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ORIGINAL ARTICLE

Associations of leptin gene polymorphisms with production traits in pigs

J. de Oliveira Peixoto[1], S.E. Facioni Guimarães[1], P. Sávio Lopes[1], M.A. Menck Soares[2], A. Vieira Pires[3], M.V. Gualberto Barbosa[4], R. de Almeida Torres[1] & M. de Almeida e Silva[5]

1 Department of Animal Science, Federal University of Viçosa (UFV), Viçosa, MG, Brazil
2 UNIOESTE, Cascavel, PR, Brazil
3 UFVJM, Diamantina, MG, Brazil
4 Embrapa-CNPGL, Juiz de Fora, MG, Brazil
5 UFMG, Belo Horizonte, MG, Brazil

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Correspondence
Jane de Oliveira Peixoto, Animal Science Department, Federal University of Viçosa, Viçosa, MG 36571-000, Brazil.
Tel.: +55 31 38992273; Fax: +55 31 38992275;
E-mail: janeop@bol.com.br

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Summary

The associations of leptin (*LEP*) gene polymorphisms C798T, T2411C, T3266G and T3469C with production traits were investigated in a F2 pig population produced by divergent crosses. The statistical model included genotype, sex, batch and genotype by sex interaction as fixed effects and sire as random effect. Polymorphism C798T was associated with variation in total teat number ($p < 0.02$) and left teat number ($p < 0.03$), and polymorphism T3469C was associated with weight at 21 days ($p < 0.03$), 42 days ($p < 0.05$), 63 days ($p < 0.02$) and 77 days of age ($p < 0.04$) as well as feed intake ($p < 0.01$), average daily gain ($p < 0.01$), feed conversion ($p < 0.01$), bacon depth ($p < 0.03$) and slaughter weight ($p < 0.03$). Phenotypic associations were also performed by combining T3469C and C798T genotypes. Interaction between C798T genotypes and sex was observed for some traits. LEP genotypes had significant influence on performance traits, and can be considered as potential genetic markers for selection. However, these results have to be validated in commercial herds.

Introduction

The protein hormone leptin (LEP) is produced and secreted almost exclusively by adipocytes. This hormone acts as a signal of satiety over the hypothalamus, thereby controlling body weight and energetic balance (Barb *et al.* 2001). Leptin may influence directly growth and body composition through physiological and endocrine mechanisms. Consequently, polymorphisms identified in this gene may be considered as potential genetic markers for growth rate, feed intake (FI) and feed conversion (FC). Traditional selection methods for these traits have been successful, however, the contribution of individual genes in these processes is still unknown, since growth and body composition are controlled by many genes and influenced by several environmental factors.

The *LEP* gene was mapped on pig chromosome 18q13–q21 (Neuenschwander *et al.* 1996). In swine, this gene is composed of three exons and two introns (Bidwell *et al.* 1997), with the coding area being formed by the second and third exons. Comparative studies showed a high homology between the porcine and human or porcine and mouse sequences. This homology across mammals suggests that the structural part of the *LEP* gene is evolutionary highly conservative.

The aim of this paper was to investigate the associations between LEP gene polymorphisms (C798T, T2411C, T3266G and T3469C) and production traits in a F2 swine population produced by different pig breeds.

Materials and methods

Animals

The study population was created by crossing two boars of the native Brazilian Piau with 18 commercial sows composed of Landrace, Large White and Pietrain breeds. From the F1 generation, 11 boars and 54 females were selected and crossed, producing the F2 population. The F2 animals were divided into five batches using date of birth as a criterion. Sixty days after birth, animals were transferred to collective cages, where they stayed until 77 days of age. Afterwards, animals were moved to individual cages in order to measure FC (FI/weight gain), average daily gain (ADG) and FI over 28 days (from 77 to 105 days of age). The investigated traits are presented in Table 1.

Genotyping

Genotyping was performed at the Animal Biotechnology Laboratory of the Animal Science Department at Federal University of Viçosa. Genomic DNA was extracted from white cells of parental, F1 and F2 animals, and then purified with phenol–chloroform according to Sambrook *et al.* (1989). DNA aliquots were diluted in TE solution (10 mmol/l Tris–HCl, pH 8.0 and 1 mmol/l EDTA, pH 8.0) at a concentration of 25 ng/μl.

