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# Association of *POU1F1/Rsa*I genotypes with carcass traits in pigs

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**Abstract**: The objective of this study was to evaluate an association between the polymorphism of the porcine pituitary-specific transcription factor gene *(POU1F1,* previously called *PIT1*) and carcass quality in $F_2$ animals (grandparents: Zlotnicka Spotted boars and Polish Large White sows) being a part of experimental material prepared for a QTL mapping project. The analysis covered a total of 188 $F_2$ offspring of 13 males and 67 females ($F_1$ generation). The *Rsa*I PCR/RFLP polymorphism of the *POU1F1* gene was identified and the least squares method was used to evaluate the significance of its effect on the value of carcass quality traits. Three *POU1F1/Rsa*I genotypes were identified in $F_2$ porkers: EE (n=32), EF (n=68) and FF (n=88). Twenty-four carcass quality traits were measured after 24 h of cooling. The *POU1F1/Rsa*I genotype proved to have a significant effect on the following traits: weight of ham bone and bacon including ribs, fat thickness at the lower back (point $K_3$), over the loin, and average fat thickness (mean of five measurements). These results confirm that the *POU1F1* gene may be linked to the gene/genes affecting fat deposition in the pig carcass. Moreover, pigs with the EE genotype had a greater loin eye area and showed a higher meat weight and content of carcass than animals of both EF and FF genotypes (unsignificant association), which suggests that a further study is necessary to confirm or exclude the effect of the *POU1F1* gene on these traits.

**Key words**: *POU1F1* (*PIT1*), PCR/RFLP, pig, carcass quality.

## Introduction

Continuous improvements in breeding programmes need special tools (e.g. molecular markers) to make them more efficient. In order to find the best markers, several candidate genes need to be tested. One of them is the *POU1F1* gene (previously called *PIT1*) encoding the pituitary-specific transcription factor, which plays an essential role in the transcription of the growth hormone, prolactin and thyrotropin β subunit (INGRAHAM et al. 1990a, b, RADOVICK et al. 1992, STEIN-

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FELDER et al. 1991). The pituitary transcription factor gene is a part of the large POU-domain gene family. Analyses of several POU-domain-specific proteins have defined roles for the POU domain in high-affinity, site-specific DNA sequence recognition and in protein-protein interactions (ROSENFELD 1991). Therefore the biological importance of *POU1F1* allows to assume a certain influence of this gene on several economically important traits, principally growth rate and carcass composition. The results obtained in an experiment performed in the Iowa State University (ISU) indicate significant associations between the polymorphism of this gene and birth weight (*PIT1/Bam*H1 and *PIT1/Rsa*I polymorphism), back fat deposition (*PIT1/Msp*I polymorphism) and *longissimus* muscle area (*PIT1/Rsa*I polymorphism) (YU et al. 1994, 1995). A growth rate QTL had been shown to be located on chromosome 13 (ANDERSSON et al. 1994). Chromosome 13 QTLs for early growth rate were mapped more than 20 cM away from the *PIT1* locus in a Hohenheim PiGMaP Wild Boar × Pietrain family (MOSER et al. 1998). WILKIE et al. (1996) have indicated the region of chromosome 13, comprising markers *Sw769* and *Sw398*, which are about 20 cM distant from locus *POU1F1*, as responsible for the size of loin eye area. A significant effect of the polymorphism *POU1F1/Msp*I on mean backfat thickness has been also shown by STANCEKOVA et al. (1999), whereas no significant differences were detected for lean-to-fat ratio between *POU1F1/Rsa*I genotypes.

In the present study the relation between *POU1F1/Rsa*I genotypes and meat and fat deposition in pig carcasses was examined.

