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Obese gene polymorphism in Pietrain and Large White pigs after a divergent selection

A.C.P. Silveira[1], R.C. Antunes[1], J.F. Almeida[2], T.F. Braga[1], P.F.A. Freitas[1], A.S.M. César[1] and E.C. Guimarães[1]

[1]Programa de Pós-Graduação em Ciências Veterinárias,
Faculdade de Medicina Veterinária,
Universidade Federal de Uberlândia, Uberlândia, MG, Brasil
[2]Laboratório de Genética Molecular,
Universidade Federal de Uberlândia,
Instituto de Genética e Bioquímica, Uberlândia, MG, Brasil

Corresponding author: A.C.P. Silveira
E-mail: anacarolina@medicaveterinaria.com

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ABSTRACT. The aim of the present study was to identify polymorphisms in the *leptin* gene of 112 pigs and compare the maternal and paternal lineage of Pietrain and Large White breeds that underwent a divergent selection for over 30 years. DNA samples extracted from the blood of these animals were amplified by polymerase chain reaction and genotyped by restriction fragment length polymorphism using the restriction enzyme *Hin*fI. The data were statistically analyzed by the chi-square test. The results showed that 87.5% of the animals were genotype TT, where 31.25% were Large White paternal lineage, 31.25% were Pietrain paternal lineage and 25% were Pietrain maternal lineage. The TC genotype appeared in 12.5%, where 10% were Large White, 0.5% were Pietrain paternal lineage and 2% were of the same maternal lineage breed; CC was not observed. As for the allele frequency, 93.75% of the T and 6.25% of the C allele were found. In Pietrain maternal and paternal lineages, it could not be determined that the *Obese* gene had been fixed in the maternal lineage. However, in the Pietrain and Large White paternal lineages there was a statistically significant difference,

suggesting that the C allele is associated with growth and daily weight gain and that the low frequency of C in the Pietrain lineage is related to low rate of body fat, a typical feature of this breed.

Key words: Quantitative trait loci; *Leptin*; Polymerase chain reaction; *Obese* gene; Restriction fragment length polymorphism

INTRODUCTION

In pigs, the *Obese* gene (or *leptin* gene) is considered to be an eligible gene to follow characteristics of economic importance, such as feed intake, pig fat thickness, growth, and reproduction (Lagonigro et al., 2003). The search for polymorphism in this gene is aimed at responding if the alterations found may be correlated to productive and reproductive features. In this species, seven *leptin* gene polymorphisms were described: C/T, A/G, C/T, G/T, A/T, T/C, and G/A at the following positions: 867, 1112, 3469, 3714, 2845, 3996, and 2728, respectively (Stratil et al., 1997; Robert et al., 1998; Jiang and Gibson, 1999; Kennes et al., 2001). Researchs such as those of Robert et al. (1998) and Jiang and Gibson (1999) suggest a relation between polymorphism and the features of economic interest. Others have found little or no statistical significance among them (Malek et al., 2001; Kennes et al., 2001; Szydlowski et al., 2004; Terman, 2005). de Oliveira Peixoto et al. (2006) affirm that the individual contribution of the *leptin* gene to productive and reproductive feature is still unknown, since growth and carcass composition are controlled by various genes and influenced by the environment.

Generally, the strategy adopted by laboratories for quantitative trait locus linking, analysis and detection map construction explores genetic and phenotype divergence among pig breeds. The breeds used for cross-reference are of three types: European or American commercial breeds (Large White, Landrace and/or Pietrain), Chinese breeds (mainly Meishan) and the wild European pig (Ellegren, 1995; Reis Filho, 2007).

Therefore, in this study, Pietrain breed was used, whose paternal genetic lineage improvement resulted from the necessity of an even more carcass quality-requiring market, showing as constitutional advantages: lower fat rate and body fat thickness, excellent fore and hind legs and a larger loin, and due to the growing use of pig husbandry technology and labor force reduction, research also focuses on the maternal ability development of the breed, such as maternal docility, high milk production and piglet vitality (Porter, 1993). Large White is the breed that offers the largest daily weight gain, reaching 90 kg at a lower age compared to Pietrain (Tibau et al., 1997), when fast and slow growth lineages, respectively, were considered.

The aim of the present study was to identify and compare *Obese* gene polymorphism frequency in paternal and maternal lineages of Pietrain breed and in Pietrain and Large White paternal lineages, which have undergone divergent selection for over 30 years.

