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Association of PIT1, GH and GHRH polymorphisms with performance and carcass traits in Landrace pigs

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Abstract. The study of candidate genes, based on physiological effects, is an important tool to identify genes to be used in marker-assisted selection programs. In this study, a group of halothane gene-free, non-castrated, male Landrace pigs was used to study the association between polymorphisms in the *PIT1* (n = 218), *GH* (n = 213) and *GHRH* (n = 206) genes and fat thickness, average daily gain, and the EPD (expected progeny difference) for fat thickness, average daily gain, and litter size. These genes are potential candidate markers because of their important physiological effects. The pigs were genotyped by PCR-RFLP, and the statistical model used to analyze the association between genotypes and the traits measured included genotypes as a fixed effect and age and weight as covariates. *PIT1* polymorphisms were associated with fat thickness (P = 0.0019), EPD for average daily gain (P = 0.0001) and EPD for fat thickness (P = 0.0001), whereas *GH* polymorphisms were associated with fat thickness (P = 0.0326) and average daily gain (P = 0.0127), and *GHRH* polymorphisms were associated with the average daily gain (P = 0.0001) and EPD for fat thickness (P = 0.0004). These results confirmed the potential usefulness of these genes in marker-assisted selection programs for pig breeding.

Key words: carcass, GH, GHRH, performance, pigs, PIT1.

Introduction

The *GH* (growth hormone) gene pathway contains various interdependent genes, such as *GH*, *IGF1* (insulin-like growth factor), *PIT1* (pituitary-specific transcription factor), *GHRH* (growth hormone releasing hormone), somatostatin, *GHRHR* (growth hormone releasing hormone receptor), *GHR* (growth hormone receptor) and others (Cogan and Phillips 1998). These genes are potential candidate markers because of their important physiological effects associated with economic traits.

The study of candidate genes based on physiological effects is simple, in contrast to investigations using anonymous DNA markers that require prolonged and demanding delineations. When an association between a DNA polymorphism and an important trait is found, this marker can be immediately included in marker-based selection programs. Such inclusions gradually increase the accuracy of selection and, consequently, improve the response, although further research is needed to understand the gene-environment interactions (de Vries et al. 1998). Some DNA polymorphisms may affect gene expression at both translational and transcriptional levels. However, the effect of a single polymorphism may be masked by an interaction with environmental and genetic factors. Such interactions may mask the effects of a given gene through a genotypic disequilibrium among genes and/or a strong environmental influence.

Fat thickness and average daily gain are very important traits in pig production because they are correlated with growth and with the quantity of

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lean meat in the carcass. Based on physiological effects, the *GH* gene and other genes in its pathway are related to these two traits.

In a study of *Dra*I and *Taq*I polymorphisms in the *GH* gene, Nielsen and Larsen (1991) observed changes in the blood GH levels that suggested that genetic variations in the *GH* locus may directly affect the expression of proteins associated with this pathway. Geldermann et al. (1996) reported the association of carcass traits with haplotypes identified in the *GH* gene. In a study of *Apa*I and *Hinp*I polymorphisms, Knorr et al. (1997) found an association between gene variants and fat traits, and concluded that the *GH* locus required additional investigation in order to assess its usefulness in marker-assisted selection programs. Similarly, Yu et al. (1995, 1999), Stancekova et al. (1999) and Brunsch et al. (2002) studied the association between *PIT1* polymorphisms and carcass and performance traits, and suggested that this gene could be a candidate gene for a QTL in pigs. Chung et al. (1998) treated porcine anterior pituitary cells in primary culture with GHRH and showed that the *PIT1* mRNA level increased 3-fold. This work shows the involvement between the *PIT1* and *GHRH* genes.

In this study, we investigated the association between polymorphisms in the genes for PIT1, GH and GHRH and the performance and carcass traits in a purebred Landrace pig population.

Material and methods

Pigs and data collection

Two hundred and eighteen Landrace pigs from Granja Rezende S/A, Brazil, were used in this study. All of the pigs were non-castrated males that were halothane-free, as confirmed by PCR-RFLP (data not shown) for the CT 1843 mutation in *HAL* gene (Fujii et al. 1991). Genomic DNA was isolated from white blood cells, and was used to genotype the *HAL*, *PIT1*, *GH*, and *GHRH* genes.

