

Relations between the polymorphism in the coding and 5'-flanking regions of the porcine *MYOD1* and *MYF5* genes and selected productive traits in Line 990 gilts*

**Paweł Urbański¹, Mariusz Pierzchała¹,
Marian Kamyczek², Marian Różycki³, Jolanta Kurył¹**

¹ Polish Academy of Sciences Institute of Genetics and Animal Breeding,
Jastrzębiec, 05-552 Wólka Kosowska, Poland

² Pig Hybridization Centre, National Research Institute of Animal Production,
Pawłowice, 64-122 Pawłowice, Poland

³ National Research Institute of Animal Production, 32-083 Balice, Poland

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Genes *MYOD1* and *MYF5* of the *MyoD* family were considered as candidate genes for growth rate and carcass traits in pigs. Products of both genes are transcription factors with domain bHLH. A relation was estimated between new polymorphisms identified in the coding and 5' flanking regions of both genes and productive traits of 216 gilts of synthetic Line 990. Statistical evaluation was performed with the model including the fixed effect of *RYRI* genotype and effect of the sire. For both genes a significant relation was identified between the novel polymorphisms and several productive traits. For some traits, heterozygotes showed the highest, while for some other the smallest values. The results suggest that novel mutations in *MYOD1* and *MYF5* genes could be more useful in pig selection than are mutations in the non-coding regions of both genes.

KEY WORDS: carcass / gene polymorphism / mutation / *MYOD1* / *MYF5* / pig

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Two methods are known of identification the quantitative traits (QTs) in animals: (1) mapping of hypothetic genes by linkage analysis, and (2) evaluation of the effect of candidate genes polymorphism on a trait of interest. In Polish literature application of both methods has been reviewed by Kurył [2000]. In the last decade many chromosomal regions were identified with QTLs for carcass traits and growth rate in pigs [Malek *et al.* 2001, Geldermann *et al.* 2003]. So far, candidate genes for pig carcass traits are: *RYR1*, growth hormone (*GH*), insulin-like growth factors (*IGFI* and *II*), genes of *MyoD* family, leptin and leptin receptor (*LEP* and *LEPR*), pituitary transcription factor 1 (*PIT1*) and several other genes – growth hormone releasing peptide (*GHRL*), melanocortin-4-receptor (*MC4R*) and high mobility group A1 protein (HMGA1) – as reviewed by Urbański [2003].

Recently, important for carcass traits were found genes of *MyoD* family: *MYOD1* (*MYF3*), *MYF5*, *MYOG* and *MYF6* (also called *MRF4* or herculin). The products of *MyoD* genes are specific transcription factors with the bHLH domain, which participate in muscle development from commitment and proliferation through fibre formation up to their postnatal maturation and function. The expression of *MyoD* genes takes place exclusively in skeletal muscles. As described by Buckingham [1994] the *MYF5* and *MYOD1* genes are expressed during proliferation of myoblasts, while *MYF6* and *MYOG* during myoblast differentiation. Postnatal expression of the *MYOD1*, *MYF5* and *MYOG* genes is characteristic of satellite cells [Koishi *et al.* 1995]. Satellite cells proliferate and differentiate, thereby enabling postnatal muscle growth [Beilharz *et al.* 1992]. Te Pas and Visscher [1994] suggest that also the *MyoD* genes could have a major effect on muscularity and growth. Of the *MyoD* family genes, *MYOD1* was the first to be identified and cloned [Weintraub *et al.* 1991]. The porcine *MYOD1* and *MYF5* genes have been mapped to chromosomes 2 and 5, respectively [Soumillion *et al.* 1997]. Both comprise three exons and two introns [Chang *et al.* 1995, Te Pas *et al.* 1999]. In the *MyoD* family genes, SNPs were identified in non-coding and 3' flanking regions (with exception of SNP in exon 2 of *MYF5* gene, but it was characteristic only of Meishan pigs [Stratil and Čepica 1999]). A relation between these mutations and carcass traits has already been reported [Cieślak *et al.* 2000, 2002, Kurył *et al.* 2002, Te Pas *et al.* 1999].

The aim of this study was to evaluate the effect of novel mutations identified earlier in the coding and 5' flanking regions of the *MYOD1* and *MYF5* genes by Urbański and Kurył [2004ab] on growth rate and carcass traits characterizing the meat deposition in pigs.

