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Association among Ryanodyn Receptor and Insulin-Like Growth Factor Genes with Production Traits in a Commercial Type Swine Population from Mexico

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Abstract: Due to consumer preferences, pork breeders are focusing to the production of leaner pigs, as well as towards to higher final weight of carcasses. Linkage analysis studies have been conducted in order to analyze associations among genetic variants of genes such as Ryanodyn Receptor (RYR-1 or porcine stress syndrome) and Insulin-like Growth Factor (IGF-2). These associations can be useful for the breeding of swine population with improved fat and weight traits. In the present study, the following production traits were analyzed in 190 F1 (filial generation), obtained from 30 P1 (paternal generation) pigs: number of animals born, weight at birth, number of dead animals at lactation, weaning weight, post weaning weight and final weight. Furthermore, carcass traits as hot-carcass weight, dorsal fat thickness and carcass yield were studied. Genotypic frequencies of RYR-1 gene were: NN (dominant homozygous, normal), 0.75; Nn (heterozygous, normal carrier), 0,24; nn (recessive homozygous, affected), 0.01. Allelic frequencies for RYR-1 were: N, 0.87; n, 0.13. For the IGF-2 gene, six different alleles were found based in their size in base pairs, with the following allele frequencies: 232 (0.06), 236 (0.03), 238 (0.6), 244 (0.07), 246 (0.01) and 248 (0.23), conforming 12 different genotypes. The highest frequencies were for genotypes 238/238 (0.35) and 238/248 (0.28). Analysis of variance of the results showed no association among the productive and carcass traits analyzed with the genotypes of the genes studies, except for weaning weight for RYR-1 ($p<0.01$), as well as for birth weight and weaning weight for IGF-2 ($p<0.05$).

Key words: Ryanodin receptor, insuline-like growth factor, production traits, swine, Mexico

INTRODUCTION

The production of pork meat has always been directed to higher weight gains and starting some years ago, it has also been focused on leaner carcasses due to consumer preferences. Therefore, research has been conducted to find association among different Quantitative Trait Loci (QTL's) and these production characteristics. On the other hand, evidence for the association among higher carcass yield, as well as leaner meat and Porcine Stress Syndrome (PSS) has been reported (Cechová *et al*., 2007). PSS is an autosomic recessive genetic disease originated by the mutation of the Ryanodyn Receptor gene (RYR-1), causing the pale-soft-exudative type meat (MacLennan *et al*., 1990, Fujii *et al*., 1991; Otsu *et al*., 1991; MacLennan, 1992). This type of meat considerably reduces meat quality and when exposed to stress, such as during transportation, the affected animals can die with the corresponding economic loss (Leach *et al*., 1996, Laville *et al*., 2009). PSS is due to a point mutation (C/T) at nucleotide 1843, causing an arginine for cisteine substitution at position 615 of the calcium release channel of muscle fiber cells. Because the mutation eliminates a restriction site for the enzyme Hin PI and creates one for Hgi AI, a Polymerase Chain Reaction restriction Fragment Length Polymorphism (PCR-RFLP) test has been devised for the determination of the genotypes of PSS (NN, homozygous dominant, normal; Nn, heterozygous, carrier; nn, homozygous recessive, affected) (Fujii *et al*., 1991; Otsu *et al*., 1991; Lee *et al*., 2002). PSS frequency is higher in lean, muscular porcine breeds, such as Pietrain (97%), Poland China (80%), Landrace (37%) and Duroc ((22%) (Rempel *et al*., 1993). Although in the past, it was common to use PSS affected animals for breeding, currently this method is discouraged and the use of the PCR-RFLP molecular test to identify and eliminate the affected animals is recommended, causing the PSS frequency to

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drop in commercial pig populations, ranging from 2.7% in countries such as the U.S.A. (Ritter *et al*., 2008) to 29.9 in Mexico (Riojas-Valdés *et al*., 2005).

A microsatellite QTL called SWC9 of the insulin-like growth factor 2 (IGF-2) has been correlated with muscular mass and fat deposition, as well as with the lean content of ham. This microsatellite is located in the 3 untranslated region (UTR 3) of IGF-2 and is very polymorphic (8 alleles), from 241-260 base pairs (bp) (Nezer *et al*., 1999, Jeon *et al*., 1999). Other studies have found correlations among QTL's and meat quality traits (Van Laere *et al*., 2003; Nezer *et al*., 2003; Markijung *et al*., 2008).

