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EFFECT OF *PPARGC-1* GENE ON BACKFAT THICKNESS IN PIGS *

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ABSTRACT

PPARGC-1 gene is assumed to be a candidate gene with a major effect on fatness and meat quality. In this study, frequency of T/A substitution at position 1378 in *PPARGC-1* gene in pigs was examined in boars of 4 different breeds. Frequency of genotypes and alleles were compared between animals with the thinnest and with the thickest backfat. Differences in genotype frequency between groups were significant in dam line of Slovenian Landrace (SL11) and sire line of Large White breed (LW66). Allele A was predominantly present in animals with thick backfat in SL11 (71.05%), LW66 (58%) and in sire line of Slovenian Landrace (67.65%). Differences in allele frequency were significant in SL11. We found a significant effect on phenotypic and breeding values for backfat in population of Large White breeds, where homozygotes TT had the thickest backfat, and heterozygotes the thinnest. In population of Slovenian Landrace breed the thickest backfat had homozygotes AA and the thinnest homozygotes TT. Similar results were obtained by analysis of gene effect on breeding values. Inconsistency could be explained by different background of allele, epistasis and intensity of selection.

Key words: pigs / backfat / genetics / *PPARGC-1* gene / polymorphism

POVEZAVA GENA *PPARGC-1* Z DEBELINO HRBTNE SLANINE †

IZVLEČEK

Gen za *PPARGC-1* je kandidatni gen z velikim učinkom na zamaščenost in kvaliteto mesa. V prispevku smo preučevali frekvence točkovnih mutacij A → T na mestu 1378 v 8. eksonu gena za *PPARGC-1* pri merjascih štirih različnih linij dveh pasem. Frekvence genotipov in alel smo primerjali med merjasci s tanjšo (skupina A) ter merjasci z debelejšo hrbtno slanino (DHS), (skupina B). Razlike v frekvencah genotipov so bile značilne med skupinama merjascev pri maternalni liniji pasme slovenska landrace (SL11) ter pri terminalni liniji pasme large white (LW66). Alela A je bila pogostejša pri živalih z debelejšo DHS pri SL11 (71,05 %), LW66 (58 %) ter pri terminalni liniji pasme slovenska landrace (67,65 %). Razlike v frekvencah alel so bile značilne v populaciji SL11. Genotip je značilno vplival na DHS v populacijah LW11 in LW66, kjer so imeli debelejšo slanino homozigoti TT in tanjšo heterozigoti. V populacijah SL11 in SL55 so imeli najdebelejšo slanino genotipi AA in najtanjšo slanino homozigoti TT.

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Nasprotujoči rezultati so lahko posledica genetskega ozadja alel, epistaze in intenzivnosti selekcije.

Ključne besede: prašiči / debelina hrbtne slanine / genetika / gen *PPARGC-1* / polimorfizem

INTRODUCTION

Although most genetic progress in pigs has been made by selection on predictor of breeding values, information, acquired from molecular genetics, can clarify some genetic factors with effect on production traits. Due to quantitative nature of production traits, the majority of genetics is oriented to identification and mapping quantitative trait locus (QTL) with effect on economically important traits. One of the most important traits is also meatiness, because in Slovenia the carcasses are still paid regarding the lean meat content.

Growth of adipose tissue is a quantitative trait regulated by many transcription factors, included in the process of adaptive thermogenesis and biosynthesis of mitochondria (Rosen *et al.*, 2000). One of them is protein *PPARGC-1* (peroxisome proliferator-activated receptor-gamma coactivator-1), which is a transcriptional coactivator of the nuclear receptor PPARγ (peroxisome proliferator-activated receptor-gamma). It is an important factor in the process of adipocyte differentiation and muscle fibre type determination (Lin *et al.*, 2002; Rosen *et al.*, 2000). *PPARGC-1* stimulates mitochondrial biogenesis and respiration in muscle cells. It induces activity of the genes included in adaptation of organism on temperature and nutritional changes (Knutti and Kralli, 2001; Puigserver *et al.*, 1998). The mechanism of adipogenesis regulation and the role of PPARγ and *PPARGC-1* were also described by Milosevic Berlic *et al.* (2004).

