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Additive and Dominance Effects of *MC4R* and *PIT1* Polymorphisms on Production and Carcass Traits in Duroc Pigs

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Abstract

This study aimed to estimate the allelic and genotypic frequencies of single nucleotide polymorphisms (SNPs) of *MC4R* (melanocortin-4-receptor gene) and *PIT1* (pituitary specific transcription factor-1) genes, and their additive dominance genetic effects on production and carcass traits in Duroc pigs. A total of 2170 tail tissue samples (1728 gilts and 442 male pigs) were collected from Dabaco Nucleus Breeding Pig Company, Bac Ninh province, Vietnam from January 2017 to June 2020. Polymorphisms in the *MC4R* and *PIT1* genes were detected using the PCR-RFLP method. For *MC4R*, the frequencies of alleles A and G were 0.430 and 0.570, respectively. For *PIT1*, the frequencies of alleles A and B were 0.460 and 0.540, respectively. The genotype frequency distribution of the *MC4R* gene was in Hardy-Weinberg equilibrium (P = 0.375), but the genotype frequency distribution of *PIT1* (P <0.001) was not. The *MC4R* and *PIT1* polymorphisms were significantly associated with final body weight and average daily gain (P <0.001). Significant additive effects were also found (P <0.05) for the two traits. The selection of individuals with the *MC4R* genotype AA and *PIT1* genotype AA could improve final body weight and average daily gain in Duroc pigs.

Keywords

MC4R, *PIT1*, polymorphisms, additive effect, Duroc pigs

Introduction

Genetic variation in quantitative or complex traits can be partitioned into many components due to the additive, dominance, and interaction effects of genes. The additive and dominant effects contribute significantly to genetic variability in pig productivity traits, with the additive genetic variance contributing from 8.8 to 43.6%, and the dominant genetic variance contributing from 2.2 to

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10.3% of the phenotypic variance (Culbertson *et al.*, 1998).

The rapid improvement in breeding pig productivity is due to a new breed selection method based on marker-assisted selection (MAS). The cell transmembrane protein receptor encoded by the *MC4R* (melanocortin-4-receptor) gene plays an important role in controlling food intake, body weight, and maintaining intracellular energy stability. Pigs with allele A of the *MC4R* gene have been shown to have a higher body weight gain than other individuals carrying allele G (Kim *et al.*, 2000; Houston *et al.*, 2004; Piórkowska *et al.*, 2010; Dvořáková *et al.*, 2011; Muñoz *et al.*, 2011; Davoli *et al.*, 2012; Tempfli *et al.*, 2015; Thuy *et al.*, 2019). Growth, carcass performance, and meat quality are influenced by the *PIT1* (pituitary specific transcription factor-1) gene. Pigs carrying either the AB or BB genotype have been shown to have higher carcass weights than those carrying the genotype AA (Yu *et al.*, 1995; Brunsch *et al.*, 2002; Franco *et al.*, 2005a; 2005b; Piórkowska *et al.*, 2013; Kim *et al.*, 2014).

In Vietnam, previous studies have been conducted to analyze *MC4R* and *PIT1* gene polymorphisms and their association with some traits of pigs. Bo *et al.* (2019a; 2019b) studied the effects of *MC4R* and *PIT1* polymorphisms on the semen quality of Duroc pigs. The effects of *MC4R* on the weight gain of Duroc pigs was confirmed in the study of Thuy *et al.* (2019), while the polymorphisms of the *MC4R* gene in wild boars were presented by Noi *et al.* (2010). Dung *et al.* (2014) worked on *PIT1* gene polymorphisms but did not mention the breed.

The objective of this study was to investigate the association of *MC4R* and *PIT1* genotypes and their additive genetic effects on the growth traits of Duroc pigs.

Materials and Methods

The experiment was carried out at the Dabaco Nucleus Breeding Pigs Company, Bac Ninh province, Vietnam from January 2017 to June 2020. In the beginning, 2170 Duroc pigs (1728 gilts and 442 male pigs) at the average age of 76.89 ± 14.02 (± sd) days were used. The pigs were fed *ad libitum* to the end of the growing stage (153.88 ± 10.60 days).

