

Additive Genetic Effects of RNF4, RBP4, and IGF2 Polymorphisms on Litter Size in Landrace and Yorkshire Sows

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Abstract

The association of the RNF4, RBP4, and IGF2 genotypes and their additive genetic effects with litter size in purebred Landrace and Yorkshire sows were studied. The results revealed significant associations between the RNF4 and RBP4 genotypes with the total number of piglets born (TNB) and number of piglets born alive (NBA) traits ($P < 0.05$). The RNF4 CC genotype had greater TNB and NBA than the TT genotype in both breeds. The RBP4 BB genotype had greater TNB and NBA than the AA genotype in the Landrace breed. Significant additive effects of the RNF4 and RBP4 genes on the TNB and NBA were detected ($P < 0.05$). No significant associations of the IGF2 genotypes and their additive effects with any reproductive traits were observed in both Landrace and Yorkshire sows ($P > 0.05$). The results suggested that the RNF4 and RBP4 genes could be useful in selection for increasing TNB and NBA traits in pigs.

Keywords

RNF4, RBP4, IGF2, additive genetic effect, litter size

Introduction

Improvement in litter size has become of great interest in the pig industry as good fecundity is directly related to a sow's productive life. Litter size is defined as the total number of piglets born (TNB), number of piglets born alive (NBA), or number weaned (NW). All these reproductive traits are expressed only in females and after sexual maturity. These biological characteristics and their low heritability limit the effectiveness of selection for prolificacy. Marker-assisted selection (MAS) could be an important tool for genetic improvement of litter size. A few candidate genes for litter size have already been identified in pigs according to their

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roles in the physiology of reproduction and/or their position within chromosomal regions containing quantitative trait loci (QTL) for reproductive traits (Distl, 2007). To use these markers in MAS, it is necessary to verify whether these markers are associated with the traits in the specific population under selection.

Ring finger protein 4 (RNF4) is a nuclear receptor coregulator that can serve as a coactivator for steroid receptor-dependent and independent promoters. Overexpression of the RNF4 gene can enhance the transcription of steroid receptors, including the glucocorticoid, progesterone, and estrogen receptors (Moilanen *et al.*, 1998; Saville *et al.*, 2002). In particular, RNF4 can stimulate transcription of rat LH β through mediate interactions between the distal and proximal gonadotropin-releasing hormone (GnRH) response regions of the LH β promoter (Curtin *et al.*, 2004). A previous study showed that porcine RNF4 (pRNF4) was expressed highly in ovaries and testis (Niu *et al.*, 2009), which suggested that the pRNF4 gene might play a role in ovulation by regulating the expression of the porcine LH β gene. The retinol-binding protein 4 (RBP4) gene codes for a member of the RBP protein family present in the uterus and in embryos during the early stages of gestation (Trout *et al.*, 1991). These proteins bind retinol, and the bound retinol is then internalized by the cells and triggers embryogenesis (Yelich *et al.*, 1997). Messer *et al.* (1996) have proposed RBP4 as a possible candidate gene associated with litter size. Subsequently, Rothschild *et al.* (2000) carried out a study on animals from six commercial lines and reported a significant effect of an intronic polymorphism, RBP4-MspI, on the total number of piglets born. Many other studies have shown the existence of a relationship between this polymorphism and litter size (Drogemuller *et al.*, 2001; Linville *et al.*, 2001; Blowe *et al.*, 2006; Spotter & Distl, 2006). The insulin-like growth factor 2 (IGF2) gene 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 be playing a role in fertility.

Genetic variation in quantitative or complex traits can be partitioned into many components due to additive, dominance, and interaction effects of genes. The most important is the additive genetic variance because it determines most of the correlation among relatives and the opportunities for genetic change by natural or artificial selection. Therefore, the objective of this study was to investigate the association of the RNF4, RBP4, and IGF2 genotypes, and their additive genetic effects on litter size of Landrace and Yorkshire pig breeds.

