five highest and five lowest TNB values and were used to amplify the DNA segments of the porcine *IL-4* gene using each primer (Table 1). The amplicons of the porcine *IL-4* gene were sequenced using the CEQ 8000 Genetic Analysis System (Beckman-Coulter, Brea, CA, USA) to find out the SNPs segregating in this pig population. To verify the SNPs in the porcine *IL-4R* gene, five non-synonymous SNPs (c.163G > A, c.242C > T, c.623G > A, c.1016G > T, and c.1577A > T) of the porcine *IL-4R* gene were selected based on the restriction enzymes available in the Ensembl database (ENSSSCT00000008566.4; http://asia.ensembl.org/index.html, accessed on 2 September 2020). These were used to verify the SNP in the Landrace pig population. The specific primers of the porcine *IL-4R* gene were designed based on relevant nucleotide sequence information (GenBank accession number: NC_010445.3), as shown in Table 2. The verified SNPs of the porcine *IL-4* and *IL-4R* genes were used purposely for association analysis in this study. These SNPs were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) assay. The PCR amplification was performed in a total volume of 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 each primer (Table 2), and 0.2 U Taq DNA polymerase (Fermentas, Hanover, MD, USA). 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 s, 58–60 °C for 30 s, and 72 °C for 30 s; and then 5 min at 72 °C to complete the reaction. The PCR products were digested with 2.5 U of the restriction enzyme (Fermentas, Hanover, MD, USA) for each fragment (Table 2) and incubated for 2 h. The digested PCR products were then separated on 6% polyacrylamide electrophoresis in 1× TBE (Tris-borate-EDTA) buffer and visualized by ethidium bromide staining.
Table 1. Primer sequences used for single nucleotide polymorphism (SNP) identification in porcine IL-4 gene. PCR, polymerase chain reaction; IL, interleukin; Ta, annealing temperature.

| Primers | Location | Primer Sequences | PCR Size (bp) | Ta (°C) |
|---------|----------|-----------------|---------------|---------|
| IL-4-1  | Exon 1   | F: 5′-GTAGCTGCTCCACAGTAAAC-3′
R: 5′-CATACAGGAGGATCCAGAAGA-3′ | 236            | 60      |
| IL-4-2  | Exon 2   | F: 5′-AAACGCTCTCTGTGCTCTCT-3′
R: 5′-TGCTCCCTGTATTCAGGAGA-3′ | 409            | 60      |
| IL-4-3  | Exon 3   | F: 5′-GCACGTGATATGAGGCTCT-3′
R: 5′-TGTTGACCGCTCCAGAAGA-3′ | 189            | 60      |
| IL-4-4  | Exon 4   | F: 5′-GGAGAGAAGCCCATGAGCAA-3′
R: 5′-GCCTATGCAAAAAGTGGACA-3′ | 227            | 60      |
| IL-4-5  | Intron 2 | F: 5′-GTGCTGCTGCACTCCACAC-3′
R: 5′-ACAGTGATACAAACAGGCG-3′ | 333            | 58      |
| IL-4-6  | Intron 3 | F: 5′-GTGCTGCTGCACTCCACAC-3′
R: 5′-ACAGTGATACAAACAGGCG-3′ | 333            | 58      |

Table 2. Primer sequences and restriction enzymes used for genotyping of porcine IL-4 and IL-4R genes. PCR, polymerase chain reaction; IL, interleukin; SNP, single nucleotide polymorphism; Ta, annealing temperature.

| SNP Position (SNP ID) | Location | Primer Sequences | PCR Size (bp) | Ta (°C) | Restriction Enzyme |
|-----------------------|----------|-----------------|---------------|---------|-------------------|
| IL-4 g.134993898T > C (rs329453960) | Intron 3 | F: 5′-GTGCTGCTGCACTCCACAC-3′
R: 5′-ACAGTGATACAAACAGGCG-3′ | 333            | 58      | BsuRI            |
| IL-4R c.163G > A (rs342744061) | Exon 1   | F: 5′-TCTTGATCACTGGGCTTCCG-3′
R: 5′-AACTCAGCGCTGCAGTTGAC-3′ | 152            | 60      | PifFlI           |
| IL-4R c.242C > T (rs334778260) | Exon 2   | F: 5′-GGTGCTGCTGCACTCCACAC-3′
R: 5′-TGGAAGGACGTCTTCACAC-3′ | 180            | 60      | AfIII            |
| IL-4R c.623G > A (rs790396006) | Exon 4   | F: 5′-TCTATACGCTTGACCTACCT-3′
R: 5′-TAACCTAGCCAGCTGACCTA-3′ | 148            | 60      | MluI             |
| IL-4R c.1016G > A (rs692527061) | Exon 8   | F: 5′-CTGGAGCTGCTGTGACCTA-3′
R: 5′-ACACTCTGGCCAGGATCT-3′ | 137            | 58      | BsuRI            |

2.3. Statistical Analysis

Allelic and genotypic frequencies were calculated. Hardy–Weinberg equilibrium (HWE) was analyzed using the chi-square test. Association analysis of porcine IL-4 and IL-4R SNP markers with the litter size traits was examined using a general linear model. The statistical model was used as follows: $Y_{ijkl} = \mu + P_i + YS_j + G_k + e_{ijkl}$, where $Y_{ijkl}$ is representative of the observations of the phenotype values, $\mu$ represents the average normalized record of populations, $P_i$ represents the fixed effect of parities ($i = 1$ and $\geq 2$), $YS_j$ represents the fixed effect of year-season ($j = 1–8$), $G_k$ is representative of the fixed effect of the genotypes for IL-4 and IL-4R ($k = 1–3$) or the accumulated favorable alleles for IL-4 and IL-4R ($k = 0–4$), and $e_{ijkl}$ represents the residual error. Moreover, the additive effect was calculated as the 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 [38]. The estimated effects were calculated using a $t$-test on significant deviations from zero. Furthermore, linear regression between the number of favorable alleles of porcine IL-4 and IL-4R genes and least square mean values of the litter size traits were analyzed.
3. Results