The performance and carcass traits assessed included fat thickness (FT), average daily gain (ADG) and expected progeny differences for average daily gain (EPD ADG), fat thickness (EPD FT) and litter size (EPD LS). FT was obtained by ultrasound at the last rib, 6.5 cm from the vertebral spine, ADG was calculated as the difference between the initial and final body weights divided by the number of days of the test, and EPD was estimated using BLUP Animal Model (Best Linear Unbiased Prediction, University of Nebraska, NE). All of the data were collected during the improvement program at Granja Rezende S/A.

PIT1 genotyping

The *PIT1* gene was genotyped by PCR-RFLP (polymerase chain reaction – restriction fragment length polymorphism). A 1745 bp fragment was amplified from intron 4 to the 3' untranslated region (UTR). The primers used were those designed by Yu et al. (1994). PCR was done using 100-200 ng of genomic DNA, 200 μM of each dNTP, 0.4 μM of each primer, 2.5 U of *Taq* polymerase, 1.5 mM of $MgCl_2$ and 1 × enzyme buffer in a final volume of 25 μL. The reactions were run in a PTC-100 thermocycler (MJ Research) using the following conditions: 95°C for 4 min prior to 30 cycles of 95°C for 1 min, 61°C for 1 min and 72°C for 3 min, followed by a final extension at 72°C for 5 min. After amplification, 15 μL of the PCR amplicon were digested using 12 U of *Rsa*I restriction enzyme by incubation overnight at 37°C, and the bands were detected in 2% agarose gels stained with ethidium bromide (10 mg mL^{-1}) and photographed under UV light. Allele *A* showed a 710 bp fragment, whereas allele *B* yielded two fragments (388 and 322 bp). Monomorphic fragments of 774, 153 and 108 bp were also seen.

GH genotyping

A pair of primers designed by Larsen and Nielsen (1993) was used to genotype the *GH* gene. PCR was done using 50 ng of genomic DNA, 100 μM of each dNTP, 0.5 μM of each primer, 1 U of *Taq* polymerase, 3 mM of $MgCl_2$ and 1 × enzyme buffer in a final volume of 20 μL. The reactions were done using the following conditions: 95°C for 3 min, prior to 35 cycles of 95°C for 45 s, 59°C for 45 s and 76°C for 1 min, with a final extension at 76°C for 4 min. After amplification, 20 μL of the PCR amplicon (605 bp) were digested with 2-4 U of *Dde*I restriction enzyme by incubation overnight at 37°C, and the genotyping was done in 2.5% agarose gels stained with ethidium bromide (10 mg mL^{-1}) and photographed under UV light. Two alleles were generated: D_1 with 335 bp, 148 bp and 122 bp fragments, and D_2 with 457 bp and 148 bp.

GHRH genotyping

A 455 bp fragment spanning exon 3 of the *GHRH* locus was obtained by PCR using 50 ng of

genomic DNA, 100 μM of each dNTP, 0.5 μM of each primer, 1 U of *Taq* polymerase, 1.5 mM of $MgCl_2$ and 1 X enzyme buffer in a final volume of 20 μL. The cycling program consisted of 95°C for 5 min, followed by 35 cycles of 95°C for 30 s, 63°C for 45 s and 72°C for 1 min, with a final extension of 72°C for 5 min. The primers were those designed by Moody et al. (1995) and used by Baskin and Pomp (1997). The PCR product was digested overnight at 37°C using 10 U of *Alu*I restriction enzyme, and genotyping was done in 3% agarose gels stained with ethidium bromide (10 mg mL^{-1}) and photographed under UV light. Two alleles were generated: allele *A*, with a polymorphic fragment of 250 bp, and allele *B*, with a polymorphic fragment of 230 bp.