The single nucleotide polymorphisms (SNPs) analysed in the present study were first detected by Soares (2001) when sequencing the *LEP* gene in the parental generation of the present study (GenBank accession number AY079082). Utilizing the sequence published by Bidwell *et al.* (1997) (GenBank accession number U66254), Soares (2001) developed the four primer pairs used in this study (Table 2) to amplify regions containing the four leptin SNPs characterized by base substitutions at positions 798 bp (C $\rightarrow$ T), 2411 bp (T $\rightarrow$ C), 3266 bp (T $\rightarrow$ G) and 3469 bp (T $\rightarrow$ C). These SNPs were detected by the enzymes *Pvu*II, *Bam*HI, *Fok*I and *Hin*fI respectively. Only the T3469C substitution was located in a coding region, in the second exon of LEP. All other polymorphisms were located in introns.

Amplification reactions were conducted in a final volume of 20 μl, containing 1 unit of *Taq* DNA polymerase, 0.2 mM of each dNTP, 0.2 μM of each primer (forward and reverse), 20 mM Tris–HCl (pH 8.3), 2.0 mM $MgCl_2$, 50 mM KCl and 25 ng DNA. Amplification results were evaluated in a 8% polyacrylamide gel electrophoresis, after visualizing specific bands using the silver nitrate protocol according to Hiss *et al.* (1994).

Table 1 Total number of animals (n) and mean ± SE for each production trait

Trait	n	Mean ± SE	Trait	n	Mean ± SE
TN (n)	815	13.17 ± 0.05	SA (days)	538	147.81 ± 0.43
RTN (n)	815	6.62 ± 0.03	SW (kg)	529	64.71 ± 0.24
LTN (n)	816	6.62 ± 0.03	CW (kg)	540	53.60 ± 0.23
BW (kg)	815	1.20 ± 0.01	THW (kg)	543	7.28 ± 0.04
W21 (kg)	673	4.91 ± 0.04	HW (kg)	543	4.99 ± 0.03
W42 (kg)	668	8.31 ± 0.07	LR (mm)	549	19.81 ± 0.21
W63 (kg)	660	16.35 ± 0.13	P2 (mm)	547	16.81 ± 0.16
W77 (kg)	616	21.45 ± 0.17	LEA (cm^2)	499	26.30 ± 0.17
W105 (kg)	602	36.48 ± 0.26	LD (mm)	495	43.80 ± 0.20
FI (kg)	618	39.90 ± 0.32	BCW (kg)	541	2.69 ± 0.02
ADG (kg)	609	0.53 ± 0.01	BCD (mm)	539	24.92 ± 0.29
FC (kg/kg)	603	2.79 ± 0.03	IMF (%)	504	1.55 ± 0.03

TN, total teat number; RTN, right teat number; LTN, left nipple number; BW, birth weight; W21, weight at 21 days; W42, weight at 42 days; W63, weight at 63 days; W77, weight at 77 days; W105, weight at 105 days; FI, feed intake from 77 to 105 days of age; ADG, average daily gain from 77 to 105 days of age; FC, feed conversion from 77 to 105 days of age; SA, slaughter age; SW, slaughter weight; CW, carcass weight; THW, total ham weight; HW, skinless and fatless ham weight; LR, midline backfat thickness at last rib; P2, backfat thickness at last rib, 6.5 cm from the midline; LEA, loin eye area; LD, loin depth; BCW, bacon weight; BCD, bacon depth; IMF, intramuscular fat.