Materials and Methods

Animals, molecular methods, and data collection

Purebred Landrace and Yorkshire breeds from the Dabaco Nucleus Breeding Pigs Company (DBC) in Bac Ninh province and the Dong Hiep Pig Farm (DH) in Hai Phong province were used in this study. The litter size traits of 834 litters, including 413 litters of Landrace and 421 litters of Yorkshire, were recorded in the first six parities.

Genomic DNA was isolated from ear tissue samples using a QIAamp DNA FFPE Tissue Kit. The concentration and purity of the DNA samples were checked on 1% agarose gel and measured at OD_{A260/A280}. Then, the DNA was diluted to a concentration of 50 ng μL^{-1} .

The PCR-RFLP procedures as described in Niu *et al.* (2009), Rothschild *et al.* (2000), and Knoll *et al.* (2000) were used to amplify the specific gene fragments of RNF4, RBP4, and IGF2, respectively (**Table 1**). The PCR programmes were performed slightly different from what was reported previously. The PCR reaction was performed using 50ng of genomic DNA, 1.5mM MgCl₂, 0.2mM dNTPs, 0.5 μM primers, 2U of Taq DNA polymerase, and PCR buffer in a 25 μL final volume. The amplification conditions followed the temperature programs of: (1) 94°C for 4min followed by 35 cycles of 94°C for 45s, 53°C for 45s, 72°C for 2 min, and ending with a final step of 72°C for 10min for RNF4; (2) 95°C for 3min followed by

Table 1. Primer sequences, endonuclease, and allele sizes of the RNF4, RBP4, and IGF2 genes

Gene	Primer sequences (5'-3')	Product size (bp)	Endonuclease	Allele size (bp)	Sources
RNF4	CGAAATGCCAGGGAAGAG CCATGCAGATCGGACAAC	937	<i>SacII</i>	T-937 C-545, 392	Niu <i>et al.</i> (2009)
RBP4	GAGCAAGATGGAATGGGTT CTCGGTGTCTGTAAAGGTG	550	<i>MspI</i>	A-190, 154, 136 B-190, 136, 125	Rothschild <i>et al.</i> (2000)
IGF2	CACAGCAGGTGCTCCATCGG GACAGGCTGTATCCTGTGGG	336	<i>NciI</i>	A-308, 28 B-208, 100, 28	Knoll <i>et al.</i> (2000)

35 cycles of 95°C for 30s, 56°C for 45s, 72°C for 45s, and ending with a final step of 72°C for 5min for RBP4; and (3) 95°C for 2min, followed by 30 cycles of 95°C for 20s, 55°C for 30s, and 72°C for 60s, with a final extension at 72°C for 7min for IGF2.

The amplified fragments were digested by the *SacII*, *MspI*, and *NciI* enzymes for RNF4, RBP4, and IGF2, respectively. An 8µL sample of each PCR product was digested at 37°C overnight in a total volume of 30µL, containing 1U of the appropriate restriction enzyme, 3µL of restriction buffer, and 18.3µL of H₂O. The obtained fragments were separated on 2% agarose gel.

The total number of piglets born (TNB), the number of piglets born alive (NBA), and the number of piglets weaned (NW) were recorded to analyze the association with the genes and predict their additive genetic effects.

Statistical analysis

The associations of the RNF4, RBP4, or IGF2 genotypes with litter size traits were analyzed according to the following model:

$$Y_{ijklm} = \mu + G_i + F_j + P_k + S_l + e_{ijklm}$$

where, Y_{ijklm} is the observed value; μ is the average normalized record of the population; G_i is the individual gene effects of RNF4, or RBP4, or IGF2 ($i = 3$); F_j is the effect of the farms ($j = 2$: DBC, DH); P_k is the effect of the parity ($k = 6$); S_l is the effect of the season ($l = 2$: winter-spring, summer-autumn); and e_{ijklm} is the residual error. Both additive and dominant effects were estimated using the GLM procedure of SAS (9.1, 2002), where the additive effect was estimated as 0.5, 0, and -0.5

for the genotypes CC/BB/BB, TC/AB/AB, and TT/AA/AA of the RNF4, RBP4, and IGF2 genes, respectively, and the dominance effects were represented as -0.5, 1, and 0.5 for CC/BB/BB, TC/AB/AB, and TT/AA/AA, respectively.