## Material and methods

### Experimental animals

The porkers included in this study were a part of experimental material prepared for QTLs mapping in pigs (ŻURKOWSKI et al. 1995). Altogether, 188 $F_2$ castrated males obtained from crossing 12 $F_1$ boars with 67 $F_1$ sows were randomly chosen for fattening and estimation of carcass performance traits after slaughter. The $F_1$ animals were the offspring of 9 Zlotnicka Spotted boars and 37 Polish Large White sows. For technical reasons only some of the porkers could be fattened in individual pens (92 animals), the remaining being placed in group pens (96 animals). Two young hogs were chosen at random from each litter for individual fattening and two for fattening in group pens. The porkers were fattened from the age of 90-100 days (initial mean live body weight of 25 kg) up to 100 kg of live body weight at the Kołbacz Experimental Farm, National Research Institute of Animal Production, according to feeding standards (Normy Żywienia Świń – Feeding Standards for Pigs 1993). Carcass quality evaluation was performed by dissecting the right carcass side after 24 h of cooling, according to the Pig Progeny Testing Station procedure. Twenty-four carcass quality traits listed in Table 1 were measured and analysed in the $F_2$ animals.

### *POU1F1* genotyping

Genomic DNA was isolated from leukocytes with the use of the WIZARD kit (Promega, Madison, WI, USA). The *POU1F1* genotyping was performed by PCR/RFLP analyses with *Rsa*I endonuclease (YU et al. 1994). An 1746-bp fragment, which was amplified within a part of exon IV, intron IV, exon V, intron V and a part of exon VI, included the *Rsa*I polymorphic site in intron V (YU et al. 1994). The PCR product was digested with *Rsa*I endonuclease, generating several fragments: three monomorphic (774, 153 and 108 bp) and three polymorphic (710 bp – E allele, 388/322 bp – F allele). One band (710 bp), two bands (388 and 322 bp) or three bands (710, 388, 322 bp) interpreted EE, FF and EF genotypes, respectively.

### Statistical analysis

The association between *POU1F1/Rsa*I genotypes and the traits measured in pigs was analysed using the least squares method of the GLM procedure (SAS 6.12; 1996) according to the following model:

$$Y_{ijklm} = \mu + G_i + S_j + D_{jk} + YST_l + \beta(w_{ijklm} - \overline{w}) + e_{ijklm}$$

where $Y_{ijklm}$ is the trait measured on *ijklm*th animal; $\mu$ is the overall mean; $G_i$ is the effect of *i*th *POU1F1/Rsa*I genotype; $S_j$ is the effect of *j*th sire; $D_{kj}$ is the effect associated with *k*th dam within *j*th sire group; $YST_l$ is the effect of *l*th year, season and type of fattening; $\beta$ is the linear regression coefficient for cold carcass weight; $w_{ijklm}$ is the cold carcass weight of *ijklm*th individual included as covariable; $\overline{w}$ is the average of cold carcass weight; and $e_{ijklm}$ is the random error.

## Results

Three *POU1F1/Rsa*I genotypes, designated according to YU et al. (1994), were found within the animals analysed. Genotype frequency estimates in the analysed group of porkers were as follows: 17.01% for EE (32 animals), 36.17% for EF (68 animals) and 48.82% for FF (88 animals). The least square mean (LSM) estimates with standard errors for *POU1F1/Rsa*I genotypes and pig carcass traits are shown in Table 1. The higher weight of ham bone was significantly associated with the EE genotype, whereas the higher weight of bacon including ribs and fat thickness measured at the lower back (tailing edge of the cross-section of *M. gluteus superficialis* – point $K_3$) and over the loin as well as average fat thickness from five measurements on the back were significantly associated with the FF genotype (Table 1). No other significant differences were observed between *POU1F1/Rsa*I genotypes in the value of the remaining traits analysed. However, the above-mentioned relationship between the FF genotype and carcass

**Table 1**. Least square mean (LSM) estimates and their standard errors for three POU1F1/RsaI genotypes and meat and fat deposition traits in pig carcass