Statistical analyses

Statistical analyses to test putative associations among LEP polymorphisms and performance traits were carried out using the SAS statistical program (SAS Institute Inc. 1998). The model was:

Table 2 Primers (forward and reverse) used for amplification of the four regions that contain polymorphisms within the leptin gene, sequence, nucleotide position and product length (bp), based on GenBank sequence U66254

Polymorphisms	Sequence 5′ $\rightarrow$ 3′	Position	Product (bp)
C798T/*Pvu*II	F: gggatagcctgaagtcgtgc	561–1161	601
	R: caacctctgaggtccggacc		
T2411C/*Bam*HI	F: gtggggtccagatatccgtt	1916–2519	604
	R: ccaggctaggggtctaatcg		
T3266G/*Fok*I	F: tgtgagaaacagacagtcgtgg	3092–3514	423
	R: tgaggatctgttggtagatcgc		
T3469C/*Hin*fI	F: aacagagggtcaccggtttg	3416–3901	486
	R: tttggaagagcagcttagcg		

$$y_{ijklm} = \alpha + G_i + S_j + GS_{ij} + L_k + p_l + (C_{ijklm} - \bar{C})b + e_{ijklm},$$

where y_{ijklm} is observation; α is general mean; G_i is fixed effect of genotype i ($i = 1, 2, 3$); S_j is fixed effect of sex j ($j = 1, 2$ with 1, castrated males and 2, females); GS_{ij} is sex by genotype interaction; L_k is fixed effect of batch k ($k = 1, 2, 3, 4$ and 5); p_l is random effect of the sire l; b is linear regression coefficient of the trait on the covariate; C_{ijklm} is observed covariate value; and e_{ijklm} is random error.

No covariate was used in analyses of the traits total teat number (TN), left teat number (LTN) and right teat number. Litter size at birth was the covariate for birth weight (BW), and litter size at weaning was the covariate for the remaining weights (W21, W42, W63, W77 and W105). Weight at 77 days (W77) was used as the covariate in analyses of traits FI, ADG and FC. The cooled right half carcass weight was used as covariate for the analyses of carcass and carcass cut traits. Mean values of individual genotypes were compared by *F*-test. Mean values of combined genotypes were compared with contrasts by *F*-test. In the case of a significant *F*-test for the genotype by sex interaction, genotype mean values within each sex were compared with a *t*-test.

Results

Genotypes in the F2 generation

Genotypic and allelic frequencies of polymorphisms in F2 animals are shown in Table 3. To simplify genotypic description, the genotypes were designated as AA (wild type), AB (heterozygote) and BB (homozygote for the SNP) for all four SNPs. In the case of polymorphism C798T the genotype CC corresponded to wild type (AA), and the genotype TT is the genotype homozygote for the mutation. For the SNP T3266G the genotype TT was the wild type and the genotype GG was the homozygote mutated. On the other hand, for polymorphism at T2411C and T3469C the genotype TT corresponded to wild type and the genotype CC was the genotype homozygote for the mutation. The frequency of the BB genotype was zero for LEP C798T and T3469C and very low (7.32%) for T3266G. Consequently, they were excluded from the association analyses.

Association between LEP and production traits

Association analyses were first carried out separately for each locus. Only genotypes C798T and T3469C were significantly associated with production traits. The results of association analyses are presented in Table 4. Genotypes C798T showed association with total ($p < 0.02$) and LTN ($p < 0.03$). Genotypes T3469C were significantly associated with weight at 21 days ($p < 0.02$), 42 days ($p < 0.04$), 63 days ($p < 0.02$) and 77 days of age ($p < 0.03$), FI ($p < 0.01$), ADG ($p < 0.001$) and FC ($p < 0.001$), bacon depth ($p < 0.03$) and slaughter weight ($p < 0.03$). In this study, allelic variants originating from Piau animals presented a negative association with the phenotypic mean values. Although genotype heterozygote at polymorphism C798T was associated with higher TN and LTN and the genotype heterozygote at polymorphism T3469C was positively associated with ADG and FI.

Other association analyses were performed combining the genotypes of the polymorphisms T3469C and C798T. It was not possible to do the associations analyses combining the four polymorphisms. When combining all four polymorphisms, 24 genotypic classes with small number of observations within the classes were noticed, consequently the statistical analyses were not consistent. Genotype combinations were settled only between polymorphisms

Table 3 The frequency of genotypes and alleles at polymorphisms within leptin gene in F2 pigs

		Genotype frequency[2]			Allelic frequency[3]	
SNP/enzyme	n[1]	AA	AB	BB	A	B
C798T/*Pvu*II	473	77.17 (365)	22.83 (108)	–	88.59 (601)	11.41 (237/364)
T2411C/*Bam*HI	445	31.46 (140)	53.26 (237)	15.28 (68)	58.09 (540/64)	41.91 (497/64/47)
T3266G/*Fok*I	369	64.23 (237)	28.45 (105)	7.32 (27)	78.45 (423)	21.55 (174/249)
T3469C/*Hin*fI	506	86.36 (437)	13.64 (69)	–	93.18 (53/433)	6.82 (53/344/89)

SNP, single nucleotide polymorphism.

