A novel polymorphism in exon 1 of the porcine *myogenin* gene

Joanna Wyszyńska-Koko, Jolanta Kurył

Institute of Genetics and Animal Breeding, Polish Academy of Sciences, Jastrzębiec, Poland

**Abstract.** Myogenin is a gene belonging to the MyoD family, which codes for the bHLH transcription factor playing a key role in myogenesis. It affects the processes of differentiation and maturation of myotubes during embryogenesis. Fragments of the porcine *myogenin* coding sequence and promoter region were amplified and subjected to MSSCP analysis. T→C transition recognised by the MaeII restriction enzyme in exon 1 was revealed, which appeared to be a silent mutation in the region of the transactivation domain. No other polymorphism was found either in the remaining coding sequence or the promoter region.

**Key words:** MSSCP, myogenin, Sus scrofa.

Myogenin (*MYOG*), together with *MYF3* (*MyoD1*), *MYF5* and *MYF6* (*MRF4, herculin*), belongs to the MyoD family genes. These genes code for the four bHLH transcription factors which control the processes of myogenesis, induce an expression of muscle specific genes (Lassar et al. 1989) and can convert various nonmuscle cells into muscle (Weintraub et al. 1989). The induction of the *MYOG* expression is associated with a rapid set-out of the myoblast differentiation program and start of specific muscle genes expression (Montarras et al. 1991; Buckingham 1992). The knock-out experiments on murine embryos revealed a crucial role of *MYOG* in myogenesis. The null myogenin-mutants in the homozygous state were stillborn and showed several muscle and skeletal defects. Muscle tissue was dramatically reduced, mononucleated myoblasts were present instead of muscle fibers, occasional myofibers showed a lowered density, disorganized structure and various stages of maturation (Hasty et al. 1993; Nabeshima et al. 1993). These results suggest the role of *MYOG* in the differentiation and maturation of myoblasts and focused the attention of scientists on *myogenin* as a possible gene affecting the muscle phenotype in pigs. The variation of the *MYOG* locus in pigs was the subject of several studies (Mendez et al. 1997; Soumillion et al. 1997), but the polymorphisms found appeared to be localised in introns or in distant flanking regions.

The aim of our study was to analyse the DNA polymorphism in the coding sequence of *MYOG* and conformation of the polymorphism described by Nowak et al. (2003), and in the promoter region of that gene.

Blood samples were collected from 432 domestic pigs of different breeds: Polish Landrace (39), Polish Large White (185), Polish commercial line L990 (24), Pietrain (15), Duroc (15), commercial lines: PIC (9), Torhyb (86) and Stamboek (38), and Polish native pig Z³otnicka Spotted (21). DNA was isolated from the whole blood using the method described by Kawasaki (1990). Primer sequences, lengths of amplified fragments, references and thermal cycling profiles are shown...
in Table 1. The primers of the MYOG coding sequence are based on Nowak et al. (2003). For the more proximal promoter fragment (p2) primers were designed on the basis of the MYOG sequence in the GenBank database (acc. no U14331) using the primer 3 program (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). For the PCR of the p1 fragment reaction mixes (12.5/10 μL) comprised 30 ng genomic DNA (3/10 μL), 1.5 mM MgCl₂ in PCR Buffer, 25 pmol of each primer, 0.25 mM of each dNTP and 0.3 U of AmpliTaq DNA Polymerase (Applied Biosystems, USA). The p2, e1, e2 and e3 reaction mixes (12.5 μL) comprised 30 ng genomic DNA (3 μL), 1.5 mM MgCl₂ in PCR Buffer, 1 × Q-Solution, 20 pmol of each primer, 0.25 mM of each dNTP and 0.3 U of HotStart Taq DNA Polymerase (Qiagen, Germany).

Fragments p1, e2 and e3 were subjected to a multitemperature single-strand conformation polymorphism (MSSCP) analysis. The PCR products were mixed (1:1) with a loading buffer (50% formamide, 0.5 M EDTA pH 8.0, 0.05% bromophenol blue) and denatured at 95°C for 5 minutes. The probes were immediately chilled on ice and loaded on 8% (and/or 12% in case of the e2 and e3 fragments) polyacrylamide gels (29 acrylamide : 1 bis-acrylamide) containing 1 × TBE buffer. The gels (180 mm × 140 mm) were run at 300 V for 3h in 2 × TBE at the temperature program: 30°C – 1h, 18°C – 1h, 8°C – 1h. The DNA conformers were visualised with the silver staining technique (Silver Stain, Kucharczyk Electrophoretical Techniques, Poland) according to the procedure described by the manufacturer. Fragments p2 and e1, too long for SSCP analysis, were subjected to the RF-MSSCP method (restriction fragments – multitemperature single-strand conformation polymorphism). The PCR products were digested overnight with 3 U restriction enzyme (p2 - HinfI, e1 - NlaIII) in a total volume of 12 μL to obtain two shorter DNA fragments. The whole volume of the probe after digestion was subjected to the same steps as nondigested p1, e2 and e3 fragments. For the described analyses the following pig breeds differing in carcass quality and growth rate traits were used: Polish commercial line 990, PIC, Torhyb, Duroc and the Polish indigenous pig Z³otnicka Spotted.