3.1. Polymorphisms of Porcine IL-4 and IL-4R Genes

To verify the polymorphisms of the porcine IL-4 gene, the DNA segments of exons and introns of the porcine IL-4 gene were sequenced. An SNP in intron 3 of the porcine IL-4 gene was found and corresponded to an SNP of porcine IL-4 g.134993898T > C (rs329453960) locus in Ensembl data (https://asia.ensembl.org/Sus_scrofa/Info/Index, accessed on 2 September 2020). It was detected with the restriction enzyme BsuRI. Two specific alleles revealed two fragments of 295 and 38 bp for allele T and three fragments of 180, 115, and 38 bp for allele C (Figure 1A). To verify the polymorphisms of the porcine IL-4R gene, five non-synonymous SNPs were selected to be examined in the Landrace pig population. A polymorphic site of c.1577A > T (rs342791614) was found in exon 8. It was a non-synonymous mutation leading to a non-conservative amino acid exchange at position 526 from glutamine to leucine (Q526L). This polymorphic site was detected with the restriction enzyme AluI. Two specific alleles revealed a 137 bp fragment for allele A and two fragments of 119 and 18 bp for allele T (Figure 1B). However, no polymorphisms of the four SNP markers (c.163G > A, c.242C > T, c.623G > A, and c.1016G > T) of the porcine IL-4R gene were observed in this study.

Figure 1. Genotyping single nucleotide polymorphisms (SNPs) of porcine IL-4 and IL-4R genes (A) at IL-4 g.134993898T > C locus with BsuRI and (B) at IL-4R c.1577A > T locus with AluI. The molecular marker of 100 bp DNA ladder (M) and the genotypes of porcine IL-4 (CC, TC, and TT) and IL-4R (AA, AT, and TT) genes are indicated at the top of each line. IL, interleukin.

3.2. Genotypic and Allelic Frequencies

The genotypic and allelic frequencies of the porcine IL-4 and IL-4R genes are shown in Table 3. Two polymorphic sites of the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T genes were found to be segregating in the Landrace sows. At the IL-4 g.134993898T > C and IL-4R c.1577A > T loci, three genotypes were observed. The IL-4 g.134993898T and IL-4R c.1577A alleles were more frequent in this pig population. Moreover, the four SNP markers of the porcine IL-4R gene at c.163, c.242, c.623, and c.1016 loci were fixed as c.163G, c.242C, c.623G, and c.1016G, respectively (data not shown). The chi-square test revealed that the genotype distributions of the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T loci within Landrace sows were in agreement with the HWE specifications (p > 0.05).
3.3. Associations of Porcine IL-4 and IL-4R Polymorphisms with Litter Size Traits

The effects of porcine IL-4 and IL-4R genotypes on litter size traits were assessed in the Landrace sows. The porcine IL-4 and IL-4R polymorphisms were associated with litter size traits of pigs. Association of the porcine IL-4 g.134993898T > C polymorphism with litter size traits is shown in Table 4. No significant association of porcine IL-4 g.134993898T > C polymorphism with any litter size traits was found in the first parity of sows. However, the porcine IL-4 g.134993898T > C polymorphism was significantly associated with the NWA trait in the later parities of sows. The sows with the TT and TC genotypes had higher NWA values than the sows with the CC genotype. The significant additive effect for the NWA trait was detected in the later parities of sows. Thus, the porcine IL-4 g.134993898T allele seems to be a favorable allele for litter size traits in pigs. Association of the porcine IL-4R c.1577A > T polymorphism with litter size traits is shown in Table 5. There was no significant association of porcine IL-4R c.1577A > T polymorphism with any litter size traits in the first parity of sows. However, the porcine IL-4R c.1577A > T polymorphism was significantly associated with the NWA trait in the later parities of sows. The sows with the TT genotype had higher NBA and NWA values than the sows with the CC genotype. The significant additive effect for the NWA trait in the later parities of sows. The sows with the TT and TC genotypes had higher NWA values than the sows with the CC genotype. Notably, the significant additive effects for the NBA and NWA traits were observed in later parities of sows. Thus, the porcine IL-4R c.1577T allele seems to be a favorable allele for litter size traits in pigs.

Table 4. Association of porcine IL-4 g.134993898T > C locus with litter size traits.

| Parity (2nd–8th parities) | Traits 1 | Genotypes (Means ± SE) 2 | Additive | Dominance |
|---------------------------|----------|--------------------------|----------|-----------|
|                           | n        | TT                       | TC       | CC        |           |
| First parity              | 132      | 9.58 ± 0.45              | 9.77 ± 0.48 | 9.68 ± 0.67 | −0.05 ± 0.25 | 0.14 ± 0.32 |
|                           |          | 8.65 ± 0.47              | 8.57 ± 0.36 | 8.08 ± 0.42 | 0.29 ± 0.27 | 0.11 ± 0.12 |
|                           |          | 8.15 ± 0.47              | 7.85 ± 0.64 | 7.28 ± 0.54 | 0.44 ± 0.22 | 0.14 ± 0.12 |
|                           |          | 1.58 ± 0.06              | 1.60 ± 0.08 | 1.67 ± 0.08 | −0.04 ± 0.02 | −0.02 ± 0.02 |
|                           |          | 6.42 ± 0.12              | 6.58 ± 0.15 | 6.52 ± 0.18 | −0.05 ± 0.05 | 0.11 ± 0.08 |
| Later parities            | 348      | 11.25 ± 0.51             | 11.03 ± 0.53 | 10.72 ± 0.55 | 0.27 ± 0.28 | 0.04 ± 0.17 |
|                           |          | 10.48 ± 0.58             | 9.92 ± 0.57 | 9.47 ± 0.75 | 0.51 ± 0.32 | 0.06 ± 0.32 |
|                           |          | 9.72 ± 0.47 b            | 9.42 ± 0.47 b | 8.45 ± 0.52 a | 0.64 ± 0.21 * | 0.33 ± 0.34 |
|                           |          | 1.58 ± 0.05              | 1.57 ± 0.04 | 1.54 ± 0.05 | 0.02 ± 0.01 | 0.01 ± 0.02 |
|                           |          | 6.62 ± 0.05              | 6.67 ± 0.05 | 6.59 ± 0.07 | 0.02 ± 0.02 | 0.06 ± 0.02 |

