ASSOCIATION OF POLYMORPHISMS IN THE RNF4, RBP4, AND IGF2 GENES WITH REPRODUCTIVE TRAITS IN LANDRACE AND YORKSHIRE SOWS

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ABSTRACT

The aim of the present study was to examine the association of polymorphisms in the small nuclear RING finger protein (RNF4), retinol binding protein 4 (RBP4) and the insulin-like growth factors 2 (IGF2) genes with reproductive traits in Landrace and Yorkshire sows. A total 393 sows (188 Landrace and 205 Yorkshire) were genotyped using the polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The results found that the polymorphic sites of two polymorphisms in the RNF4 and RBP4 genes were found in both breeds (RNF4: TT, TC and CC; RBP4: AA, AB, BB genotype; IGF2: AB and BB), except the genotype AA of IGF2 was not observed in Yorkshire sows. The polymorphisms of RNF4 showed a significant association with total number of piglets born and the number of piglets born alive in both Landrace and Yorkshire sows ($P < 0.05$). Sows with the CC genotype produced more piglets than TT genotype. A significant association of the RNF4 polymorphism with total litter weight of piglets born was also observed in the Landrace sows ($P < 0.05$). For the RBP4 gene, there was a significant association between the genotypes with the number of piglets borm, total number born alive, and total litter birth weight in Landrace sows ($P < 0.05$). No significant association of the IGF2 polymorphism with any reproductive traits was observed in either Landrace or Yorkshire sows ($P > 0.05$). These results indicated that the RNF4 and RBP4 genes can be used as candidate genes for improvement of litter size traits in pigs.

Keywords: IGF2, Landrace, reproductive performance, RNF4, RBP4, Yorkshire.

Mối liên hệ giữa đa hình gen RNF4, RBP4 và IGF2 với các tính trạng sinh sản của lợn nái Landrace và Yorkshire

TÓM TẮT

Nghiên cứu nhằm xác định mối liên hệ giữa đa hình gen RNF4, RBP4 và IGF2 với các tính trạng sinh sản của lợn nái Landrace và Yorkshire. Tổng số 393 nái (188 Landrace và 205 Yorkshire) được sử dụng để phân tích đa hình các gen bằng phương pháp PCR - RFLP. Kết quả cho thấy, cả 3 kiểu gen của RNF4 (TT, TC và CC), RBP4 (AA, AB và BB) được tìm thấy ở cả 2 giống lợn. Gen IGF2 có 3 kiểu gen AA, AB và BB xuất hiện ở quần thể lợn Landrace, nhưng chỉ 2 kiểu gen AB và BB được tìm thấy trong quần thể lợn Yorkshire. Đa hình gen RNF4 có ảnh hưởng rõ rệt với các tính trạng số con sơ sinh/ổ, số con sơ sinh sống/ổ ở cả 2 giống lợn Landrace và Yorkshire ($P < 0,05$). Lợn nái mang kiểu gen CC có số con sơ sinh/ổ, số con sơ sinh sống/ổ cao hơn những lợn nái mang kiểu kiên TT. Riêng ở lợn nái Landrace, khối lượng sơ sinh/ổ giữa các lợn nái mang kiểu gen khác nhau có sự sai khác có ý nghĩa thống kê. Lợn nái CC có khối lượng sơ sinh/ổ cao hơn lợn nái mang kiểu gen TT. Ở gen RBP4, sự sai khác rõ rệt về số con sơ sinh/ổ, số con sơ sinh sống/ổ giữa giữa các kiểu gen chỉ quan sát được ở lợn nái Landrace. Lợn nái Landrace BB có số con sơ sinh/ổ, số con sơ sinh sống/ổ và khối lượng sơ sinh/ổ cao hơn rõ rệt lợn nái mang kiểu gen AA ($P < 0,05$). Đa hình gen IGF2 không ảnh hưởng đến các tính trạng sinh sản của cả 2 giống lợn nghiên cứu ($P > 0,05$). Như vậy, gen RNF4 và RBP4 có thể được sử dụng như những gen ứng cử viên nhằm nâng cao tính trạng sinh sản ở lợn.

