Association of *NR4A1* and *GNB2L1* genes with reproductive traits in commercial pig breeds

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ABSTRACT. The nuclear receptor subfamily 4, group A, member 1 (*NR4A1*) and guanine nucleotide binding protein beta polypeptide 2 like-1 (*GNB2L1*) genes are expressed during the ovulatory process and in early pregnancy in pigs. The objective of this study was to analyze the effects of *NR4A1* and *GNB2L1* gene variants on reproductive traits in commercial pig breeds. Two single nucleotide polymorphisms (SNPs) of *NR4A1* and *GNB2L1* were identified by a polymerase chain reaction-restriction fragment length polymorphism method. Analysis of the association of these two SNPs with reproductive traits was evaluated in 515 commercial sows (273 Large White and 242 Landrace). The SNP *NR4A1* g.3952A>G showed a significant association with the total number of piglets born, the number of piglets born alive, the number of piglets weaned alive, and the litter weight at weaning in the Landrace sows (P < 0.05). A significant association of SNP *GNB2L1* g.2373T>C with the litter weight at birth was observed in the Large White

sows (P < 0.05). These results indicate that the porcine *NR4A1* and *GNB2L1* can be used as candidate genes for improvement of litter size traits in pigs.

Key words: *NR4A1*; *GNB2L1*; Reproductive trait; Litter size; Pig breeds

INTRODUCTION

Reproductive traits have a major effect on costs and economic returns in pig production (Martínez-Giner et al., 2013). Previous studies have indicated that long-term selection for ovulation rate and embryonic survival can successfully increase litter size in pigs (Johnson et al., 1999; Bertani et al., 2004; Gladney et al., 2004). Various genomic approaches have been used to identify candidate genes for increased ovulation rate and embryonic survival. These studies have shown that candidate genes for reproductive traits are typically expressed in ovarian follicles and the anterior pituitary gland (Bertani et al., 2004; Gladney et al., 2004). Five genes, follistatin (*FST*), nuclear receptor subfamily 4, group A, member 1 (*NR4A1*), guanine nucleotide binding protein beta polypeptide 2 like-1 (*GNB2L1*), ferritin heavy-chain (*FTH*), and follicle stimulation hormone beta subunit (*FSHB*), are known to be differentially expressed between pig lines selected for improved reproductive traits and non-selected lines (Bertani et al., 2004; Gladney et al., 2004). To date, a few studies have used *FST*, *FTH*, and *FSHB* genes as a marker for determining reproductive improvement in pigs (Linville et al., 2001; Blowe et al., 2006; Lin et al., 2006; Pripwai and Mekchay, 2012). However, information on the association of *NR4A1* and *GNB2L1* with reproductive traits is limited. The present study was initiated to remedy this information deficit by examining the relationships of *NR4A1* and *GNB2L1* with reproductive traits in sows.

NR4A1 (also known as nerve growth factor induced-B, NGFI-B) is an orphan nuclear receptor for a transcription factor, and plays a major role in the ovulatory process (Liu et al., 2011). The gene is rapidly and transiently induced by a luteinizing hormone surge in granulosa cells of preovulatory follicles in rats (Park et al., 2001) and humans (Havelock et al., 2005). The porcine *NR4A1* gene is highly expressed in the ovary and uterus (Liu et al., 2011). Moreover, selection for increased levels of *NR4A1* gene expression in ovarian follicles is related to an enhanced rate of ovulation and improved survival of embryos in pigs (Gladney et al., 2004). The porcine *NR4A1* gene is localized on chromosome SSC5 (Genini et al., 2006) and contains six exons and five introns that encode a 202-amino acid protein (Liu et al., 2011). An A/G polymorphism in intron 5 of porcine *NR4A1* was found to be significantly associated with the total number of piglets born (TNB) and number of piglets born alive (NBA) traits in pigs (Liu et al., 2011).

