



Polymorphisms of Candidate Genes Associated with Growth and Carcass Traits in Canadian Duroc Pigs

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ABSTRACT

Background: Selection based on traits such as average daily gain (ADG) and carcass quality in commercial pig populations have drawn attention of swine breeders due to the correlation with growth and consumer preference for carcass composition, respectively. The association between melanocortin 4 receptor (MC4R) gene, which encodes the G-protein-coupled receptor and ADG and carcass quality, have been well documented. The study was conducted to determine the effects of MC4R/*TaqI* and PIT-1/*RsaI* polymorphisms on growth and carcass quality traits in total of 576 Duroc pigs at national breeding farms of Vietnam.

Methods: After the performance test, the desired traits including ADG, IF and LD were measured. ADG (g/day) was calculated as the live weight divided by the number of days from birth to 100 kg, BF and LD can be captured at position P2 (6-8 cm away from body midline at the last rib level) with Ultrasound machine Aloka SSD 500V. Genomic DNA were collected and genotyped to observe the polymorphism of MC4R and PIT-1.

Result: Three genotypes AA, AG and GG of MC4R gene and AA, AB and BB of PIT-1 gene were found in the studied pig population. The observed frequencies of AA, AG and GG were 0.09, 0.41 and 0.50 (MC4R gene) and for AA, AB and BB were 0.37, 0.47 and 0.16, respectively. The G allele (MC4R) and B allele (PIT-1) have more positive effects on traits of ADG, BF and LD. Specifically, in MC4R gene, the individuals carrying GG genotype had higher ADG by 50 gram and lower BF by 1.4 mm than AA genotype. In PIT-1 gene, pigs carrying BB genotype had higher ADG and LD than AA genotype by 37 gram and by 1.9 mm, respectively. Therefore, the increased selection of G allele, GG genotype (MC4R) and of B allele, BB genotype (PIT-1) should be considered to contribute to the improvement of ADG, BF and LD traits in Duroc population in current study.

Key words: Average daily gain, Backfat thickness, MC4R, Loin depth, PIT-1.

INTRODUCTION

Pork is the most popular meat globally and contributes a remarkable source in human nutrition. To cope with the increasing demand for meat in the next fifty years, the pig meat production needs to be tripled. Because of the intensification of this request, introduction of methods that can evaluate animals with economically and agriculturally elite traits are highly required (Alexandratos and Bruinsma, 2012). Recently, selection based on traits such as average daily gain (ADG) and carcass quality in commercial pig populations have drawn attention of swine breeders due to the correlation with growth and consumer preference for carcass composition, respectively (Kang *et al.*, 2015).

The genetic markers approach has been widely used to augment the efficiency of determining the superiority of individuals animals among the population (Groenen *et al.*, 2012). Previous genomic studies have revealed numerous candidate genes with significant variations in the growth and carcass quality (Rothschild, 2000). Generally, selection based on candidate gene allow rapid screening and identification of traits whose phenotypes are difficult to measure.

The association between melanocortin 4 receptor (MC4R) gene, which encodes the G-protein-coupled receptor and ADG and carcass quality have been well documented (Dvorakova *et al.*, 2011; Klimenko *et al.*, 2014; Szyndler-Nędza *et al.*, 2010). Since its proven role in

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mediating energy homeostasis, it was suggested that the MC4R regulates the feed intake and metabolism in mammals (Hirose *et al.*, 2013). In pigs, MC4R gene was physically mapped to chromosome 1 (SSC1) q22-q27. It was indicated that there exists a strong correlation between the MC4R gene polymorphism and the growth and meat quality (Kim *et al.*, 2000). The most well-documented missense mutation Asp298Asn in highly conserved region of MC4R protein sequence disrupts the MC4R signaling pathway. Nevertheless, the effects of this mutation on the phenotype were not uniform across the breeds rather a line-dependent phenotype effects on ADG and carcass traits in Large White,

Landrace, Pietrain, crossbred populations and indigenous breeds in various countries (Davoli *et al.*, 2012; Hongyan Zhu *et al.*, 2017; Klimenko *et al.*, 2014; Valluzzi *et al.*, 2019).