The porcine *PPARGC-1* gene (GenBank acc. no. AY346131) is located on chromosome 8 (Milosevic Berlic, 2002; Jacobs *et al.*, 2006). The sequencing, polymorphisms analysis and mapping of porcine *PPARGC-1* gene were reported by Jacobs *et al.* (2006). Substitution T/A at nucleotide position 1378 causes amino acid substitution (Cys→Ser) at position 430. According to Jacobs *et al.* (2006) Cys/Ser substitution could have major effects because of influences on disulfide bridges that might be present in the protein.

Frequency distribution of mutation A1378T in *PPARGC-1* was studied in Chinese and Western pig breeds because of remarkable differences in fatness (Kunej *et al.*, 2005). Allele T was associated with fat type Chinese pig breeds, whereas the A allele was more frequent in lean type Western pigs (Kunej *et al.*, 2005). Jacobs *et al.* (2006) studied the *PPARGC-1* gene effect on performance in Meishan x White Composite resource population and the study did not reveal the effect of A1378T on performance. They found a significant correlation between SNP in exon 9 (A1747C) and leaf fat weight, backfat, and weight of the belly. Several QTLs with effect on backfat, located on chromosome 8 were reported (Bidanel and Rothschild, 2002; Rohrer, 2000; Bidanel *et al.*, 2001). Some of these regions correspond to the location of *PPARGC-1*. *PPARGC-1* is assumed to be a candidate gene with a major effect on backfat and other characteristic associated with fatness. This gene can be also considered as a candidate gene for meat quality (Knutti and Kralli, 2001).

The purpose of this study was to find the frequency of mutation of *PPARGC-1* gene in boars of different phenotype for backfat. The effect of *PPARGC-1* gene on phenotypic and breeding values was analyzed using a linear model.

MATERIAL AND METHODS

Data Preparation

In this study, boars of dam and sire line of Slovenian Landrace (SL11 and SL55) and Large White (LW22 and LW66) breed were included. Boars were selected at intermediate selection stage in the year 2003 in the performance test and finished the test at 100 kg. Animals were divided in two groups based on backfat thickness (Table 1). Animals of each breed with the thinnest backfat were assigned to group A and those with the thickest backfat to group B. The total number of genotyped animals was 166. The differences between groups are evident (Table 1).

Table 1. Statistics for backfat in group A and B
Preglednica 1. Osnovna statistika za debelino hrbtne slanine v skupinah A in B

	A				B			
Breed Pasma	n	$\bar{x}$	min	max	n	$\bar{x}$	min	max
LW22	25	7.97	6.67	8.67	21	12.48	11.33	15.00
LW66	19	7.51	6.33	8.67	25	10.29	9.33	12.67
SL11	22	7.55	6.00	8.00	19	13.26	12.33	16.67
SL55	18	8.63	7.67	9.33	17	15.86	14.67	17.67

n – number / število; $\bar{x}$ – mean / povprečje; min – minimum / najmanj; max – maximum / največ;

PCR-RFLP analysis

Genomic DNA was isolated from skin tissue samples by standard phenol-chloroform-isoamyl alcohol (25:24:1) extraction (Ausubel *et al.*, 2000). The 200-bp fragment of *PPARGC-1* gene was amplified with PCR reaction, which was carried out in a volume of 10 µl: 2 µl of template DNA, 1 x PCR buffer (Fermentas, Vilnius, Lithuania), 1mM $MgCl_2$, 200 µM dNTP, 0.5 U *Taq* DNA polymerase (Fermentas, Vilnius, Lithuania) and 5 pmol of each primer. The primers used for amplification of target sequences were:

PPARGC-1 – SSCP.F (5'- TAAAGATGCCGCCTCTGACT - 3')
PPARGC-1 – SSCP.R (5'- CTGCTTCGTCGTCAAAAACA - 3').

The following amplification parameters were applied: 95 °C for 5 min followed by 31 cycles: 95 °C for 1 min, 56 °C for 1 min and 72 °C for 1 min. The reaction was completed by the final synthesis at 72 °C for 7 min. The PCR products were digested with 2U of *Alu*I (Fermentas, Vilnius, Lithuania) at 37 °C overnight and analyzed on 2% agarose gels.

