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Determination of PPARGC1 Cys430Ser polymorphism and MHS genotype in Croatian autochthonous pig breeds

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ABSTRACT

The aim of the research was to determine PPARGC1 and MHS genetic status of two Croatian autochthonous pig breeds. The research was carried out on 20 Black Slavonian pigs and 20 Turopolje pigs. Genomic DNA was extracted from the blood. PCR was performed and PCR products genotyped by Restriction Fragment Length Polymorphism (RFLP). Allele frequency for PPRGC1 gene was determined by the Hardy-Weinberg equilibrium principle. Research results showed that A allele frequency in Black Slavonian pig was 44.44%, and of the T allele 55.56%. Homozygous genotypes (Ser/Ser) were dominant in Turopolje pig breeds (A=100.00%). Determined MHS status proved both pig breeds to be completely MHS resistant. Our results point out the necessity to further investigate genetic status of both pig breeds in order to improve constitution and quality traits of muscle tissue in pure pig breeds.
(Keywords: PPARGC1, MHS, Black Slavonian pig, Turopolje pig, allele frequency)

INTRODUCTION

Croatia is a home of two autochthonous pig breeds, of Black Slavonian pig and Turopolje pig. The main characteristics of this two breeds are late maturity and high fat content. They were of importance for local economy in the past. However, as the production focused on lean pig breeds, these two breeds gradually lost on their importance to become endangered and consequently included in the Endangered Breeds Protection Program of Central and Eastern Europe. Previous researches on these pig breeds focused primarily on determination of their productivity and slaughtering traits, the results of which pointed out excellent quality of muscle tissue of both Black Slavonian (*Kralik et al*., 1988; *Petričević et al*., 1988) and Turopolje breed (*Đikić et al*., 2002). It is expected that in the near future the two pig breeds will gain on their relevance in the improvement of constitution and quality of muscle tissue in pure pig breeds.

Intensive selection of lean pig breeds resulted in poor muscle tissue quality traits of some breeds. Scientific journals published numerous candidate genes for production traits or just molecular markers related to phenotypic diversity (*Houde, et al*., 2001). Peroxime Proliferator-Activated Receptor-Gamma Coactivator-1 (PPArGC1) and Malignant Hyperthermia Syndrome (MHS) genes are candidate genes for fattening and quality traits of muscle tissue. Recently, a few studies was carried out on PPARGC1 gene in pigs, because of its central role in adypocite differentiation (*Lowel* and *Spiegelman*, 2000) and impact of fat deposition and distribution in pig's body. It is also plays an important role as a transcriptional coactivator and metabolism regulation

(*Puigserver* and *Spiegelman*, 2003). *Kunej et al*. (2005) determined differences between Chinese and Western pig breeds in the T allele frequency (a T/A substitution at position 1378 in the PPARGC1 gene causing a Cys430Ser amino acid substitution). The conclusion was made that these differences could be connected with differences in fat and muscle tissue deposition and distribution between Western and Chinese pig breeds. Further studies on PPARGC1 gene are needed for better understanding of its role and function in order to determine its effect on fattening and slaughtering traits of lean pig breeds.

MHS gene (RYR1 gene) affects stress susceptibility in pigs mutation of that gene occurs at the nucleotide position 1843, resulting in cytosine/thymine substitution (*Fuji et al*., 1991). Mutation of that gene is common in lean pig populations and causes disturbance of muscle tissue quality. Despite of its negative effects, it is still one of the most important candidate genes in determination of slaughtering traits of carcasses and quality of muscle tissue. Many authors found out that MHS-carriers (recessive homozygotes) had more muscle tissue in carcass (*Aalhus et al*., 1991; *Pommier et al*., 1992; *Rosner et al*., 2003), but these traits were clearly related to stress susceptibility of pigs causing poor quality of meat (*Denborough*, 1998; *Rűbensam*, 2000; *Houde et al*., 2001). Relation among MHS genotypes and good quality traits of carcasses can be important in improvement of those breeds which traits were disturbed during the course of intensive selection. The aim of this research was to determine PPARGC1 and MHS status of Black Slavonian and Turopolje pig, and to elaborate their possible exploitation in improvement of production and slaughtering traits of lean pig breeds.