| Traits | POU1F1/RsaI genotype | | |
|---|---|---|---|
| | EE | EF | FF |
| Weight of tender loin (kg) | 0.408 ± 0.027 | 0.389 ± 0.016 | 0.402 ± 0.016 |
| Weight of loin with fat (kg) | 7.269 ± 0.279 | 7.680 ± 0.167 | 7.638 ± 0.169 |
| Weight of loin without fat (kg) | 3.361 ± 0.182 | 3.572 ± 0.109 | 3.650 ± 0.110 |
| Weight of m. longissimus dorsi (kg) | 1.939 ± 0.270 | 1.954 ± 0.161 | 1.775 ± 0.164 |
| Width of loin eye (cm) | 7.386 ± 0.285 | 7.313 ± 0.170 | 7.131 ± 0.173 |
| Height of loin eye (cm) | 4.256 ± 0.217 | 4.061 ± 0.130 | 4.060 ± 0.132 |
| Loin eye area ($cm^2$) | 24.868 ± 1.787 | 23.510 ± 1.067 | 22.969 ± 1.084 |
| Weight of ham (kg) | 8.967 ± 0.228 | 8.713 ± 0.136 | 8.657 ± 0.139 |
| Weight of ham meat (kg) | 5.366 ± 0.168 | 5.238 ± 0.100 | 5.146 ± 0.102 |
| Weight of ham bone (kg) | 0.703 ± 0.032$^{a}$ | 0.629 ± 0.019$^{b}$ | 0.625 ± 0.019$^{b}$ |
| Weight of bacon with ribs (kg) | 5.845 ± 0.258$^{a}$ | 6.344 ± 0.154$^{b}$ | 6.401 ± 0.157$^{b}$ |
| Weight of abdominal fat (kg) | 1.242 ± 0.105 | 1.294 ± 0.063 | 1.269 ± 0.064 |
| Fat thickness over shoulder (cm) | 4.195 ± 0.219 | 4.346 ± 0.131 | 4.413 ± 0.133 |
| Backfat thickness (cm) | 2.756 ± 0.206 | 2.826 ± 0.123 | 2.914 ± 0.125 |
| Fat thickness at lower back -K1 (cm) | 2.871 ± 0.239 | 2.976 ± 0.143 | 3.086 ± 0.145 |
| Fat thickness at lower back -K2 (cm) | 2.604 ± 0.239 | 2.864 ± 0.143 | 3.005 ± 0.145 |
| Fat thickness at lower back -K3 (cm) | 2.383 ± 0.219$^{A}$ | 2.905 ± 0.131$^{aB}$ | 3.155 ± 0.133$^{bB}$ |
| Average fat thickness from five measurements (cm) | 2.961 ± 0.172$^{a}$ | 3.183 ± 0.102$^{ab}$ | 3.311 ± 0.104$^{b}$ |
| Weight of ham fat (kg) | 2.875 ± 0.166 | 2.831 ± 0.099 | 2.883 ± 0.101 |
| Weight of loin fat (kg) | 3.908 ± 0.266 | 4.108 ± 0.159 | 3.989 ± 0.161 |
| Fat thickness over loin (kg) | 2.446 ± 0.221$^{a}$ | 2.700 ± 0.132$^{ab}$ | 2.904 ± 0.134$^{b}$ |
| Fat thickness at side of loin (kg) | 2.639 ± 0.177 | 2.769 ± 0.106 | 2.868 ± 0.107 |
| Weight of meat in carcass (kg) | 17.121 ± 0.454 | 17.016 ± 0.271 | 16.780 ± 0.276 |
| Meat content of carcass (%) | 42.236 ± 1.104 | 41.971 ± 0.660 | 41.271 ± 0.670 |

$^{a, b}$ unequal indexes indicate significant differences ($P < 0.05$)
$^{A, B}$ unequal indexes indicate significant differences ($P < 0.01$)