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 least square means ± standard error. TT, TC, and CC are genotypes of porcine IL-4 g.134993898T > C locus. Values in each row with differing superscripts are considered significantly different (\( p < 0.05 \)).
NBA, NWA, and MWW traits were found. Furthermore, the results of linear regression analysis revealed that the increased accumulated favorable alleles were highly positively correlated with the least square mean values of the TNB, NBA, and NWA traits in later parities of sows (Figure 2A–C). However, lower correlations between the accumulated favorable alleles and the least square mean values of litter size traits were observed in the first parity, as well as the MBW and MWW traits in the later parities of sows (result not shown). Notably, the largest number of favorable alleles (TTTT) of porcine IL-4 and IL-4R genotypes seemed to have advantageous effects on litter size traits. On the other hand, the smallest number of favorable alleles (CCAA) of porcine IL-4 genotypes seemed to have disadvantageous effects on these traits.

Table 5. Association of porcine IL-4 R.1577A > T locus with litter size traits.

| Parity               | Traits | Genotypes (Means ± SE) | Additive | Dominance |
|----------------------|--------|------------------------|----------|-----------|
|                      | n      | AA                     | AT       | TT        |
| First parity         |        |                        |          |           |
| TNB                  | 89     | 10.03 ± 0.76           | 9.27 ± 0.58 | 0.11 ± 0.32 | 0.65 ± 0.35 |
| NBA                  | 176    | 8.55 ± 0.32            | 8.19 ± 0.67 | 0.03 ± 0.32 | 0.33 ± 0.47 |
| NWA                  | 53     | 7.57 ± 0.61            | 7.30 ± 0.49 | 0.08 ± 0.35 | 0.20 ± 0.42 |
| MBW                  | 53     | 1.58 ± 0.08            | 1.67 ± 0.08 | −0.06 ± 0.02 | −0.02 ± 0.03 |
| MWW                  | 53     | 6.39 ± 0.10            | 6.55 ± 0.18 | −0.06 ± 0.05 | 0.09 ± 0.07 |
| Later parities       |        |                        |          |           |
| (2nd–8th parities)   |        |                        |          |           |
| TNB                  | 133    | 10.55 ± 0.43           | 11.54 ± 0.68 | −0.52 ± 0.28 | −0.47 ± 0.32 |
| NBA                  | 133    | 9.43 ± 0.47 a          | 10.71 ± 0.64 b | −0.75 ± 0.28 * | −0.53 ± 0.36 |
| NWA                  | 133    | 9.09 ± 0.46 a          | 10.20 ± 0.58 b | −0.80 ± 0.28 ** | −0.32 ± 0.31 |
| MBW                  | 133    | 1.60 ± 0.04            | 1.57 ± 0.05 | −0.01 ± 0.02 | 0.04 ± 0.02 |
| MWW                  | 133    | 6.63 ± 0.06            | 6.60 ± 0.06 | 0.01 ± 0.02 | 0.02 ± 0.03 |

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 least square means ± standard error. AA, AT, and TT are genotypes of porcine IL-4 R.1577A > T locus. Values in each row with differing superscripts are considered significantly different (ab p < 0.05), * p < 0.05, ** p < 0.01.

Table 6. Association of accumulated favorable alleles of porcine IL-4 g.134993898T > C and IL-4 R.1577A > T loci, with litter size traits.

| Parity               | Traits | Number of Favorable Alleles (Means ± SE) |       |       |       |       |
|----------------------|--------|-----------------------------------------|-------|-------|-------|-------|
|                      | n      | 1                                       | 2     | 3     | 4     |       |
| First parity         |        | 7                                       | 59    | 144   | 89    | 18    |
| TNB                  | 10.72 ± 1.42 | 9.58 ± 0.57                              | 10.12 ± 0.54 | 10.15 ± 0.52 | 8.68 ± 0.72 |
| NBA                  | 8.84 ± 1.25 | 8.24 ± 0.58                              | 8.74 ± 0.52 | 9.15 ± 0.67 | 8.12 ± 0.95 |
| NWA                  | 7.23 ± 1.27 | 7.25 ± 0.73                              | 7.88 ± 0.64 | 8.57 ± 0.71 | 7.82 ± 0.84 |
| MBW                  | 1.48 ± 0.16 | 1.58 ± 0.05                              | 1.57 ± 0.04 | 1.56 ± 0.05 | 1.58 ± 0.08 |
| MWW                  | 6.52 ± 0.32 | 6.41 ± 0.12                              | 6.55 ± 0.10 | 6.43 ± 0.08 | 6.64 ± 0.19 |
| Later parities       |        | 18                                      | 152   | 371   | 225   | 48    |
| (2nd–8th parities)   |        |                                         |       |       |       |       |
| TNB                  | 9.94 ± 1.14 | 10.21 ± 0.62                              | 10.87 ± 0.51 | 10.68 ± 0.43 | 11.71 ± 0.78 |
| NBA                  | 8.81 ± 1.17 ab | 8.75 ± 0.55 a                             | 9.91 ± 0.46 b | 9.85 ± 0.47 b | 10.95 ± 0.77 b |
| NWA                  | 7.81 ± 1.11 ab | 8.34 ± 0.55 a                             | 9.51 ± 0.43 bc | 9.30 ± 0.47 bc | 10.52 ± 0.72 c |
| MBW                  | 1.65 ± 0.07 | 1.61 ± 0.05                              | 1.63 ± 0.04 | 1.66 ± 0.05 | 1.58 ± 0.07 |
| MWW                  | 6.21 ± 0.11 a | 6.64 ± 0.04 b                             | 6.65 ± 0.03 b | 6.63 ± 0.03 b | 6.65 ± 0.05 b |

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 least square means ± standard error. Values in each row with differing superscripts are considered significantly different (ab c p < 0.05). Number of favorable alleles is accumulated favorable alleles of the porcine IL-4 g.134993898T and IL-4 R.1577A alleles.
Figure 2. Linear regression analysis of the number of favorable alleles of porcine IL-4 and IL-4R genes and least square mean values of (A) TNB: total number born, (B) NBA: number born alive, and (C) NWA: number of piglets weaned alive traits in later parities of pigs. The y indicates means phenotypic values of litter size traits, x indicates the number of accumulated favorable alleles, and $R^2$ indicates the coefficient of determination for the linear regression equation.