Statistical Analysis

Frequency distribution of genotypes and alleles were calculated. The statistical package SAS was used (SAS Inst. Inc., 2001). Genotype and allele frequencies between groups were tested using the multinomial model in proc logistic (SAS Inst. Inc., 2001).

The effect of *PPARGC-1* genotype on phenotypic values was analyzed with the following statistical model:

$$y_{ijk} = \mu + G_i + S_j + b(x_{ijk} - \bar{x}) + e_{ijk} \quad [1]$$

For analysis the effect of PPARGC-1 genotype on breeding values the following statistical model was used:

$$y_{ij} = \mu + G_i + e_{ij} \quad [2]$$

where y_{ijk} is the phenotypic respectively breeding value for backfat, μ is intercept, G_i is PPARGC-1 genotype (i=1, 2, 3), S_j is the season of selection at intermediate selection stage in performance test (j=1, 2, 3, …, 11, 12), b is the regression coefficient for body weight at the end of performance test, and e_{ijk} is a residual.

RESULTS AND DISCUSSION

Following digestion of PCR product with *Alu*I, the T allele was cut into fragments of 121, 27, 31 and 21 bp (Figure 1). Allele A had one restriction site more at position 1379 and 121 bp band was cleaved into 61 and 60 bp band, consecutive the allele A had: 61, 60, 27, 31 and 21 bp bands. On agarose gels were defined: genotype AA had ≈60 and ≈30 bp, genotype TT 121 and ≈30 bp and heterozygote AT had 3 bands: 121, ≈60 and ≈30 bp.

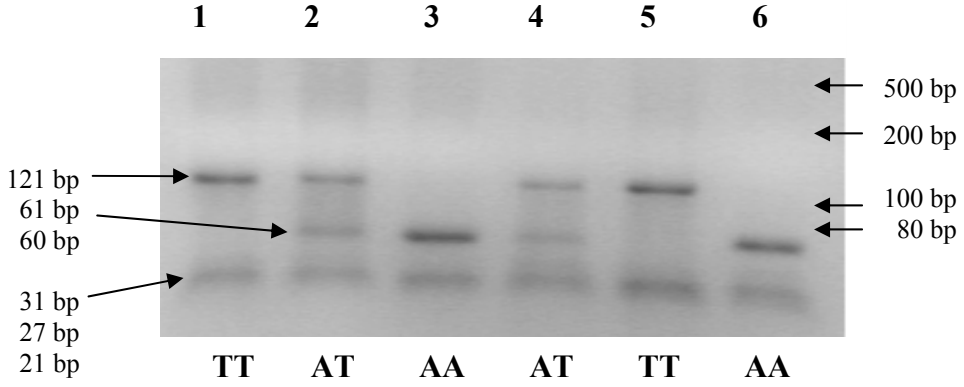

Figure 1. Restriction patterns of samples.
Slika 1. Restrikcijska analiza vzorcev.

Three genotypes were identified in the studied populations: AA, TT and AT (Table 2). The distribution of *PPARGC-1* genotypes frequencies demonstrated significant differences between group A and group B in dam line of Slovenian Landrace (SL11) and sire line of Large White breed (LW66). In the populations of SL11 the frequency of heterozygote was 54.5% in group A and 57.9% in group B. The frequency of TT was 22% in group A, whereas no genotype TT was found in group B. In populations of LW66, the difference of frequency of AT between groups was larger (78.9% in group A and 44% in group B). In group B genotype TT was present in 20%, whereas in group A in 5.3%.

Group A and B of dam line of Large White (LW22) had a similar distribution of genotypes (Table 2). In both groups genotype AA was the most frequent (80 and 81%) and the frequency of heterozygote was similar (20% and 18%). In populations of SL55 the most frequent was genotype AT (50% and 64.7%). In group A the frequency of genotype TT was 11%, whereas in group B was not present.