Results

Association of the RNF4 genotypes and their additive effects with the litter size traits

The least square means additive effects and dominant effects for litter size are presented in **Table 2**. There were significant differences in the TNB and NBA traits among the TT, TC, and CC genotypes in both the Landrace and Yorkshire sows ($P < 0.05$). The CC sows in the Landrace population outperformed the TT genotype by 1.25 piglets born and 1.27 piglets born alive ($P < 0.05$). The CC genotype in Yorkshire produced 1.68 piglets born and 1.26 piglets born alive more than the TT sows ($P < 0.05$). Under this study, we did not find any substantial differences among the RNF4 genotypes with regard to the NW trait in both the Landrace and Yorkshire populations ($P > 0.05$).

The significant additive effects of 0.62 ± 0.22 piglets/litter for the TNB and 0.64 ± 0.21 piglets/litter for the NBA were detected in the Landrace population ($P < 0.05$). Similarly, the significant additive effects of 0.83 ± 0.37 piglets/litter for the TNB and 0.63 ± 0.23 piglets/litter for the NBA were detected in the Yorkshire population ($P < 0.05$). No significant dominant effects were found for most of the studied traits ($P > 0.05$), except for the NW in the Yorkshire population ($P < 0.05$).

Table 2. Association of the RNF4 genotypes and additive and dominance effects with the litter size traits

Traits	RNF4 genotype (LSM ± SE)			Effect (LSM ± SE)	
	TT	TC	CC	Additive	Dominance
<i>Landrace population</i>					
	<i>233</i>	<i>133</i>	<i>47</i>		
TNB	11.51 ± 0.24 ^b	11.85 ± 0.23 ^{ab}	12.76 ± 0.39 ^a	0.62 ± 0.22*	-0.29 ± 0.33
	<i>230</i>	<i>133</i>	<i>47</i>		
NBA	10.21 ± 0.23 ^b	10.59 ± 0.23 ^{ab}	11.48 ± 0.38 ^a	0.64 ± 0.21*	-0.26 ± 0.32
	<i>138</i>	<i>106</i>	<i>42</i>		
NW	9.47 ± 0.14	9.48 ± 0.13	9.40 ± 0.21	-0.03 ± 0.12	0.05 ± 0.19
<i>Yorkshire population</i>					
	<i>34</i>	<i>143</i>	<i>244</i>		
TNB	11.46 ± 0.54 ^b	12.79 ± 0.26 ^{ab}	13.14 ± 0.22 ^a	0.83 ± 0.37*	0.49 ± 0.37
	<i>34</i>	<i>143</i>	<i>244</i>		
NBA	9.76 ± 0.47 ^b	10.94 ± 0.23 ^{ab}	11.02 ± 0.19 ^a	0.63 ± 0.23*	0.55 ± 0.32
	<i>23</i>	<i>108</i>	<i>198</i>		
NW	9.14 ± 0.26	9.62 ± 0.12	9.35 ± 0.10	0.10 ± 0.13	0.37 ± 0.18*

Note: Values in each row of each breed with different superscripts are significantly different ($P < 0.05$). * $p < 0.05$. Number of records denoted by an italic number. TNB (total number of piglets born); NBA (number of piglets born alive); NW (number weaned).

Association of the RBP4 genotypes and their additive effects with litter size traits

For the RBP4 gene, the association and genetic variance effects with litter size are shown in **Table 3**. The homozygote BB significantly differed from the homozygous AA for the TNB and NBA traits ($P < 0.05$) in the Landrace population. The sows with the BB genotype had an advantage of 0.77 piglets for the TNB and 0.62 piglets for the NBA as compared with the AA sows. In addition, significant additive RBP4 effects of 0.39 ± 0.13 piglets/litter for the TNB and 0.31 ± 0.13 piglets/litter for the NBA were detected in the Landrace population for the B allele ($P < 0.05$). There were no significant differences among genotypes of the RBP4 gene for all the study traits in the Yorkshire population ($P > 0.05$). No significant additive effects for all traits ($P > 0.05$) were found while significant dominant effects were found for the TNB and NBA traits in Yorkshire ($P < 0.05$).