4. Discussion

Increasing litter size is of significant economic interest in the pig industry [1–3]. Embryonic survival is the main factor in determining litter size in pigs and depends on maternal recognition of pregnancy, embryo attachment, implantation, placental development, uterine capacity, and the maintenance of pregnancy [4,39]. Numerous studies have demonstrated the role of different cytokines in the channel of communication established between the trophoblast and maternal endometrium, which contributes to embryo implantation and the pregnancy of pigs [8,40,41].

IL-4 is an anti-inflammatory Th2 cytokine that can bind IL-4R to regulate IgE production, immune response, and embryonic implantation [13,29,42]. Several studies have reported that polymorphisms of IL-4 and/or IL-4R genes are associated with allergic diseases [23,24], diabetes [43], cancers [36], and pregnancy disorders [25–27]. In the present study, we have verified and elucidated polymorphisms of the porcine IL-4 and IL-4R genes with respect to reproductive performance traits in commercial pigs. The porcine IL-4 g.134993898T > C and IL-4R c.1577A > T polymorphisms were found to be segregating in Landrace sows. At the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T loci, three possible genotypes were observed in pigs. The IL-4 g.134993898T and IL-4R c.1577A were major alleles in this pig population. The results of the chi-square test revealed that the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T polymorphisms in these sows met the HWE specifications. This result indicates that the porcine IL-4 g.134993898T > C and IL-4R c.1577A > T polymorphisms were under homeostasis when accompanied by the effects of artificial selection.

The porcine IL-4 is mapped on SSC2 at position 134.9 Mb. Recently, a conducted genome-wide association study (GWAS) revealed that the porcine IL-4 gene is located within QTLs regions for the TNB (139.9–140.9 Mb) and piglet mortality traits (138.7–143.7 Mb) in pigs [21]. Moreover, the expression levels of the porcine IL-4 gene are upregulated...
in the endometrium during implantation [14] and involved in the gene networks for pregnancy establishment in pigs [15]. Furthermore, the porcine IL-4 protein concentrations are increased in serum of pregnant sows, as well as in maternal and fetal placenta during pregnancy periods [8]. These evidences indicate that the IL-4 gene plays a crucial role in embryo implantation and may be important for litter size traits.

In this study, the polymorphism of the porcine IL-4 gene had a significant association with litter size traits in pigs. A positive effect of the favorable IL-4 g.134993898T allele on litter size traits was found. The IL-4 g.134993898T > C polymorphism was found to be located in the non-coding sequence of the porcine IL-4 gene. We hypothesize that this SNP might be in linkage disequilibrium with other causal polymorphisms that may be located in another region of the porcine IL-4 gene. The association of the porcine IL-4 gene with litter size traits may be attributed to the balance of cytokines in endometrium during implantation period and the regulation of endometrial cytokines by IL-4. The IL-4 polymorphisms may be implicated in the impaired Th1/Th2 cytokine balance and may influence implantation defects and pregnancy disorders. Previous studies have demonstrated that increased anti-inflammatory Th2 cytokines (e.g., IL-4, IL-10) and decreased pro-inflammatory Th1 cytokines (e.g., IL-2, TNF-α, IFN-γ) levels in endometrium are important for successful pregnancy [6,15]. However, the increased Th1/Th2 cytokine ratios may increase cytotoxicity against embryos and lead to implantation failure [44,45]. Moreover, the IL-4 polymorphisms are associated with the recurrent abortion and pregnancy specific disorder of pre-eclampsia in humans [26,27]. Furthermore, the IL-4 polymorphisms may influence the other endometrial cytokines in promoting embryo implantation and placental development during pregnancy. Previous studies have reported that the endometrial cytokines of leukemia inhibitory factor (LIF) and vascular endothelial growth factor (VEGF) are upregulated by IL-4 [46–48]. The LIF is an endometrial requirement for implantation and embryo development [47] and the VEGF is involved in endometrial angiogenesis [14]. The decreased VEGF and IL-4 expression levels are associated with recurrent spontaneous miscarriage or recurrent abortion in women [49], and the decreased LIF and IL-4 cytokines of decidual T cells are associated with unexplained recurrent abortions [47]. Moreover, the polymorphisms of LIF and VEGF genes are associated with litter size traits in pigs [10,14]. This evidence suggested that the IL-4 gene may contribute to embryonic implantation and be related to litter size traits in pigs.

The porcine IL-4R gene is mapped on SSC3 at position 19.5 Mb and is closely located to the QTL regions for total number born (15.3–20.0 Mb) and some reproductive performance traits (11.9–33.7 Mb) in pigs [20,22]. It has recently been reported that the porcine IL-4R gene is involved in the gene-transcription factor networks for TNB trait in pigs [32]. Moreover, the IL-4R mRNAs are expressed in the endometrium, embryonic disc, and trophectoderm in pigs [30,31]. This evidence indicates that the IL-4R gene plays an important role in embryo implantation and may be related to litter size traits in pigs.