Table 2. Frequency of genotypes of *PPARGC-1* gene and test of differences between groups
Preglednica 2. Frekvence genotipov gena *PPARGC-1* in test razlik med skupinama

Breed Pasma	Group Skupina	Total	AA		AT		TT		Test
		n	n	frequency %	n	frequency %	n	frequency %	P-value
LW22	A	25	20	80.0	5	20.0	0	0.0	1.0000
	B	21	17	81.0	3	18.0	1	1.0	
LW66	A	19	3	15.8	15	78.9	1	5.3	0.0772
	B	25	9	36.0	11	44.0	5	20.0	
SL11	A	22	5	22.7	12	54.5	5	22.7	0.0472
	B	19	8	42.1	11	57.9	0	0.0	
SL55	A	18	7	38.9	9	50.0	2	11.0	0.8330
	B	17	6	35.3	11	64.7	0	0.0	

Table 3 Frequency of alleles of *PPARGC-1* gene and test of differences between groups
Preglednica 3 Frekvenca alelov gena *PPARGC-1* in test razlik med skupinama

Breed Pasma	Group Skupina	Total	Allele A		Allele T		Test
		n	n	frequency %	n	frequency %	P-value
LW22	A	50	45	90.00	5	10.00	0.7703
	B	42	37	88.10	5	11.90	
LW66	A	38	21	55.26	17	44.74	0.7974
	B	50	29	58.00	21	42.00	
SL11	A	44	22	50.00	22	50.00	0.0550
	B	38	27	71.05	11	28.95	
SL55	A	36	23	63.89	13	36.11	0.7407
	B	34	23	67.65	11	32.35	

Allele A was predominantly present in animals with thick backfat (group B) in SL11 (71.05%), LW66 (58%) and in 67.65% was present in SL55 (Table 3). In LW22 allele A was more frequent in animals with thin backfat. Differences in frequency of alleles were significant in SL11.

The analysis revealed significant differences in frequency distribution of genotypes and alleles of *PPARGC-1* gene between animals with different phenotype for backfat in populations

of SL11 and LW66. These results support the assumption that T/A substitution at 1378 nucleotide position in *PPARGC-1* gene influences the fatness characteristics (Kunej *et al.*, 2005).

Table 4 Least square means (LSM) and standard errors (SE) for phenotypic values for backfat for different breeds

Preglednica 4 Ocenjene srednje vrednosti (LSM) in standardne napake (SE) za debelino hrbtne slanine (v mm) po pasmah in genotipih

Breed Pasma	Trait Lastnost	Analysis Analiza	AA	AT	TT	P-value
LW22	BF-2	1	10.34 ± 0.39	8.94 ± 0.82	10.95 ± 2.54	0.3006
		2	10.30 ± 0.38	8.89 ± 0.82		0.1339
	BF-3	1	10.15 ± 0.36	8.62 ± 0.76	10.17 ± 2.35	0.2104
		2	10.11 ± 0.35	8.58 ± 0.76		0.0809
LW66	BF-2	1	10.20 ± 0.59	8.67 ± 0.34	10.40 ± 0.70	0.0248
		2	10.00 ± 0.67	8.46 ± 0.34		0.0735
	BF-3	1	9.92 ± 0.54	8.46 ± 0.32	10.13 ± 0.65	0.0184
		2	9.73 ± 0.61	8.46 ± 0.34		0.0615
SL11	BF-2	1	11.16 ± 0.83	9.92 ± 0.66	8.38 ± 1.30	0.1624
		2	11.13 ± 0.86	9.93 ± 0.68		0.2666
	BF-3	1	11.24 ± 0.84	9.91 ± 0.66	8.44 ± 1.32	0.1603
		2	11.22 ± 0.88	9.92 ± 0.69		0.2370
SL55	BF-2	1	11.30 ± 1.34	11.73 ± 1.13	7.30 ± 2.67	0.2928
		2	11.38 ± 1.41	11.70 ± 1.16		0.8754
	BF-3	1	11.56 ± 1.39	12.17 ± 1.17	7.60 ± 2.77	0.3010
		2	11.67 ± 1.46	12.12 ± 1.20		0.8281

LW22 – dam line of Large White breed; LW66 – sire line of Large White; SL11 – dam line of Slovenian Landrace; SL55 – sire line of Slovenian Landrace; BF–2 – average of two measurements of backfat; BF-3 – average of 3 measurements of backfat; Analysis 1 – least square means for different genotypes AA, AT, and TT; Analysis 2 – comparison between homozygotes AA and heterozygotes AT;
LW22 – ženska linija pasme Large White; LW66 – moška linija pasme Large White; SL11 – ženska linija slovenske landrace; SL55 – moška linija slovenske landrace; BF–2 – povprečje dveh meritev hrbtne slanine; BF-3 – povprečje treh meritev hrbtne slanine; Analiza 1 – povprečja najmanjših kvadratov za različne genotipe AA, AT in TT; Analiza 2 – primerjava homozigotov AA in heterozigotov AT;