Association of the IGF2 genotypes and their additive effects with litter size traits

For the IGF2 gene, the three genotypes of

AA, AB, and BB were found in the Landrace population while only two genotypes, AB and BB, were observed in the Yorkshire population (**Table 4**). Our results showed that there were not any substantial differences among the IGF2 genotypes with regard to litter size traits in both the Landrace and Yorkshire populations ($P > 0.05$). Consequently, no additive effects or dominant effects were detected in the Landrace population ($P > 0.05$) for all the traits.

Discussion

Genetic markers allow the identification of animals carrying beneficial or harmful alleles early in life, thereby improving the accuracy, reducing the generation interval, and accelerating the genetic improvement of a trait. In this study, RNF4, RBP4, and IGF2 were selected as candidate genes on the basis of their physiological roles in ovulation, implantation, and embryonic development, and allelic variation in these genes were identified and tested for associations with litter size traits in the Landrace and Yorkshire populations.

For the RNF4 gene, we found that RNF4

Table 3. Association between the RBP4 genotypes and additive and dominance effects with the litter size traits

Traits	RBP4 genotype (LSM ± SE)			Effect (LSM ± SE)	
	AA	AB	BB	Additive	Dominance
<i>Landrace population</i>					
	<i>360</i>	<i>177</i>	<i>157</i>		
TNB	11.24 ± 0.22 ^b	11.52 ± 0.22 ^{ab}	12.01 ± 0.26 ^a	0.39 ± 0.13*	-0.10 ± 0.25
	<i>357</i>	<i>174</i>	<i>156</i>		
NBA	10.03 ± 0.22 ^b	10.39 ± 0.23 ^{ab}	10.65 ± 0.26 ^a	0.31 ± 0.13*	0.06 ± 0.25
	<i>198</i>	<i>121</i>	<i>110</i>		
NW	9.47 ± 0.12	9.56 ± 0.11	9.62 ± 0.14	0.07 ± 0.07	0.02 ± 0.14
<i>Yorkshire population</i>					
	<i>245</i>	<i>270</i>	<i>271</i>		
TNB	12.72 ± 0.25	12.08 ± 0.25	12.48 ± 0.27	-0.12 ± 0.14	-0.52 ± 0.24*
	<i>245</i>	<i>270</i>	<i>261</i>		
NBA	10.72 ± 0.23	10.21 ± 0.23	10.50 ± 0.25	-0.11 ± 0.13	-0.39 ± 0.22*
	<i>165</i>	<i>188</i>	<i>144</i>		
NW	9.43 ± 0.11	9.31 ± 0.11	9.41 ± 0.12	-0.01 ± 0.13	-0.10 ± 0.11

Note: Values in each row of each breed with different superscripts are significantly different ($P < 0.05$). * $p < 0.05$. Number of records denoted by an italic number. TNB (number of piglets born); NBA (number of piglets born alive); NW (number weaned).

Table 4. Association between the IGF2 genotypes and additive and dominance effects with the litter size traits

Traits	IGF2 genotype (LSM ± SE)			Effect (LSM ± SE)	
	AA	AB	BB	Additive	Dominance
<i>Landrace population</i>					
	<i>17</i>	<i>190</i>	<i>281</i>		
TNB	12.03 ± 0.68	11.46 ± 0.20	12.06 ± 0.21	0.02 ± 0.34	-0.59 ± 0.41
	<i>17</i>	<i>190</i>	<i>278</i>		
NBA	10.84 ± 0.66	10.14 ± 0.20	10.74 ± 0.21	-0.05 ± 0.32	-0.65 ± 0.39
	<i>13</i>	<i>124</i>	<i>190</i>		
NW	9.62 ± 0.40	9.45 ± 0.13	9.42 ± 0.12	-0.10 ± 0.19	-0.07 ± 0.25
<i>Yorkshire population</i>					
		<i>52</i>	<i>367</i>		
TNB		12.72 ± 0.42	13.33 ± 0.27	-	-
		<i>51</i>	<i>367</i>		
NBA		10.66 ± 0.39	11.06 ± 0.25	-	-
		<i>43</i>	<i>276</i>		
NW		9.73 ± 0.20	9.63 ± 0.13	-	-