The body weight at the beginning (IBW) and the end of the experiment (FBW) were individually recorded. The average daily gain (ADG) during the fattening period was calculated. Backfat thickness (BF) and depth of the *longissimus dorsal* (LD) muscle between the third and fourth last rib were measured with an ultrasound device (AgroScan AL with a linear probe ALAL350) according to the methods described by Youssao *et al.* (2002). Lean meat percentage (LM) was estimated from the BF and LD using the regression equation recommended by the Ministère des classes moyennes et de l'agriculture de Belgique (1999).

At birth, the piglets were tattooed individually on the ear, and their tails were docked. The tail tissue samples were collected and stored at -20^{o}C in a centrifuge tube (1.5mL) for DNA extraction. The DNA extraction and polymorphism characterization of *MC4R* and *PIT1* were performed at the Center for Gene Technology, Dabaco Nucleus Breeding Pig Company, Vietnam. Genomic DNA was extracted by a G-spin™ Total DNA Extraction Kit (INTRON Biotechnology). The concentration and purity of the DNA were checked on 1% agarose gel and measured for their ODA260/A280 ratio, respectively. The DNA was diluted to a concentration of 50 ng/µL. To amplify the fragments of the *MC4R* and *PIT1* genes, primers according to Kim *et al.* (2000) and Stančeková *et al.* (1999) were used, respectively.

PCR reactions were carried out in a total volume of 25µL containing 50ng of genomic DNA, 1.5 mM MgCl2, 0.2 mM dNTPs, 0.5 µM primers, 2U of Taq DNA polymerase, and PCR buffer. Conditions of the *MC4R* amplifications were 94°C for 2min, followed by 35 cycles of 94°C, 20s; 56.5°C, 10s; and 72°C, 20s; and ending with a final step of 72^{o}C for 5min. The conditions for *PIT1* were 94°C for 3min, followed by 30 cycles of 94°C, 20s; 54.5°C, 20s; 72°C, 1min and 50s; and ending with a final step of 72^{o}C for 5min.

Genotypes of the genes were determined by the PCR-RFLP method. The PCR products were digested with 5U of *Taq*I (for *MC4R*) and *Rsa*I

(for *PIT1*), and then, the fragments were separated on a 2.5% agarose gel to allow 226bp fragments to be observed for the AA genotype; 226bp, 156bp, and 70bp fragments for the AG genotype; and 150bp and 70bp for the GG genotype for *MC4R*; and 774bp, 710bp, 103bp, and 108bp for the AA genotype; 774bp, 710bp, 388bp, 322bp, 103bp, and 108bp for the AB genotype; and 774bp, 388bp, 322bp, 103bp, and 108bp for the BB genotype for *PIT1*.

Hardy-Weinberg equilibrium was performed using the Chi-square test. The effects of the *MC4R* and *PIT1* genotypes on the production traits were evaluated with the general linear model (GLM) procedure of SAS version 9.0. The statistical values presented in the tables are sample size (n), least-squares mean (LSM), and standard error (SE). Pairwise comparisons between LSM were performed by Tukey's test. The statistical model was $Y_{ijk} = \mu + G_i + S_j + G_i * S_j + \varepsilon_{ijk}$, where Y_{ijk} = observed values, μ = overall mean, G_i = genotype effects of *MC4R* or *PIT1*, S_j = effect of sex, G_i*S_j = interaction between genotype, and sex, e_{ijk} = the residual error. Additive and dominance effects were estimated using the GLM procedure of SAS, where the additive effects were estimated as 0.5, 0, and -0.5 for homozygous dominant, heterozygous, and homozygous recessive of each gene, respectively (AA, AG, and GG for *MC4R*; AA, AB, and BB for *PIT1*).