MATERIAL AND METHODS

Biological material and sample collection

Blood samples were collected from the jugular vein of 112 pigs, where 36 were Pietrain paternal lineage (8 males and 28 females), 30 were Pietrain maternal lineage (11 males

and 19 females) and 46 Large White paternal lineage (11 males and 35 females); all supplied by the nucleus farm of a breeding company, located in Rio Verde, Goiás, Brazil.

Genotyping at the *Obese* locus

Genotyping at the *Obese* gene was performed on animal blood by polymerase chain reaction (PCR)-restriction fragment length polymorphism, according to Stratil et al. (1997), using a pair of primers with the following sequences: 5'TGCAGTCTGTCTCCTCCAAA3' (forward) and 5'CGATAATTGGATCACATTTCTG3' (reverse), which amplifies an amplicon of 152 bp. PCR was carried out in a PTC-MJ Research thermocycler. After preheating at 95°C for 2 min, amplification was done using 34 cycles at 95°C for 1 min, 55°C for 1 min, and 72°C for 1 min. For the PCR assays, 1 U Taq DNA polymerase, 10X PCR buffer, 3.0 mM $MgCl_2$, 200 µM of each dNTP, 10 pM of each primer and 200 ng genomic DNA in a final volume of 20 µL were used. After amplification, 10 µL of the PCR amplicon was digested with 2 U *Hin*fI restriction enzyme for 8 h at 37°C; genotyping was performed on a 3.5% agarose gel stained with ethidium bromide (10 mg/mL) and photographed under UV illumination.

Statistical analysis

The genotypes obtained were statistically analyzed by the χ^2 (chi-square) test at a significance level of 0.05.

RESULTS

Genotyping at the *Obese* locus

Figure 1 shows the genotype patterns. Allele T contained one 152-bp fragment, whereas allele C had two fragments (84 and 68 bp).

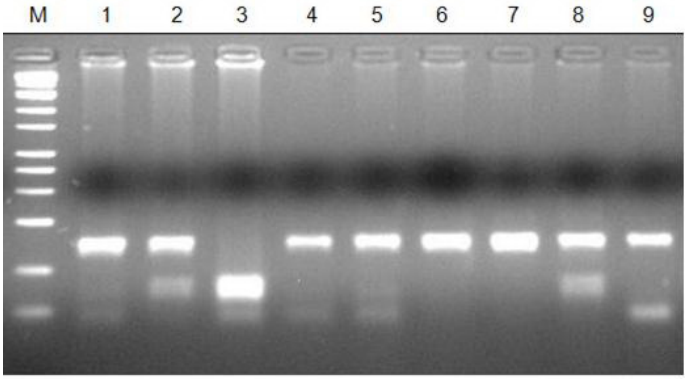

Figure 1. *Hin*fI polymorphisms in the *Obese* gene detected by polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR products were run on a 3.5% agarose gel. M = 50-bp ladder of molecular weight markers (Invitrogen); lane 1 = TT genotype pattern sample; lane 2 = TC genotype pattern sample; lane 3 = CC genotype pattern sample; lanes 4-6, 7, 9 = TT samples; lane 8 = TC sample.

Allele genotype frequencies

After genotyping the 112 Large White pigs (paternal lineage) and Pietrain (maternal and paternal lineages), the genotype (Table 1) frequencies and the *Obese* gene allele frequency (Table 2) were determined. When comparing the genotype frequency of paternal and maternal Pietrain lineages, statistically significant differences were not found ($P > 0.05$.) However, when analyzing the paternal Pietrain and Large White lineages, statistically significant differences were found ($P < 0.05$).

Table 1. Determination of *Obese* gene genotype frequency of 112 Pietrain breed pigs (maternal and paternal lineages) and Large White (paternal lineage).

Breed	Genotype frequency							
	TC	(%)	TT	(%)	CC	(%)	Total	(%)
Pietrain (maternal lineage)	2	(1.8%)	28	(25%)	0	(0%)	30	(26.8%)
Pietrain (paternal lineage)	1	(0.9%)	35	(31.25%)	0	(0%)	36	(32.15%)
Large White (paternal lineage)	11	(9.8%)	35	(31.25%)	0	(0%)	46	(41.05%)
Total	14	(12.5%)	98	(87.5%)	0	(0%)	112	(100%)

Table 2. Determination of *Obese* gene allele frequency of 112 Pietrain breed pigs (maternal and paternal lineages) and Large White (paternal lineage).