GNB2L1 encodes a guanine-nucleotide binding protein that acts as a receptor for activated C kinase proteins and has also been named RACK1 (Chou et al., 1999; Uenishi et al., 2004). The porcine *GNB2L1* gene (GenBank accession No. NM_214332.1) has been successfully characterized. It is located on chromosome SSC5 (NCBI database, www.ncbi.nlm.nih.gov) and consists of eight exons and seven introns that encode a 317-amino acid protein (Chou et al., 1999). *GNB2L1* is expressed in the pituitary gland, testis, ovaries, spleen, thymus, liver, kidney, and adrenal gland in mammalian species (Chou et al., 1999; Martínez-Giner et al., 2013). It has also been shown to be expressed in oocytes and preimplantation embryos of mice (Luria et al., 2000; Dehghani and Hahnel, 2005). Thus, it may be involved in oocyte and embryo development in mammals.

The information from previous studies indicates that *NR4A1* and *GNB2L1* could be candidate genes that could act as markers of reproductive traits in pigs. The aim of this study was to

confirm this possibility by using *NR4A1* and *GNB2L1* polymorphisms to examine the association of these genes with reproductive traits in commercial pig breeds.

MATERIAL AND METHODS

Animals and genomic DNA extraction

Blood samples were taken from a total of 515 sows of the commercial pig breeds Large White (N = 273) and Landrace (N = 242). All sows were obtained from Betagro Hybrid International Company (Thailand). Reproductive performance of the sows was assessed in 2293 litters and was recorded in terms of TNB, NBA, number of piglets weaned alive (NWA), litter weight at birth (LWB, kg), and litter weight at weaning (21 days, LWW, kg). Genomic DNA samples were extracted by the Chelex method according to Walsh et al. (1991) and were kept at 4°C until analysis.

Identification of single nucleotide polymorphisms (SNP) and genotyping

SNP in the porcine *NR4A1* gene was detected as described by Liu et al. (2011). To identify the SNP in the porcine *GNB2L1* gene, primers were designed using nucleotide sequence information (GenBank accession No. AF146043; see Table 1). The primers covered the 5'-flanking region, exons and 3'-flanking region of the porcine *GNB2L1* gene. Six DNA samples were selected from Large White sows with the three highest and three lowest TNB values and were amplified using each primer. Each amplicon was then directly sequenced using the CEQ 8000 Genetic Analysis System (Beckman-Coulter, USA) to identify SNPs in *GNB2L1*. The identified SNP (g.2373T>C) in *GNB2L1* was used for the association study. Genotyping the SNPs of *NR4A1* and *GNB2L1* was carried out using the PCR-RFLP method. The PCR products were amplified in a total 20-µL volume containing 50 ng genomic DNA sample, 1X $(NH_4)_2 SO_4$ buffer (Fermentas), 1.5 mM $MgCl_2$, 0.2 mM dNTPs, 0.4 µM of each primer (Table 2), and 0.2 U *Taq* DNA polymerase (Fermentas). The amplification conditions were as follows: 94°C for 3 min for initial denaturing and 40 cycles at 94°C for 30 s, 58°-62°C for 30 s, 72°C for 45 s, and a final extension at 72°C for 5 min. The PCR fragments were digested with 0.25 U restriction enzymes (Fermentas) as shown in Table 2. The digested PCR fragments were separated by electrophoresis on 6% polyacrylamide gels in 1X TBE buffer. The gels were visualized by ethidium bromide staining.

Table 1. Primer sequences used for SNP identification in the porcine *GNB2L1* gene.

Primers	Nucleotide sequences	Position* (Region)	Product size (bp)	Tm (°C)
GNB2L1-1	F: 5'-ACTCTCCACAATACCTTAGG-3' R: 5'-TGCTAAGGTGAACTATTAAAC-3'	1402-1885 (5'-flanking region)	484	58°
GNB2L1-2	F: 5'-CAGAGCATAAACCTCTCCAG-3' R: 5'-TGAGGAAAAGACCGAGCAG-3'	1809-2341 (5'-flanking region)	533	60°
GNB2L1-3	F: 5'-TTGAGCAGGAGTGCATTGTG-3' R: 5'-ACGCACGGGTCAGCTCAC-3'	2251-2668 (Exon 1)	418	58°
GNB2L1-4	F: 5'-AATCACCTTGAGGTATCCTG-3' R: 5'-CTCTTACACAGTGAACCTGA-3'	3363-3734 (Exon 2)	372	60°
GNB2L1-5	F: 5'-ATCCATAGTGTAACCAAGTC-3' R: 5'-TATAAAAGGCCTACAACCTG-3'	4120-4663 (Exon 3-4)	544	58°
GNB2L1-6	F: 5'-CAGGTTTTTCACCTGTGACC-3' R: 5'-CAGGTATTTGGCCTCAACTG-3'	5146-5810 (Exon 5-6)	665	60°
GNB2L1-7	F: 5'-AACTCTTGGCATCACAGCAC-3' R: 5'-GACTACACTAACCTGATGAG-3'	6340-7059 (Exon 7)	720	58°