Porcine pituitary-specific positive transcription factor 1 (PIT-1) gene is localized on chromosome 13 and belongs to POU domain protein family. The PIT1 mediates the expression of growth hormone prolactin and thyroid-stimulating hormone genes. PIT-1 also is a promising candidate gene associated with desired traits (Franco *et al.*, 2005; Song *et al.*, 2007). PIT-1 genomic polymorphisms have been illustrated in numerous reports depicting its close association with ADG and carcass composition traits in different breeds, including Landrace, Pietrain, Yorkshire, Duroc and other breeds (Brunsch *et al.*, 2002; Franco *et al.*, 2005; Silveira *et al.*, 2009; Yu *et al.*, 1994).

The Duroc breed is highly recommended as one of the top terminal sire line in crossbreeding system. In Vietnam, Canadian Duroc breeds were widely utilized in industrial pig farming due to the positive effects on growth and viability on their progenies. This study aimed to correlate the effects of the genetic variability of MC4R and PIT-1 on growth (ADG), and various carcass quality traits including backfat thickness (BT) and loin depth (LD) on Canadian Duroc pig reared in the South of Vietnam.

MATERIALS AND METHODS

Ethics statement

Samples collected were used only for routine diagnostic purpose of the breeding programs and not specifically for the purpose of this project. Therefore, approval of an ethics committee was not mandatory. Sample collection and data recording were conducted strictly according to the Vietnamese law on animal protection and welfare.

Animals

A total of 576 Canadian Duroc pigs of the same genetic line were used. They were reared in groups of 12-14 individuals under the closed-house system, with an ambient temperature of 28 to 30 °C and relative humidity of 60% to 70% at the nucleus farm belong to the Institute of Animal Science for Southern Vietnam. Pigs were started putting on the performance test at the age between 65 and 70 days old with the body weight of around 25 kg per each. Measurements of live-weight, backfat thickness and loin depth were done at off test time between 145 and 155 days of age (body weight of around 100 kg). The desired traits in

this study including average daily gain (ADG), backfat thickness (BF) and loin depth (LD). ADG (g/day) was calculated as the live weight at off test time minus the weight started test and then divided by the number of days from starting to finishing test. BF and LD can be captured at point P2 (6-8 cm away from body midline at the tenth rib location) with an Ultrasound machine Aloka SSD 500V at off test time.

Genotyping

Genomic DNA was extracted from 3-5ml lyophilized blood using the Kit Gene Jet Whole Blood Genomic DNA Purification (Thermo Fisher Scientific). Primer pairs used to amplify the MC4R and PIT-1 fragments were presented in Table 1. PCR amplification of target genes were carried out in a 25 µL reaction mix with 1 µL 100-500ng of genomic DNA, 2.5µL of each dNTPs (200µM), 2.5 µL of each primer (10pM), 2.5 µL MgCl₂ (1.5mM), 0.5 µL Tag Polymerase, 5 µL 10X buffer and the double-distilled water. PCR conditions were programmed as follows: pre-denaturation at 94°C in 5 min; 35 cycles of denaturation at 94°C for 30 s, annealing at 62°C for 30 s and extension at 72°C for 30s; and final extension at 72°C for 7 min. The PCR products were confirmed on 1.5% agarose gel and subsequently digested with restriction enzymes containing 10 µL PCR reaction mixture, 2µL buffer G, 1-2 µL restriction enzyme (*TaqI* or *RsaI*) and the nuclease-free water were adjusted to 25 µL. The incubation was carried at 65°C overnight and the digested products were observed on 3% agarose gel.

Statistical analysis

MC4R and PIT-1 frequencies were calculated according to Hardy-Weinberg equilibrium principle. The following linear mixed model was employed to analyze the association between genotypes and their effects on ADG, BF and LD:

$$Y_{ijk} = \mu + S_i + G_j + e_{ijk}$$

Where

Y_{ijk} : value of the observed traits (ADG, BF or LD), μ : overall mean, S_i : effect of sex, G_j : effect of genotype (MC4R or PIT-1), e_{ijk} : random residual effect.

The results of the measured traits were illustrated as mean \pm standard deviation (SD). Statistical analyses were performed using SPSS software version 20 (SPSS Inc., Chicago, IL, USA). Student's *t*-test with $P < 0.05$ were considered statistically significant.