In this study, the porcine IL-4R c.1577A > T polymorphism was significantly associated with the NBA and NWA traits. A positive effect of the favorable IL-4R c.1577T allele on litter size traits was found. Remarkably, the porcine IL-4R c.1577A > T polymorphism revealed a non-synonymous mutation and exhibited a changing amino acid residue at position Q526L in the cytoplasmic domain of the IL-4R structural molecule. This domain region is important to the IL-4 signaling transduction response and exhibits several key functions to regulate the tyrosine phosphorylation and intracellular signal transduction pathways. It contains numerous tyrosine (Y) residues (at positions Y504, Y583, Y610, Y638, and Y718) and sequence motifs of the binding sites for the insulin receptor substrate-1 (IRS-1), IRS-2, and signal transducer and activator of transcription 6 (STAT6) in terms of the growth and gene expression of the regulation domains [50]. Moreover, a high degree of homology of the porcine IL-4R tyrosine residues and their surrounding amino acid residues with human IL-4R amino acid sequence [16,50] was observed in this study (Figure 3). It can be expected that the porcine IL-4R molecule has functions that are similar to the human IL-4R molecule. Therefore, the changing of Q526L was located in the growing
domain of the cytoplasmic region that was close to the tyrosine-phosphorylation of the IRS sequence motif and may have affected the intracellular signal transduction pathways and the function of cells. Furthermore, four specific DNA motifs (\textsuperscript{1549}CCCAGAGCC\textsuperscript{1427}, \textsuperscript{1448}CCCAGAGCC\textsuperscript{1456}, \textsuperscript{1448}CCCAGAGCT\textsuperscript{1456}, and \textsuperscript{1560}CCCAGAGCT\textsuperscript{1577}) were observed in the cytoplasmic region of the porcine IL-4R gene. Interestingly, the polymorphism of IL-4R c.1577A > T (Q526L) was also located at the nucleotide position 1577 on the \textsuperscript{1569}CCCAGAGCT\textsuperscript{1577} motif of the porcine IL-4R mRNA sequence (Genbank accession no. NM_214340.1). These sequence motifs might have affected its expression or relationships to functional variations. However, the variant Q526L of the porcine IL-4R gene has not yet been functionally identified. It has been hypothesized that the observed amino acid change of Q526L might serve as the functional explanation of these traits, although the possibility of mutations inside or outside the coding region in close linkage disequilibrium with porcine IL-4R c.1577A > T (Q526L) should not be excluded.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{alignment.png}
\caption{Alignment of porcine IL-4R amino acid sequence (GenBank Accession no. NP_999505.1) and human IL-4R amino acid sequence (NP_000409.1). Tyrosine (Y) residues of porcine IL-4R amino acid sequence are localized in the cytoplasmic region at positions Y504, Y583, Y610, Y638, and Y718, corresponding to human IL-4R sequence at positions Y497, Y575, Y603, Y631, and Y713. Sequence motifs of binding sites for insulin receptor substrate (IRS) and signal transducer and activator of transcription 6 (STAT6) are indicated by boxed and shaded areas. \(| = \) identity, \(\blacktriangle = \) the porcine IL-4R polymorphism at position Q526L (c.1577A > T). SNP, single nucleotide polymorphism.}
\end{figure}

There is limited literature on the association of the IL-4R gene with litter size traits in pigs. However, several studies have demonstrated that the polymorphisms of IL4R gene are associated with the recurrent abortion, spontaneous preterm birth, and pre-eclampsia in humans \([27,33–35]\). Moreover, the decreased circulating IL-4R protein levels in serum were associated with pre-eclampsia in Danish women \([51]\). In contrast, no association of IL-4R variants with the recurrent abortion has been reported in Iranian women \([52]\). Furthermore, the polymorphisms of the IL-4R gene are associated with LIF mRNAs and protein expression levels \([48]\) and variations of LIF gene are association with the recurrent abortions in women \([47]\) and litter size traits in sows \([10]\). This evidence suggests that the IL-4R gene may be implicated in litter size traits in pigs.

In order to establish an elite breeding stock with high prolificacy traits in pigs, there is an essential procedure to accumulate identified genes or combine more affected genes from multiple parents into a superior genotype of the breeding stock, which is called gene pyramiding schema \([53]\). The gene pyramiding approach can be achieved through improving genetic values with marker-assisted selection (MAS) to accumulate favorable alleles from different loci for increasing the frequency of favorable alleles of genes controlling trait of interest in a population \([53]\). Thus, identification of the effects of combined genotypes or accumulated favorable alleles on litter size traits is required. There are numerous studies demonstrating that the accumulated favorable alleles, combined genotypes, or epistatic interactions of two or more loci QTLs or candidate genes have more beneficial effects on litter size traits in pigs \([10,12,54,55]\).
Several studies have reported that the interactions of the IL-4 and IL-4R genotypes are associated with diabetes, cancer, and pemphigus foliaceus in humans [43,56,57]. Unfortunately, no interaction effects of porcine IL-4 and IL-4R genotypes on litter size traits were observed in this study (data not shown). Therefore, it was explored whether the accumulated favorable alleles of the porcine IL-4 and IL-4R genotypes are associated with litter size traits in pigs. Interestingly, the significant effect of the accumulated favorable alleles (IL-4 g.134993898T and IL-4R c.1577T) of porcine IL-4 and IL-4R genes on litter size traits was exhibited. The increased numbers of favorable alleles were positively correlated with the TNB, NBA, and NWA traits in sows (Figure 2). Moreover, the accumulated favorable alleles (TTTT) were characterized as an advantageous genotype with the highest values for the NBA, NWA, and MWW traits. Meanwhile, the accumulated unfavorable alleles (CCAA) were characterized as a disadvantageous genotype with the lowest values for these traits (Table 6). This evidence indicates that there are strong additive effects of the accumulated favorable alleles of porcine IL-4 and IL-4R genes on the litter size traits and the increasing numbers of favorable alleles seem to enhance litter size traits in these sows. Therefore, the accumulated favorable alleles (TTTT) can be used in marker-assisted selection to select the individuals with higher litter size traits. These findings suggest the gene pyramiding effects of porcine IL-4 and IL-4R genes may provide advantages for the breeding industry. The results in this study indicate that the porcine IL-4 and IL-4R genes could be expected to be involved in the reproductive processes of pigs, especially with regard to litter size traits. Further studies are required to confirm the association of these SNPs with litter size traits in larger population samples and various commercial pig breeds.