Material and methods

The study covered 216 gilts of synthetic Line 990. The animals were kept at the Pig Hybridization Centre, Pawłowice, of the National Research Institute of Animal Production, Cracow, Poland. Between 25 and 100 kg body weight (BW) the gilts were fed *ad libitum* a commercial mixed feed, and then slaughtered at 100 kg BW. Right

carcass sides were divided into cuts and dissected into meat, fat and bone according to the procedure used in Polish Pig Testing Stations, as described by Różycki [1996].

Genomic DNA was isolated from leukocytes according to Kawasaki [1990]. The *RYR1/HinP1* genotypes were established using sequence of primers given by Kamiński *et al.* [2001], whereas those at *MYOD1* and *MYF5* loci according to Urbański and Kurył [2004ab]. Statistical evaluation of results was performed with the least squares method of the GLM procedure [SAS 2001] according to the following model:

$$Y_{ijklm} = \mu + G_i + G_j + RYR1_k + O_l + (G_i G_j)_{ij} + \beta(x_{ijklm} - x) + e_{ijklm}$$

where:

- y_{ijklm} – trait measured on *ijklm*-th animal;
- μ – overall mean;
- G_i – effect of *MYOD1* genotype;
- G_j – effect of *MYF5* genotype;
- $RYR1_k$ – fixed effect of *RYR1* genotype;
- O_l – sire effect;
- $(G_i G_j)_{ij}$ – effect of interaction *MYOD1* × *MYF5* genotype;
- $\beta(x_{ijklm} - x)$ – linear regression for weight of right carcass side (for carcass traits) and age at slaughter (for growth rate traits);
- e_{ijklm} – random error.

Differences between pig groups (with greatest and smallest meat content of carcass and greatest and smallest loin eye area) for the frequency of alleles at the *MYOD1* and *MYF5* loci were evaluated according to Weber [1986].

Results and discussion

In the earlier authors' papers [Urbański and Kurył 2004ab] seven SNP novel mutations in both genes of interest were reported – three in *MYOD1* and four in *MYF5* genes. Three of them were found in exons, one in 5' UTR region, and three in promoter region.

Frequency of genotypes at the *MYOD1*, *MYF5* and *RYR1* loci found in this study in Line 990 gilts is shown in Table 1. At the locus *MYF5*, mutations *C580T* and *C613T* were linked [Urbański and Kurył 2004b], and therefore the frequency of alleles at both mutations amounted to the same value. An absence of genotype *TT* and a low frequency of heterozygotes was observed. A similar distribution of *MYF5* genotypes has been found in Polish Landrace and Polish Large White pigs by Urbański *et al.* [2005, unpublished].

Our earlier studies as well as those by other authors showed that *RYR1* genotype

Table 1. Frequency of genotypes at loci *MYOD1*, *MYF5* and *RYR1* in Lina 990 gilts (L990)

Gene/mutation/localization	Genotype	L990 (n=216)
<i>MYOD1</i>	GG	24.9
G302A	GA	44.8
5' UTR	AA	28.3
<i>MYOD1</i>	CC	15.5
C489T	CT	32.4
Exon1	TT	32.1
<i>MYOD1</i>	GG	44.9
G544C	GC	47.1
Exon1	CC	8.0
<i>MYF5</i>	AA	14.9
A65C	AC	31.2
5'-flanking region	CC	33.9
<i>MYF5</i>	CC	97.7
C580T	CT	2.3
5'-flanking region	TT	0.0
<i>MYF5</i>	CC	97.7
C613T	CT	2.3
5'-flanking region	TT	0.0
<i>MYF5</i>	CC	55.9
C2931T	CT	32.3
Exon3	TT	11.8
<i>RYR1</i>	CC	31.02
C1843T	CT	41.11
Exon17	TT	7.9

affects the carcass traits significantly and may modify the effect of other genes [Kuryl 1998, a review]. Therefore, in the present study, an effect of *RYR1* genotype was included into the statistical model applied. The frequency of heterozygotes and homozygotes *TT* at locus *RYR1* was found relatively high. Only 30% of animals were free of *RYR1^T* allele (Tab. 1). An effect of *RYR1* genotype was identified on weight of sirloin ($P<0.05$), weight of ham ($P<0.05$), meat content of valuable cuts ($P<0.01$) and of the whole carcass ($P<0.01$) – Table 2.