Từ khóa: IGF2, Landrace, năng suất sinh sản, RNF4, RBP4, Yorkshire.

1. INTRODUCTION

Reproductive rate, especially litter size is one of the most economically important traits in pig production. Hence, it would be advantageous for pig producers to be able to select replacement gilts that had the potential to have larger litters (Campbell *et al.*, 2008). However, enhancement of reproductive traits by traditional selective methods has proven to be difficult due to low heritability for litter size, narrowing the genetic base of a population, and high costs (Naqvi, 2007). Therefore, the identification of major genes controlling reproduction could bring a possibility of utilization of these genes in marker-assisted selection (MAS) programs in pigs to improve reproductive efficiency. Up to date, a number of polymorphic genes, including RNF4, RBP4 and IGF2, with significant effects on litter size have been identified in pigs (Buske *et al.*, 2006).

The small nuclear RING finger protein (RNF4) is a steroid receptor coregulator, which can activate transcription from a steroid-independent promoter (Kaiser *et al.*, 2003; Poukka *et al.*, 2000). RNF4 can stimulate the rat Luteinizing Hormone-β promoter (Curtin *et al.*, 2004), and overexpression of RNF4 can enhance the transcription of glucocorticoid, progesterone, and estrogen receptors (Moilanen *et al.*, 1998; Saville *et al.*, 2002), as well as oocyte fetal germ cell and granulosa cell maturation (Hirvonen-Santti *et al.*, 2004). When studing the association of the RNF4 gene with reproductive traits of pigs, Niu *et al.* (2009) indicated that a RNF4 polymorphism was found to be significantly associated with the total piglets born and piglets born alive traits in pigs. The retinol binding protein 4 (RBP4) gene is localized on chromosome 14 in pigs. Harney *et al.* (1993) have shown that there is an increase in RBP4 gene expression in gravid porcine endometrium from day 10 to 12. Their results support an important role for this vitamin A transport protein in uterine and conceptus physiology during the establishment of pregnancy. Therefore, RBP4 was investigated as a candidate gene for litter size owing to its role at the time of high embryonic mortality rate. In pigs, the insulin-like growth factors 2 (IGF2) gene, localised on chromosome 2, appears maternally imprinted and expressed only via the sire (Nezer *et al.*, 1999). This gene was marked as a candidate gene for muscle mass (skeletal and cardiac) and fat deposition (Jeon *et al.*, 1999; Nezer *et al.*, 1999). However, Horák *et al.* (2001) reported that IGF2 could play a role in fertility.

In Vietnam, at present, the application of molecular genetics in breeding selection to improve pig production has obtained certain achievements. These studies have mainly focused on the effects of some genes such as halothane, RN, MC4R, and HFABF on pig production, but there are few or no studies evaluating the association of RNF4, RBP4, and IGF2 with reproductive traits. Therefore, the aim of this study was to evaluate the association of polymorphisms of RNF4, RBP4, and IGF2 with reproductive traits as a means for future genetic improvements in pig breeds in Vietnam.

2. MATERIALS AND METHODS

2.1. Animals and DNA extraction

The breeds used in this study were purebred Landrace and Yorkshire from the Dabaco Nucleus Breeding Pigs Company (DBC), Bac Ninh province and Dong Hiep Pig Farm (DH) in Hai Phong province. A total of 393 breeding sows, including 188 Landrace sows and 208 Yorkshire sows, were used. Ear notching was done on new born pigs for the purpose of identification. All pigs were fed with the same diet.

Ear tissue samples of Landrace and Yorkshire pigs were collected, genomic DNA was extracted by the QIAamp DNA FFPE TISSUE Kit. The concentration and purity of DNA was checked on 1% agarose gel and measured $OD_{A260/A280}$. Then, DNA was diluted to a concentration of 50 ng/µl.