*Nucleotide position based on sequence information in GenBank accession No. AF146043.

Table 2. Primer sequences, PCR conditions, and restriction enzymes used for genotyping of porcine *NR4A1* and *GNB2L1* genes.

SNPs	Primer sequences	Tm (°C)	Product size (bp)	Restriction enzyme
NR4A1[a]	F: 5'-TTCCTCTGGGTCACAACG-3' R: 5'-CTCACAGAGTCAATGCCG-3'	58°	128	*Dde*I
GNB2L1[b]	F: 5'-TTGAGCAGGAGTGCATTGTG-3' R: 5'-ACCGGATAGCTCAGAGAGTC-3'	62°	143	*Taq*I

[a]Liu et al. (2011), [b]Mismatch base is underlined to generate a recognition site of the restriction enzyme *Taq*I for genotyping.

Statistical analysis

Genotype and allele frequencies were calculated. Hardy-Weinberg equilibrium was tested using the chi-square analysis. The analysis of the association of the *NR4A1* and *GNB2L1* genes with reproductive traits was performed using a general linear model (GLM) with the SAS software, version 6.08 (SAS Institute, Inc., Carry, NC, USA). The following statistical model was used: $Y_{ijkl} = \mu + P_i + YS_j + G_k + e_{ijkl}$, where Y_{ijkl} is representative of the observations of the phenotype values, μ represents the average normalized record of populations, P_i represents the fixed effect of parities (i = 1-5), YS_j represents the fixed effect of year-season (j = 1-8), G_k is representative of the fixed effect of the genotypes (k = 1-3), and e_{ijkl} represents the residual error. Additive and dominant effects were calculated according to Lin et al. (2006). Significance of the effects was tested by *t*-tests for deviation from zero.

RESULTS

Polymorphisms of *NR4A1* and *GNB2L1* genes

The A>G polymorphism in intron 5 of the porcine *NR4A1* gene was reported in a previous study (Liu et al., 2011) and, using the standard mutation nomenclature, named g.3952A>G based on the available nucleotide sequence information (GenBank accession No. NG_013208.1). The *NR4A1* g.3952A>G SNP was digested with the restriction enzyme *Dde*I and two specific alleles were identified, a 128-bp fragment for allele A, and two fragments of 93 and 35 bp for allele G. A novel SNP *GNB2L1* g.2373T>C was found in the 5'-flanking region and was also detected with the restriction enzyme *Taq*I. Two specific alleles were found to be a 143-bp fragment for allele T and two fragments of 122 and 21 bp for allele C. These two polymorphisms were segregating in the commercial pig breeds. The three different genotype patterns for SNPs *NR4A1* g.3952A>G and *GNB2L1* g.2373T>C loci among commercial pig breeds are shown in Figures 1 and 2, respectively.
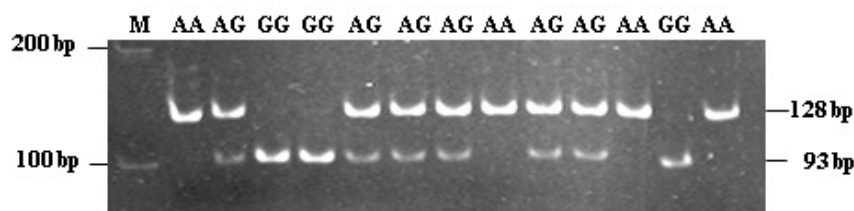