A significant relation was observed in gilts between several production traits and the genotype at the *MYOD1* and *MYF5* loci (Tab. 2). The transition G302A in region 5' UTR of *MYOD1* gene appeared to be significant for weight of loin. Heterozygotes showed a significantly lower weight of loin than AA homozygotes (by 0.24 kg). The polymorphism C489T (in *MYOD1*) and A65C (in *MYF5*) both appeared to be significant for weight of loin and sirloin ($P<0.01$). Homozygotes *TT* and *CC* at these loci, respectively, showed lower values of the two carcass traits mentioned than homozygotes *CC* and *AA*, respectively. As already mentioned, the effect of *RYR1* genotype on the weight of sirloin was significant ($P<0.05$), whereas that of A65C polymorphism at

the *MYF5* locus – highly significant ($P < 0.01$) – Table 2. An influence of interactions *MYOD1* × *RYR1* and *MYF5* × *RYR1* on meat deposition traits of carcass appeared not significant.

The linked mutations – *C580T* and *C613T* – identified in the promoter region of *MYF5* gene appeared to be significant for weight of ham, height and area of loin eye and meat content of carcass. Homozygotes *CC* showed the highest values of these traits. These results, however, must be considered as only preliminary since no homozygotes *TT* within analysed gilts were found and only five gilts were of *CT* genotype. Statistical evaluation of results showed that mean daily BW gain and weight of right carcass-side were both significantly related to mutation *C2931T* in exon 3 of gene *MYF5*. Heterozygotes showed the highest values of both traits. The difference in mean daily BW gain between the heterozygotes *CT* and homozygotes *CC* amounted to 36.5 g/day ($P \leq 0.05$).

No significant relation was found between the polymorphism identified in exon 1 (*G566C*) of the *MYOD1* gene and productive traits in gilts. In two pig breeds analysed earlier – Polish Large White and Polish Landrace – homozygotes of allele *G* at the 566 nt of *MYOD1* gene were the most profitable for loin traits as well as for meat content of both the valuable carcass cuts and carcass [Urbański *et al.* 2005, unpublished]. Those studies might be continued on other breeds, as the mutation changes amino acid sequence, similarly to the transition *C2931T* found by us in exon 3 of *MYF5* gene (Arg→Pro and Leu→Pro, respectively). The effect of proline upon protein folding is due to its ring structure. Moreover, proline disturbs the formation of α -helix because of the absence of a hydrogen atom in its amide group, which could compose a hydrogen bond [Frankel and Pabo 1988]. In the studies by Cieślak *et al.* [2000, 2002] and Kurył *et al.* [2002] the relation was evaluated between the polymorphism in non-coding regions of both *MYOD1* and *MYF5* genes and porcine carcass traits. A significant relation was identified between loin eye area, meat content of ham and meat content of loin and polymorphism *C1264A* in intron 1 of *MYOD1* gene.

Te Pas *et al.* [1999] and Cieślak *et al.* [2002] did not find significant relation between polymorphism identified in intron 1 or 2 of *MYF5* gene and growth rate or carcass traits. But weight of ham meat, meat content of ham, meat and fat content of loin and loin eye area were related to the genotype *MYF5/HinfI* [Cieślak *et al.* 2002]. The heterozygotes showed the highest value of these traits.

From gilts analysed two groups of animals were selected: with the greatest (>62%) and the smallest (<55%) meat content of carcass. The frequency of individual *MYOD1* and *MYF5* genotypes was calculated in both groups. The same was done for loin eye area where the pigs were distributed between the greatest (>60 cm²) and the smallest (<49 cm²) value of the trait. The frequency of genotypes regarding each mutation in *MYOD1* and *MYF5* genes was compared between animals with greatest and smallest value of these traits. The observed differences in the frequency of genotypes regarding analysed SNPs appeared not significant (Tab. 3). A similar situation was observed within Polish Large White and Polish Landrace pigs [Urbański *et al.* 2005, unpublished] but

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Table 3. Frequency of individual *MYOD1* and *MYF5* genotypes within group of L990 gilts of highest and lowest carcass meat content and greatest and smallest loin eye area