Results

Polymorphisms of *MC4R* and *PIT1*

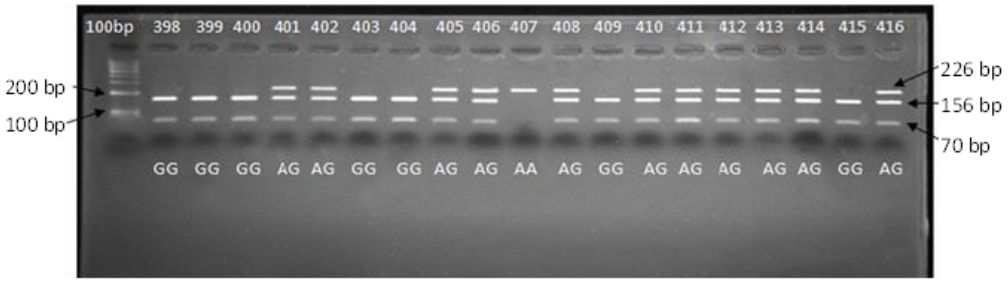
Three different genotypes (AA, AG, and GG) were generated after cutting the PCR products of the *MC4R* gene with the *Taq*I enzyme. AA had a single fragment of 226bp; AG had three fragments (226, 156, and 70bp); and GG had two fragments (156 and 70bp) (**Figure 1**).

The genotypes AA, AB, and BB were produced when the PCR products of the *PIT1* gene were cut with the *Rsa*I enzyme. AA presented four bands (774, 710, 103, and 108bp); AB had six bands (774, 710, 388, 322, 103, and 108bp); and BB had five bands (774, 388, 322, 103, and 108bp) (**Figure 2**).

For *MC4R*, the allele frequencies were 0.43 for allele A and 0.57 for allele G (**Table 1**). This locus was in Hardy-Weinberg equilibrium (P = 0.375). For *PIT1*, alleles A and B appeared with the frequencies of 0.46 and 0.54, respectively (**Table 1**). The genotype frequency distribution of *PIT1* was not in Hardy-Weinberg equilibrium (P <0.001).

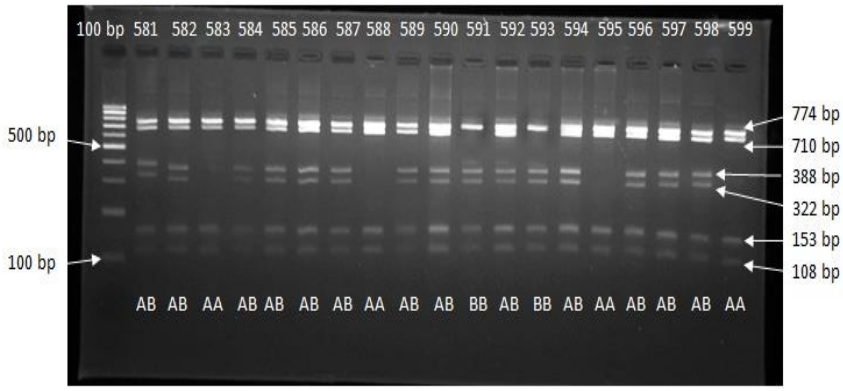
Effects of the MC4R and PIT1 genotypes on growth and carcass traits

For *MC4R*, the analysis showed that this locus was associated with the growth traits of Duroc pigs (**Table 2**). Pigs with the AA genotype had higher FBW, ADG, and LD values than pigs with the GG genotype (P <0.05), whereas pigs with the GG genotype had higher LM than those with the AA genotype (P <0.05). Significant additive effects of 0.836kg, 3.36kg, and 41.5g on the IBW, FBW, and ADG, respectively, were detected (P <0.001) in Duroc pigs in this study. The dominance effects were significant for the ADG, LD, and LM (P <0.05).



MC4R gene (A)

Figure 1. PCR fragments of the *MC4R* gene digested by *Taq*I on 2.5% agarose gel



PIT1 gene (B)

Figure 2. PCR fragments of the *PIT1* gene digested by *Rsa*I on 2.5% agarose gel

Table 1. Genotype and allele frequencies of *MC4R* and *PIT1* in Duroc pigs

Genes		n	Observed frequency	Expected frequency	P (Hardy-Weinberg)
MC4R					0.375
Genotype	AA	408	0.188	0.181	
	AG	1029	0.474	0.489	
	GG	733	0.338	0.330	
Allele	A		0.430		
	G		0.570		
PIT1					<0.001
Genotype	AA	435	0.245	0.211	
	AB	760	0.428	0.497	
	BB	580	0.327	0.293	
Allele	A		0.460		
	B		0.540		