Statistical analyses

The association between the genotypes of each of the three loci and the traits measured in the pigs and EPD data was examined using a linear model and SAS software (1992). The general linear model used was assumed to be:

$$Y_{ij} = \mu + Genotype_i + \gamma_1(X_{ij1}-X..._1) + \gamma_2(X_{ij2}-X..._2) + e_{ij}$$

where Y_{ij} corresponds to the traits observed (FT, ADG, EPD for ADG, EPD for FT, and EPD for LS) in the i-th genotype group, μ is the population mean of the measurements, $Genotype_i$ represents the *PIT1* (i = *AA*, *AB*), *GH* (i = D_1D_1, D_1D_2), and *GHRH* (i = *AA*, *AB*, *BB*) genotypes, γ_1 and γ_2 are the covariate effects (X_{ij1} age and X_{ij2} weight of the pig), and e_{ij} is the random error effect of the ij^{th} pig, with normal distribution, and independently distributed with zero mean and variance σ^2.

Pig age and weight at slaughter were used as covariates to correct the trait values since all of the economic traits were highly influenced by these two covariables. The pairwise test used was the *t* test.

The Chi-square test was used to assess whether the frequencies of genotypes were in the Hardy-Weinberg proportions, with the Yates correction being applied to *PIT1* and *GH*.

Results

A total of 218, 213, and 206 pigs were genotyped for polymorphisms in the *PIT1*, *GH*, and *GHRH* genes, respectively. The genotype and allelic frequencies found for the three genes are shown in Table 1. Chi-square analysis has showed that the genotypic frequencies for the *PIT1* ($p < 0.05$) and *GH* ($p<0.01$) genes were not in the Hardy-Weinberg equilibrium. The heterozygote genotypes for these genes seem to be overrepresented according to Hardy-Weinberg law.

Table 1. Genotypic and allelic frequencies for the *PIT1*, *GHRH* and *GH* genes

Gene	Genotype	Genotype frequency	Allele	Allelic frequency
PIT1	AA (n = 165)	0.757	A	0.878
	AB (n = 53)	0.243	B	0.122
GH	D_1D_1 (n = 141)	0.662	D_1	0.831
	D_1D_2 (n = 72)	0.338	D_2	0.169
GHRH	AA (n = 26)	0.126	A	0.374
	AB (n = 102)	0.495	B	0.626
	BB (n = 78)	0.379	–	–

The least square means of the traits and the standard errors for each genotype are shown in Table 2. Significant differences were observed among the *PIT1* genotypes for all traits, except for the variables ADG and EPD LS. The *GHRH* geno-

Table 2. Least square means and standard error (in parentheses) of each trait according to the genotypic effect

Trait	PIT1		GHRH			GH	
	AA n=165	AB n=53	AA n=26	AB n=102	BB n=78	D_1D_1 n=141	D_1D_2 n=72
FT (mm)	11.521(0.132)^{A}	10.846(0.234)^{B}	11.048(0.330)	11.257(0.167)	11.801(0.191)	11.640(0.142)^{a}	10.981(0.199)^{b}
ADG (kg/day)	0.868(0.004)	0.873(0.007)	0.901(0.009)^{A}	0.858(0.004)^{B}	0.864(0.005)^{B}	0.868(0.004)^{a}	0.876(0.006)^{b}
EPD ADG (g)	52.710(1.005)^{A}	46.091(1.776)^{B}	51.914(2.510)	50.687(1.271)	51.465(1.455)	51.562(1.104)	51.363(1.546)
EPD FT (mm)	0.017(0.018)^{A}	–0.131(0.031)^{B}	–0.091(0.045)^{A}	–0.065(0.022)^{A}	0.090(0.026)^{B}	0.011(0.019)	–0.045(0.027)
EPD LS (no. of piglets)	0.389(0.010)	0.384(0.018)	0.399(0.025)	0.411(0.012)	0.364(0.014)	0.374(0.011)	0.411(0.015)

Different letters in the same row indicate a significant difference for each genotype
^{a,b} $P<0.05$
^{A,B} $P<0.01$

types showed significant differences for ADG and EPD FT, whereas the *GH* genotypes showed significant differences for FT and ADG.