MATERIALS AND METHODS

The research was carried out on 20 Black Slavonian and 20 Turopolje pigs. Blood samples were collected on family farms in Eastern Croatia and in the area of Turopolje countryside. Genomic DNA was extracted from blood samples using standard phenol-chloroform-isoamyl alcohol (25:24:21) extraction protocol (*Ausubel et al*., 2000). Polymerase chain reaction (PCR) for PPARGC1 gene was carried out in a total volume of 20 µl containing 100 ng of genomic DNA, 1 x PCR buffer, 200 µM dNTP, 1 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Litva) and 5 pmol of each oligonucleotide. The sequences of the oligonucleotides, covering 200 bp of the exon 8 central region, were as follows: PGC1-SSCP.F (5'-TAAAGATGCCGCCTCTGACT-3') and PGC1-SSCP.R (5'-CTGCTTCGTCGTCAAAAACA-3'). PCR was performed under following conditions: initial denaturation at 95 °C for 5 minutes, followed by 31 cycles of denaturation at 95 °C (40 s), annealing at 59 °C (40 s) and extension at 72 °C (50 s) with final extension step at 72 °C for 7 min. PCR products were checked on 2% agarose gel.

Digestion of PCR products was carried out in a final volume of 20 µl containing 13 µl of PCR product, 1x reaction buffer and 1 U of *Alu*I restriction enzyme (Fermentas, Lithuania). Reactions were incubated at 37 °C for 2 h and resolved on a 3% agarose gel.

Sequencing of PCR products was performed on the ABI sequencing machine (Perkin Elmer). Sequences were aligned in ClustalW and checked for A/T polymorphism at nucleotide position 1378.

PCR for MHS gene was carried out in a total volume of 20 µl containing 100 ng of genomic DNA, 1 x PCR buffer, 200 µM dNTP, 1 mM $MgCl_2$, 1 U Taq DNA polymerase (Fermentas, Lithuania) and 10 pmol of each primer. Primer sequences were as follows: RYR1.F (5'-CTGGTGACATAGTTGATGAGGTTTG-3') and RYR1.R (5'-

GTGCTGGATGTCCTGTGTTCCCT-3'). PCR was performed according to the following protocol: initial denaturation at 95 °C for 5 min, followed by 35 cycles of denaturation at 95 °C (1 min), annealing at 56 °C (1 min) extension at 72 °C (1 min) with final extension step at 72 °C for 2 min. PCR products were checked on 1.5% agarose gel. Restriction digestion of PCR products was performed with *Hin*6I (Fermentas, Lithuania) for 2 h at 37 °C and analyzed on 2.5% agarose gel. Allele frequency for each pig breed was calculated by the Hardy-Weinberg equilibrium principle, $p^2+2pq+q^2=100$. All analyses were carried out in the genetic laboratory of the Zootechnical department, Biotechnical Faculty in Ljubljana, Slovenia.

RESULTS AND DISCUSSION

The *Figure 1* presents restriction results of PPArGC1 gene by AluI restriction enzyme on 3% agarose gel. Animals homozygous for A allele (Ser) have 4 restriction sites resulting in 5 bands (61 bp, 60 bp, 31 bp, 27 bp and 21 bp). T allele homozygotes (Cys) have lost one restriction site resulting in 4 bands (121 bp, 31 bp, 27 bp and 21 bp). In heterozygous animals (AT), restriction digestion results in 6 bands. Seqence analysis of central region of the exon 8 PPARGC1 gene in Black Slavonian and Turopolje pig breeds exhibits nucleotide polymorphism at position 1378 (*Figure 2*).

RFLP analysis of PPARGC1 gene showed presence of only AA genotype in Turopolje pig (*Table 1*). This could be consequence of high proportion of inbreeding in this population of pig. Allele frequencies of PPARGC1 gene in Turopolje breed do not correspond with results obtained by *Kunej et al*. (2005) that reported the T allele to be dominant in fatty pig breeds, and the A allele to be dominant in lean breeds. The A allele frequency of Black Slavonian pig was 44.44%, and the T allele 55.56%.