5. Conclusions

In this study, we found that polymorphisms of porcine IL-4 and IL-4R genes are associated with the reproductive traits of pigs. The porcine IL-4 g.134993898T > C and IL-4R c.1577A > T polymorphisms had clear effects on NBA, NWA, and MWW traits in commercial pigs. Moreover, the accumulation of favorable alleles of porcine IL-4 and IL-4R genes is positively correlated with the litter size traits. These findings emphasize the importance of the porcine IL-4 and IL-4R genes in the reproductive traits of pigs. Therefore, the porcine IL-4 and IL-4R genes may be potential candidate genes for the genetic improvement of litter size traits in the pig breeding industry.

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References

1. Metodev, S.; Thekkoot, D.M.; Young, J.M.; Onteru, S.; Rothschild, M.F.; Dekkers, J.C.M. A whole-genome association study for litter size and litter weight traits in pigs. *Livest. Sci.* 2018, 211, 87–97. [CrossRef] [PubMed]
2. An, S.M.; Kwon, S.; Hwang, J.H.; Yu, G.E.; Kang, D.G.; Park, D.H.; Kim, T.W.; Park, H.C.; Ha, J.; Kim, C.W. Hypomethylation in the promoter region of ZBP1 as a potential litter size indicator in Berkshire pigs. *Arch. Anim. Breed.* 2019, 62, 69–76. [CrossRef] [PubMed]
3. Schneider, J.F.; Rempel, L.A.; Snelling, W.M.; Weidmann, R.T.; Nonneman, D.J.; Rohrer, G.A. Genome-wide association study of swine farrowing traits. Part II Bayesian analysis of marker data. *J. Anim. Sci.* 2012, 90, 3360–3367. [CrossRef] [PubMed]
4. Spötter, A.; Distl, O. Genetic approaches to the improvement of fertility traits in the pig. *Vet. J.* 2006, 172, 234–247. [CrossRef] [PubMed]
5. Fu, Y.; Fu, J.; Wang, A. Association of EphA4 polymorphism with swine reproductive traits and mRNA expression of EphA4 during embryo implantation. *Mol. Biol. Rep.* 2012, 39, 2689–2696. [CrossRef] [PubMed]
6. Saini, V.; Arora, S.; Yadav, A.; Bhattacharjee, J. Cytokines in recurrent pregnancy loss. *Clin. Chim. Acta* 2011, 411, 702–708. [CrossRef]
7. Geisert, R.D.; Johnson, G.A.; Burghardt, R.C. Implantation and establishment of pregnancy in the pig. *Advo. Anat. Embryol. Cell Biol.* 2015, 216, 137–163.
8. Vélez, C.; Clauzure, M.; Williamson, D.; Koncurat, M.A.; Santa-Coloma, T.A.; Barbeito, C. IL-1β, IL-2 and IL-4 concentration during porcine gestation. *Theriogenology* 2019, 128, 133–139. [CrossRef] [PubMed]
9. Niu, B.Y.; Ye, L.Z.; Li, F.E.; Deng, C.Y.; Jiang, S.W.; Lei, M.G.; Xiong, Y.Z. Identification of polymorphism and association analysis with reproductive traits in the porcine RNF4 gene. *Anim. Reprod. Sci.* 2009, 110, 283–292. [CrossRef]
10. Lin, H.C.; Liu, G.F.; Wang, A.G.; Kong, L.J.; Wang, X.F.; Fu, J.L. Effect of polymorphism in the leukemia inhibitory factor gene on litter size and litter weight traits in pigs. *BMC Genet.* 2011, 2, 187–191.
11. Yang, L.; Fu, J.; Fu, Y.; Wang, A. Association analysis between a polymorphism in the 5′-regulatory region of the IL-6 gene and litter size in pigs. *J. Anim. Sci. Biotechnol.* 2014, 5, 156. [CrossRef] [PubMed]
12. Kumchoo, T.; Mekchay, S. Association of non-synonymous SNPs of OPN gene with litter size traits in pigs. *Arch. Anim. Breed.* 2015, 58, 317–323. [CrossRef]
13. Gu, T.; Zhu, M.J.; Schroyen, M.; Qu, L.; Nettleton, D.; Kuhar, D.; Ross, J.W.; Zhao, S.H.; Tuggle, C.K. Endometrial gene expression profiling in pregnant Meishan and Yorkshire pigs on day 12 of gestation. *BMC Genom.* 2014, 15, 18. [CrossRef] [PubMed]
14. Chen, X.; Li, A.; Chen, W.; Wei, J.; Fu, J.; Wang, A. Differential gene expression in uterine endometrium during implantation in pigs. *Biol. Reprod.* 2015, 92, 52. [CrossRef] [PubMed]
15. Lim, K.J.; Odukoya, O.A.; Ajian, R.A.; Li, T.C.; Weetman, A.P.; Cooke, I.D. Profile of cytokine mRNA expression in peri-implantation human endometrium. *Mol. Hum. Reprod.* 1998, 4, 77–81. [CrossRef] [PubMed]
16. Ryan, J.J.; McReynolds, L.J.; Keegan, A.; Wang, L.H.; Garfén, E.; Rothman, P.; Nelsms, K.; Paul, W.E. Growth and gene expression are predominantly controlled by distinct regions of the human IL-4 receptor. *Immunity* 1996, 4, 123–132. [CrossRef] [PubMed]
17. Arababadi, M.K.; Mosavi, R.; Ravari, A.; Teimori, H.; Hassanshahi, G. Association of interleukin-4 polymorphisms with multiple sclerosis in southeastern Iranian patients. *Ann. Saudi Med.* 2012, 32, 127–130. [CrossRef]
18. Chatterjee, P.; Chiasson, V.L.; Bounds, K.R.; Mitchell, B.M. Regulation of the anti-inflammatory cytokines interleukin-4 and interleukin-10 during pregnancy. *Front. Immunol.* 2014, 27, 253. [CrossRef]
19. Onteru, S.K.; Fan, B.; Du, Z.Q.; Garrick, D.J.; Stalder, K.J.; Rothschild, M.F. A whole-genome association study for pig reproductive traits. *Anim. Genet.* 2012, 43, 18–26. [CrossRef] [PubMed]
20. Schneider, J.F.; Miles, J.R.; Brown-Brandl, T.M.; Nienaber, J.A.; Rohrer, G.A.; Vallet, J.L. Genomewide association analyses for average birth interval and stillbirth in swine. *J. Anim. Sci.* 2015, 93, 529–540. [CrossRef]
21. Guo, X.; Su, G.; Christensen, O.F.; Janss, L.; Lund, M.S. Genome-wide association analyses using a Bayesian approach for litter size and piglet mortality in Danish Landrace and Yorkshire pigs. *BMC Genom.* 2016, 17, 468. [CrossRef] [PubMed]
22. Nonneman, D.J.; Schneider, J.F.; Lents, C.A.; Wiedmann, R.T.; Vallet, J.L.; Rohrer, G.A. Genome-wide association and identification of candidate genes for age at puberty in swine. *BMC Genet.* 2016, 17, 50. [CrossRef]
23. Zhu, S.; Chan-Yeung, M.; Becker, A.B.; Dimich-Ward, H.; Ferguson, A.C.; Manfreda, J.; Watson, W.T.; Paré, P.D.; Sandford, A.J. Polymorphisms of the IL-4, TNF-alpha, and Epsilon Ribeta genes and the risk of allergic disorders in at-risk infants. *Am. J. Respir. Crit. Care Med.* 2000, 161, 1635–1639. [CrossRef] [PubMed]