polymorphisms are significantly associated with TNB and NBA in both the Landrace and Yorkshire breeds. The CC sows had higher TNB and NBA than the TT sows. The effect of the RNF4 polymorphisms on litter size in these two individual populations with different genetic backgrounds suggested that RNF4 might

be a good candidate gene for reproductive traits and/or play a role in reproduction. The additive effect of the C allele on the TNB and NBA were 0.62 and 0.64 piglets/litter, respectively, in Landrace sows. Thus, selection of the C allele could contribute to a higher TNB and NBA traits in the population. This result is consistent

with a previous study of Niu *et al.* (2009) who reported that CC sows in the Qingping population outperformed the TT genotype by 1.74 piglets born and 2.02 piglets born alive, and outperformed the TC genotype by 0.99 piglets born alive. Significant additive effects on the TNB and NBA were also detected. The additive genetic effect is the most important genetic variation because it is the only value that informs us about what can be passed from one generation to the next generation, so selection based on additive variance is highly accurate and stable over generations. The results of this study suggest the selection of RNF4 CC Landrace and Yorkshire sows to pair for mating could contribute to a higher TNB and NBA traits.

Significant effects of the loci of RBP4 on the TNB and NBA for the Landrace breed were also found under this study. The Landrace sows with the BB genotype had significantly higher TNB and NBA values than those of the sows with the AA genotype. Additionally, an additive effect of the RBP4 B allele in Landrace was also detected. The results of several previous studies with RBP4 as a candidate gene for litter size are in agreement with our results and reported an additive gene effect of the favorable B allele (Wang *et al.*, 2006; Terman *et al.*, 2007). Wang *et al.* (2006) showed that sows with the BB genotype of the RBP4 locus had more piglets per litter than sows with the AA or AB genotypes. Terman *et al.* (2007) reported that sows with the BB genotype produced more piglets than sows with the AA or AB genotypes and this result was confirmed statistically in the first and second parities. However, Linville *et al.* (2001) and Blowe *et al.* (2006) did not find a significant difference between Landrace and Large White in their studies of RBP4. Also, Drogemuller *et al.* (2001) were not able to detect significant effects on litter size in a synthetic line for the RBP4 genotypes. Or in contrast, several other studies indicated that the AA genotype has higher TNB and NBA compared to the other genotypes (Rothschild *et al.*, 2000; Omelka *et al.*, 2008, Spotter *et al.*, 2009; Marantidis *et al.*, 2015). Rothschild *et al.* (2000) found an additive effect of allele A on

TNB and NBA in several commercial lines, but no statistically significant effect of any allele on litter size in the synthetic line. Similarly, no effect of the individual alleles was reported in the studies of Drogemuller *et al.* (2001) and Linville *et al.* (2001). Inconsistent with these findings, our results indicated that there was an association of the RBP4 gene and the significant additive effect with TNB and NBA in the Landrace breed. Hence the selection of sows carrying the BB genotype could contribute to higher TNB and NBA traits.

No significant association was found between the polymorphisms of the IGF2 gene and litter size traits, and also, no significant additive effects were detected in both the Landrace and Yorkshire populations. This result is inconsistent with a previous study, which indicated that the IGF2 gene in Black Pied Poestice sows of the genotypes AB and BB had larger litters than the AA genotype (Horák *et al.*, 2001). What needs pointing out is that the number of observations or the background genetics of each different pig breed could lead to different results.

Conclusions

The results in this study demonstrated that the litter sizes (TNB and NBA) of Landrace and Yorkshire sows carrying the RNF4 CC genotype, and Landrace sows carrying the RBP4 BB genotype were greater than other genotypes. In addition, significant additive genetic effects for the TNB and NBA were found. Hence, the selection of Landrace and Yorkshire sows carrying the RBP4 BB and CC genotypes could contribute to higher TNB and NBA traits.

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