Figure 1. PCR-RFLP test for the SNP *NR4A1* g.3952A>G locus after digestion with the restriction enzyme *Dde*I. *Lane M* = 100-bp DNA ladder. *NR4A1* genotypes are given at the top of each line.
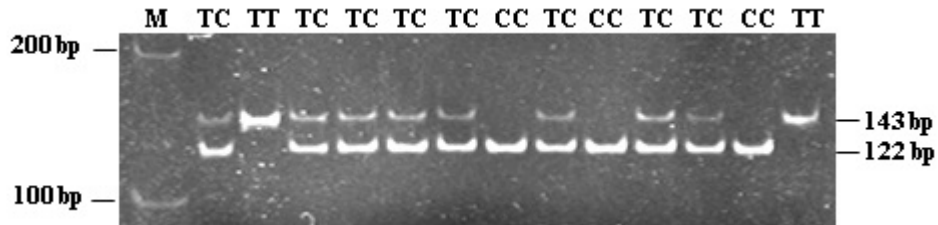
Figure 2. PCR-RFLP test for the SNP *GNB2L1* g.2373T>C locus after digestion with the restriction enzyme *Taq*I. *Lane M* = 100-bp DNA ladder. *GNB2L1* genotypes are given at the top of each line.

Genotype and allele frequencies

All genotype and allele frequencies of the porcine *NR4A1* and *GNB2L1* genes of two commercial pig breeds are shown in Table 3. The chi-square (χ^2) test showed that all genotypes for each locus of the porcine *NR4A1* and *GNB2L1* genes in the two commercial pig breeds were in Hardy-Weinberg equilibrium ($P > 0.05$), except for the SNP *NR4A1* g.3952A>G of the Large White pigs, which deviated significantly from the Hardy-Weinberg equilibrium ($P < 0.05$). At the *NR4A1* g.3952A>G locus, the *NR4A1* g.3952A allele was more frequent in the two commercial pig breeds. At the *GNB2L1* g.2373T>C locus, the *GNB2L1* g.2373T allele was the major allele in Landrace sows. The *GNB2L1* g.2373T allele was found to be a minor allele among Large White sows.

Table 3. Genotype and allele frequencies of the porcine *NR4A1* and *GNB2L1* genes in commercial pig breeds.

SNPs	Breed	Genotype frequency			Allele frequency[1]		P value[2] (χ^2)
		AA	AB	BB	f(A)	f(B)	
NR4A1 (g.3952A>G)	Large white	0.44	0.37	0.19	0.62	0.38	0.039*
	Landrace	0.69	0.28	0.03	0.83	0.17	0.839
GNB2L1 (g.2373T>C)	Large white	0.12	0.45	0.43	0.35	0.65	0.994
	Landrace	0.38	0.46	0.16	0.61	0.39	0.931

[1]Allele A represents wild-type alleles of the *NR4A1* g.3952A and *GNB2L1* g.2373T loci, respectively and allele B represents mutant alleles of the *NR4A1* g.3952G and *GNB2L1* g.2373C loci, respectively. [2]The P value is considered a significant level of the chi-square (χ^2) test for Hardy-Weinberg equilibrium of each locus in different pig breeds, *$P < 0.05$.

Associations of *NR4A1* and *GNB2L1* polymorphisms with reproductive traits

The association of the *NR4A1* and *GNB2L1* genes with reproductive traits is shown in Tables 4 and 5. There was no significant association of the *NR4A1* g.3952A>G SNP with any reproductive trait in Large White and Landrace sows at first parity. At later parities, the *NR4A1* g.3952A>G SNP was significantly associated with the TNB, NBA, NWA, and LWW traits in the Landrace sows ($P < 0.05$). Sows with the AA and AG genotypes had significantly higher TNB, NBA, NWA, and LWW values than with the GG genotype. Moreover, significant additive and dominant effects on the reproductive traits were observed in the Landrace sows (Table 4). No association was found in Large White sows for the *NR4A1* g.3952A>G SNP and any reproductive trait. No association of the *GNB2L1* g.2373T>C SNP was found with any reproductive trait in both breeds at first parity. *GNB2L1* g.2373T>C did show a significant association with the LWB trait at later parities in Large White sows ($P < 0.05$). Sows with the CC genotype had significantly higher LWB values

than those of sows with the TT and TC genotypes. Moreover, significant additive and dominant effects of the *GNB2L1* g.2373T>C SNP were found for reproductive traits in Large White and Landrace sows (Table 5).