Discussion

Pig age and weight, which were used as covariates to correct all trait values, showed significant effects on the traits, thus justifying their use in the model. The chi-square test showed the deviations from the Hardy-Weinberg equilibrium at *PIT1* and *GH* loci. This population was under selection, as confirmed by the chi-square results, and the significant mean differences for most traits in the *PIT1* and *GH* genotypes (Table 2) suggested that these genes may be important candidates for use as molecular markers in selection programs.

The administration of *GH* decreases the size and number of adipocytes (fat-storage cells) (Rehfeldt et al. 1994), reduces the activity of lipogenic enzymes (Magri et al. 1990), and accelerates fat degradation (De Feo et al. 1989). Etherton (2000) also reported that the administration of porcine somatotropin (PST) to growing pigs increased the muscle tissue by 50% and decreased the fat tissue deposit.

The *AB PIT1* pigs have a lower FT ($P = 0.0019$), EPD ADG ($P = 0.0001$), and EPD FT ($P = 0.0001$) (Table 2). Our previous work with this population showed that this *PIT1* polymorphism was correlated with *GH* mRNA levels, with *AB* pigs having higher levels of mRNA for this hormone (Franco et al. 2005). These findings could explain why *AB* pigs had a lower FT and EPD FT, and also agreed with the important physiological role of GH on fat metabolism mentioned by Te Pas et al. (2001).

Our results therefore provide strong support for the physiological effects of GH on carcass composition, since pigs with a higher *GH* mRNA expression (Franco et al. 2005) had a lower fat thickness. The lower EPD FT seen in *AB PIT1* pigs is of great relevance for selection programs, since it indicates that animals carrying the *B* allele may have a higher potential for transferring the lower fat thickness trait (which is normally correlated with a higher lean meat content) to the next generation. *AB PIT1* pigs also had a lower EPD ADG, and could therefore transfer a lower capacity of ADG to the next generation.

The higher frequency of *AA PIT1* pigs (Table 1) in this population may reflect the positive selection towards a higher EPD ADG. On the other hand, there is also possibility that allele *B* is being introduced through selection.

Clutter and Brascamp (1998) commented that animals with limited nutritional feeding direct most of their intake energy towards lean meat gain and minimize their gain in fat. In contrast, with *ad libitum* feeding, much of the food consumed is used to accumulate fat. The accumulation of lean meat in the carcass may reach a plateau, whereas the accumulation of fat continues to increase (Whittemore 1986). Therefore, *AB PIT1* pigs may be close to the lean meat gain plateau, but with the excess food not being transformed into fatness, unlike *AA PIT1* pigs, which have a higher FT, EPD ADG and EPD FT.

In nutritionally restricted feeding, there is a negative correlation between ADG and FT, whereas *ad libitum* feeding results in a positive correlation between ADG and FT (Clutter and Brascamp 1998). Our results with *ad libitum* feeding agree with those obtained by these authors. Pigs with a higher food intake may have a higher ADG and FT because they have exceeded their nutritional limit and now transform food into fat.

Our results also agree with those given by Brunsch et al. (2002), who reported an association between *Rsa*I polymorphism and several performance traits. In contrast, *Msp*I polymorphism for *PIT1* has been associated with several performance traits, but not with *Rsa*I (Yu et al. 1995; Stancekova et al. 1999). That disagreement among these studies probably resulted from the fact that different breeds were used, as discussed by Sun et al. (2002) and Yu et al. (1999) and/or small or moderate effects of markers on phenotypic traits (Yu et al. 1999).

At least four hypotheses could explain the association between the *PIT1* polymorphism and economic traits: (1) This polymorphism could be involved in processing *PIT1* mRNA to generate an RNA molecule with variations covering exons 4, 5 and 6 in this region, although we have previously shown that this is not the case (Franco et al. 2005); (2) This polymorphism can be involved in alternative transcript production to produce variations in other regions of the gene. Various transcripts have been described for the *PIT1* gene (Tuggle and Trenke 1996); (3) This polymorphism could be involved in expressing its own *PIT1* gene (we have not tested the latter two hypotheses); and (4) The *PIT1* gene is linked to one or more genes that are also involved in these traits. This hypothesis is supported by the polygenic inheritance of quantitative traits, as indicated by Yu et al. (1999).

