Investigation of Polymorphisms in MC4R and GPX5 Genes in Greek Pigs

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Abstract. Recent studies have reported that gene polymorphisms in melanocortin 4 receptor (MC4R) and glutathione peroxidase 5 (GPX5) genes are associated with litter size in pig and can be used as genetic markers in gene assisted selection programs for the improvement of reproductive performance. The objective of this study was to investigate the existence of these polymorphisms in pigs raised in Greece. One hundred pigs raised in Greece were included in the study. DNA was extracted and genotyping was performed using RFLP- PCR. For MC4R, genotype GG had a frequency of 0.25, GA 0.55 and AA 0.20, while the frequency of allele G was 0.52 and of A 0.48. For GPX5, genotype AA had a frequency of 0.22, AB 0.48 and BB 0.30, with frequencies of alleles A and B being 0.46 and 0.54, respectively. The molecular results indicated that all genotypes of the two genes were present in the investigated population.

Keywords: gene polymorphisms, pig, litter size, reproduction

1 Introduction

One of the most important economically traits in pig production is reproductive rate, and especially litter size, as an increase in the number of pigs weaned per sow will increase economic returns for pig producers with minimal additional inputs (Rothschild, 1996). Therefore, the genetical improvement of litter size in swine is of expanding interest for pig producers. However, most selection programmes, are almost only based on phenotypical traits, which are laborious, expensive and especially in pig production time consuming.

During the last years, advances in molecular techniques can now be used to increase rate of response to selection and marker assisted selection (MAS), employed in conjunction with traditional selection methods, has been in progress to increase

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litter size in swine. It has been proposed that candidate gene analyses can be used to identify individual genes responsible for traits of economic importance (Rothschild and Soller, 1997).

The glutathione peroxidase 5 gene (GPX5) on SSC7 is located in a chromosomal region in which several quantitative trait loci (QTL) for reproductive traits in swine, such as uterine capacity, ovulation rate and litter size have been detected. Linkage analyses of GPX5 showed that this gene is closely linked to the major histocompatibility complex (MHC), which has been suggested to have an effect on reproductive traits in swine (Vaiman et al. 1998; Buske et al. 2005).

The melanocortin 4 receptor (MC4R) gene codes for a G-protein-coupled receptor and it was demonstrated to be important in the control of energy balance in humans and rodents. Energy balance is maintained by controlling energy intake, i.e. feed intake, and energy expenditure by physical activity and metabolism. The response of the melanocortin 4 receptor to leptin signalling can thus be considered as a link between feed intake and body weight and body composition (Marsh et al. 1999; Wikberg et al. 2000). Consequently, MC4R is a strong candidate for growth and body composition in pigs, as well as reproductive performance.

Therefore, the aim of the present study was to establish GPX5 and MC4R genes allele and genotypes frequencies, in a Greek swine population.

2 Materials and Methods

Population description

The study was conducted in a large commercial pig farm in Greece. One hundred pigs were included in the study.

Genotyping procedure

DNA was extracted from whole blood samples using the NucleoSpin Blood kit (Macherey-Nagel, Germany) according to manufacturer's instructions. The integrity of the DNA samples was examined by electrophoresis through a 1.5% agarose gel. For genotyping of the MC4R gene a 483 bp fragment was amplified by PCR using the primer pair: 5′-ACAGTTAAGCGGGTTGGAAT-3′ and 5′-CAGGGGATAGCAACAGATGA-3′. PCR amplification was performed using approximately 300ng of genomic DNA as template, 200 nM primers each, 1 mM dNTPs and 1 unit Taq DNA Polymerase Recombinant in 25 μl total volume reaction. PCR conditions were 94°C for 3 min, 35 cycles of 94°C for 30 sec, 56°C for 30 sec, 72°C for 30 sec and a final extension period at 72°C for 10 min. PCR products were digested using *TaqI* restriction enzyme and resolved by electrophoresis on 1.5% agarose gels, visualised with ethidium bromide and imaged under UV illumination.
For genotyping of the GPX5 gene a 501 bp fragment was amplified by PCR using the primer pair: 5′- TTCATGTAGAACTTATTTCTG -3′ and 5′-TGACTTACCCATTCTTCAG-3′. PCR amplification was performed using approximately 300ng of genomic DNA as template, 200 nM primers each, 1 mM

dNTPs and 1 unit Taq DNA Polymerase Recombinant in 25 µl total volume reaction. PCR conditions were 94°C for 3 min, 35 cycles of 94°C for 30 sec, 51°C for 30 sec, 72°C for 30 sec and a final extension period at 72°C for 10 min. PCR products were digested using *Hinfl*, restriction enzyme and resolved by electrophoresis on 1.5% agarose gels, visualised with ethidium bromide and imaged under UV illumination.

Once genotypes were determined, allelic frequencies at each gene locus were calculated by gene counting. Deviations from Hardy-Weinberg equilibrium were examined for each locus using chi-squared tests.