Figure 1

PCR-RFLP fragments of T1378A polymorphism in PPARGC1 gene

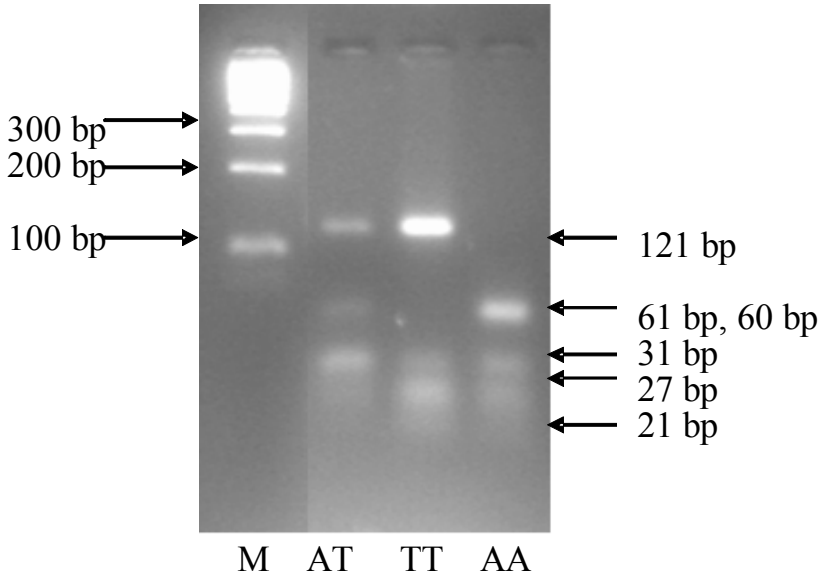


Figure 2

Nucleotide polymorphism at position 1378 in the central region of the exon 8

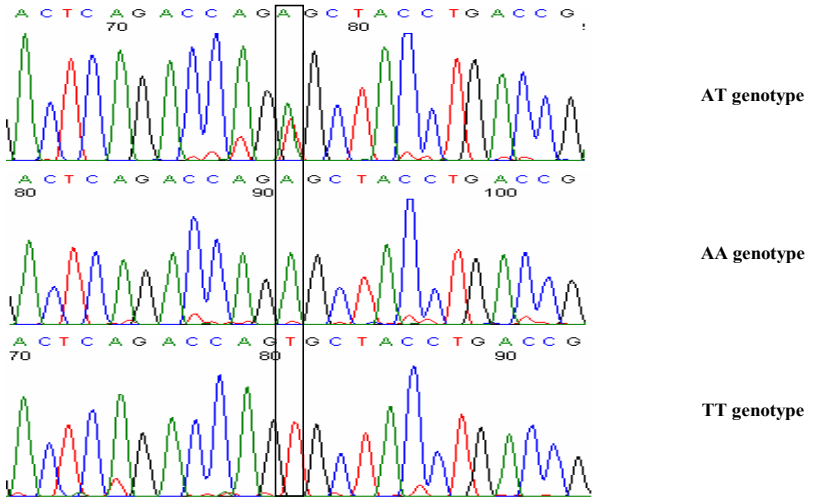


Table 1

Allele frequency of PPARGC1 gene in Black Slavonian and Turopolje pig

Pig breed	Cys430Ser genotypes				Allele frequency[2]	
	n	AA[1]	AT[1]	TT[1]	A	T
Black Slavonian	20	5	7	8	44.44	55.56
Turopolje	20	20	-	-	100.00	-

[1]Genotype AA:Ser/Ser, AT:Ser/Cys, TT:Cys/Cys.
[2]Allele frequency calculated according to Hardy-Weinberg equilibrium principle.