2.2. PCR amplification and genotyping

Primers for RNF4 (Niu *et al.*, 2009), RBP4 (Rothschild *et al.*, 2000), and IGF2 (Knoll *et al.*, 2000) were used to amplify the specific gene

fragments (Table 1). The PCR program was slightly different from what was reported previously. The PCR reaction was performed using 50ng of genomic DNA, 1.5mM MgCl2, 0.2 mM dNTPs, 0.5 µM primers, and 2U of Taq DNA polymerase and PCR buffer for a 25 µl final volume. Amplification conditions were: (1) RNF4 followed the temprature program: 94°C for 4 min, followed by 35 cycles of 94°C for 45 sec, 53°C for 45 sec, and 72°C for 2 min, and ending with a final step of 72°C for 10 min; (2) RBP4 was: 95°C for 3 min, followed by 35 cycles of 95°C for 30 sec, 56°C for 45 sec, and 72°C for 45 sec, and ending with a final step of 72°C for 5 min; and (3) IGF2 was: 95°C for 2 min, followed by 30 cycles of 95°C for 20 sec, 55°C for 30 sec, and 72°C for 60 sec, with a final extension at 72°C for 7 min. The length of the PCR products were 336 bp, 550 bp, and 937 bp for IGF2, RBP4 and RNF4, respectively.

The amplified fragments were digested by SacII, MspI, and NciI enzymes for RNF4, RBP4 and IGF2, respectively. 8µL of each PCR product was digested at 37°C overnight in a total volume of 30µL, containing 1 U of the appropriate restriction enzyme, 3µL of restriction buffer, and 18.3µL of H_2O. The obtained fragments were separated on 2% agarose gel.

2.3. Data collection

Data of reproduction traits, which included age at first service (AFS), age at first farrowing (AFF), farrowing interval (FI), number born (NB), number born alive (NBA), number weaned (NW), birth rate (BR), weaning rate (WR), birth weight (BW), weaning weight (WW), litter birth weight (LBW), and litter weaning weight (LWW), were collected from the breeding farms.

2.4. Statistical analysis

The association of RNF4, RBP4, or IGF2 genotypes with reproductive performance was analyzed according to the following model: $Yijklm = \mu + Gi + Fj + Pk + Sl + eijklm$, where Yijklm is the observed values, µ is the average normalize record of the population, Gi is individual gene effects of RNF4, RBP4, or IGF2 (i = 3: 1, 2, 3), Fj is the effect of farms (j = 2: DBC, DH), Pk is the effect of parities (k = 6: 1, 2,..., 6), Sl is the effect of seasons (l = 2: 1, 2), and eijklm is the residual error.

3. RESULTS

3.1. Polymorphisms of the RNF4, RBP4, and IGF2 genes

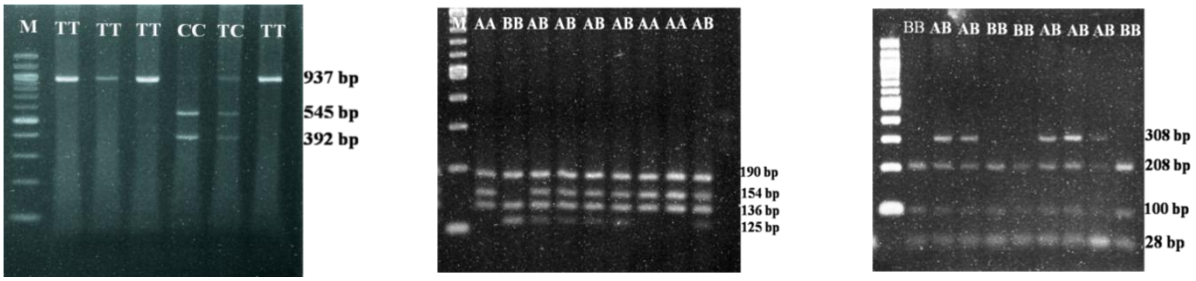
Banding patterns of RNF4 and IGF2 were consistent with what has been previously reported by Niu *et al.* (2009) and Knoll *et al.* (2000). Two alelle specific patterns of the RNF4 gene were obtained after SacII digestion, including an uncut 937bp fragment for allele T and two fragments of 545 bp and 392 bp for allele C; and consequently resulting in three genotypes, TT (937 bp), CC (545 and 392 bp), and TC (937, 545 and 392 bp) (Figure 1). For IGF2, two alleles (A and B) and three genotypes (AA, AB, and BB) were indentified in Landrace sows while there were only two genotypes (AB and BB) in Yorkshire sows. Allele A is characterized by digestion of the 336 bp PCR product into fragments of 308 and 28 bp, while allele B with a polymorphic restriction site is represented by fragments of 208, 100, and 28 bp. In the present study the banding patterns of RBP4 were not entirely consistent with what Rothschild *et al.* (2000) reported. The banding patterns can be classified into AA (190, 154, and 136 bp), BB (190, 136, and 125 bp) and AB (190, 154, 136, and 125 bp) (Figure 1).

