

PIT1 gene polymorphism in Pietrain and Large White pigs after divergent selection

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ABSTRACT. We examined the polymorphisms in the PIT1 gene of 103 pigs and compared their frequencies in the maternal and paternal lineages of the Pietrain and Large White breeds, which have undergone divergent selection for over 30 years. DNA samples extracted from the blood of these animals were amplified by PCR and genotyped by RFLP, using the restriction enzyme *RsaI*. The data were analyzed with the chi-square test. We found that 57.3% of the animals were genotype AB, among which 26.2% were of the Large White paternal lineage, 18.5% the Pietrain paternal lineage and 12.6% the Pietrain maternal lineage. The AA genotype appeared in 20.4%, of which 7.8% were Large White, 4.8% the Pietrain paternal lineage and 7.8% the Pietrain maternal lineage. BB was observed in 22.3% (6.8% were of the Large White paternal lineage, 9.7% of the Pietrain paternal lineage and 5.8% of the Pietrain maternal lineage). The allele frequencies were 49.0% A and 51.0% B allele. When we examined the Pietrain maternal and paternal lineages, we found that the PIT1 gene had been fixed in the paternal lineage, suggesting that the B allele is associated with low body fat and improved muscle development when

compared to the maternal lineage. However, no significant differences were found between the Pietrain and Large White paternal lineages.

Key words: POU1F; Candidate gene; PCR-RFLP; Genotyping

INTRODUCTION

The PIT1 gene is located on chromosome 13 in pigs, and studies of association between characteristics of performance and carcass quality in pigs and polymorphisms in this gene show that it can be considered a candidate gene for QTLs in these animals (Yu et al., 1995, 1999; Stancekova et al., 1999; Brunsch et al., 2002). The expression of the PIT1 gene is related to the rate of growth and low amount of fat on carcasses of pigs (Andersson et al., 1994; Wilkie et al., 1996; Moser et al., 1998).

In this study, the Pietrain breed was used, whose paternal genetic lineage improvement came about from the need of an even more carcass-quality-requiring market, demonstrating as constitutional advantages: lower fat rate and body fat width, excellent fore- and hindlegs and a larger loin; other contributing factors were advances in pig husbandry, with growing technology use, labor force reduction, and research focusing on the development of maternal ability of the breed, such as matrix docility, high milk production and piglet vitality (Porter, 1993). Large White is the breed that offers the largest daily weight gain and lowest age to reach 90 kg compared to Pietrain (Tibau et al., 1997), where fast and slow growth lineages were respectively considered.

The aim of this study was to identify and compare the *RsaI* polymorphism of the PIT1 gene in paternal and maternal lineages of the Pietrain breed and in Pietrain and Large White paternal lineage breeds, which had undergone divergent selection for over 30 years.

MATERIAL AND METHODS

Biological material and sample collection

Blood samples were collected from the jugular vein of 103 pigs, where 34 were of the Pietrain paternal lineage (8 males and 26 females), 27 of the Pietrain maternal lineage (9 males and 18 females) and 42 of the Large White paternal lineage (11 males and 31 females) all supplied by a nucleus farm from a genetic improvement company, located in Rio Verde, Goiás, Brazil.

Genotyping at the PIT1 locus

Genotyping at the PIT1 gene was performed with animal blood by polymerase chain reactions (PCR)-restriction fragment length polymorphism (RFLP), according to Yu et al. (1994), using a pair of primers with the following sequences: 5' AGTG TAGCCAGAGCATCT 3' (forward) and 5' ACCACATCTGCACACTCA 3' (reverse), which amplify a region of 1745 bp. PCRs were carried out in a PTC-MJ Research thermocycler. After preheating at 95°C for 4 min, amplification was done using 30 cycles at 95°C for 1 min, 61°C for 1 min, and 72°C for 3 min. The final incubation was done at 72°C for 5 min. For the PCR assays, 2.5 U Taq DNA polymerase, 1X PCR buffer, 1.5 mM MgCl₂, 200 µM of each dNTP, 10 pM each

of primer and 200 ng genomic DNA in a final volume of 25 μ L were used. After amplification, 15 μ L of the PCR amplicon was digested with 6 U *RsaI* restriction enzyme for 8 h at 37°C; genotyping was performed on 2.0% agarose gel stained with ethidium bromide (10 mg/mL) and photographed under UV illumination.

