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Genetic diversity of porcine *PRLR* gene and its relationship to litter size in Large White pigs

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Original article

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Improving the litter performance of sows is one of the main challenges in the current pig industry. In this study, single nucleotide polymorphisms (SNPs) of the *PRLR* gene were performed, in order to test whether they are associated with the reproductive performance of Large White pigs. In total, we discovered nine SNP loci (g.C260G, g.C362T, g.C527G, g.A540G, g.A584G, g.A673T, g.A745G, g.C765T and g.A934G) in exon 10 of PRLR. The result showed that genotypes CC and CT at the g.C362T locus and genotype AG at g.A584G could significantly increase the litter size of different strains of Large White pigs ($p < 0.05$). In addition, the genotype CC at the g.C765T locus and the genotype AA at g.A934G could also increase the litter size (TNB could be increased by 1.5 piglets per year; while NBA could be increased by 0.98 piglets per year, $p < 0.01$). Furthermore, the haplotype combinations of H2H7 and H4H4 were the dominant combinations and contributed to larger litter sizes in the Large White pigs. In conclusion, there were dominant genotypes in the related SNP loci in the *PRLR* gene that were beneficial to improving the litter traits of sows. These findings will provide a reference for screening the molecular markers of a high reproductive performance in sows, and are helpful for genetic breeding and the reproductive improvement of pigs.

Key words: sow, prolactin, polymorphism, reproduction, association analysis.

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The reproductive performance of sows is closely related to the economic success of large-scale pig farms. The factors that can affect an evaluation of the reproductive performance of sows are diverse (Tummaruk *et al.* 2009). The total number born (TNB) and number born alive (NBA) are the key indicators used to evaluate the reproductive performance of sows (Bakoev *et al.* 2020). However, low heritability is a reproductive trait of pigs (Zhang *et al.* 2020; Plaengkaeo *et al*. 2021). Improving traits with a low heritability with conventional breeding programs is a very slow process. Unlike conventional breeding, molecular mark-assisted selection has triggered faster progress, where a single nucleotide polymorphism (SNP) is the most widely used molecular marker technology (Tian *et al.* 2015; Xu *et al.* 2017). SNPs are widespread in animal and plant genomes, and sometimes, mutations in a single nucleotide of specific genes will cause changes in the reproductive performance. Recently, an SNP molecular labelling technology has been widely used to study the quantitative traits of pigs (Wu *et al.* 2018).

Prolactin (PRL) is a protein hormone secreted by the anterior pituitary gland's eosinophils. Its primary role is to promote the development of an animal's gonads and mammary glands (Schennink *et al.* 2013). When combined with the corresponding receptor on the target cells (prolactin receptor, PRLR), PRL causes a series of physiological responses (Schennink *et al.* 2015). The *PRLR* gene is closely related to the animal's reproductive performance. For example, *PRLR* knockout mice were found to be infertile due to an embryo implantation failure and showed reproductive disorders such as cycle disorder and a reduced fertilisation rate (Ormandy *et al.* 1997). Previous studies also found that polymorphisms of the *PRLR* gene can affect the reproductive performance. In some poultry breeds, the SNP of the exons of the *PRLR* gene was found to result in significant differences in the egg-laying, growth and development between the different genotypes (Chen *et al.* 2012; Liang *et al.* 2019). Jiang *et al.* (2005) identified SNP (A9026G) on exon 3 of the chicken *PRLR* gene, which leads to a nucleotide conversion in the 5'-untranslated region of the *PRLR* gene. The *PRLR* gene polymorphism was associated with the number of lambs (An *et al.* 2015). Furthermore, it has been shown that porcine *PRLR* is localised to chromosome 16 that contains ten exons and nine introns, in which the 10th exon region harbours many mutations and has some associations with the reproductive performance of pigs (Hu *et al.* 1999).

The Large White pig is also known as the Yorkshire pig. It has the excellent characteristics of a high yield, fast growth rate, high feed utilisation rate, high carcass lean meat percentage, strong adaptability, high feed conversion rate and a high slaughter rate. It is also the world's most famous and widely-distributed dominantly lean pig species. Its related reproductive performance is inseparable from the development of animal husbandry (Alam *et al.* 2021). Hence, ways to improve the breeding traits of Large White pigs have become a hot topic in the field of animal husbandry. The modern Large White pig has undergone many improvements and different countries have conducted breeding according to their needs. While retaining the characteristics of the Large White pig, on the whole, the animals in different countries now have unique characteristics and form different strains. Therefore, when this study was designed, American, Danish and Canadian Large White pigs were selected to research the effects of different SNP sites in the *PRLR* gene on the sow reproductive performance and to establish the basis for a molecular marker foundation for sows, in order to increase the litter size.

Materials and Methods

Sample information and DNA extraction

A total of 201 back hair samples were collected from American Large White pigs (AC, n = 134), Canadian Large White pigs (CD, n = 52) and Danish Large White pigs (DN, n = 15). All of these pigs were selfed progeny of the corresponding breeds and were selected from the farms of Tianren Agriculture and Animal Husbandry Technology Co., Ltd. located in Leshan City, Sichuan Province, China. We recorded the total number born (TNB) and the number of born alive (NBA) of the first and second litter for each sow. Genomic DNA was extracted with the DP316 DNA kit (Tiangen Biochemistry Technology Co., Ltd., Beijing, China) from twenty hairs with follicles of the Large White pigs. The DNA purity was checked on 1% agarose gel electrophoresis and its quality was assessed by spectrophotometry (Thermo Fisher NanoDrop 2000/2000c).