3.2. Associations of RNF4, RBP4 and IGF2 polymorphisms with reproductive traits

Tables 2, 3, and 4 summarize the results concerning the association of the genes, parities, farms, and seasons with reproductive traits in the Landrace and Yorkshire sows. The results showed that parities and farms affected almost all the reproductive traits of commercial breeds ($P<0.05$). Seasons affected only a few traits at weaning time in Landrace sows but affected almost traits in Yorkshire sows. The polymorphisms of the RNF4 and RBP4 genes had an association with litter size traits while no significant association was found between the polymorphism of the IGF2 gene with any reproductive traits.

Table 1. Primer sequences, PCR product sizes and restriction enzymes of RNF4, RBP4, and IGF2

Gene	Region	Primer sequences (5'-3')	Product size (bp)	Restriction enzyme	Sources
RNF4	Intron 5	CGAAATGCCAGGGAAGAG CCATGCAGATCGGACAACT	937	*SacII*	Niu *et al.* (2009)
RBP4	Exon 2	GAGCAAGATGGAATGGGTT CTCGGTGTCTGTAAAGGTG	550	*MspI*	Rothschild *et al.* (2000)
IGF2	Intron 7	CACAGCAGGTGCTCCATCGG GACAGGCTGTCATCCTGTGGG	336	*NciI*	Knoll *et al.* (2000)



RNF4 gene RBP4 gene IGF2 gene

Figure 1. Genotyping of RNF4, RBP4, and IGF2 after digestion with the restriction enzyme SacII, MspI, and Nci I, respectively. The molecular marker of 100 bp DNA ladder (M) and the RNF4, RBP4, and IGF2 genotypes are indicated at the top of each lane

The RNF4 gene was significantly associated with the NB and NBA traits in both Landrace and Yorkshire sows ($P<0.05$). Sows with the CC genotype had significantly higher NB and NBA values than with the TT genotype. A signigicant difference was also observed for the LBW trait in Landrace sows ($P<0.001$). The sows with the CC genotype had a greater LBW value compared to those with the TT genotype. In the RBP4 locus, the genotypes were significantly associated with the NB, NBA, and LBW traits in Landrace sows ($P<0.05$). The BB sows had greater NB, NBA, and LBW values than with AA. The FI and WR traits of Yorkshire sows were significant different among genotypes. Sows with the AB genotype had shorter FI values than BB and had higher WR values than AA. No significant association was found of the RBP4 genes' genotypes with litter size in Yorkshire sows. For the IGF2 gene, there was no significant association of genotypes with any reproductive traits in either Landrace or Yorkshire sows ($P>0.05$).

4. DISCUSSION

The development of porcine genome maps offers the opportunity to identify individual genes controlling reproduction (Drogemuller *et al.*, 2001). Applications of MAS will increase as more associations between markers and traits are identified (Rothschild, 1998). Reproduction traits plays an important role in the profitability of the pig industry (Marantidis *et al.*, 2015). Identification of a single gene with large effects on quantitative traits provides a clear opportunity to improve the accuracy of selection for litter size (Short *et al.*, 1997). In the present study, we examined polymorphisms of RNF4, RBP4, and IGF2 with reproductive traits of two comercial breeds, Landrace and Yorkshire sows. The polymorphisms of RNF4 and RBP4 were found to segregate in the Landrace and Yorkshire sows. All three possible genotypes of both genes (TT, TC, and CC of RNF4; and AA, AB, and BB of RBP4) were observed in both commercial breeds. However, in the IGF2 gene, three genotypes (AA, AB and BB) were found in Landrace sows, but no AA genotype was detected in Yorkshire sows.