Results of analysis of the effect of PPARGC-1 genotype are shown as the least square means (Table 4). The analysis 1 was done to compare all three genotypes (AA, AT, and TT), whereas in the analysis 2 homozygotes TT were excluded because of the low number of observations and the estimates of mean values were less reliable. In population of Large White boars the thinnest backfat had heterozygotes, what could be the consequence of non-additive effects. The thickest backfat had homozygotes TT, what was in agreement with results obtained by Kunej *et al.*

(2005). In population of Slovenian Landrace boars the homozygotes had the thinnest backfat, whereas the homozygotes AA had the thickest backfat. A significant effect of PPARGC-1 genotype was found in population of Large White boars.

The estimation of *PPARGC-1* gene effect on breeding values revealed similar results (Table 5). The heterozygotes had the smallest predicted breeding values in Large White populations but in population of Slovenian Landrace boars the best predicted breeding values had homozygotes TT. We found a significant effect of *PPARGC-1* gene on breeding values for backfat in population of sire line of Large White breed.

Table 5 Least square means (LSM) and standard errors (SE) for breeding values for backfat for different breeds and genotypes

Preglednica 5 Ocenjene srednje vrednosti (LSM) in standardne napake (SE) za plemenske vrednosti za debelino hrbtne slanine po pasmah in genotipih

Breed Pasma	Analysis Analiza	AA	AT	TT	P-value
LW22	1	–1.0987 ± 0.1586	–1.6916 ± 0.3411	0.0580 ± 0.9648	0.1374
	2	–1.0987 ± 0.1586	–1.6916 ± 0.3411		0.1223
LW66	1	0.4397 ± 0.1846	–0.1128 ± 0.1254	0.2308 ± 0.2611	0.0491
	2	0.4398 ± 0.1864	–0.1128 ± 0.1266		0.0192
SL11	1	–1.9898 ± 0.4490	–2.7588 ± 0.3376	–3.8344 ± 0.7241	0.0980
	2	–1.9898 ± 0.4742	–2.7589 ± 0.3565		0.2036
SL55	1	–0.5046 ± 0.5650	–0.2923 ± 0.4555	–3.0280 ± 1.4403	0.2097
	2	–0.5046 ± 0.5739	–0.2923 ± 0.4627		0.7752

LW22 – dam line of Large White breed; LW66 – sire line of Large White; SL11 – dam line of Slovenian Landrace; SL55 –sire line of Slovenian Landrace; Analysis 1 – least square means for different genotypes AA, AT, and TT; Analysis 2 – comparison between homozygotes AA and heterozygotes AT;
LW22 – ženska linija pasme Large White; LW66 – moška linija pasme Large White; SL11 – ženska linija slovenske landrace; SL55 – moška linija slovenske landrace; Analiza 1 – povprečja najmanjših kvadratov za različne genotipe AA, AT in TT; Analiza 2 – primerjava homozigotov AA in heterozigotov AT;

CONCLUSIONS

The comparison of the genotype frequency between groups of the thinnest (group A) and the thickest (group B) revealed differences in population of sire line Large White breed and dam line of Slovenian Landrace breed, but the distribution of allele differed between group only in population of dam line of Slovenian Landrace breed. The estimation of *PPARGC-1* gene effect showed a significant effect of *PPARGC-1* gene on phenotypic values in population of Large White boars, but the analysis of gene effect on breeding values revealed a significant effect only in sire line of Large White breed. Such conflicting results may be the consequences of different background of allele, epistasis, intensity of selection, and deficiency of experiment design. Although these results did not confirmed the effect of allele A to be involved in inhibited fat deposition in both populations this type of analysis (especially as data size increases) with porcine candidate genes provides useful information for better understanding of the process of fat deposition. Further evaluations and researches of effect of T1378A substitution in porcine

PPARGC-1 gene should be studied on performance traits in different commercial pig populations.

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