Primer design, PCR amplification and SNP identification

The corresponding primers were designed according to the pig *PRLR* gene sequence (DQ458765.1) using Primer Premier 5.0 software and were synthesised by Chengdu Qingke Zixi Biotechnology Co., Ltd. The primer sequences were 5'-TAAGTAGTGGGATGTTAGGAA-3' and 5'-TGTTGTGGAGAAAGAGGC-3'. PCR was performed in a total volume of 30 µl using the following mixture: 2 × Taq Master Mix 15.0 µl, 1.5 µl of primers, 1.0 µl for the DNA template and ddH_2O 11.0 µl. The PCR amplification condition was as follows: an initial denaturing step at 94°C for 90 s, followed by 30 cycles of denaturing at 94°C for 20 s, annealing at 60°C for 20 s and extending at 72°C for 60 s, with a final extension step at 72°C for 5 min. The PCR products were sequenced by Chengdu Qingke Zixi Biotechnology Co., Ltd. The sequencing data was analysed with Bioedit software to search for the SNPs.

Data analyses

The Hardy-Weinberg equilibrium (HWE) and the Polymorphism Information Content (*PIC*) were analysed with SPSS 22.0 software. The allele and genotype frequencies were also calculated. A linkage disequilibrium analysis was performed using Haploview 4.2 software, while a haplotype construction of the SNP locus was performed using Phase 2.0. The association analysis between the SNP locus and the litter size traits was analysed with SAS

8.0 software. The model was designed as follows:

$$Yij = \mu + Gi + Pj + eij,$$

where *Yij* is the reproductive trait recording value; μ is the population mean; *Gi* was the i-th genotype effect; *Pj* is the parity effect; and *eij* is the random residual effect. The analysis was expressed as the least squares mean and standard error of the mean (LSM ± SEM), with $p < 0.05$ indicating a significant difference and $p < 0.01$ indicating an extremely significant difference. SAS 8.0 software was used for all the statistical analyses.

Results

Polymorphism and the genetic parameters of the *PRLR* gene

The PCR amplification products were sequenced, and the sequencing results were compared with the existing *PRLR* gene sequences in the gene bank. Nine SNPs were found in the exon region of the *PRLR* gene (Fig 1). The allele frequency, genotype frequency, *PIC* and χ^2 *t*ext of the nine SNPs polymorphic loci of the *PRLR* gene in the 201 tested pigs were calculated, and the results are shown in Table 1. According to the genetic parameters, the nine mutation sites in the

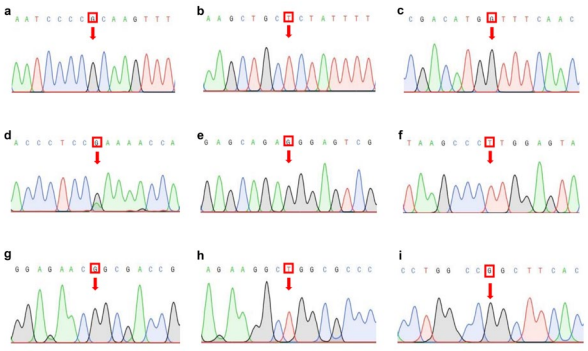

Fig. 1. Sequencing maps for the *PRLR* gene. Nine SNPs (a: g.C260G; b: g.C362T; c: g.C527G; d: g.A540G; e: g.A584G; f: g.A673T; g: g.A745G; h: g.C765T; i: g.A934G) were detected in the *PRLR* gene.

Table 1

Genotype frequencies, allele frequencies and Hardy-Weinberg equilibrium (HWE) test of *PRLR* SNPs locus in Large White pigs

SNPs	Genotype frequency / Allele frequency	Totality	AC	DN	CD	χ^2	*PIC*
C260G	CC/CG/GG	0.836/0.159/0.005	0.784/0.209/0.007	1.000/0.000/0.000	0.923/0.077/0.000	0.16	0.1429
	C/G	0.916/0.084	0.888/0.112	1.000/0.000	0.961/0.039		
C362T	CC/CT/TT	0.109/0.493/0.398	0.127/0.463/0.410	0.133/0.534/0.333	0.058/0.558/0.384	1.12	0.3533
	C/T	0.355/0.645	0.358/0.642	0.4000/0.600	0.337/0.663		
C527G	CC/CG/GG	0.836/0.159/0.005	0.784/0.209/0.007	1.000/0.000/0.000	0.923/0.077/0.000	0.16	0.1429
	C/G	0.916/0.084	0.888/0.112	1.000/0.000	0.961/0.039		
A540G	AA/AG/GG	0.915/0.085/0.000	0.881/0.119/0.000	1.000/0.000/0.000	0.981/0.019/0.000	0.39	0.0777
	A/G	0.958/0.042	0.941/0.059	1.000/0.000	0.990/0.010		
A584G	AA/AG/GG	0.453/0.468/0.079	0.485/0.433/0.082	0.333/0.534/0.133	0.404/0.538/0.058	1.51	0.3378
	A/G	0.687/0.313	0.701/0.299	0.600/0.400	0.673/0.327		
A673T	AA/AT/TT	0.463/0.458/0.079	0.485/0.433/0.082	0.467/0.400/0.133	0.404/0.538/0.058	1.07	0.3356
	A/T	0.692/0.308	0.702/0.298	0.667/0.333	0.673/0.327		
A745G	AA/AG/GG	0.005/0.169/0.826	0.007/0.209/0.784	0.000/0.133/0.967	0.000/0.077/0.923	0.28	0.1498
	A/G	0.089/0.911	0.112/0.888	0.067/0.933	0.039/0.961		
C765T	CC/CT/TT	0.294/0.482/0.224	0.254/0.478/0.268	0.867/0.133/0.000	0.231/0.596/0.173	0.18	0.3738
	C/T	0.535/0.465	0.493/0.507	0.067/0.933	0.529/0.471		
A934G	AA/AG/GG	0.294/0.482/0.224	0.254/0.478/0.268	0.867/0.133/0.000	0.231/0.596/0.173	0.18	0.3738
	A/G	0.535/0.465	0.493/0.507	0.067/0.933	0.529/0.471		

χ^2 (HWE): Hardy-Weinberg equilibrium χ^2 value. Large White pig strains: AC – American Large White pigs (n=134), DN – Danish Large White pigs (n=15), CD – Canadian Large White pigs (n=52). PIC – ploymorphism information content.