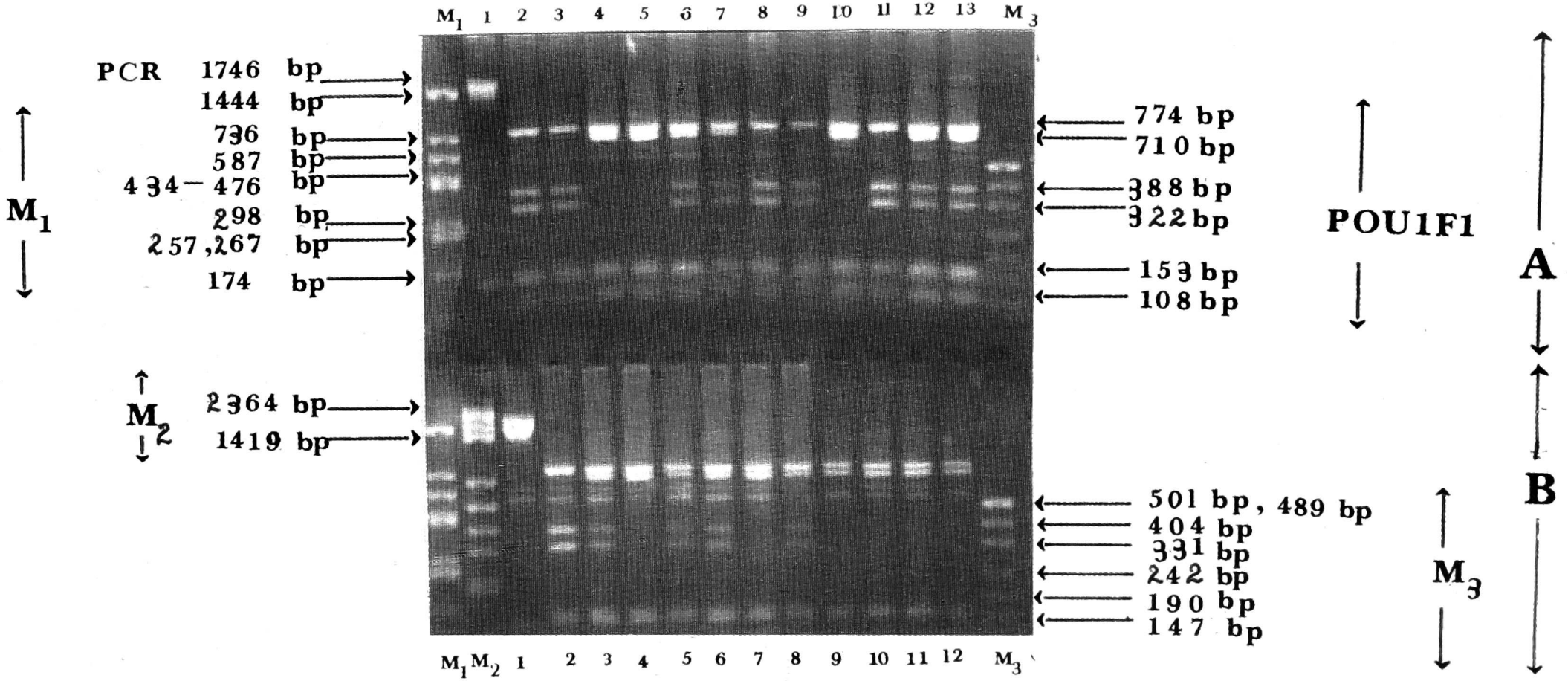


Figure 1. *Rsa*I polymorphism in *POU1F1* intron V: genotype EE, 710 bp fragment; genotype FF, 388 and 322 bp fragments; genotype EF, 710, 388 and 322 bp fragments. Monomorphic fragments of 774, 153 and 108 bp are also indicated; $M_1$, pUC19/*Hae*III marker (11-1444 bp); $M_2$, pUC19/*Rsa*I/*Hin*fI/*Pvu*II marker (65-2364 bp); $M_3$, pUC19/*Msp*I marker (26-501 bp); lines 1 are PCR products; lines 4, 5, 10 (part A) and lines 4, 7, 9-12 (part B) are homozygotes EE; lines 2, 3, 8, 9, 11(part A) and line 2 (part B) are homozygotes FF; lines 6, 7, 12, 13 (part A) and lines 3, 5, 6, 8 (part B) are heterozygotes EF

value always resulted in a higher adiposity. Fat thickness at certain measurement points was higher in pigs with the FF genotype than in EF and EE genotypes [backfat thickness, fat thicknes over the shoulder, at the lower back (points $K_1$ and $K_2$) and at the side of the loin]. Moreover, pigs with the EE genotype had a greater loin eye area and showed a higher meat deposition in some carcass cuts (weight of ham meat, meat weight and content of carcass) than animals of both EF and FF genotypes.