Statistical analysis

The genotypes obtained were statistically analyzed by the chi-square test at a significance level of 0.05.

RESULTS

Genotyping at the PIT1 locus

The pattern of bands found was in accordance with Yu et al. (1994), as expected, since the primers used were the same. The genotyping of animals was carried out based on this standard, where it can be seen that all individuals had monomorphic fragments of 774, 153 and 108 bp. The ones with AA genotype still showed a band of 710 bp, the three AB bands of 710, 388 and 322 bp, and the BB genotype bands of 388 and 322 bp.

Allele genotype frequencies

After genotyping the 103 Large White (paternal lineage) and Pietrain (maternal and paternal lineages) pigs, the genotype frequencies (Table 1) and the PIT1 gene allele frequency (Table 2) were determined.

Table 1. Determination of PIT1 gene genotype frequency of 103 Pietrain pigs (maternal and paternal lineages) and Large White pigs (paternal lineage).

Breed	Genotype frequency			
	AB	BB	AA	Total
Pietrain (maternal lineage)	13 (12.6 %)	6 (5.8%)	8 (7.8%)	27 (26.2%)
Pietrain (paternal lineage)	19 (18.5%)	10 (9.7%)	5 (4.8%)	34 (33.0%)
Large White (paternal lineage)	27 (26.2%)	7 (6.8%)	8 (7.8%)	42 (40.8%)
Total	59 (57.3%)	23 (22.3%)	21 (20.4%)	103 (100%)

Data are reported as number with percent in parentheses.

Table 2. Determination of PIT1 gene allele frequency of 103 Pietrain pigs (maternal and paternal lineages) and Large White pigs (paternal lineage).

Breed	Allele frequency		
	A	B	Total
Pietrain (maternal lineage)	29 (14.1%)	25 (12.1%)	54 (26.2%)
Pietrain (paternal lineage)	29 (14.1%)	39 (19.0%)	68 (33.1%)
Large White (paternal lineage)	43 (20.8%)	41 (19.9%)	84 (40.7%)
Total	101 (49.0%)	106 (51.0%)	206 (100%)

Data are reported as number with percent in parentheses.

When comparing the genotype frequency of paternal and maternal Pietrain lineages, statistically significant differences were found ($P < 0.05$). However, when analyzing the paternal Pietrain and Large White lineages, such differences were not found ($P > 0.05$.)

DISCUSSION

Pierzchala et al. (2003) studied crossings that included the Pietrain and Large White races and observed the following genotypic frequencies; 42.5% of AA, 48.8% of AB and 8.7% of BB. When comparing the genotypic frequencies of the paternal and maternal Pietrain lineages, there was a statistically significant difference, in which the paternal lineage showed a higher frequency of the B allele, compared to the mother of A. These results suggest that the B allele is associated with the development of muscle mass, because the selection of divergent animals of paternal lineage tends to yield higher muscle rates in the carcass and lower rates of fat than that of the maternal lineage.

Between the paternal lines of Large White and Pietrain, there was no significant difference. It is believed that the expression of the PIT1 gene would be greater in the Large White race, since this offers rapid growth, while that of the Pietrain is slow-growing, suggesting that the expression of this gene relates to growth rate in Pietrain and the development of the muscle mass in Large White, characteristics of these races.

Several authors have associated the PIT1 gene with growth rate and low amount of fat in swine carcass. They have been reports of a significant effect on the PIT1 genotype and the circulating levels of growth hormone, as well as a positive correlation between the mRNA of this gene and the plasma concentration of growth hormone (Sun et al., 2002). Correlations between polymorphisms of PIT1 and characteristics of fat deposition in meat of swine have also been found.

CONCLUSION

The divergent selection between paternal and maternal lineages of Pietrain swine changed the genotypic frequency of PIT1 in these animals, and allele B was the best one established in the paternal lineage, leading to a greater muscle development and low levels of fat compared to the maternal lineage. Among the paternal lineage of Pietrain and Large White the frequency did not change, and in the former, the gene expresses itself through muscle development and in the latter by the rate of growth, considered to be fast in this race.

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