PRLR gene were indicated in the Hardy-Weinberg equilibrium by the χ^2 text. A medium polymorphism was found in g.C362T, g.A584G, g.A673T, g.C765T and g.A934G ($0.25 < PIC < 0.50$), while a low polymorphism was revealed in g.C260G, g.C527G, g.A540G and g.A745G ($0.00 < PIC < 0.25$).

Linkage disequilibrium relationships and haplotype analysis between the SNPs of the *PRLR* gene

Haploview 4.2 software was used to analyse the linkage disequilibrium (LD) among the nine mutation sites of the *PRLR* gene in the three Large White pigs lines (Fig. 2). The results showed a strong linkage disequilibrium among the SNP loci in the American Large White pigs. In the Canadian Large White pigs, there was strong linkage disequilibrium among the following loci: g.C360T, g.A584G and g.A673T, g.C765T, and g.A934G. In the Danish Large White pigs, probably due to the small sample size, there was no strong linkage disequilibrium. According to the results of the total samples, it was found that there was a strong linkage imbalance among the other loci, except for the g. A540G loci.

Nine SNPs from the *PRLR* genes detected from 201 Large White pig individuals were analysed for the haplotype, and the results are shown in Table 2. The detected nine SNPs constituted nine haplotypes in the 201 Large White pigs, including five haplotypes with a frequency above 3%; the three highest frequencies were H2, H1 and H4, at 41.64%, 22.79% and 19.51%, respectively, while the haplotypes with the lowest frequencies were H8 and H9.

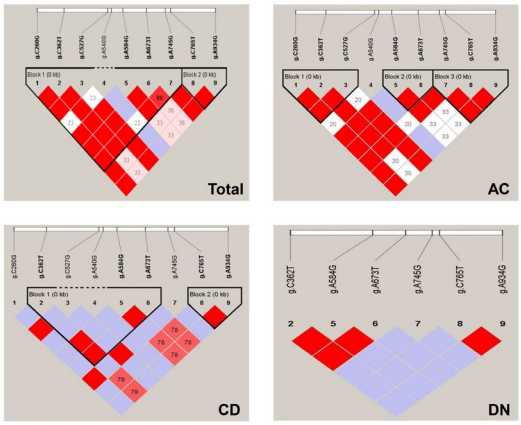

Fig. 2. Linkage disequilibrium plot of the three strains using the SNPs of porcine PRLR. 1231234Large White pig strains: AC – American Large White pigs; CD – Canadian Large White pigs; DN – Danish Large White pigs; Total – all of the Large White pigs.

Association analysis of the *PRLR* gene with litter traits

The association analyses of the nine SNPs of the *PRLR* in different strains of Large White pigs are shown in Table 3. In the analysis of the association between the *PRLR* gene SNP loci and the litter size of Large White pigs, both the CC genotype at g.C765T and the AA genotype at g.A934G significantly increased the total number born and the number born alive at first parity ($p < 0.01$), which could also significantly increase the total number born at multiparous parity ($p < 0.05$). There were no significant differences between the genotypes at the remaining loci.

Table 2

Haplotype frequencies of porcine SNPs in the *PRLR* gene

Haplotype	g.C260G	g.C362T	g.C527G	g.A540G	g.A584G	g.A673T	g.A745G	g.C765T	g.A934G	Frequency (%)
H1	C	T	C	A	A	A	G	T	G	22.79
H2	C	T	C	A	A	A	G	C	A	41.64
H3	C	C	C	A	G	A	A	C	A	0.50
H4	C	C	C	A	G	T	G	T	G	19.51
H5	C	C	C	A	G	T	G	C	A	2.88
H6	C	C	C	G	A	A	G	T	G	4.22
H7	G	C	G	A	G	T	A	C	A	8.46
H8	C	C	C	G	A	A	G	C	A	0.01
H9	G	C	G	A	G	T	A	T	G	0.00

Table 3

Associations between the SNPs of the *PRLR* and the litter traits in Large White pigs