24. Kabesch, M.; Tzotcheva, I.; Carr, D.; Höfler, C.; Weiland, S.K.; Fritzsch, C.; von Mutius, E.; Martinez, F.D. A complete screening of the IL4 gene: Novel polymorphisms and their association with asthma and IgE in childhood. *J. Allergy Clin. Immunol.* 2003, 112, 893–898. [CrossRef] [PubMed]

25. Chatterjee, P.; Kopriva, S.E.; Chiasson, V.L.; Young, K.J.; Tobin, R.P.; Newell-Rogers, K.; Mitchell, B.M. Interleukin-4 deficiency induces mild preeclampsia in mice. *J. Hypertens.* 2013, 31, 1414–1423. [CrossRef]

26. Salimi, S.; Khorasani, M.; Yaghmaei, M.; Mokhtari, M.; Moossavi, M. Possible association of IL-4 VNTR polymorphism with susceptibility to preeclampsia. *Biomed. Res. Int.* 2014, 2014, 497031. [CrossRef] [PubMed]

27. Golovatyuk, K.P. Role of gene polymorphism of IL-4 and IL-17 in recurrent miscarriage, came in art cycles. *Reprod. Endocrinol.* 2017, 33, 26–31. [CrossRef]

28. Wang, H.; Zamorano, J.; Keegan, A. A role for the insulin-interleukin (IL)-4 receptor motif of the IL-4 receptor α-chain in regulating activation of the insulin receptor substrate 2 and signal transducer and activator of transcription 6 pathways. *J. Biol. Chem.* 1998, 273, 9898–9905. [CrossRef]

29. Kruse, S.; Japha, T.; Tedner, M.; Sparholt, S.H.; Forster, J.; Kuehr, J.; Deichmann, K.A. The polymorphisms S503P and Q576R in the interleukin-4 receptor alpha gene are associated with atopy and influence the signal transduction. *Immunology* 1999, 96, 365–371. [CrossRef]

30. Huang, J.; Liu, R.; Su, L.; Xiao, Q.; Yu, M. Transcriptome analysis revealed the embryo-induced gene expression patterns in the endometrium from Meishan and Yorkshire pigs. *Int. J. Mol. Sci.* 2015, 169, 22692–22710. [CrossRef]

31. Isom, S.C.; Spollen, W.G.; Blake, S.M.; Bauer, B.K.; Springer, G.K.; Prather, R.S. Transcriptional profiling of day 12 porcine embryonic disc and trophectoderm samples using ultra-deep sequencing technologies. *Mol. Reprod. Dev.* 2010, 77, 812–819. [CrossRef] [PubMed]

32. Verardo, L.; Lopes, M.S.; Mathur, P.; Madsen, O.; Silva, F.F.; Groenen, M.A.M.; Knol, E.F.; Lopes, P.S.; Guimarães, S.E.F. Gene networks for total number born in pigs across divergent environments. *Mamm. Genome* 2017, 28, 426–435. [CrossRef] [PubMed]

33. Goddard, K.A.; Tromp, G.; Romero, R.; Olson, J.M.; Lu, Q.; Xu, Z.; Parimi, N.; Nien, J.K.; Gomez, R.; Behnke, E.; et al. Candidate-gene association study of mothers with pre-eclampsia, and their infants, analyzing 775 SNPs in 190 genes. *Hum. Hered.* 2007, 63, 1–16. [CrossRef] [PubMed]

34. Menon, R.; Pearce, B.; Velez, D.R.; Meriali, M.; Williams, S.M.; Fortunato, S.J.; Thorsen, P. Racial disparity in pathophysiological pathways of preterm birth based on genetic variants. *Reprod. Biol. Endocrinol.* 2009, 7, 62. [CrossRef]

35. Romero, R.; Velez Edwards, D.R.; Kusanovic, J.P.; Hassan, S.S.; Mazaki-Tovi, S.; Vaisbuch, E.; Kim, C.J.; Chaiworapongsa, T. Pregnancy and spontaneous fetal loss: A pig perspective. *Reprod. Biol. Endocrinol.* 2003, 18, 34. [CrossRef] [PubMed]

36. Walsh, P.S.; Metzger, D.A.; Higuchi, R. Chelex 100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. *Biotechniques* 2013, 54, 134–139. [CrossRef] [PubMed]

37. Muñoz, G.; Ovilo, C.; Estellé, J.; Silió, L.; Fernández, A.; Rodriguez, C. Association with litter size of new polymorphisms on ESRI and ESRI2 genes in a Chinese-European pig line. *Genet. Sel. Evol.* 2007, 39, 195–206. [CrossRef] [PubMed]

38. Spencer, T.E.; Bazer, F.W. Conceptus signals for establishment and maintenance of pregnancy. *Reprod. Biol. Endocrinol.* 2004, 2, 49. [CrossRef] [PubMed]