Table 2. Additive and dominance effects of *MC4R* polymorphisms on production and carcass traits in Duroc pigs

Traits	*MC4R* genotype									Genetic effect			
	AA			AG			GG			Additive		Dominance	
	n	LSM	SE	n	LSM	SE	n	LSM	SE	LSM	SE	LSM	SE
IBW	408	33.49	0.47	1029	33.29	0.33	733	31.50	0.37	0.84**	0.25	0.70	0.36
FBW	400	99.84^{a}	0.54	1004	96.10^{b}	0.38	722	92.65^{c}	0.42	3.36***	0.29	-0.20	0.41
ADG	399	872.73^{a}	6.68	1001	825.53^{b}	4.68	721	788.77^{c}	5.18	41.47***	3.55	-10.33*	5.06
BF	80	11.96	0.34	229	11.95	0.21	170	11.08	0.25	0.34	0.17	0.26	0.24
LD	80	63.07^{a}	0.77	229	60.83^{b}	0.47	170	61.34^{ab}	0.58	0.60	0.40	-1.26*	0.56
LM	80	61.68^{ab}	0.36	229	61.18^{b}	0.22	170	62.21^{a}	0.27	-0.22	0.18	-0.56*	0.26

*Note: LSM in each row with different superscripts are significantly different (P <0.05); *: P <0.05; **: P <0.01, ***: P <0.001. IBW: initial body weight (kg); FBW: final body weight (kg); ADG: average daily gain (g); BF: backfat thickness (mm); LD: depth of longissimus dorsal (mm); LM: lean meat percentage (%).*

For the *Rsa*I locus of *PIT1*, there was an association between the gene polymorphisms and FBW and ADG (P <0.05). The pigs with genotype AA had higher FBW and ADG values

than pigs carrying the genotype BB (**Table 3**). In addition, significant additive effects of 1.53kg on FBW and 22.4g on ADG were found (P <0.001). However, no significant dominance effects were detected for all the studied traits (P >0.05) except for IBW (P <0.05).

Discussion

For *MC4R*, the allele frequency distribution in the population in this study was similar to the previous results of Thuy *et al.* (2019) who reported that the frequencies of A and G were 0.414 and 0.586, respectively, in Duroc pigs. However, previous studies have reported different A and G allele frequency distributions in Duroc pig populations. The study of Hong *et al.* (2015) showed that the frequency of allele G (0.1) was lower than that of allele A (0.9) in a Duroc pig population raised in Korea. The allele A frequency (0.754) was also predominantly higher than that of allele G (0.246) in Italian Duroc pigs (Davoli *et al.*, 2012). The work of Piórkowska *et al.* (2010) showed that the frequencies of genotype GG (0.103) and allele G (0.315) were lower than those of genotype AA (0.472) and allele A (0.685) for Duroc pig populations raised in Poland. In contrast, a lower frequency of the A allele (0.235) compared to the G allele (0.765) was found in Duroc × Landrace/ Large White crossbred pigs in Spain (Galve *et al.*, 2012). In addition, the variation of the allele A frequency in the population also depended on the pig breed. Particularly, the allele A frequencies in DIV2, Yorkshire, Landrace, Meishan, and Bamei pig breeds raised at the experimental farm of Huazhong Agricultural University, China were 0.873, 0.429, 0.508, 1,000, and 0.613, respectively (Chao *et al.*, 2012). The frequency of allele A in a Duroc pig population raised in Denmark increased from 0.59 to 0.96 over the period of 1990-2002 (Bruun *et al.*, 2006). The studies above show that allele A appears with different frequencies in different pig populations and under different selection conditions. This suggests that different pigs and selection targets alter the frequency of the occurrence of allele A in a population. The results of our study are consistent with the previous study of Thuy *et al.* (2019) who reported that Duroc pigs with the genotype AA had a higher ADG (853.30g) as compared to pigs with the genotype GG (790.40g). Kim *et al.* (2000) indicated that *MC4R* polymorphisms were not associated with BF, but were statistically significant for the LM and ADG for Duroc pigs. At present, the *Taq*I locus of the *MC4R* gene has been applied to the Danish breeding program to improve the ADG for four breeds of pigs, namely Hamshire, Duroc, Landrace, and Yorkshire (Bruun *et al.*, 2006).