SNPs	Breed	Genotype	TNB1	NBA1	TNB2	NBA2
g.C260G	AC	CC	10.31 ± 0.26	9.49 ± 0.29	10.88 ± 0.28	10.01 ± 0.28
		CG	11.03 ± 0.51	9.86 ± 0.55	10.68 ± 0.53	10.32 ± 0.55
		GG	15.00 ± 2.70	15.00 ± 2.94	11.00 ± 2.83	10.00 ± 2.89
	DN	CC	11.80 ± 0.91	11.20 ± 0.83	12.87 ± 1.07	11.67 ± 0.95
	CD	CC	11.46 ± 0.35	10.33 ± 0.39	/	/
		CG	12.25 ± 1.21	10.25 ± 1.34	/	/
	Total	CC	10.77 ± 0.21	9.88 ± 0.23	11.13 ± 0.28	10.22 ± 0.27
		CG	11.19 ± 0.48	9.91 ± 0.52	10.68 ± 0.57	10.32 ± 0.57
		GG	15.00 ± 2.74	15.00 ± 2.92	11.00 ± 3.03	10.00 ± 3.00
g.C362T	AC	CC	11.59 ± 0.66	11.00 ± 0.71a	10.82 ± 0.68	10.53 ± 0.70
		CT	10.37 ± 0.34	9.45 ± 0.37ab	10.66 ± 0.36	9.90 ± 0.37
		TT	10.31 ± 0.37	9.35 ± 0.39b	11.04 ± 0.38	10.13 ± 0.39
	DN	CC	11.50 ± 2.40	11.50 ± 2.05ab	9.00 ± 2.79	8.00 ± 2.39
		CT	13.13 ± 1.20	12.63 ± 1.03a	14.50 ± 1.39	13.25 ± 1.19
		TT	9.80 ± 1.52	8.80 ± 1.30b	11.80 ± 1.76	10.60 ± 1.51
	CD	CC	11.00 ± 1.37	10.67 ± 1.54	/	/
		CT	11.03 ± 0.44	9.93 ± 0.49	/	/
		TT	12.30 ± 0.53	10.85 ± 0.60	/	/
	Total	CC	11.50 ± 0.59	11.00 ± 0.62	10.63 ± 0.70	10.26 ± 0.69
		CT	10.79 ± 0.28	9.85 ± 0.29	11.10 ± 0.36	10.29 ± 0.36
		TT	10.78 ± 0.31	9.69 ± 0.33	11.10 ± 0.39	10.17 ± 0.39
g.C527G	AC	CC	10.31 ± 0.26	9.49 ± 0.29	10.88 ± 0.28	10.01 ± 0.28
		CG	11.03 ± 0.51	9.86 ± 0.55	10.68 ± 0.53	10.32 ± 0.55
		GG	15.00 ± 2.70	15.00 ± 2.92	11.00 ± 2.83	10.00 ± 2.89
	DN	CC	11.80 ± 0.91	11.20 ± 0.83	12.87 ± 1.07	11.67 ± 0.95
	CD	CC	11.46 ± 0.35	10.33 ± 0.39	/	/
		CG	12.25 ± 1.21	10.25 ± 1.34	/	/
	Total	CC	10.77 ± 0.21	9.88 ± 0.23	11.13 ± 0.28	10.22 ± 0.27
		CG	11.19 ± 0.48	9.91 ± 0.52	10.68 ± 0.57	10.32 ± 0.57
		GG	15.00 ± 2.74	15.00 ± 2.92	11.00 ± 3.03	10.00 ± 3.01
g.A540G	AC	AA	10.47 ± 0.25	9.56 ± 0.27	10.85 ± 0.26	10.05 ± 0.27
		AG	10.68 ± 0.68	9.94 ± 0.74	10.75 ± 0.70	10.25 ± 0.72
	DN	AA	11.80 ± 0.91	11.20 ± 0.83	12.87 ± 1.07	11.67 ± 0.95
	CD	AA	11.53 ± 0.34	10.33 ± 0.37	/	/
		AG	11.00 ± 2.43	10.00 ± 2.67	/	/
	Total	AA	10.88 ± 0.20	9.91 ± 0.22	11.08 ± 0.26	10.23 ± 0.26
		AG	10.71 ± 0.67	9.94 ± 0.71	10.75 ± 0.76	10.25 ± 0.75
g.A584G	AC	AA	10.24 ± 0.34	9.29 ± 0.37	11.12 ± 0.35	10.23 ± 0.35
		AG	10.62 ± 0.36	9.74 ± 0.39	10.36 ± 0.37	9.69 ± 0.38
		GG	11.36 ± 0.82	10.73 ± 0.89	11.64 ± 0.84	11.18 ± 0.86
	DN	AA	9.80 ± 1.52	8.80 ± 1.30b	11.80 ± 1.76	10.60 ± 1.51
		AG	13.13 ± 1.20	12.63 ± 1.03a	14.50 ± 1.39	13.25 ± 1.19
		GG	11.50 ± 2.40	11.50 ± 2.05ab	9.00 ± 2.80	8.00 ± 2.39
	CD	AA	12.24 ± 0.52	10.81 ± 0.58	/	/
		AG	11.04 ± 0.45	9.93 ± 0.50	/	/
		GG	11.00 ± 1.37	10.67 ± 1.54	/	/
	Total	AA	10.68 ± 0.29	9.62 ± 0.31	11.17 ± 0.36	10.26 ± 0.36
		AG	10.96 ± 0.28	10.04 ± 0.30	10.86 ± 0.37	10.12 ± 0.37
		GG	11.31 ± 0.69	10.81 ± 0.73	11.23 ± 0.84	10.69 ± 0.83

Table 3 Cont.