3 Results

As illustrated in Figures 1 and 2 (representative of some samples), PCR amplification of the MC4R and GPX5 genes was successful, using the conditions detailed in materials and methods. Furthermore, RFLP-PCR analysis performed in the PCR products, using the restriction enzymes for each SNP, as described in materials and methods, revealed the genotype of each animal, for each gene locus (Figures 3 and 4, representative of some animals, for each gene).

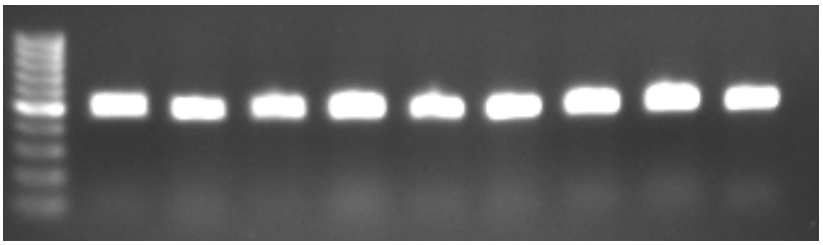

Fig. 1. PCR amplification of the MC4R gene locus.

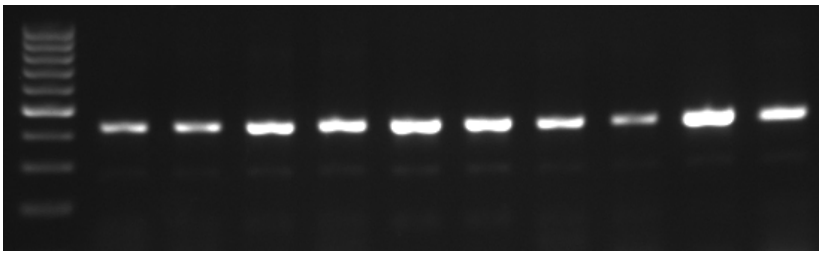

Fig. 2. PCR amplification of the GPX5 gene locus.

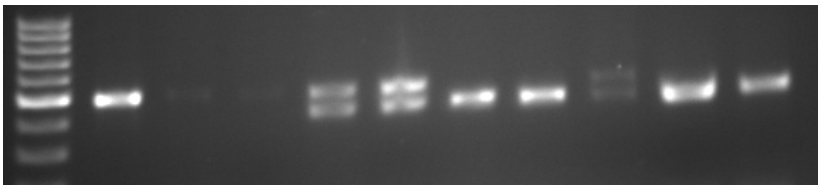

Fig. 3. RFLP-PCR analysis for the MC4R gene polymorphism. PCR products were digested with *TaqI*.

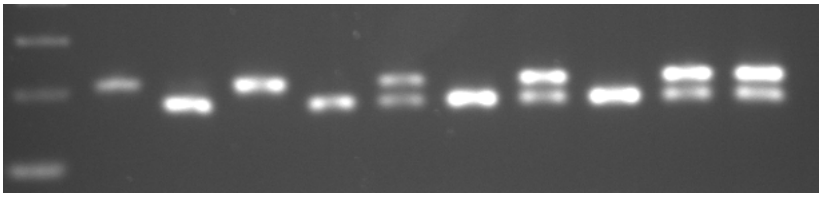

Fig. 4. RFLP-PCR analysis for the GPX5 gene polymorphism. PCR products were digested with *Hinfl*.

Genotypic and allelic frequencies estimated for the 100 examined pigs, for the two gene loci are presented in Table 1.

Table 1. Genotypic and allelic frequencies (%) in the two studied gene loci

Gene Locus	Allele		Genotypic Frequency (%)			Allelic frequency (%)	
	0	+	00	0+	++	0	+
MC4R	G	A	0.25	0.55	0.20	0.52	0.48
GPX5	A	B	0.22	0.48	0.30	0.46	0.54

As illustrated in Table 1, for MC4R, genotype GG had a frequency of 0.25, GA 0.55 and AA 0.20, while the frequency of allele G was 0.52 and of A 0.48. For GPX5, genotype AA had a frequency of 0.22, AB 0.48 and BB 0.30, with frequencies of alleles A and B being 0.46 and 0.54, respectively. Both MC4R and GPX5 gene loci were found to be in Hardy-Weinberg equilibrium in the studied population.

4 Discussion

In the present study we investigated the genotypes of the MC4R and GPX5 gene polymorphisms in pigs raised in Greece. Since these two single nucleotide polymorphisms have been reportedly associated with reproductive traits such as litter size it was considered important to determine whether the desired genotypes were present in Greek pigs.

Allelic frequencies of the two investigated gene locus estimated in the present study were similar to those reported by previous studies (Buske et al. 2006; Jokubka et al. 2005). This suggests that the desired genotypes are present in Greek pigs, and would be very interesting to perform association studies between these SNPs and reproductive parameters. Therefore, further work is needed in order to confirm these associations in pigs raised in Greece. Once confirmed, these SNPs could be incorporated in a larger panel of markers to assist breeders in selecting pigs with improved reproductive traits, such as litter size.

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