39. Ross, J.W.; Ashworth, M.D.; Hurst, A.G.; Malayer, J.R.; Geisert, R.D. Analysis and characterization of differential gene expression during rapid trophoblastic elongation in the pig using suppression subtractive hybridization. *Reprod. Biol. Endocrinol.* 2003, 14, 23. [CrossRef]

40. Bidarimath, M.; Tayade, C. Pregnancy and spontaneous fetal loss: A pig perspective. *Mol. Reprod. Dev.* 2017, 84, 856–869. [CrossRef]

41. Takeda, K.; Kishimoto, T.; Akira, S. STAT6: Its role in interleukin-4-mediated biological functions. *J. Mol. Med.* 1997, 75, 317–326. [CrossRef]

42. Bugawan, T.L.; Mirel, D.B.; Valdes, A.M.; Panelo, A.; Pozzilli, P.; Erlich, H.A. Association and interaction of the IL4R, IL4, and IL13 loci with type 1 diabetes among Filipinos. *Am. J. Hum. Genet.* 2003, 72, 1415–1514. [CrossRef] [PubMed]

43. Ghasemnejad-Berenji, H.; Ghaffari Novin, M.; Hajshafiha, M.; Nazarian, H.; Hashemi, S.M.; Ilkhanizadeh, B.; Ghasemnejad, T.; Sadeghpour, S.; Ghasemnejad-Berenji, M. Immunomodulatory effects of hydroxychloquine on Th1/Th2 balance in women with repeated implantation failure. *Biomed. Pharmacother.* 2018, 107, 1277–1285. [CrossRef]

44. Kwak-Kim, J.Y.; Chung-Bang, H.S.; Ng, S.C.; Ntrivalas, E.I.; Mangubat, C.P.; Beaman, K.D.; Beer, A.E.; Gilman-Sachs, A. Increased T helper 1 cytokine responses by circulating T cells are present in women with recurrent pregnancy losses and in infertile women with multiple implantation failures after IVF. *Hum. Reprod.* 2003, 18, 767–773. [CrossRef] [PubMed]

45. Faffe, D.S.; Flynt, L.; Bourgeois, K.; Panettieri, R.A., Jr.; Shore, S.A. Interleukin-13 and interleukin-4 induce vascular endothelial growth factor release from airway smooth muscle cells: Role of vascular endothelial growth factor genotype. *Am. J. Respir. Cell Mol. Biol.* 2006, 34, 213–218. [CrossRef] [PubMed]
47. Piccinni, M.P.; Beloni, L.; Livi, C.; Maggi, E.; Scarselli, G.; Romagnani, S. Defective production of both leukemia inhibitory factor and type 2 T-helper cytokines by decidual T cells in unexplained recurrent abortions. Nat. Med. 1998, 49, 1020–1024. [CrossRef] [PubMed]

48. Saleh, A.; Stathopoulou, M.G.; Dadé, S.; Ndiaye, N.C.; Azimi-Nezhad, M.; Murray, H.; Masson, C.; Lamont, J.; Fitzgerald, P.; Visvikis-Siest, S. Angiogenesis related genes NOS3, CD14, MMP3 and IL4R are associated to VEGF gene expression and circulating levels in healthy adults. BMC Med. Genet. 2015, 16, 90. [CrossRef]

49. Banerjee, P.; Jana, S.K.; Pasricha, P.; Ghosh, S.; Chakravarty, B.; Chaudhury, K. Proinflammatory cytokines induced altered expression of cyclooxygenase-2 gene results in unreceptive endometrium in women with idiopathic recurrent spontaneous miscarriage. Fertil. Steril. 2013, 99, 179–187. [CrossRef] [PubMed]

50. Ryan, J.J.; McReynolds, L.J.; Huang, H.; Nelms, K.; Paul, W.E. Characterization of a mobile Stat6 activation motif in the human IL-4 receptor. J. Immunol. 1998, 161, 1811–1821.

51. Taylor, B.D.; Tang, G.; Ness, R.B.; Olsen, J.; Hougaard, D.M.; Skogstrand, K.; Roberts, J.M.; Haggerty, C.L. Mid-pregnancy circulating immune biomarkers in women with preeclampsia and normotensive controls. Pregnancy Hypertens. 2016, 6, 72–78. [CrossRef] [PubMed]

52. Tavasolian, F.; Abdollahi, E.; Samadi, M. Association of the IL4R single-nucleotide polymorphism I50V with recurrent spontaneous abortion (RSA). J. Assist. Reprod. Genet. 2014, 31, 851–856. [CrossRef] [PubMed]

53. Servin, B.; Martin, O.C.; Mézard, M.; Hospital, F. Toward a theory of marker-assisted gene pyramiding. Genetics 2004, 168, 513–523. [CrossRef]

54. Wang, X.; Wang, A.; Fu, J.; Lin, H. Effects of ESR1, FSHB and RBP4 genes on litter size in a Large White and Landrace herds. Arch. Anim. Breed. 2006, 49, 64–70. [CrossRef]

55. Noguera, J.L.; Rodríguez, C.; Varona, L.; Tomás, A.; Muñoz, G.; Ramírez, O.; Barragán, C.; Arqué, M.; Bidanel, J.P.; Amills, M.; et al. A bi-dimensional genome scan for prolificacy traits in pigs shows the existence of multiple epistatic QTL. BMC Genom. 2009, 10, 636. [CrossRef]

56. Landi, S.; Bottari, F.; Gemignani, F.; Gioia-Particola, L.; Guino, E.; Osorio, A.; de Oca, J.; Capella, G.; Canzian, F.; Moreno, V.; et al. Interleukin-4 and interleukin-4 receptor polymorphisms and colorectal cancer risk. Eur. J. Cancer 2007, 43, 762–768. [CrossRef]

57. Toumi, A.; Abida, O.; Ben Ayed, M.; Masmoudi, A.; Turki, H.; Masmoudi, H. Cytokine gene polymorphisms in Tunisian endemic pemphigus foliaceus: A possible role of il-4 variants. Hum. Immunol. 2003, 74, 658–665. [CrossRef]