[1]Number of animals genotyped for each polymorphism.

[2]Percentage of animals with specified genotype of a given polymorphism. The total number of animals genotyped in parentheses.

[3]The fragment size in base pairs for each allele after restriction fragment length polymorphism reaction is in parentheses.

Table 4 Association analyses between production traits in F2 pigs and individual polymorphisms C798T and T3469C within leptin gene, significance level of the genotypic effect, number of animals genotyped (n) and least square mean (LSM) ± SE

			Genotype AA		Genotype AB	
Trait	SNP	p-value	n	LSM ± SE	n	LSM ± SE
TN (n)	C798T	0.02	363	13.09 ± 0.07	94	13.42 ± 0.12
LTN (n)	C798T	0.03	363	6.53 ± 0.04	94	6.72 ± 0.06
W21 (kg)	T3469C	0.02	391	5.22 ± 0.06	64	4.84 ± 0.11
W42 (kg)	T3469C	0.04	403	8.71 ± 0.09	60	8.15 ± 0.20
W63 (kg)	T3469C	0.02	406	16.89 ± 0.17	59	15.76 ± 0.32
W77 (kg)	T3469C	0.03	394	21.87 ± 0.22	60	20.56 ± 0.41
FI (kg)	T3469C	0.01	397	39.37 ± 0.41	68	41.79 ± 0.92
ADG (kg)	T3469C	0.001	393	0.51 ± 0.01	68	0.58 ± 0.01
FC (kg/kg)	T3469C	0.001	388	2.91 ± 0.04	68	2.63 ± 0.07
BCD (mm)	T3469C	0.03	338	24.77 ± 0.35	54	26.76 ± 0.85
SW (kg)	T3469C	0.03	295	64.00 ± 0.29	46	65.96 ± 0.78

SNP, single nucleotide polymorphism; TN, total teat number; LTN, left teat number; W21, weight at 21 days; W42, weight at 42 days; W63, weight at 63 days; W77, weight at 77 days; FI, feed intake from 77 to 105 days of age; ADG, average daily gain from 77 to 105 days of age; FC, feed conversion from 77 to 105 days of age; BCD, bacon depth; SW, slaughter weight.

T3469C and C798T and their frequencies are presented in Table 5. The significance of the associations analyses, along with the mean and standard error for production traits and the combined genotypes T3469C/C798T are presented in Table 6. It was observed that LEP combined genotypes were statistically associated with TN ($p < 0.06$), LTN ($p < 0.03$), weight at birth ($p < 0.04$), weight at 21 days ($p < 0.07$) and weight at 63 days ($p < 0.04$), total ham weight ($p < 0.04$), skinless and fatless ham weight ($p < 0.07$). The combined genotypes 12 and 22 appeared to be favourable for TN and LTN. The combined genotypes 11 and 12 presented a favourable association with weight at birth, weight at 21 days and weight at 63 days.

Table 5 Leptin genotype combinations at T3469C/C798T and their observed frequencies in F2 pigs

T3469C	C798T	Combined genotypes	Frequency	Per cent
11	11	11	107	55.73
11	12	12	54	28.13
12	11	21	21	10.94
12	12	22	10	5.21

Combined genotypes: 11, homozygote (–/–) for both SNPs; 12, homozygote (–/–) at T3469C and heterozygote (+/–) at C798T; 21, heterozygote (+/–) at T3469C and homozygote (–/–) at C798T.