These authors studied seven polymorphisms (including four microsatellites) in the *PIT1* region and observed an association between microsatellite and other polymorphisms and FT. As a result, they concluded that the *PIT1* gene could be a QTL for this trait. In addition, an interval mapping study provided evidence of a QTL for FT approximately 20 cM from the *PIT1* gene.

Based on the known physiological effects of the *PIT1* gene and on the results of the present study and our previous report (Franco et al. 2005), we suggest that the polymorphism within *PIT1* affects the traits studied here by controlling *GH* expression.

With regard to the *GH* gene D_1D_2 pigs have a lower FT (P = 0.0326) and a higher ADG (P = 0.0127) (Table 2). Since GH is involved in fat metabolism (Etherton 2000), a possible explanation for this result could be that this polymorphism, which changes an amino acid in the protein sequence, could interfere with the efficiency or quantity of GH secretion.

The population was in the Hardy-Weinberg equilibrium with respect to *GHRH* locus, and the association analysis revealed that pigs with the *Alu*I *AA* genotype had a higher ADG (P = 0.0001), whereas the *Alu*I *BB* genotype was associated with a higher EPD FT (P = 0.0004) (Table 2). The Hardy-Weinberg equilibrium at the *GHRH* locus suggested a very small effect of this gene on the traits or that the linkage between *GHRH* and the QTL was very weak, although this could reflect the small number of *AA* pigs used in the analysis. Despite its role in stimulating GH secretion by the pituitary, Arbona et al. (1990) showed that the anterior pituitary cells of neonatal pigs (3–5 days old) produced more GH after exposure to GHRH than cells of 3-month-old pigs. Since we studied older pigs (around puberty), the GH secretion would be less dependent on GHRH. Moreover, Farmer et al. (1993) commented about the possible involvement of IGF1 in controlling GH secretion.

Conclusions

In conclusion, our results indicate that these three polymorphisms contributed to the variation in the traits analysed and reinforce the possibility of using these polymorphisms in molecular marker-assisted selection and breeding programs. Additional research with a bigger population of the same breed in order to have more animals of each genotype is required. Moreover, a study with other commercial breeds is also needed to determine the general effects of these polymorphisms, particularly since commercial pigs are obtained from crosses of two or more breeds.

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REFERENCES

Arbona JR, Salfen BE, Marple DN, Mulvaney DR, Kuhlers DL, Sartin JL, et al., 1990. Effects of age, sex and selection for growth on growth hormone secretion by individually dispersed porcine anterior pituitary cells. J Anim Sci 68 (Suppl. 1): 302 (Abstr.).

Baskin LC, Pomp D, 1997. Restriction fragment length polymorphism in amplification products of the porcine growth hormone-releasing hormone gene. J Anim Sci 75: 2285.

Brunsch C, Sternstein I, Reinecke P, Bieniek J, 2002. Analysis of associations of PIT1 genotypes with growth, meat quality and carcass composition traits in pigs. J Appl Genet 43: 85–91.

Clutter AC, Brascamp EW, 1998. Genetics of performance traits. In: Rothschild MF, Ruvinsky A, eds. The Genetics of the Pig. Cab International Wallingford UK: 427–462.

Cogan JD, Phillips JA, 1998. Growth disorders caused by genetic defects in the growth hormone pathway. Adv Pediatr 45: 337–361.

De Feo P, Perriello G, Trolone E, Ventura MM, Santeusanio F, Brunetti P, et al., 1989. Demonstration of a role for growth hormone in glucose counterregulation. Am J Physiol 256: E835–E843.

De Vries AG, Sosnicki A, Garnier JP, Plastow GS, 1998. The role of major genes and DNA technology in selection for meat quality in pigs. Meat Sci 49: S245–S255.

Etherton TD, 2000. The biology of somatotropin in adipose tissue growth and nutrient partitioning. J Nutr 130: 2623–2625.

Farmer C, Pommier SA, Brazeau P, 1993. Validation of a culture system for porcine pituitary cells: effects of growth hormone-releasing factor and(or) somatostatin on growth hormone secretion. J Anim Sci 71: 923–929.