Table 2. Association of RNF4, parities, farms, and seasons with reproductive traits in Landrace and Yorkshire sows

Traits	Landrace							Yorkshire						
	RNF4			Level of significance				RNF4			Level of significance			
	TT	TC	CC	RNF4	Farm	Parity	Season	TT	TC	CC	RNF4	Farm	Parity	Season
AFS	249.18 ± 6.70	255.68 ± 7.76	272.69 ± 11.76	ns	ns	-	ns	251.14 ± 16.00	260.56 ± 6.41	250.36 ± 7.02	ns	**	-	ns
	27	17	6					3	18	17				
AFF	366.36 ± 6.71	372.02 ± 7.77	389.5 ± 11.77	ns	*	-	ns	368.07 ± 15.74	376.71 ± 6.31	366.09 ± 6.90	ns	**	-	ns
	27	17	6					3	18	17				
FI	157.11 ± 2.14	151.3 ± 1.93	151.58 ± 2.97	ns	*	ns	ns	155.32 ± 1.54	149.57 ± 2.43	150.70 ± 1.77	ns	ns	ns	ns
	38	67	17					38	23	23				
NB	11.51 ± 0.24^{b}	11.85 ± 0.23^{ab}	12.76 ± 0.39^{a}	*	*	***	ns	11.46 ± 0.54^{b}	12.79 ± 0.26^{ab}	13.14 ± 0.22^{a}	**	*	*	ns
	233	133	47					34	143	244				
NBA	10.21 ± 0.23^{b}	10.59 ± 0.23^{ab}	11.48 ± 0.38^{a}	*	**	ns	ns	9.76 ± 0.47^{b}	10.94 ± 0.23^{b}	11.02 ± 0.19^{a}	*	***	ns	**
	230	133	47					34	143	244				
BR	88.8 ± 1.00	89.42 ± 0.98	90.69 ± 1.66	ns	ns	***	*	86.73 ± 1.97	86.8 ± 0.96	84.66 ± 0.82	ns	***	***	**
	230	133	47					34	143	244				
LBW	16.71 ± 0.33^{b}	17.59 ± 0.32^{ab}	18.96 ± 0.54^{b}	***	***	***	ns	15.26 ± 0.78	16.81 ± 0.37	16.99 ± 0.31	ns	***	***	*
	213	130	46					30	134	231				
BW	1.47 ± 0.02	1.48 ± 0.02	1.5 ± 0.03	ns	ns	***	ns	1.34 ± 0.04	1.32 ± 0.02	1.31 ± 0.02	ns	***	**	ns
	213	130	46					30	134	231				
NW	9.47 ± 0.14	9.48 ± 0.13	9.4 ± 0.21	ns	***	ns	ns	9.14 ± 0.26	9.62 ± 0.12	9.35 ± 0.10	ns	***	ns	***
	138	106	42					23	108	198				
WR	84.47 ± 1.40	85.38 ± 1.32	83.05 ± 2.10	ns	ns	*	ns	86.22 ± 2.93	84.76 ± 1.32	84.68 ± 1.10	ns	**	**	ns
	135	106	42					23	107	198				
LWW	64.87 ± 1.46	62.87 ± 1.38	65.78 ± 2.22	ns	***	ns	*	59.79 ± 2.96	61.28 ± 1.31	59.3 ± 1.11	ns	***	ns	***
	121	98	37					21	101	168				
WW	6.85 ± 0.13	6.63 ± 0.12	7.05 ± 0.19	ns	ns	*	ns	6.56 ± 0.25	6.39 ± 0.11	6.39 ± 0.09	ns	**	*	*
	121	98	37					21	101	168				

Note: Least square means ± SE (denoted by bold number) and number of records (denoted by italic number) of age at first service (AFS, day), age at first farrowing (AFF, day), farrowing interval (FI, day), number born (NB, piglet), number born alive (NBA, piglet), number weaned (NW, piglet), birth rate (BR, %), weaning rate (WR, %), birth weight (BW, kg), weaning weight (WW, kg), litter birth weight (LBW, kg), and litter weaning weight (LWW, kg),
*Value in each row of each breed with different superscripts are significantly different (P < 0.05). ns: P≥ 0.05; *: P< 0.05; **: P < 0.01; ***: P< 0.001.*