Table 1: Primers for amplifying MC4R, PIT-1 and information about their respective products.

Gene	Polymorphism	Primer	PCR fragment	Restriction enzyme	RFLP fragments (bp)
MC4R	G892A	TACCCTGACCATCTTGATTGAT AGCAACAGATGATCTCTTTG	226bp	<i>TaqI</i>	226-156-70
PIT-1	C14702G	AGTGTAGCCAGAGCATCTACC ACATCTGCACACTCA	1745bp	<i>RsaI</i>	710- 388-322

RESULTS AND DISCUSSION

Allelic distribution of MC4R and PIT-1

The MC4R gene was represented by the fragments of AA, AG and GG (Fig 1), while the PIT-1 gene was marked by the fragments of AA, AB and BB (Fig 2). The genotype and allele frequency of MC4R and PIT-1 were displayed in Table 2 and Table 3. The frequencies of the allele A and allele G were 0.3 and 0.7, respectively. The most frequent genotype was GG (50%) and the least was AA (9%). Among the genotypes of PIT-1 gene AB was abundant (47%). Allele A portrayed a higher frequency than allele B (0.6 vs 0.4). Based on the probability of proportion test, the observed frequencies of MC4R and PIT-1 were in Hardy-Weinberg equilibrium.

Association between MC4R, PIT-1 and studied traits

Association analysis indicated that MC4R polymorphism had remarkable effects on ADG, BF and LD in Canadian Duroc populations (Table 4). The GG genotype showed the highest ADG (856 g/day) and LD (56.1mm) and differed significantly with AA and AG. Animals of AA genotype had highest BF (13.2mm) than AG and GG genotypes. On the other hand, the phenotypic analysis also exhibited the correlation of PIT-1 polymorphisms on three traits in this study (Table 4). Our results demonstrated that the genotype BB was associated with higher ADG and LD (882g/day and 56.7mm, respectively) whereas the genotype AA showed the longest BF phenotype (56.7mm).

In our Canadian Duroc population, it was revealed that allele G dominated allele A and all three genotypes were found with genotype GG was more abundant than genotype AA. Previous studies also confirmed our results. For instance, in the Philippines, Octura *et al.* (2014) showed that genotypic frequency in indigenous pigs for

AA, AG and GG was 16%, 43% and 41%, respectively (Octura *et al.*, 2014). In agreement with Octura *et al.*, other authors also confirmed that the abundance of allele G over allele A in their lines (Dvorakova *et al.*, 2011; Valluzzi *et al.*, 2019). However, Hirose *et al.* (2014) illustrated that the frequency for allele A and G in Duroc lines was 87.9% and 12.1%, respectively. Other indistinguishable results were also obtained that were in line with the data of Hirose *et al.* (HongyanZhu *et al.*, 2017; Kenchawong *et al.*, 2019; Lyubov *et al.*, 2016). Conclusively, the frequency of MC4R at the Asp298Asn mutation site were breed dependent.

In the PIT-1 gene, we demonstrated that genotype AA predominated genotype BB, with allele A was 60% and allele B was 40% in the population. Our results provide strong support for other previous research, such as Lyubov *et al.* (2016), identified the significant effect of genotype

Table 2: Genotypic and allelic frequencies of MC4R.

	Genotype and frequency			Allele frequency	
	AA	AG	GG	A	G
Number of pigs	54	236	286	0.3	0.7
Observed frequency	0.09	0.41	0.5		
Expected frequency	0.09	0.42	0.49		

Table 3: Genotypic and allelic frequencies of PIT-1.

	Genotype and frequency			Allele frequency	
	AA	AB	BB	A	B
Number of pigs	217	276	95	0.6	0.4
Observed frequency	0.37	0.47	0.16		
Expected frequency	0.36	0.48	0.16		

Table 4: Association between MC4R, PIT-1 and ADG, BF and LD..