For *PIT1*, the allele A frequency (0.247) was lower than that of allele B (0.753) for a Pietrain pig population, however, the frequencies of allele A for Landrace (0.662) and Large White (0.835) were higher than that of B, respectively 0.338 and 0.165 (Piórkowska *et al.*, 2013). Similarly,

Table 3. Additive and dominance effects of *PIT1* polymorphisms on production and carcass traits in Duroc pigs

Traits	*PIT1* genotype									Genetic effect			
	AA			AB			BB			Additive		Dominance	
	n	LSM	SE	n	LSM	SE	n	LSM	SE	LSM	SE	LSM	SE
IBW	435	31.58	0.29	760	32.53	0.21	580	31.98	0.25	-0.24	0.14	0.52*	0.22
FBW	430	96.69^{a}	0.57	753	95.93^{a}	0.40	575	93.63^{b}	0.49	1.53***	0.28	0.58	0.43
ADG	430	835.26^{a}	6.72	752	817.03^{a}	4.81	575	796.18^{b}	5.80	22.44***	3.33	-4.70	5.07
BF	46	11.31	1.34	118	12.47	0.48	70	11.43	0.68	0.35	0.25	0.32	0.35
LD	46	59.09	2.64	118	60.75	0.95	70	59.72	1.34	1.08*	0.49	0.39	0.69
LM	46	61.46	1.45	118	60.61	0.53	70	61.47	0.74	-0.13	0.27	-0.25	0.38

*Note: LSM in each row with different superscripts are significant different (P <0.05); *: P <0.05; **: P <0.01, ***: P <0.001. IBW: initial body weight (kg); FBW: final body weight (kg); ADG: average daily gain (g); BF: backfat thickness (mm); LD: depth of longgissimus dorsal (mm); LM: lean meat percentage (%).*

the allele A frequency in Landrace pigs (0.878) was higher than that of B (0.122) (Franco *et al.*, 2005a; b). In contrast, the allele A frequency of the *PIT1* gene in a Pietrain pig population (0.393) was lower than that of allele B (0.607) (Brunsch *et al.*, 2002). Yu *et al.* (1995) did not detect allele B in Meishan and Minzhu pig breeds, however, alleles A and B appeared with the same frequency (0.5) in Duroc pigs. They also reported that in Hampshire pigs, the frequency of allele A (0.38) was lower than that of allele B (0.62), and in Landrace, allele A appeared with a higher frequency (0.88) than allele B (0.12). Thus, phenotypic selection may change the allelic frequency in a population. The results of the present study are inconsistent with previous studies on the relationship between *PIT1* polymorphisms and growth traits. Final body weights were not significantly different between pigs with AA (92.60kg) and BB (89.23kg) genotypes in Landrace pigs (Franco *et al.*, 2005a). Another study by Franco *et al.* (2005b) found Landrace pigs carrying the genotype AA had no differences in their ADG (868g) compared to pigs with the genotype BB (873g). Piórkowska *et al.* (2010) showed that *PIT1* gene polymorphisms did not affect the BF and LD but affected the LM of Polish Large White pigs. Pigs with the *PIT1* genotype AA had a higher LM (59.30%) than pigs with the genotype BB (58.50%). Piórkowska *et al.* (2013) also indicated that no significant additive or dominance effects of *PIT1* gene polymorphisms on the BF (-0.003 and 0.06) or LM (-0.25 and -0.29) were found (P >0.05).

Conclusions

There were associations between *MC4R* and *PIT1* gene polymorphisms and the FBW and ADG. The *MC4R* genotype AA and the *PIT1* genotype AA Duroc pigs had greater FBW and ADG values. In addition, significant additive genetic effects on FBW and ADG were found in both genes. The obtained results suggest the selection of *MC4R* and *PIT1* genotype AA pigs could improve the FBW and ADG traits.

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