Gene/ localisation	Mutation	Genotype	Frequency of <i>MYOD1</i> and <i>MYF5</i> genotypes within pig groups of different meat content of carcass			Frequency of <i>MYOD1</i> and <i>MYF5</i> genotypes within pig groups of different loin eye area		
			L990			L990		
			<55%	>42%	P	<49 cm ²	>60 cm ²	P
<i>MYOD1</i> 5' UTR	G302A	GG	21.4	42.9		29.7	33.3	
		GA	44.3	57.1	ns	40.4	44.4	ns
		AA	14.3	0.00		29.7	22.2	
<i>MYOD1</i> exon1	C489T	CC	7.7	20.0		13.9	14.1	
		CT	43.4	50.0	ns	55.8	58.1	ns
		TT	24.9	30.0		37.2	25.8	
<i>MYOD1</i> exon1	G544C	GG	44.0	55.0		37.2	45.2	
		GC	52.0	35.0	ns	51.2	38.7	ns
		CC	4.0	10.0		11.4	14.1	
<i>MYF5</i> promoter	A43C	AA	5.4	11.1		11.1	13.1	
		AC	41.1	55.4	ns	50.0	54.5	ns
		CC	33.3	33.3		38.9	30.4	
	C580T	CC	89.3	95.0		92.5	100.0	
		CT	10.7	5.0	ns	7.5	0.00	ns
		TT	0.00	0.00		0.00	0.00	
C413T	CC	89.3	95.0		92.5	100.0		
	CT	10.7	5.0	ns	7.5	0.00	ns	
	TT	0.00	0.00		0.00	0.00		
<i>MYF5</i> exon3	C2931T	CC	33.3	48.8		45.5	43.2	
		CT	44.7	18.8	ns	18.2	15.8	ns
		TT	20.0	12.4		34.3	21.0	

ns - P>0.05.

only concerning a frequency of *MYF5* genotypes. However, the frequency of genotypes *MYOD1* was significantly different between breeds and highly significantly different between groups of gilts with greatest and smallest value of meat content of carcass and loin eye area.

In the present study, heterozygotes as regards certain mutations in *MYOD1* and *MYF5* genes showed greatest or smallest value for carcass traits compared to both homozygotes. A negative molecular heterosis, described for some human genes by Comings and MacMurray [2000], may possibly explain this phenomenon. Those authors suggested that in the case of regulation of the dose-dependent gene, the presence of a regulatory sequences in a heterozygous state could modify the gene function.

Summarizing the results presented here, one may conclude that an effect of genotype at the *MYOD1* and *MYF5* loci on daily live weight gain and carcass traits of Line

990 gilts depended on the particular mutation being either profitable or not for an individual trait. These genotypes could be useful in selection aiming at increasing the meat content of carcass in Line 990 pigs.

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Paweł Urbański, Mariusz Pierzchała,
Marian Kamyczek, Marian Różycki, Jolanta Kurył

Zależność między polimorfizmem w rejonach kodujących i 5' flankujących genów *MYOD1* i *MYF5* a wybranymi cechami produkcyjnymi loszek linii 990

Streszczenie

Analizowano dwa geny z rodziny *MyoD* – *MYOD1* i *MYF5* – jako kandydujące dla tempa wzrostu i cech mięsności tuszy świń. Produktami tych genów są czynniki transkrypcyjne z domeną bHLH. Ocenę zależności między nowym polimorfizmem, zidentyfikowanym wcześniej w rejonach kodujących i 5' flankujących obu genów, a cechami produkcyjnymi przeprowadzono na materiale obejmującym 216 loszek syntetycznej linii 990, tuczonych do masy ciała 100 kg. W modelu statystycznym dodatkowo uwzględniono stały efekt genotypu *RYRI* i efekt ojca. Stwierdzono istotne zależności między nowymi polimorfizmami w badanych genach a niektórymi cechami produkcyjnymi loszek. Względem pewnych cech, heterozygoty charakteryzowały się największymi bądź najmniejszymi wartościami. Uzyskane wyniki mogą sugerować, że nowe mutacje zidentyfikowane w rejonach kodujących i 5' flankujących genów *MYOD1* i *MYF5* mogą być bardziej przydatne w selekcji świń, aniżeli znane wcześniej mutacje w rejonach niekodujących obu genów.