Genotype by sex interaction

No significant genotype by sex interactions at T3469C were observed for any of the performance traits. On the other hand, a significant genotype by sex interaction was observed at C798T for weight at 42 ($p < 0.03$) and 63 days ($p < 0.01$), FI ($p < 0.04$), ADG ($p < 0.02$) and FC ($p < 0.05$). These results are presented in Table 7. The effect genotype by sex interaction on individual performance trait becomes apparent when genotypes are compared within each sex. For instance, within males there was significant difference between AA and AB genotypes of polymorphism C798T for ADG and FC, whereas within females this effect of genotypes was not observed.

Discussion

This study demonstrates the potential for genetic analyses in native Brazilian pigs, which has not yet been properly explored. The Brazilian native pig breeds originated from breeds introduced by Portuguese settlers in the XVI century has also some influence of Dutch pigs and African pig breeds (Vianna 1985). These animals were used for breeding in small farms, having as their main traits rusticity, adaptability to poor conditions of management and feeding and a great resistance to diseases. All these old breeds are considered to be of the fat type, supplying the farmers with meat, but also with a large amount of fat. However, in the last decades, owing to the changes in the consumer habit and the low production efficiency, these native pigs are on their way to extinction (Lopes *et al.* 2002).

Two hypotheses may explain the genotype/trait associations. The first suggests polymorphisms to be in linkage disequilibrium with another SNP which should be the true causal site of the observed variation in the traits. Swine chromosome 18 (SSC18) according to the USDA-MARC_current map (average sex), described at http://www.thearkdb.org/arkdb/do/getMappingDetails?accession=ARKLGP00000280 (accessed on 25 October 2006) represents one of the lower marker density and higher mapping interval among the chromosomes in the USDA-MARC linkage map (Campbell *et al.* 2001). In agreement with SSC18 mapping, the neuropeptide Y locus (18q24) is located near LEP (18q13–21). The former acts against leptin and is also considered as a candidate gene for pig performance. *LEP* gene may also be in linkage disequilibrium with other loci that have potential biological action over animal performance, such as IGF-binding protein 3 and growth

Table 6 Association analyses between production traits in F2 pigs and combined genotypes T3469C/C798T within leptin gene, significance level of the genotypic effect and least square mean (LSM) ± SE

		Combined genotypes T3469C/C798T (LSM ± SE)[2]			
Trait[1]	p-value	11	12	21	22
TN (n)	0.06	13.07[a] ± 0.13	13.30[a] ± 0.18	12.44[b] ± 0.28	13.23[a] ± 0.40
LTN (n)	0.03	6.49[b] ± 0.08	6.72[a] ± 0.11	6.17[b] ± 0.16	6.67[a] ± 0.24
BW (kg)	0.04	1.20[a] ± 0.02	1.20[a] ± 0.03	1.09[b] ± 0.05	1.08[b] ± 0.07
W21 (kg)	0.07	5.05[a] ± 0.12	5.24[a] ± 0.16	4.65[b] ± 0.23	4.52[b] ± 0.34
W63 (kg)	0.04	17.00[a] ± 0.40	17.14[a] ± 0.53	16.14[a] ± 0.82	13.87[b] ± 1.16
THW (kg)	0.04	7.46[a] ± 0.09	7.27[b] ± 0.09	7.49[a] ± 0.10	7.28[b] ± 0.10
HW (kg)	0.07	5.00[a] ± 0.13	4.78[b] ± 0.11	5.17[b] ± 0.15	4.97[b] ± 0.15

[1]TN, total teat number; LTN, left teat number; BW, weight at birth; W21, weight at 21 days; W63, weight at 63 days; THW, total ham weight; HW, skinless and fatless ham weight.
[2]Mean values followed by different letter, in the same line are different by *F*-test ($p < 0.05$).

Table 7 Significance of the genotype by sex interaction effect over polymorphism C798T and it unfolding within each sex

		Male mean[3]		Female mean[3]	
Trait [1]	p-value[2]	AA	AB	AA	BB
W42 (kg)	0.03	8.45[a]	8.88[a]	9.11[a]	8.58[b]
W63 (kg)	0.01	15.64[a]	17.53[a]	17.78[a]	16.74[b]
FI (kg)	0.04	40.04[a]	39.10[a]	38.49[b]	40.93[a]
ADG (kg)	0.02	0.53[a]	0.49[b]	0.52[a]	0.55[a]
FC (kg/kg)	0.05	2.76[b]	3.03[a]	2.75[a]	2.70[a]