Breed	Allele frequency					
	T	(%)	C	(%)	Total	(%)
Pietrain (maternal lineage)	58	(25.9%)	2	(0.9%)	60	(26.8%)
Pietrain (paternal lineage)	71	(31.7%)	1	(0.45%)	72	(32.15%)
Large White (paternal lineage)	81	(36.15%)	11	(4.9%)	92	(41.05%)
Total	210	(93.75%)	14	(6.25%)	224	(100%)

DISCUSSION

The genotype frequencies observed were consistent with those reported by Borges and Goulart (2002) who, when genotyping 80 Large White pigs, observed 82.5% TT, 17.5% TC and no CC, and when analyzing 53 Pietrain animals, found 90.56% TT genotypes, 7.54% TC and 1.88% CC. The same allele frequencies were found by Borges and Goulart (2002), Stratil et al. (1997), Korwin-Kossakowska et al. (2001), Kulig et al. (2001), and Szydlowski et al. (2004) when studying other pig breeds.

Since no statistically significant difference was found between Pietrain breed maternal and paternal lineage genotypes, it is not possible to affirm that the *Obese* gene has been fixed more frequently on the maternal lineage than on the paternal lineage. The *Obese* gene was believed to be more often fixed on the maternal lineage, since due to the growing use of pig husbandry technology and the reduction in labor force, research has focused on ability development such as high milk production, which is related to body fat in female pigs (Porter, 1993), and on the fact that Chinese hyper-proliferative breeds such as Meishan are obese, while in the paternal lineage a lower fat rate and body fat thickness is desired (Porter, 1993).

Jiang and Gibson (1999) found a positive correlation between C allele and the body fat rate in the Meishan breed. Borges and Goulart (2002) suggested that the C allele may be associated with fat accumulation. Later, Kuryl et al. (2003) concluded that the TT genotype may be more advantageous since it is associated with lower fat deposition in carcass when compared to TC. The research of Urban et al. (2002) demonstrated that in Landrace breed animals, the homozygote (TT) showed a lower daily weight gain compared to the heterozygote (TC).

The differences between the paternal and maternal lineages of pigs impact the growth and development of these animals, which may be mediated by the metabolism and partition of nutrients between fat and muscle tissues (Sinclair et al., 1998). These energy metabolism variations may be attributed, in part, to the endocrine profile of the different maternal and paternal lineages and environmental conditions (Leininger et al., 2000.) Litten et al. (2004) argue that differences in growth, feed intake and carcass quality between maternal and paternal lineages of pig breeds are mainly due to genetics, with little influence of the endocrine factors of such animals.

Yet the higher C allele frequency found in Large White paternal lineage in relation to the Pietrain paternal lineage suggests that this is linked to growth and daily weight gain, since the growth of Large White is considered to be fast, while that of the Pietrain is slow. Houseknecht et al. (1998) argue that feed intake regulation and the energy balance in the body are important in optimizing animal growth. Moreover, the fact that *leptin* regulates growth hormone secretion by stimulating it has been observed (Barb et al., 1998.)

The Large White breed is also associated with high prolificacy (Porter, 1993) and best average daily weight gain (Pires et al., 2002.) It is, therefore, possible that the C allele is related to growth and daily weight gain and that no significant difference between maternal and paternal lineages of Pietrain was found due to the size of the sample. However, Szydlowski et al. (2004) found no association between R3469C polymorphism of the *leptin* gene and intramuscular fat characteristics in Large White when analyzing 135 animals.

It is possible that the low C allele frequency in Pietrain is linked to breed characteristics, cited by Rothschild and Ruvinsky (1998) as the high percentage of lean meat, low fat rates, high loin thickness, and greater conformation of carcass in hind legs. Also, Gregory et al. (1977) found higher circulating levels of insulin in Large White pigs than in Pietrain, which are more sensitive to stress and are producers of greater lean mass.

de Oliveira Peixoto et al. (2006) cited two cases that enable an associated explanation between the *leptin* gene and productive and reproductive characteristics in pigs. The first suggests that the detected polymorphisms are in imbalance with another single nucleotide polymorphism, which could be the true cause of the observed variations. The second states that there may be false positives for a limited number of observations of some genotypes or combinations.

CONCLUSIONS

The divergent selection of Pietrain breed maternal and paternal lineages did not alter the genotype and allele frequency of the *Obese* gene, as well as between genders. However, among Large White and Pietrain breed paternal lineages, the C allele was better fixed in the latter, suggesting that it is linked to growth and daily weight gain or that the lower frequency found in Pietrain correlates with its characteristics.

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