Traits	MC4R genotypes			PIT-1 genotypes		
	AA	AG	GG	AA	AB	BB
Number of pigs	54	236	286	217	276	95
ADG (g/day)	806 ^b ±143	838 ^b ±158	856 ^a ± 53	845 ^b ± 158	840 ^b ± 156	882 ^a ± 125
BF (mm)	13.2 ^a ±1.2	12.8 ^{ab} ±1.6	11.8 ^b ± 9.1	13.2 ^a ± 1.3	12.1 ^b ± 2.3	12.8 ^{ab} ± 2.1
LD (mm)	55.7 ^a ±10.2	56.2 ^{ab} ±8.7	56.1 ^b ± 9.1	54.7 ^b ± 12.2	55.3 ^b ± 11.1	56.7 ^a ± 13.7

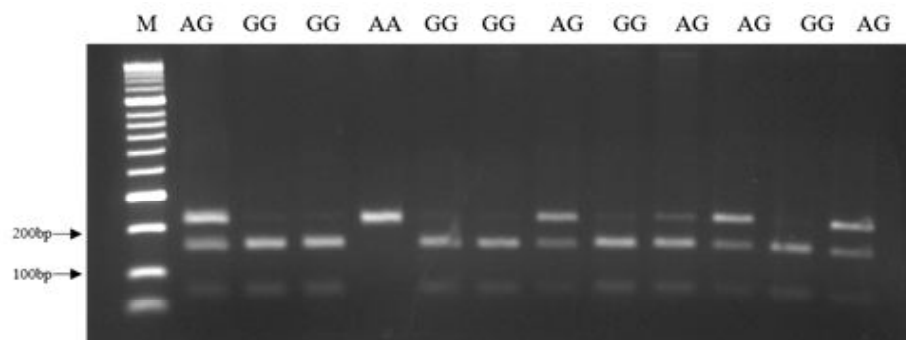


Fig 1: PCR-RFLP genotypes of MC4R/TaqI

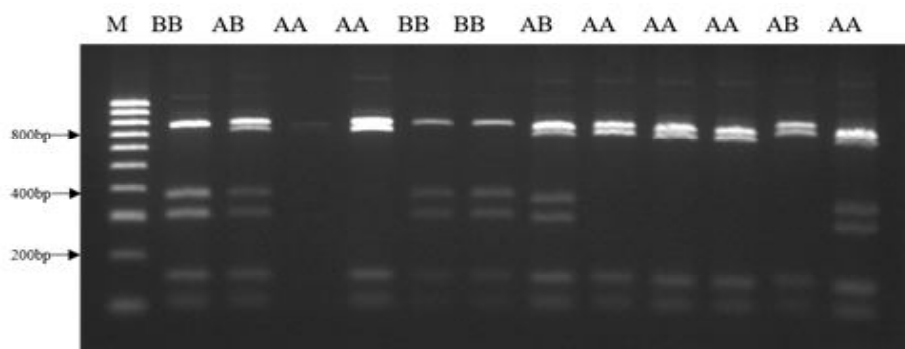


Fig 2: PCR-RFLP genotypes of PIT-1/*RsaI*

AA/PIT-1 on ADG and LD (Lyubov *et al.*, 2016). Moreover, in European Wild Boar, Pietrain and Meishan pigs, genotype AA was also recorded with a close association with performance and carcass traits (Brunsch *et al.*, 2002). However, a study conducted by Stancekova *et al.* (1999) revealed that the association between PIT-1/*RsaI* and traits in this study was not found (Stanèková *et al.*, 1999). This discrepancy among these results could be attributed to the extreme origin of the breeds, as illustrated in Sun *et al.* (2002).

The higher frequency of G in MC4R in this study may portray the positive trend of selection toward higher ADG and LD (allele G). On the other hand, there was dominance of allele A over allele B in the PIT-1 polymorphism due to the preference of increase BF. Hence, we postulate that there is a possibility of the introduction of allele A of PIT-1 and the simultaneous incorporating allele G of MC4R in the population to improve the ADG, LD and BF, respectively.

CONCLUSION

In conclusion, our data confirmed the influence of MC4R and PIT-1 polymorphism on ADG, BF and LD on Canadian Duroc and their feasibility of their application on breeding programs. In the Canadian Duroc lines, the GG genotype of MC4R and the genotype BB of PIT-1 have the positive effect on ADG and LD, however, at the expense of BF. Therefore, we suggested that MC4R and PIT-1 markers can be applied in breeding programs to improve growth and carcass qualities in swine at Vietnam animal husbandry conditions.

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