Table 3. Association of RBP4, parities, farms, and seasons with reproductive traits in Landrace and Yorkshire sows

Traits	Landrace							Yorkshire						
	RBP4			Level of significance				RBP4			Level of significance			
	AA	AB	BB	RBP4	Farm	Parity	Season	AA	AB	BB	RBP4	Farm	Parity	Season
AFS	259.56 ± 4.65	244.69 ± 5.35	258.23 ± 6.16	ns	***	-	ns	262.99 ± 5.54	248.87 ± 4.85	249.88 ± 7.05	ns	***	-	ns
	58	*21*	*19*					*20*	*29*	*13*				
AFF	376.52 ± 4.72	362.36 ± 5.51	374.94 ± 6.2	ns	***	-	ns	376.24 ± 5.58	366.64 ± 4.89	365.96 ± 7.10	ns	***	-	ns
	57	*20*	*19*					*20*	*29*	*13*				
FI	155.21 ± 2.48	150.67 ± 2.11	152.04 ± 2.72	ns	ns	ns	ns	156.14 ± 3.26^{ab}	149.71 ± 2.55^{b}	158.72 ± 3.48^{a}	*	ns	***	ns
	27	*47*	*24*					*19*	*36*	*15*				
NB	11.24 ± 0.22^{b}	11.52 ± 0.22^{ab}	12.01 ± 0.26^{a}	*	**	***	ns	12.72 ± 0.25	12.08 ± 0.25	12.48 ± 0.27	ns	***	ns	ns
	360	*177*	*156*					*245*	*270*	*271*				
NBA	10.03 ± 0.22^{b}	10.39 ± 0.22^{ab}	10.65 ± 0.26^{a}	*	*	**	*	10.72 ± 0.23	10.21 ± 0.23	10.5 ± 0.25	ns	***	ns	ns
	357	*174*	*156*					*245*	*270*	*216*				
BR	89.61 ± 0.97	90.31 ± 0.97	89.01 ± 1.15	ns	ns	ns	***	85.14 ± 0.94	85.74 ± 0.93	84.96 ± 1.02	ns	***	***	*
	357	*174*	*156*					*245*	*270*	*216*				
LBW	16.61 ± 0.31^{b}	16.98 ± 0.31^{ab}	17.52 ± 0.37^{a}	*	***	***	ns	16.20 ± 0.35	15.55 ± 0.35	16.34 ± 0.38	ns	***	***	ns
	327	*168*	*150*					*231*	*252*	*202*				
BW	1.47 ± 0.01	1.48 ± 0.02	1.48 ± 0.02	ns	ns	***	ns	1.29 ± 0.01	1.31 ± 0.01	1.33 ± 0.02	ns	***	***	ns
	327	*168*	*150*					*231*	*252*	*202*				
NW	9.47 ± 0.12	9.56 ± 0.11	9.62 ± 0.14	ns	***	ns	ns	9.43 ± 0.11	9.31 ± 0.11	9.41 ± 0.12	ns	***	ns	***
	198	*121*	*110*					*165*	*288*	*144*				
WR	85.59 ± 1.29	86.56 ± 1.22	87.6 ± 1.48	ns	ns	*	ns	83.83 ± 1.19^{b}	87.23 ± 1.16^{a}	86.07 ± 1.32^{ab}	*	*	*	ns
	195	*119*	*110*					*165*	*188*	*143*				
LWW	64.3 ± 1.34	63.72 ± 1.24	63.86 ± 1.56	ns	***	ns	**	59.65 ± 1.29	58.88 ± 1.25	61.03 ± 1.46	ns	***	**	***
	178	*116*	*95*					*153*	*177*	*123*				
WW	6.83 ± 0.12	6.70 ± 0.11	6.64 ± 0.14	ns	ns	**	ns	6.36 ± 0.11	6.38 ± 0.10	6.53 ± 0.12	ns	**	**	*
	178	*116*	*95*					*153*	*177*	*123*				