SNPs	Breed	Genotype	TNB1	NBA1	TNB2	NBA2
g.A673T	AC	AA	10.24 ± 0.34	9.29 ± 0.37	11.12 ± 0.35	10.23 ± 0.35
		AT	10.62 ± 0.36	9.74 ± 0.39	10.36 ± 0.37	9.69 ± 0.38
		TT	11.36 ± 0.82	10.73 ± 0.89	11.64 ± 0.84	11.18 ± 0.86
	DN	AA	10.71 ± 1.35	9.86 ± 1.20	12.71 ± 1.55	11.71 ± 1.36
		AT	13.17 ± 1.46	12.67 ± 1.29	14.33 ± 1.67	12.83 ± 1.46
		TT	11.50 ± 2.52	11.50 ± 2.24	9.00 ± 2.89	8.00 ± 2.53
	CD	AA	12.24 ± 0.52	10.81 ± 0.58	/	/
		AT	11.04 ± 0.45	9.93 ± 0.50	/	/
		TT	11.00 ± 1.37	10.67 ± 1.54	/	/
	Total	AA	10.73 ± 0.29	9.68 ± 0.30	11.28 ± 0.36	10.38 ± 0.35
		AT	10.91 ± 0.29	9.99 ± 0.30	10.73 ± 0.38	9.98 ± 0.37
		TT	11.31 ± 0.69	10.81 ± 0.73	11.23 ± 0.84	10.69 ± 0.83
g.A745G	AC	AA	15.00 ± 2.70	15.00 ± 2.94	11.00 ± 2.83	10.00 ± 2.89
		AG	11.04 ± 0.51	9.86 ± 0.55	10.68 ± 0.53	10.32 ± 0.55
		GG	10.31 ± 0.26	9.49 ± 0.29	10.88 ± 0.28	10.01 ± 0.28
	DN	AG	13.00 ± 2.55	12.50 ± 2.34	15.00 ± 3.00	14.50 ± 2.56
		GG	11.62 ± 1.00	11.00 ± 0.92	12.54 ± 1.17	11.23 ± 1.01
	CD	AG	12.25 ± 1.21	10.25 ± 1.34	/	/
		GG	11.46 ± 0.35	10.33 ± 0.39	/	/
	Total	AA	15.00 ± 2.73	15.00 ± 2.92	11.00 ± 3.04	10.00 ± 3.00
		AG	11.29 ± 0.47	10.06 ± 0.50	10.97 ± 0.55	10.60 ± 0.55
		GG	10.75 ± 0.21	9.85 ± 0.23	11.06 ± 0.28	10.14 ± 0.28
g.C765T	AC	CC	11.03 ± 0.47	10.15 ± 0.51	11.21 ± 0.48	10.32 ± 0.49
		CT	10.50 ± 0.34	9.39 ± 0.37	10.91 ± 0.35	10.14 ± 0.36
		TT	10.00 ± 0.45	9.47 ± 0.49	10.36 ± 0.47	9.72 ± 0.48
	DN	CC	11.85 ± 1.01	11.23 ± 0.93	13.15 ± 1.18	11.85 ± 1.05
		CT	11.50 ± 2.57	11.00 ± 2.37	11.00 ± 3.01	10.50 ± 2.68
	CD	CC	12.92 ± 0.66a	11.25 ± 0.76	/	/
		CT	11.29 ± 0.41b	10.03 ± 0.48	/	/
		TT	10.44 ± 0.77b	10.11 ± 0.88	/	/
	Total	CC	11.59 ± 0.35A	10.61 ± 0.38A	11.74 ± 0.44a	10.74 ± 0.43
		CT	10.77 ± 0.27AB	9.63 ± 0.30B	10.90 ± 0.37ab	10.15 ± 0.37
		TT	10.09 ± 0.40B	9.60 ± 0.43AB	10.36 ± 0.50b	9.72 ± 0.50
g.A934G	AC	AA	11.03 ± 0.47	10.51 ± 0.51	11.21 ± 0.48	10.32 ± 0.49
		AG	10.50 ± 0.34	9.39 ± 0.37	10.91 ± 0.35	10.14 ± 0.36
		GG	10.00 ± 0.45	9.47 ± 0.49	10.36 ± 0.47	9.72 ± 0.48
	DN	AA	11.85 ± 1.01	11.23 ± 0.93	13.15 ± 1.18	11.85 ± 1.05
		AG	11.50 ± 2.57	11.00 ± 2.37	11.00 ± 3.01	10.50 ± 2.68
	CD	AA	12.92 ± 0.66a	11.25 ± 0.76	/	/
		AG	11.29 ± 0.41b	10.03 ± 0.48	/	/
		GG	10.44 ± 0.77b	10.11 ± 0.88	/	/
	Total	AA	11.59 ± 0.35A	10.61 ± 0.38a	11.74 ± 0.44a	10.74 ± 0.43
		AG	10.77 ± 0.27AB	9.63 ± 0.30b	10.91 ± 0.37ab	10.15 ± 0.37
		GG	10.09 ± 0.40B	9.60 ± 0.43ab	10.36 ± 0.50b	9.72 ± 0.50

The different lowercase superscript of LSM of the same trait at the same locus indicating significant difference ($p < 0.05$). The different uppercase supersFcript of LSM of the same trait at the same locus indicating very significant difference ($p < 0.01$). TNB: Total number born; NBA: Number born alive. TNB1: Total number born at first parity; NBA1: Number born alive at first parity. TNB2: Total number born at multiparous parity; NBA2: Number born alive at multiparous parity. Values are the least squares mean and standard error of mean (LSM ± SEM)

Table 4

Correlations of haplotype combinations on the SNPs of the *PRLR* with the litter traits in Large White pigs

Haplotype combination	Frequency	TNB1	NBA1	TNB2	NBA2
H1H1	4.48%	8.44 ± 0.89	7.44 ± 0.94	11.11 ± 0.95	10.89 ± 0.96
H1H2	18.41%	11.11 ± 0.44	9.92 ± 0.46	10.85 ± 0.56	9.85 ± 0.56
H1H4	12.94%	10.38 ± 0.52	10.08 ± 0.55	9.50 ± 0.64	8.65 ± 0.64
H2H2	16.92%	11.03 ± 0.46	10.03 ± 0.48	11.36 ± 0.57	10.24 ± 0.58
H2H4	16.92%	10.68 ± 0.46	9.53 ± 0.48	11.44 ± 0.72	10.50 ± 0.72
H2H6	4.48%	10.44 ± 0.89	9.55 ± 0.94	11.63 ± 1.01	10.88 ± 1.02
H2H7	7.46%	12.07 ± 0.69	10.67 ± 0.73	11.42 ± 0.83	10.83 ± 0.83
H4H4	3.98%	11.50 ± 0.94	11.13 ± 1.00	12.00 ± 1.28	11.60 ± 1.29

TNB: Total number born; NBA: Number born alive. TNB1: Total number born at first litter. NBA1: Number born alive at first litter. TNB2: Total number born at multiparous litter. NBA2: Number born alive at multiparous litter. Values are the least squares mean and standard error of mean (LSM ± SEM).