The objective of this research was to determine if there is an association among the allelic variants of RYR-1 and IGF-2 genes with the production traits meat yield and dorsal fat by analyzing segregation of alleles in a commercial-type swine population.

MATERIALS AND METHODS

A total of 220 animals were included in the study, 30 parents (4 males and 26 females) and 190 F1. DNA was extracted by the standard phenol-chloroform technique. For the RYR-1 gene, PCR protocol was based in MacLennan (1992). The PCR product expected was a 659 bp fragment of exon 17 from RYR-1 gene. Primer pairs were: RYR-1 forward, 5'-TTC AGT TTG CCA CAG GTC CTA CCA-3'; RYR-1 reverse, 5'-ATT CAC CGG AGT GGA GTC TCT GAG-3'.

PCR conditions were: initial denaturing at 94°C for 3 min, 34 cycles of denaturing at 94°C, 1 min, annealing at 53°C for 2 min, extension at 72°C, 3 min and a final extension at 72°C for 5 min. A 2.5 μL sample of genomic DNA diluted at 20 ng μL^{-1} was added to the reaction mix composed of 2.5 μL of PCR buffer (1X), 1 μL of dNTP's (0.2 mM), 0.5 μL of MgCl (1 mM), 0.5 μL of each primer (0.2 μM), 1.25 U DNA Taq polymerase and ultra pure water for a final volume of 25 μL reaction were carried out in a heated-lid thermocycler (M.J. research PTC-100 R.T.) and the products of amplification were visualized by agarose gel electrophoresis, always using a negative control and a molecular weight marker. RFLP analysis was performed under the following conditions: 10 U μL^{-1} restriction enzyme Bsi HKA 1, 10x buffer, 10x BSA, 7.2 μL PCR product and 8 μL ultrapure water.

This mix was incubated at 37°C for 4 h and the products were visualized in high resolution agarose gel electrophoresis (2%), always using molecular weight marker Bulk No. VI. Results were documented in a photo documenter (Fluor-S, Biorad). The expected PCR-RFLP products were: dominant homozygous (NN), two fragments of 524 and 135 bp; heterozygous (Nn), four fragments of 524, 358, 166 and 135 bp; recessive homozygous (nn), three fragments of 358, 166 and 136 bp (MacLennan, 1992).

For the IGF-2 gene, amplification of a microsatellite sequence located at 3' UTR of the gene was performed according to Jeon *et al*. (1999), using the following PCR primers: IGF2 forward, 5-GTT TCT CCT GTA CCC ACA CGC ATC CC-3; IGF-2 reverse, 5'- CTA CAT AGC TGG GCT CAG GG -3'.

The 5 end of reverse primer was labeled with FAM. PCR conditions were as follows: an initial denaturing step at 94°C for 10 min, 32 cycles of denaturing at 94°C for 15 sec, annealing al 56°C for 30 sec, extension at 72°C for 1 min and a final extension at 72°C for 30 min. Reaction mix was composed of 1 X buffer, 1 mM MgCl, 0.2 mM dNTP's, 0.2 each primer, 0.5 U polymerase Taq and 18.2 μL ultrapure water for a final volume of 25 μL. PCR product was genotyped with a ABI Prism 373 DNA sequencer (Perkin Elmer). There are six alleles known, the shorter of 241 bp and the longest of 258 bp

RESULTS

Genotype frequencies were 0.75 for NN (165 animals, 3 males, 18 females and 144 F1); 0.24 for Nn (52 animals, 1 male, 9 females and 43 F1); 0.01 for nn (3 F1 animals). Allelic frequency was N = 0.87 and n = 0.13. Twelve different genotypes were observed, which included 6 different alleles.

The higher frequency was for genotypes 238/238 (78 animals) and 238/248 (61 animals). Allele sizes were 232, 236, 238, 244 246 and 248 bp The alleles with the higher frequencies were 238 (0.6) and 248 (0.23), whereas the one with the lowest frequency was 246 (0.01).

It was observed that animals with the genotype nn for RYR-1 had the highest birth weight (1.727 kg) but the lowest post weaning weight (5.5 kg) and dorsal fat (15 mm). Individuals heterozygous for RYR-1 had the highest post weaning weight (6.4 kg) and dorsal fat (18 mm).