Note: Least square means ± SE (denoted by bold number) and number of records (denoted by italic number) of age at first service (AFS, day), age at first farrowing (AFF, day), farrowing interval (FI, day), number born (NB, piglet), number born alive (NBA, piglet), number weaned (NW, piglet), birth rate (BR, %), weaning rate (WR, %), birth weight (BW, kg), weaning weight (WW, kg), litter birth weight (LBW, kg), and litter weaning weight (LWW, kg),
*Value in each row of each breed with different superscripts are significantly different (P < 0.05). ns: P≥ 0.05; *: P< 0.05; **: P < 0.01; ***: P< 0.001*

Table 4. Association of IGF2, parities, farms, and seasons with reproductive traits in Landrace and Yorkshire sows

Traits	Landrace							Yorkshire					
	IGF2			Level of significance				IGF2		Level of significance			
	AA	AB	BB	IGF2	Farm	Parity	Season	AB	BB	IGF2	Farm	Parity	Season
AFS	267.14 ± 16.21	252.76 ± 5.41	252.18 ± 5.20	ns	***	-	*	251.67 ± 9.64	258.54 ± 5.87	ns	***	-	ns
	3	27	37					10	24				
AFF	385.05 ± 16.34	368.99 ± 5.62	368.85 ± 5.25	ns	***	-	*	366.89 ± 9.52	374.77 ± 5.80	ns	***	-	ns
	3	*26*	*37*					*10*	*24*				
FI	155.74 ± 5.72	157.69 ± 2.56	150.71 ± 2.12	ns	ns	ns	ns	150.31 ± 2.67	153.32 ± 2.08	ns	ns	ns	ns
	6	51	44					24	34				
NB	12.03 ± 0.68	11.46 ± 0.2	12.06 ± 0.21	ns	ns	***	ns	12.72 ± 0.42	13.33 ± 0.27	ns	ns	**	ns
	17	190	281					52	367				
NBA	10.84 ± 0.66	10.14 ± 0.2	10.74 ± 0.21	ns	ns	***	ns	10.66 ± 0.39	11.06 ± 0.25	ns	*	*	ns
	17	189	278					51	367				
BR	90.91 ± 2.95	88.65 ± 0.88	89.29 ± 0.92	ns	ns	ns	ns	84.47 ± 1.50	83.51 ± 0.97	ns	***	***	ns
	17	*189*	*278*					*51*	*367*				
LBW	16.75 ± 0.95	16.78 ± 0.29	17.43 ± 0.30	ns	*	***	ns	16.25 ± 0.61	16.69 ± 0.39	ns	***	***	ns
	17	183	264					51	350				
BW	1.41 ± 0.05	1.48 ± 0.02	1.45 ± 0.02	ns	ns	***	ns	1.29 ± 0.03	1.26 ± 0.02	ns	***	**	ns
	17	183	264					51	350				
NW	9.62 ± 0.40	9.45 ± 0.13	9.42 ± 0.12	ns	***	ns	*	9.73 ± 0.20	9.63 ± 0.13	ns	***	ns	*
	13	124	190					43	276				
WR	90.83 ± 3.94	84.39 ± 1.26	85.79 ± 1.22	ns	ns	*	ns	87.56 ± 2.10	84.76 ± 1.28	ns	ns	***	ns
	13	123	187					42	276				
LWW	66.5 ± 4.68	63.25 ± 1.32	63.61 ± 1.26	ns	***	*	**	60.83 ± 2.21	60.39 ± 1.37	ns	**	*	***
	9	113	171					43	244				
WW	6.84 ± 0.40	6.67 ± 0.11	6.81 ± 0.11	ns	ns	*	ns	6.29 ± 0.19	6.27 ± 0.12	ns	ns	*	**
	9	113	171					43	244				

Note: Least square means ± SE (denoted by bold number) and number of records (denoted by italic number) of age at first service (AFS, day), age at first farrowing (AFF, day), farrowing interval (FI, day), number born (NB, piglet), number born alive (NBA, piglet), number weaned (NW, piglet), birth rate (BR, %), weaning rate (WR, %), birth weight (BW, kg), weaning weight (WW, kg), litter birth weight (LBW, kg), and litter weaning weight (LWW, kg),