Nine SNP loci were used for the haplotype combination and sixteen haplotype combinations were obtained, including eight haplotype combinations with frequencies of more than 3% (Table 4). Furthermore, among the eight haplotype combinations, the highest frequency combination was H1H2, with a frequency of 18.41%. In the sows, H2H7 was the dominant combination of the total number born at first parity (TNB1, 12.07 ± 0.69). Meanwhile, H4H4 was the dominant combination of the number born alive at first parity (NBA1, 11.13 ± 1.00), total number born (TNB2, 12.00 ± 1.28) and the number born alive at multiparous parity (NBA2, 11.60 ± 1.29). H1H1 was the inferior combination of TNB1 (8.44 ± 0.89) and NBA1 (7.44 ± 0.94), and H1H4 was the inferior combination of TNB1 (9.50 ± 0.64) and NBA1 (8.65 ± 0.64).

Discussion

Recently, molecular markers have been widely used to advance quantitative, functional and evolutionary genomics (Jiang *et al.* 2016). More and more SNPs are being used to explore the litter traits in pigs (Guo *et al.* 2017). For example, Wang *et al.* (2018) detected 41 suggestive significant SNPs associated with six reproductive traits in Large White pigs. Through a genome-wide association study of four reproductive traits in a Duroc pig herd, Zhang *et al.* (2019) detected 20 SNPs that were potentially associated with these traits of interest. The SNP loci identified in each experiment could lay the foundation for using molecular markers to increase litter size in sows. In addition, genetic variations in the functional genes could increase the number of litter births, TNB and the NBA (Laliotis *et al.* 2017). In this study, a correlation was sought between the associated polymorphism loci in the *PRLR* gene and the litter traits in Large White pigs.

Many studies have found that the *PRLR* gene plays an essential regulatory role in animal reproduction; for example, the prolactin receptor can maintain the corpus luteum and plays an important role in maternal pregnancy and foetal development (Zi *et al.* 2012) mRNA expression of prolactin receptor (PRLR. A number of polymorphisms have been identified in the porcine *PRLR* gene. Tomás *et al.* (2006) sequenced the complete coding region of the porcine *PRLR* gene and found 6 nonconservative SNPs. Skrzypczak *et al.* (2015) found three genotypes (AA, AT and TT) of *PRLR* by digestion with the *NcoI* restriction enzyme. Terman (2005) used the restriction enzyme *Alu I* to detect *PRLR* gene polymorphisms in Polish pigs and identified two alleles. Furthermore, Drogemuller *et al.* (2001) found that the *PRLR* gene polymorphism was associated with the reproductive trait of the live litter size in sows. In this study, nine SNPs were detected in exon 10 of the *PRLR* gene of Large White pigs (g.C260g, g.C362T, g.C527g, g.a540g, g.A584G, g.A673T and g.A745g). This will provide more reference sites for the molecular labelling of the *PRLR* gene.

Related studies have shown that the litter size is affected by various factors, such as the season (Mayorga *et al.* 2019; Caamaño *et al.* 2021), semen quality (Peña *et al.* 2005; Belstra *et al.* 2020), gilts (nulliparous) and multiparous sows (Peltoniemi *et al.* 2016).

Genetic variations of the *PRLR* gene in pigs can also cause changes in the litter performance. Researchers have studied different pig breeds successively and found that, in some studies of foreign breeding pigs and local pig breeds in China, the A allele is related to an excellent reproductive performance (Van Rens *et al.* 2003). Linville found that the *PRLR* gene affected the ovulation number of sows and the number of piglets produced in the first litter (Linville *et al.* 2001), and it was found that the number of individuals with the BB genotype of the *PRLR* gene was significantly higher than that of other genotypes (Mikhaĭlov *et al.* 2014). Isler found that the *PRLR* gene affects the average number of uterine horn foetuses in AA, AB and BB sows, and showed that AA > AB > BB, among which the type B gene is the favourable gene (Isler *et al.* 2002). In our study, the CC genotype at the g.C765T loci and the AA genotype at the g.A934G loci very significantly increased the TNB and NBA at first parity, as well as the TNB at multiparous parity in Large White pigs. In the haplotype construction and haplotype combination association analysis, H2H7 was the dominant combination of TNB at first parity (12.07 ± 0.69). On the other hand, H4H4 was the dominant combination of NBA at first parity (11.13 ± 1.00), TNB at multiparous parity (12.00 ± 1.28) and NBA at multiparous parity (11.60 ± 1.29). However, due to the limited sample size in this study, further investigation is required.

Conclusions

In conclusion, we found nine porcine SNPs in the *PRLR* gene, which confirmed the existence of its polymorphism. The correlation between the *PRLR* polymorphisms and the litter traits of the Large White pigs was verified. The g.C362T and g.A584G loci had significant effects on the litter traits ($p < 0.05$), while the g.C765T and g.A934G loci were also correlated with litter traits ($p < 0.01$). Therefore, the polymorphisms of *PRLR* were correlated with litter traits that have a selective value for the genetic breeding improvement of Large White pigs.