Wit regard to genotypes for IGF-2 in F1 animals, individuals with genotypes 244/248 and 248/248 had the lowest birth weight (1.88 kg), whereas the highest were for genotypes 238/246 (1.727 kg) and 238/244 (1.653 kg). Highest post weaning weight were for genotypes 238/248 (5.993 kg) and 238/244 (6.443 kg) and the lowest was for genotype 244/248 (4.36 kg) (Table 1). Highest final

Table 1: Genotype and allelic frequencies for IGF-2

Genotypes IGF-2	Number of animals	Genotype frequencies
232/238	13	0.06
232/244	4	0.02
232/248	11	0.05
236/238	12	0.05
236/248	1	0.004
238/238	78	0.35
238/244	17	0.08
238/246	5	0.022
244/248	9	0.04
246/248	1	0.004
248/248	8	0.04
Alleles IGF-2	Number of observations	Allelic frequency
232	28	0.06
236	13	0.03
238	264	0.6
244	30	0.07
246	6	0.01
248	99	0.23

Table 2: Significance values for correlation among productive parameters and RYR-1 and IGF-2 genotypes

Parameters	RYR-1	IGF-2
Birth weight	0.3093	0.000**
Weaning weigth	0.0110**	0.0420*
Post-weaning weigth	0.4551	0.1334
End of development weigth	0.8922	0.9880
End of weaning weigth	0.4147	0.2630
Hot carcass weigth	0.5198	0.2917
Dorsal fat (mm)	0.1289	0.1858

*p<0.05, **p<0.01

weights were for genotypes 232/244 (118.0 kg), 238/244 (108.29 kg) and 238/246 (107.6 kg), whereas the lowest was for genotype 244/248 (54.8 kg). According to dorsal fat, individuals with genotypes 232/244 had the highest values (22 mm), followed by genotypes 238/248 (18 mm), 238/238 and 238/248 (16 mm). Lowest dorsal fat values were for genotypes 236/238, 244/248 and 248/248. The last two genotypes had also the lowest weights at all stages of development but carcass yield was similar to the other genotypes (Table 1). Average, lowest and highest weights values, standard deviation and degree of significance in a variance statistical analysis (SPSS version 12 program) are shown in Table 2. Only weaning weight ($p<0.01$) and birth weight for RYR-1, as well as weaning weight ($p<0.05$) for IGF-2 had significant differences.

DISCUSSION

The results (25% of animals with the mutant allele) agree with a previous study of RYR-1 gene frequency in Nuevo Leon, México (Riojas-Valdés *et al*., 2005), in which the mutant allele was found in 29.9% of the animals sampled but are higher than the results reported in other studies (ranging from 2.7-12.2%) (Ritter *et al*., 2008). A report with individuals of Pietrain and Large White found a much lower frequency (4.14%) as compared with the cross breed animals sampled in the present study, used for production (Lee *et al*., 2002).

At the same time, gene and allele frequencies were very similar in both studies in the state of Nuevo León, which validates these frequencies in farms located near the Monterrey city metropolitan area. On the other hand, previous studies indicating a relationship among low levels of dorsal fat and the mutant allele (n) were confirmed in this study, since both the heterozygous and the recessive homozygote genotypes had the lowest levels (Leach *et al*., 1996). With regard to weight gain, the results agree with reports that indicate that the better values were found in the heterozygous (Cechová *et al*., 2007).

Three new alleles for the IGF-2 gene are reported (232, 236 and 238 bp). Previous studies informed of a relationship among QTL's for dorsal fat and muscular growth with IGF-2 alleles, which agree with the findings of such a relationship among IGF-2 genotype and highest weaning weights (Nezer *et al*., 1999; Jeon *et al*., 1999; DeKoning *et al*., 2000; Stinckens *et al*., 2009).

CONCLUSION

These results confirm the presence of the mutant allele (n), which causes the porcine stress syndrome and deaths in swine populations from Mexico. With regards to the alleles of IGF-2 gene, it can be concluded that selection for bigger muscular mass and leaner carcasses is not straightforward, although the biggest final weights were found among animals with genotype 232/244 but with high values of dorsal fat.

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