## Discussion

Several significant associations have been described between the *POU1F1* genotype and some carcass traits. YU et al. (1995) showed that polymorphisms of the *PIT1* gene, identified with *Bam*HI and *Msp*I restriction enzymes, were found only in Chinese breeds, whereas *Rsa*I polymorphism, within U.S. breeds (Duroc, Hampshire, Landrace). They documented that in synthetic lines involving Chinese germplasm, *PIT1*/*Msp*I polymorphism may be useful for marker-assisted selection to reduce backfat thickness. The pigs of the $F_2$ generation being homozygous for allele C (*PIT*1/*Msp*I), detected only in Chinese breeds (Meishan and Minzhu), were the fattest at market weight, as compared with all the other genotypes. Moreover, pigs with EE and EF genotypes (PIT1/RsaI) had significantly greater *longissimus* muscle area than pigs with genotype FF. What is interesting in the last association, is that allele F was detected only in U.S. grandparents (Duroc, Hampshire, Landrace) and was absent in Chinese breeds (Meishan and Minzhu).

The experimental material for this study originated from crosses of Zlotnicka Spotted boars and Polish Large White sows. Zlotnicka Spotted is an indigenous Polish fat pig, preserved as a genetic resource of alleles absent or showing a very low frequency in other breeds (KURYŁ et al. 1997, KLUKOWSKA et al. 1999). Only generation $F_2$ was tested for *POU1F1*/*Rsa*I polymorphism because DNA samples from grandparents and the majority of $F_1$ animals were exhausted in earlier studies. Both homozygous genotypes, EE and FF, were identified among several tested $F_1$ animals, which suggested that both *POU1F1*/*Rsa*I alleles, E and F, were present among grandparents of both breeds.

The responsibility of the region of chromosome 13 (comprising *PIT1 locus*) for the size of loin eye area was documented by WILKIE et al. (1996). YU et al. (1995) reported that pigs with EE and EF genotypes (*PIT1*/*Rsa*I) had 3.9 $cm^2$ more *longissimus* muscle area than pigs with genotype FF ($P < 0.05$). In the present study pigs with genotype EE had a greater loin eye area (about 1.9 $cm^2$) than pigs with genotype FF but that difference was not significant. The results obtained revealed that pigs with genotype EE, compared with those of genotype FF or EF, had a significantly heavier ham bone and lower value of the following fat deposition traits: weight of bacon including ribs, fat thickness at the lower back (point $K_3$), over the loin at the last rib, and average fat thickness from five measurements.

YU et al. (1995) have not observed any significant effect of *POU1F1/Rsa*I polymorphism on fat thickness measured at different points of the back. No significant differences between various *POU1F1/Rsa*I genotypes were observed for both average backfat and lean content in Large White and Large White × Landrace pigs reared in the Slovakia (STANCEKOVA et al. 1999).

YU et al. (1999), comparing interval mapping analyses of chromosome 13 and the results published earlier (YU et al. 1995), suggested a linkage disequilibrium between the *PIT1* locus and the backfat QTLs on pig chromosome 13. They concluded also that backfat QTLs are located at least 20 cM away from the *PIT1* locus and rather close to the *Swr1008* locus. The same authors suggested that the differences in mapping QTLs affecting fat depth to chromosome 13 between various laboratories could be due to the difference in breed origins or may be caused by the fact that the effects of QTLs located on pig chromosome 13 are small and hence were detected in some populations but not others. In our opinion it is also possible that the linkage phase between QTLs and markers varies across resource families. YU et al. (1995) showed that pigs with the *Msp*I CC genotype were fatter than those with CD and DD genotypes, whereas the study of STANCEKOVA et al. (1999) revealed that *Msp*I DD genotypes were significantly associated with a greater mean backfat and lower lean content than both CC and CD genotypes. A comparison of the results by YU et al. (1995, 1999) and STANCEKOVA et al. (1999), seems to confirm the conclusion drawn by YU et al. (1999) that fat deposition in the pig carcass is affected by the gene/genes linked to the *POU1F1* gene.

The results of the present investigation confirmed that the porcine *POU1F*1 gene, or genes closely linked to it, affect fat thickness in pig carcasses. However, further studies on other breeds or lines are necessary to confirm or exclude the influence of the *POU1F1* gene on meat deposition in the pig carcass.

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