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Author Contributions

Research concept and design: L.N; Collection and/or assembly of data: J.X.; Data analysis and interpretation: Y.W., J.X.; Writing the article: Y.W.; Critical revision of the article: T.Z., L.S, L.N.; Final approval of article: T.Z., Y.Z., L.C., M.G., S.Z., L.Z., L.N.

Conflict of Interest

The authors declare no conflict of interest.

References

Alam M., Chang H. K., Lee S. S., Choi T. J. 2021. Genetic Analysis of Major Production and Reproduction Traits of Korean Duroc, Landrace and Yorkshire Pigs. Animals (Basel) **11**: 1321. https://doi.org/10.3390/ani11051321

An X., Hou J., Gao T., Lei Y., Li G., Song Y., Wang J., Cao B. 2015. Single-nucleotide polymorphisms g.151435C>T and g.173057T>C in PRLR gene regulated by bta-miR-302a are associated with litter size in goats. Theriogenology **83**: 1477-1483.e1471. https://doi.org/10.1016/j.theriogenology.2015.01.030

Bakoev S., Getmantseva L., Bakoev F., Kolosova M., Gabova V., Kolosov A., Kostyunina O. 2020. Survey of SNPs Associated with Total Number Born and Total Number Born Alive in Pig. Genes (Basel) **11**: 491. https://doi.org/10.3390/genes11050491

Belstra B. A., Willenburg K. L., Gómez-López D. H., Knox R. V., Stewart K. R. 2020. Effects of the number of sperm and site of uterine semen deposition on conception rate and the number of embryos in weaned sows receiving a single fixed-time insemination. J. Anim. Sci. **98**: skaa260. https://doi.org/10.1093/jas/skaa260

Caamaño J. N., Tamargo C., Parrilla I., Martínez-Pastor F., Padilla L., Salman A., Fueyo C., Fernández Á., Merino M. J., Iglesias T., Hidalgo C. O. 2021. Post-Thaw Sperm Quality and Functionality in the Autochthonous Pig Breed Gochu Asturcelta. Animals (Basel) **11**: 1885. https://doi.org/10.3390/ani11071885

Chen J., Liu H., Cai Y., Wang G., Liu H., Li J. 2012. Mutations in the exon 10 of prolactin receptor gene change the egg production performance in Wanjiang white goose. Mol. Biol. Rep. **39**: 475-483. https://doi.org/10.1007/s11033-011-0761-y

Drogemuller C., Hamann H., Distl O. 2001. Candidate gene markers for litter size in different German pig lines. J. Anim. Sci. **79**: 2565-2570. https://doi.org/10.2527/2001.79102565x

Guo Y., Huang Y., Hou L., Ma J., Chen C., Ai H., Huang L., Ren J. 2017. Genome-wide detection of genetic markers associated with growth and fatness in four pig populations using four approaches. Genet. Sel. Evol. **49**: 21. https://doi.org/10.1186/s12711-017-0295-4

Hu Z. Z., Zhuang L., Meng J., Leondires M., Dufau M. L. 1999. The human prolactin receptor gene structure and alternative promoter utilization: the generic promoter hPIII and a novel human promoter hP(N). J. Clin. Endocrinol. Metab. **84**: 1153-1156. https://doi.org/10.1210/jcem.84.3.5659

Isler B. J., Irvin K. M., Neal S. M., Moeller S. J., Davis M. E. 2002. Examination of the relationship between the estrogen receptor gene and reproductive traits in swine. J. Anim. Sci. **80**: 2334-2339. https://doi.org/10.2527/2002.8092334x

Jiang R. S., Xu G. Y., Zhang X. Q., Yang N. 2005. Association of polymorphisms for prolactin and prolactin receptor genes with broody traits in chickens. Poult. Sci. **84**: 839-845. https://doi.org/10.1093/ps/84.6.839

Jiang Z., Wang H., Michal J. J., Zhou X., Liu B., Woods L. C., Fuchs R. A. 2016. Genome Wide Sampling Sequencing for SNP Genotyping: Methods, Challenges and Future Development. Int. J. Biol. Sci. **12**: 100-108. https://doi.org/10.7150/ijbs.13498

Laliotis G. P., Marantidis A., Avdi M. 2017. Association of BF, RBP4, and ESR2 Genotypes with Litter Size in an Autochthonous Pig Population. Anim. Biotechnol. **28**: 138-143. https://doi.org/10.1080/10495398.2016.1242490

Liang K., Wang X., Tian X., Geng R., Li W., Jing Z., Han R., Tian Y., Liu X., Kang X., Li Z. 2019. Molecular characterization and an 80-bp indel polymorphism within the prolactin receptor (PRLR) gene and its associations with chicken growth and carcass traits. 3 Biotech. **9**: 296. https://doi.org/10.1007/s13205-019-1827-0

Linville R. C., Pomp D., Johnson R. K., Rothschild M. F. 2001. Candidate gene analysis for loci affecting litter size and ovulation rate in swine. J. Anim. Sci. **79**: 60-67. https://doi.org/10.2527/2001.79160x

Mayorga E. J., Renaudeau D., Ramirez B. C., Ross J. W., Baumgard L. H. 2019. Heat stress adaptations in pigs. Anim. Front. **9**: 54-61. https://doi.org/10.1093/af/vfy035

Mikhaĭlov N. V., Usatov A. V., Getmantseva L. V., Bakoev S. 2014. Associations of PRLR/AluI gene polymorphism with reproductive, growth and meat quality traits in pigs. Cytol. Genet. 48, 323-326. https://doi.org/10.3103/S0095452714050053