[1]W42, weight at 42 days; W63, weight at 63 days; FI, feed intake from 77 to 105 days of age; ADG, average daily gain from 77 to 105 days of age; FC, feed conversion from 77 to 105 days of age.
[2]Significance of genotype by sex interaction by the *F*-test.
[3]Mean values followed by a different letter within the same line and sex are significantly different ($p < 0.10$) by a *t*-test.

hormone-releasing hormone receptor. Both were mapped at position q24 on chromosome 18. If this hypothesis is true, these LEP polymorphisms could serve as indirect markers for the true causal site of the variations.

The second hypothesis for the polymorphism \associations would be the existence of false-positive associations between the genotypes and the traits. One reason for false-positive associations would be the limited number of observations for some genotypes or combined genotype.

In this study, pigs with the TT genotype at position T3469C showed the highest weights at 21, 42, 63 and 77 days of age. However, heterozygous CT pigs had the highest ADG and FC from 77 to 105 days of age. Over the last years, several SNPs have been identified at LEP gene. Some few reports are available regarding the association between individual LEP gene polymorphisms and productions traits in pigs. Jiang & Gibson (1999) studying four LEP polymorphism suggested a possible association between C allele of the 3469 polymorphism and fat deposition. Kennes *et al.* (2001) reported a negative effect of the C allele on FI in Landrace pigs ($p = 0.0078$). However, Szydlowski *et al.* (2004) did not find association between T3469C polymorphism and growth traits in Polish Large White, Landrace and a synthetic pig line. Van der Lende *et al.* (2005) reviewing about *LEP* gene polymorphisms and their phenotypic associations in different species indicated that in pigs the *LEP* gene can be associated with variation in FI, ADG, carcass traits (backfat/leanness) and reproduction traits. Leptin receptor gene polymorphisms have also drawn attention from animal scientists for their possible implications in physiology of economically important traits. Chen *et al.* (2004) found significant associations between leptin receptor genotypes and litter size in Duroc and Yorkshire, associations with feed efficiency in Duroc and backfat thickness in Landrace and Yorkshire breeds. Data concerning association between genotypes and production traits are frequently controversial in the literature. This may be partly due to differences in the genetic background of the analysed breeds. Therefore, this associations need to be validated in another studies before effective conclusions can be drawn.

The genotype by sex interaction is a particular case of genotype by environment interaction. In this case, the intrinsic difference between sexes represents a distinct environmental condition to gene expression. Divergent effects of LEP genotypes in males and females may be explained by differential expression of the LEP protein in each sex. In humans, sex has a very significant effect on LEP expression: women secrete three times more LEP than men for the same quantity of body fat (Martin *et al.* 2002). In males, circulatory levels of LEP decrease after reaching puberty, it seems that testosterone is able to inhibit LEP synthesis, and oestrogen is able to stimulate it. In the present study males were castrated at 10 days of age, so they do not have enough testosterone to

control LEP levels. Nevertheless, considering that LEP expression is controlled by levels of other hormones like oestrogen, the increased circulatory oestrogen levels in females probably explain the existence of genotype by sex interaction. Considering the high level of LEP structural similarity among mammalian species (Ramsay *et al.* 1998), it is possible that this differentiated pattern of expression between sexes also occurs in pigs. The existence of interaction between genotypes and sex indicates that a marker can be more efficient to select in one sex than in another. Therefore, the effect of genotype by sex interaction should be tested in statistical models for candidate gene analyses.

All the analyses conducted here have shown consistent results that LEP genotypes are related to performance traits. Individually, C798T was associated with variation in teat number, and T3469C with several growth traits like weight at 21, 42, 63 and 77 days of age and FI traits (FI, ADG and FC). The same traits were shown to be associated with LEP genotypes in the other analyses that were carried out, i.e. W42, W63, FI, ADG and FC significant for genotype by sex interaction and TN, LTN, BW, W21 and W63 for combined genotypic association. These results, confirm that LEP is a potential candidate gene for growth and FI in pigs, however, the associations were detected in an experimental population and need to be validated in commercial herds.

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