*Value in each row of each breed with different superscripts are significantly different (P < 0.05). ns: P≥ 0.05; *: P< 0.05; **: P < 0.01; ***: P< 0.001.*

The present study indicated that RNF4 influenced NB and NBA in both Landrace and Yorkshire sows. In Landrace, the CC sows had an average of 1.25 more piglets for NB and 1.27 more piglets for NBA than TT sows; in Yorkshire, the sows with CC produced 1.68 and 1.26 more piglets for NB and NBA, respectively, as compared to TT sows. These results are consistent with a previous study which reproted that CC animals in a Qingping population had more NB (+1.74 piglets) and NBA (+2.02 piglets) than sows with the TT genotype (Niu *et al.*, 2009). RNF4 contains multiple functional domains, one of those acts on the ovaries and testes to stimulate production of the sex steroids estrogen, testosterone, and progesterone, and it can interact with steroid receptors and modulate their activity in ovarian cells (Hirvonen-Santti *et al.*, 2004). In the present study, the significant association identified between RNF4 and litter size traits indicated that this gene would be expected to play an improtant function in the litter size traits of pigs.

The RBP4 polymorphism showed a significant association with NB and NBA in Landrace sows but did not significantly affect these traits in Yorkshire sows. The Landrace sows with BB genotypes had significantly higher NB (0.77 piglets), NBA (0.62 piglets), and LWB (0.91 more kg) values than those of sows with the AA genotype. There are numerous studies on the effect of RBP4 gene polymorphisms on the reproductive performance of sows (Rothschild *et al.*, 2000; Drogemuller *et al.*, 2001; Spötter and Distl, 2006; Wang *et al.*, 2006; Terman *et al.*, 2007; Omelka *et al.*, 2008; Spotter *et al.*, 2009; Marantidis *et al.*, 2015). There are, however, two distinct trends in genotypes that have a positive effect on litter size. A greater number of studies have shown that the BB genotype has higher NB and NBA than those of the AA genotype (Drogemuller *et al.*, 2001; Wang *et al.*, 2006; Terman *et al.*, 2007; Spotter *et al.,* 2009). Several other studies indicated that the AA genotype has higher NB and NBA compared to the others genotypes (Omelka *et al.*, 2008; Marantidis *et al.*, 2015). Wang *et al.* (2006) showed that sows with the BB genotype of the RBP4 locus had more piglets per litter than sows with AA and AB genotypes. The results of the present study confirmed that there were interaction effects among genes with NB and NBA. The BB genotype had higher NB and NBA values than the AA genotype in Landrace sows. Therefore, RBP4 was investigated as a candidate gene for litter size owing to its integral role at the time of high embryonic mortality rate.

No significant association was found between the polymorphism of the IGF2 gene and reproductive traits in both Landrace and Yorkshire sows. Litter size of commercial breeds under this study were not significantly different in sows of genotypes AB and BB in comparison with those of AA genotype. This result is inconsistent with a previous study, which indicated that IGF2 in Black Pied Poestice sows of the genotypes AB and BB had larger litters than the AA genotype (Horák *et al.*, 2001). In pigs, the IGF2 gene is localized on chromosome 2. This gene was marked as a candidate gene for muscle mass (skeletal and cardiac) and fat deposition (Jeon *et al.*, 1999; Nezer *et al.*, 1999) but other possible effects of this polymorphism on reproductive traits have been evaluated (Horák *et al.*, 2001). The result of the present study disagrees with that of previous study of Horák *et al.* (2001). What needs pointing out is that the number of observations or to the background genetics of each pig breed is different.

5. CONCLUSION

The polymorphisms of the RNF4 and RBP4 genes had clear associations with NB, NBA, and LBW traits. Landrace and Yorkshire sows with the CC genotype of the RNF4 gene, and Landrace sows with the BB genotype of the RBP4 gene produced more piglets. Therefore, these two genes should be considered for use as candidate marker genes for genetic improvement of litter size in pig breeds.

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