Ormandy C. J., Camus A., Barra J., Damotte D., Lucas B., Buteau H., Edery M., Brousse N., Babinet C., Binart N., Kelly P. A. 1997. Null mutation of the prolactin receptor gene produces multiple reproductive defects in the mouse. Genes Dev. **11**: 167-178. https://doi.org/10.1101/gad.11.2.167

Peltoniemi O., Björkman S., Oliviero C. 2016. Parturition effects on reproductive health in the gilt and sow. Reprod. Domest. Anim. **51**: 36-47. https://doi.org/10.1111/rda.12798

Peña F. J., Saravia F., García-Herreros M., Núñez-Martínez I., Tapia J. A., Johannisson A., Wallgren M., Rodríguez-Martínez H. 2005. Identification of sperm morphometric subpopulations in two different portions of the boar ejaculate and its relation to postthaw quality. J. Androl. **26**: 716-723. https://doi.org/10.2164/jandrol.05030

Plaengkaeo S., Duangjinda M., Stalder K. J. 2021. Longevity and lifetime reproductive trait genetic parameter estimates from Thai Landrace and Large White pig populations. Trop. Anim. Health Prod. **53**: 319. https://doi.org/10.1007/s11250-021-02579-5

Schennink A., Trott J. F., Freking B. A., Hovey R. C. 2013. A novel first exon directs hormone-sensitive transcription of the pig prolactin receptor. J. Mol. Endocrinol. **51**: 1-13. https://doi.org/10.1530/jme-12-0234

Schennink A., Trott J. F., Manjarin R., Lemay D. G., Freking B. A., Hovey R. C. 2015. Comparative genomics reveals tissue-specific regulation of prolactin receptor gene expression. J. Mol. Endocrinol. **54**: 1-15. https://doi.org/10.1530/jme-14-0212

Skrzypczak E., Babicz M., Pastwa M. 2015. Effect of Prolactin Receptor (PRLR) and Beta-Casein (CSN2) Gene Polymorphism on the Chemical Composition of Milk Sows. Folia Biol. (Krakow) **63**: 135-144. https://doi.org/10.3409/fb63_2.135

Terman A. 2005. Effect of the polymorphism of prolactin receptor (PRLR) and leptin (LEP) genes on litter size in Polish pigs. J. Anim. Breed. Genet. **122**: 400-404. https://doi.org/10.1111/j.1439-0388.2005.00547.x

Tian H. L., Wang F. G., Zhao J. R., Yi H. M., Wang L., Wang R., Yang Y., Song W. 2015. Development of maizeSNP3072, a high-throughput compatible SNP array, for DNA fingerprinting identification of Chinese maize varieties. Mol. Breed. **35**: 136. https://doi.org/10.1007/s11032-015-0335-0

Tomás A., Casellas J., Ramírez O., Muñoz G., Noguera J. L., Sánchez A. 2006. High amino acid variation in the intracellular domain of the pig prolactin receptor (PRLR) and its relation to ovulation rate and piglet survival traits. J. Anim. Sci. **84**: 1991-1998. https://doi.org/10.2527/jas.2005-664

Tummaruk P., Tantasuparuk W., Techakumphu M., Kunavongkrit A. 2009. The association between growth rate, body weight, backfat thickness and age at first observed oestrus in crossbred Landrace x Yorkshire gilts. Anim. Reprod. Sci. **110**: 108-122. https://doi.org/10.1016/j.anireprosci.2008.01.004

Van Rens B. T., Evans G. J., Van Der Lende T. 2003. Components of litter size in gilts with different prolactin receptor genotypes. Theriogenology **59**: 915-926. https://doi.org/10.1016/s0093-691x(02)01155-x

Wang Y., Ding X., Tan Z., Xing K., Yang T., Wang Y., Sun D., Wang C. 2018. Genome-wide association study for reproductive traits in a Large White pig population. Anim. Genet. **49**: 127-131. https://doi.org/10.1111/age.12638

Wu P., Yang Q., Wang K., Zhou J., Ma J., Tang Q., Jin L., Xiao W., Jiang A., Jiang Y., Zhu L., Li X., Tang G. 2018. Single step genome-wide association studies based on genotyping by sequence data reveals novel loci for the litter traits of domestic pigs. Genomics **110**: 171-179. https://doi.org/10.1016/j.ygeno.2017.09.009

Xu C., Ren Y., Jian Y., Guo Z., Zhang Y., Xie C., Fu J., Wang H., Wang G., Xu Y., Li P., Zou C. 2017. Development of a maize 55 K SNP array with improved genome coverage for molecular breeding. Mol. Breed. **37**: 20. https://doi.org/10.1007/s11032-017-0622-z

Zhang S., Zhang J., Olasege B. S., Ma P., Qiu X., Gao H., Wang C., Wang Y., Zhang Q., Yang H., Wang Z., Ding X., Pan Y. 2020. Estimation of genetic parameters for reproductive traits in connectedness groups of Duroc, Landrace and Yorkshire pigs in China. J. Anim. Breed. Genet. **137**: 211-222. https://doi.org/10.1111/jbg.12431

Zhang Z., Chen Z., Ye S., He Y., Huang S., Yuan X., Chen Z., Zhang H., Li J. 2019. Genome-Wide Association Study for Reproductive Traits in a Duroc Pig Population. Animals (Basel) **9**: 732. https://doi.org/10.3390/ani9100732

Zi X. D., Chen D. W., Wang H. M. 2012. Molecular characterization, mRNA expression of prolactin receptor (PRLR) gene during pregnancy, nonpregnancy in the yak (*Bos grunniens*). Gen. Comp. Endocrinol. **175**: 384-388. https://doi.org/10.1016/j.ygcen.2011.12.004