Table 4. Association of the g.3952A>G locus of the porcine *NR4A1* gene with reproductive traits in commercial pig breeds.

Breed	Parity	Trait[1]	Genotype (means ± SE)[2]			Additive	Dominant
			AA	AG	GG		
Large White	First parity	TNB	10.89 ± 0.46	11.43 ± 0.49	11.66 ± 0.58	-0.38 ± 0.37	0.15 ± 0.59
		NBA	9.27 ± 0.45	9.92 ± 0.48	9.92 ± 0.66	-0.32 ± 0.37	0.32 ± 0.58
		NWA	8.57 ± 0.43	8.90 ± 0.46	8.87 ± 0.63	-0.15 ± 0.35	0.18 ± 0.55
		LWB	12.81 ± 0.59	13.48 ± 0.64	13.62 ± 0.86	-0.40 ± 0.48	0.27 ± 0.76
		LWW	57.48 ± 2.74	59.34 ± 2.92	58.22 ± 3.99	-0.37 ± 2.23	1.49 ± 3.49
	Later parities	TNB	11.11 ± 0.67	11.19 ± 0.64	11.52 ± 0.77	-0.21 ± 0.28	-0.13 ± 0.42
		NBA	9.83 ± 0.61	9.64 ± 0.58	10.16 ± 0.70	-0.17 ± 0.25	-0.36 ± 0.38
		NWA	9.00 ± 0.59	8.79 ± 0.56	9.52 ± 0.67	-0.26 ± 0.24	-0.46 ± 0.37
		LWB	14.65 ± 0.86	14.04 ± 0.81	14.98 ± 0.98	-0.17 ± 0.35	-0.78 ± 0.54
		LWW	59.65 ± 3.75	57.94 ± 3.56	62.15 ± 4.30	-1.25 ± 1.56	-2.96 ± 2.36
Landrace	First parity	TNB	9.76 ± 0.47	9.22 ± 0.50	9.23 ± 1.09	0.27 ± 0.52	-0.28 ± 0.64
		NBA	8.53 ± 0.52	7.94 ± 0.55	8.35 ± 1.21	0.09 ± 0.58	-0.50 ± 0.71
		NWA	7.86 ± 0.51	7.62 ± 0.54	7.67 ± 1.19	0.09 ± 0.56	-0.15 ± 0.69
		LWB	13.39 ± 0.67	12.46 ± 0.71	12.86 ± 1.55	0.26 ± 0.74	-0.66 ± 0.91
		LWW	52.94 ± 2.90	50.40 ± 3.08	50.27 ± 6.70	1.34 ± 3.19	-1.20 ± 3.95
	Later parities	TNB	11.00 ± 0.33[b]	11.51 ± 0.36[b]	9.37 ± 0.84[a]	0.82 ± 0.40*	1.32 ± 0.48**
		NBA	9.51 ± 0.35[b]	10.01 ± 0.38[b]	7.59 ± 0.88[a]	0.96 ± 0.42*	1.46 ± 0.50**
		NWA	8.71 ± 0.33[b]	9.46 ± 0.36[c]	6.94 ± 0.83[a]	0.88 ± 0.39*	1.64 ± 0.47***
		LWB	15.31 ± 0.47	16.18 ± 0.51	13.20 ± 1.22	1.06 ± 0.59	1.92 ± 0.69
		LWW	58.05 ± 2.00[b]	63.46 ± 2.17[c]	47.78 ± 5.17[a]	5.14 ± 2.48*	10.54 ± 2.92***

[1]LWB and LWW traits are reported in kg. [2]Means ± SE represent least square means ± standard error. Values in each row with different superscripts are significantly different (P < 0.05), *P < 0.05; **P < 0.01; ***P < 0.001.

Table 5. Association of the g.2373T>C locus of the porcine *GNB2L1* gene with reproductive traits in commercial pig breeds.