Franco MM, Antunes RC, Oliveira KM, Pereira CD, Biase FH, Nunes FMF, Goulart LR, 2005. Association of a PIT1 gene polymorphism with growth hormone mRNA levels in pig pituitary glands. Genet Mol Biol 28: in press.

Fujii J, Otsu K, Zorzato F, De Leon S, Khanna VK, Weiler JE, et al., 1991. Identification of a mutation in porcine ryanodine receptor associated with malignant hyperthermia. Science 253: 448–451.

Geldermann H, Muller E, Beeckmann P, Knorr C, Yue G, Moser G, 1996. Mapping of quantitative

trait loci by means of marker genes in F_2 generations of wild boar, Pietrain and Meishan pigs. J Anim Breed Genet 113:381-387.

Chung HO, Kato T, Tomizawa K, Kato Y, 1998. Molecular cloning of pit-1 cDNA from porcine anterior pituitary and its involvement in pituitary stimulation by growth hormone-releasing factor. Exp Clin Endocrinol Diabetes 106(3): 203–210.

Knorr C, Moser G, Muller E, Geldermann H, 1997. Associations of GH gene variants with performance traits in F_2 generations of European wild boar, Pietrain and Meishan pigs. Anim Genet 28: 124–128.

Larsen NJ, Nielsen VH, 1993. Apa I and Cfo I polymorphisms in the porcine growth hormone gene. Anim Genet 24: 71.

Magri KA, Adamo M, Leroith D, Etherton TD, 1990. The inhibition of insulin action and glucose metabolism by porcine growth hormone in porcine adipocytes is not the result of any decrease in insulin binding or receptor kinase activity. Biochem J 266: 107–113.

Moody DE, Pomp D, Barendse W, 1995. Restriction fragment length polymorphism in amplification products of the bovine growth hormone-releasing hormone gene. J Anim Sci 73: 3789.

Nielsen VH, Larsen NJ, 1991. Restriction fragment length polymorphisms at the growth hormone gene in pigs. Anim Genet 22: 291–294.

Rehfeldt C, Nurnberg K, Ender K, 1994. Effects of exogenous porcine somatotropin on the development of fat cells and fatty acid composition in backfat of live finishing pigs. Meat Sci 36: 321–331.

SAS. SAS User' s Guide: Statistical Analysis Systems. SAS Institute, Inc, Cary, NC, 1992.

Stancekova K, Vasicek D, Peskovicova D, Bulla J, Kubek A, 1999. Effect of genetic variability of the porcine pituitary-specific transcription factor (PIT1) on carcass traits in pigs. Anim Genet 30: 313–315.

Sun HS, Anderson LL, Yu TP, Kim KS, Klindt J, Tuggle CK, 2002. Neonatal Meishan pigs show POU1F1 genotype effects on plasma GH and PRL concentration. Anim Reprod Sci 69: 223–237.

Te Pas MFW, Freriksen JWM, Van Bijnen AJHM, Gerritsen CLM, Van Den Bosch TJ, Harders FH, et al., 2001. Selection for growth rate or against back fat thickness in pigs is associated with changes in growth hormone axis plasma protein concentration and mRNA level. Domest Anim Endocrinol 20: 165–184.

Tuggle CK, Trenkle A, 1996. Control of growth hormone synthesis. Domest Anim Endocrinol 13: 1–33.

Whittemore CT, 1986. An approach to pig growth modeling. J Anim Sci 63: 615–621.

Yu TP, Schmitz CB, Rothschild MF, Tuggle CK, 1994. Expression pattern, genomic cloning and RFLP analyses of the swine PIT1 gene. Anim Genet 25: 229–233.

Yu TP, Tuggle CK, Schmitz CB, Rothschild MF, 1995. Association of PIT 1 polymorphisms with growth and carcass traits in pigs. J Anim Sci 73: 1282–1288.

Yu TP, Wang L, Tuggle CK, Rothschild MF, 1999. Mapping genes for fatness and growth on pig chromosome 13: a search in the region close to the pig PIT1 gene. J Anim Breed Genet 116: 269–280.