Figure 3

PCR-RFLP fragments of PPARGC1 gene in Turopolje breed

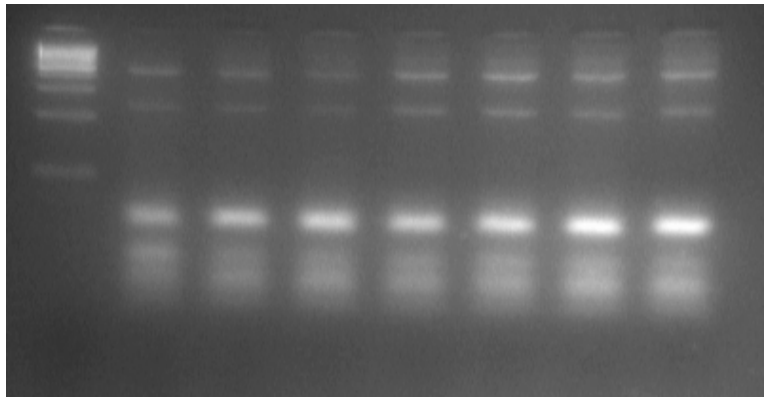
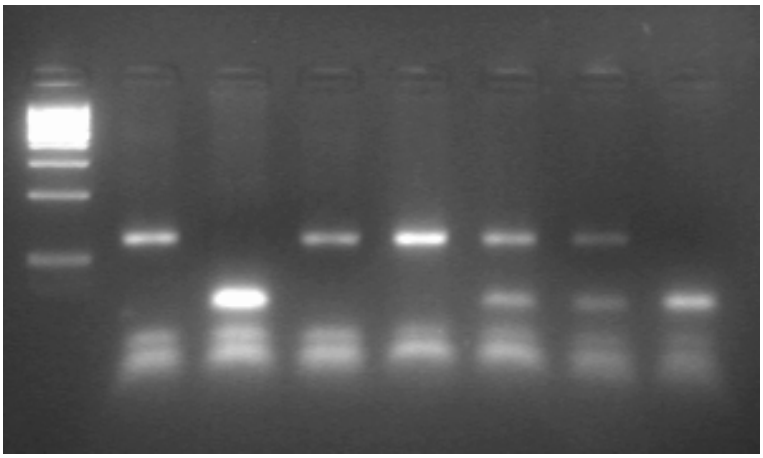


Figure 4

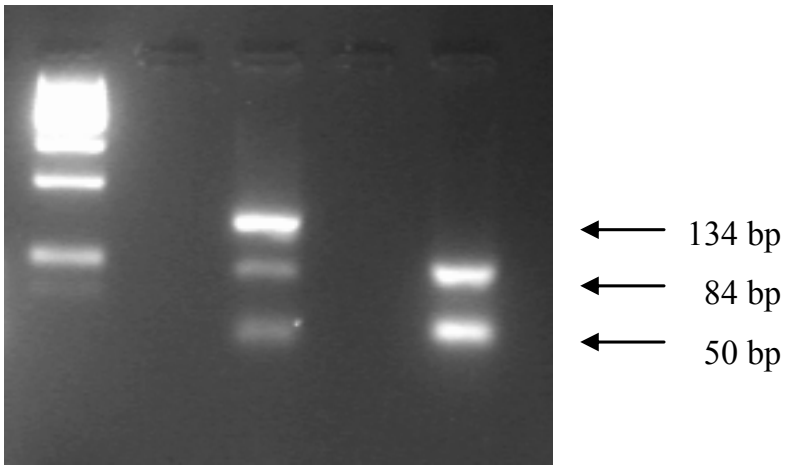
PCR-RFLP fragments of PPARGC1 gene in Black Slavonian breed



Restriction digestion of the MHS gene with Hin6I restriction enzyme results in two bands (84 bp and 50 bp) in homozygous animals. Recessive homozygotes exhibit only one band (134 bp), while in heterozygotes, digestion results in 3 bands on gel (134 bp, 84 bp and 50 bp). Restriction results in this research proved that pigs of Black Slavonian and Turopolje breeds were completely MHS resistant (*Figure 3*). Very good quality traits of meat, as well as the fact that pigs of Black Slavonian and Turopolje breeds are MHS resistant, points out the need to carry out more detailed research into genetic status of these breeds in order to determine their role in improvement of constitution and quality of muscle tissue in pure pig breeds in the near future.

Figure 5

PCR-RFLP fragments in MHS gene



CONCLUSION

Restriction analysis of PPARGC1 gene showed that homozygous genotypes (Ser/Ser) were dominant in Turopolje pig breeds (A=100.00%). The A allele frequency of Black Slavonian pig is 44.44%, and the T allele 55.56%

Determined MHS status of Black Slavonian and Turopolje pigs proved their complete resistance to stress, which points out their possible importance in improvement of quality traits of muscle tissue in lean pig breeds.

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