Breed	Parity	Trait[1]	Genotype (means ± SE)[2]			Additive	Dominant
			TT	TC	CC		
Large White	First parity	TNB	12.12 ± 0.82	10.86 ± 0.44	11.33 ± 0.45	0.40 ± 0.45	-0.86 ± 0.60
		NBA	10.93 ± 0.79	9.06 ± 0.43	9.79 ± 0.43	0.57 ± 0.43	-1.30 ± 0.58*
		NWA	9.60 ± 0.76	8.27 ± 0.41	8.86 ± 0.41	0.37 ± 0.41	-0.96 ± 0.55
		LWB	14.67 ± 1.03	12.54 ± 0.56	13.40 ± 0.57	0.63 ± 0.56	-1.50 ± 0.76*
		LWW	63.74 ± 4.72	55.05 ± 2.56	58.84 ± 2.63	2.45 ± 2.58	-6.25 ± 3.47
	Later parities	TNB	10.04 ± 0.83	10.85 ± 0.64	11.68 ± 0.63	-0.82 ± 0.32**	-0.01 ± 0.43
		NBA	8.89 ± 0.77	9.55 ± 0.60	10.12 ± 0.58	-0.61 ± 0.30*	0.04 ± 0.40
		NWA	8.47 ± 0.75	8.65 ± 0.58	9.23 ± 0.56	-0.38 ± 0.29	-0.20 ± 0.39
		LWB	13.18 ± 1.07[a]	13.84 ± 0.83[a]	14.96 ± 0.81[b]	-0.89 ± 0.41*	-0.23 ± 0.55
		LWW	55.48 ± 4.79	56.77 ± 3.69	61.07 ± 3.59	-2.79 ± 1.86	-1.50 ± 2.48
Landrace	First parity	TNB	9.62 ± 0.51	9.68 ± 0.49	8.87 ± 0.57	0.37 ± 0.28	0.43 ± 0.41
		NBA	8.37 ± 0.57	8.48 ± 0.55	7.62 ± 0.64	0.38 ± 0.32	0.48 ± 0.45
		NWA	7.96 ± 0.56	7.94 ± 0.53	6.97 ± 0.63	0.50 ± 0.31	0.48 ± 0.44
		LWB	13.06 ± 0.73	13.16 ± 0.70	12.19 ± 0.83	0.44 ± 0.42	0.54 ± 0.59
		LWW	52.13 ± 3.20	51.76 ± 3.04	49.47 ± 3.72	1.33 ± 1.90	0.97 ± 2.62
	Later parities	TNB	11.26 ± 0.35	11.34 ± 0.36	10.59 ± 0.45	0.34 ± 0.22	0.41 ± 0.30
		NBA	9.65 ± 0.36	9.81 ± 0.38	9.26 ± 0.46	0.19 ± 0.23	0.35 ± 0.32
		NWA	8.78 ± 0.35	9.13 ± 0.36	8.70 ± 0.44	0.04 ± 0.21	0.39 ± 0.30
		LWB	15.82 ± 0.49	15.86 ± 0.51	14.31 ± 0.62	0.76 ± 0.30**	0.80 ± 0.43
		LWW	60.07 ± 2.10	60.44 ± 2.20	57.31 ± 2.67	1.38 ± 1.30	1.75 ± 1.84

[1]LWB and LWW traits are reported in kg. [2]Means ± SE represent least square means ± standard error. Values in each row with different superscripts are significantly different (P < 0.05), *P < 0.05; **P < 0.01.

DISCUSSION

Litter size plays an important role in the profitability of the pig industry (Marantidis et al., 2013). Identification of single genes with large effects on quantitative traits provides a clear opportunity to improve the accuracy of selection for litter size (Short et al., 1997). Several association analyses have been carried out and candidate genes (*BF*, *ESR*, *FSHB*, *FST*, *LIF*, *PPARγ*, *PRLR*, and *RBP4*) with effects on reproductive traits have been identified in commercial pig breeds (Rothschild et al., 1996, 2000; Drögemüller et al., 2001; Buske et al., 2005; Spötter et al., 2005, 2009; Blowe et al., 2006; Lin et al., 2006; Wang et al., 2011; Marantidis et al., 2013). In the present study, we examined polymorphisms of the porcine *NR4A1* and *GNB2L1* genes with respect to reproductive traits. The *NR4A1* g.3952A>G and *GNB2L1* g.2373T>C SNPs were found to segregate in the Large White and Landrace sows. All three possible genotypes were observed in both commercial pig breeds. *NR4A1* g.3952A was the major allele in the Large White and Landrace sows. This result is consistent with a previous study, which showed that the *NR4A1* g.3952A allele was dominant among Large White pigs and DIV (a synthetic line) sows (Liu et al., 2011). *GNB2L1* g.2373T was the major allele in Landrace sows but was a minor allele in Large White sows. The results of the chi-square test showed that the *NR4A1* g.3952A>G SNP in Landrace sows met the Hardy-Weinberg equilibrium specifications whereas among the Large White sows it showed a significant deviation from Hardy-Weinberg equilibrium. *GNB2L1* g.2373T>C was in Hardy-Weinberg equilibrium in both Large White and Landrace sows. The results indicated that there was a large selection pressure on *NR4A1* g.3952A>G in the Large White sows, and that *GNB2L1* g.2373T>C of Large White and Landrace sows was under homeostasis when accompanied by artificial selection.

The association study showed that *NR4A1* and *GNB2L1* influenced reproductive traits. *NR4A1* g.3952A>G showed significant associations with TNB, NBA, NWA, and LWW traits in Landrace sows at later parities. Landrace sows with AA or AG genotypes had larger litter sizes. No association was found between *NR4A1* g.3952A>G and any reproductive trait in Large White sows. These results are inconsistent with a previous study, which indicated that the *NR4A1* g.3952A>G had significant associations with TNB and NBA traits in the Large White pig and DIV sows. Sows with AG or GG genotypes had larger litter sizes (Liu et al., 2011). The inconsistent results of these studies indicate that different linkage relationships exist across populations. Moreover, *NR4A1* g.3952A>G may have lower linkage disequilibrium with the causative mutation in this Large White population. The *NR4A1* gene is a nuclear steroid hormone receptor and is expressed in the ovarian follicle and ovary (Park et al., 2001) and plays an important role in the regulation of steroid signaling and follicle maturation (Havelock et al., 2005; Li et al., 2010; Dai et al., 2012). It has been reported that increased expression of *NR4A1* in pigs under selection for enhanced ovulation rate and embryonic survival may be correlated with an increased sensitivity to steroid hormones in ovarian follicles (Gladney et al., 2004). In the present study, the significant association identified between *NR4A1* and litter size traits indicated that this gene would be expected to play an important function in the litter size traits of pigs.

The *GNB2L1* g.2373T>C SNP showed significant association with the LWB trait in Large White sows. This SNP also displayed significant additive and dominant effects for TNB, NBA, and LWB traits in Large White sows. Similarly, *GNB2L1* g.2373T>C showed a significant additive effect for the LWB trait in Landrace sows. The *GNB2L1* gene is a receptor for activated C kinase proteins (Chou et al., 1999). It is expressed in oocytes and preimplantation embryos of mammals (Luria et al.,

2000; Dehghani and Hahnel, 2005). Selection for enhanced ovulation rate and embryonic survival caused a decrease in the expression level of *GNB2L1* in the pituitary gland of sows (Bertani et al., 2004). However, there is no evidence that long-term selection for increased ovulation rate and embryo survival altered the expression level of the *GNB2L1* gene in porcine ovarian follicles. The present study showed the association of *GNB2L1* g.2373T>C with reproductive traits in pigs. Our results indicate that the *GNB2L1* gene plays an important role in the function of the reproductive traits of pigs. Further association analyses on *NR4A1* and *GNB2L1* genes in a larger population sample are required. Moreover, the effects of the *GNB2L1* polymorphisms on mRNA and protein expression levels in target tissues need to be examined.

In conclusion, we found that polymorphisms of porcine *NR4A1* and *GNB2L1* genes have different patterns of association with reproductive traits. The SNPs *NR4A1* g.3952A>G and *GNB2L1* g.2373T>C had clear effects on TNB, NBA, NWA, LWB, and LWW traits in commercial pig breeds. The results indicate the importance of the *NR4A1* and *GNB2L1* genes in the reproductive traits of pigs. Therefore, these two SNPs may be used as candidate marker genes for genetic improvement of litter size in pig breeding.

Conflicts of interest

The authors declare no conflict of interest.

ACKNOWLEDGMENTS

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