Sequencing was performed for the DNA samples of different SSCP patterns in exon 1. For sequencing an ABI Prism 377 automatic sequencer and ABI Prism BigDye Terminator Cycle Sequencing Kit was used, according to the procedure described by the manufacturer. The obtained sequences of e1 DNA samples differing in the MSSCP pattern were subjected to in silico restriction analysis using the AnnHyb software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3_www.cgi). Basing on its results, the RFLP analysis was performed. The reaction mix of a total volume 10 μL contained 5 μL of the PCR product and 2.5 U of MaeIII restriction enzyme (New England Biolabs). The overnight digestion was followed by 3% agarose gel electrophoresis (Sigma).

Five primer pairs were used to amplify myogenin fragments. The p1 fragment covered the promoter in the region from –830 to –466 (nu-
cleotide numbers from the putative start site). The fragment marked as p2 covered the region comprising the transcription and translation start site and partly overlapped with p1 (nucleotides from –552 to +43). Primer pairs for exonic sequences were described by Nowak et al. (2003) and they amplified the fragments corresponding to three exons of MYOG excluding 5′ and 3′ UTR.

PCR products amplified by the five primer pairs were subjected to MSSCP or RF-MSSCP analyses. Fragments p1, e2 and e3 appeared to be monomorphic when 8% and/or 12% acrylamide gels were used. In exon 1 of the MYOG gene two different conformer patterns were observed using the RF-MSSCP analysis (Figure 1a). The sequencing analysis of the samples differing in the patterns was performed, which revealed a C→T transition in the region coding for the transactivation domain of the MYOG gene (Schwarz et al. 1992). The novel polymorphism we describe here is the only one confirmed by a RFLP analysis in the MYOG coding sequence. The mutation is in the third position of the Gly codon and does not change the amino acid. The in silico restriction analysis found an enzyme MaeIII to recognise the restriction site created by a T allele. The digestion with MaeIII of the e1 PCR product was performed and 3% agarose electrophoresis was run (Figure 1b). The sequence with a marked variation was deposited in the GenBank database under accession number AY642156. The groups of pig breeds differing in growth rate and carcass quality were genotyped. The allele frequency analyses showed a big predominance of the wild type C allele in all the breeds (Table 2). The breeds of Polish Landrace, Duroc, Pietrain and Polish commercial line 990 were monomorphic with only C allele present. The other breeds have a very low T allele frequency. There was only one homozygote TT found in the Polish Large White breed. A minimal frequency of the T allele in Line 990 should be expected, as the Polish Large White breed is its component, but here the sample of the animals was too small to detect it (Table 2).

Soumillion et al. (1997) described three MspI polymorphisms, all in noncoding regions. The one in the promoter region was found only in the Meishan pig. The polymorphism in the second intron was predominantly frequent also in the Chinese pig, only two heterozygous Pietrains were found by Cieślak et al. (2000). The only one mutation in the distal 3′flanking region appeared to have a high frequency in all the genotypes (Soumillion et al. 1997; Cieślak et al. 2000). However, in association with production traits, when using the statistical model including the RYRI gene effect, the mutation appeared not to have any effect on carcass quality traits (Cieślak et al. 2002). Mendez et al. (1997) communicated

![Figure 1. a) The RF-MSSCP result of exon 1 (e1 fragment) of the myogenin gene. Two conformer patterns are shown, marked as 1 and 2. Marker: pUC 19/MspI (26–501 bp); b) The homozygote CC and heterozygote CT observed for the porcine myogenin gene exon 1 (fragment e1) after MaeIII digestion: homozygote CC – not cut, fragment 431 bp; heterozygote CT, fragments: 431 + 348 + 83 bp. Marker: pUC 19/MspI (26–501 bp) (BTL, Poland).](image)

<table>
<thead>
<tr>
<th>Breed</th>
<th>No. of tested pigs</th>
<th>Allelic frequency</th>
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<tbody>
<tr>
<td>Polish Landrace</td>
<td>39</td>
<td>C 1.00 T 0.00</td>
</tr>
<tr>
<td>Polish Large White</td>
<td>185</td>
<td>C 0.95 T 0.05</td>
</tr>
<tr>
<td>L990</td>
<td>24</td>
<td>C 1.00 T 0.00</td>
</tr>
<tr>
<td>Pietrain</td>
<td>15</td>
<td>C 1.00 T 0.00</td>
</tr>
<tr>
<td>Duroc</td>
<td>15</td>
<td>C 1.00 T 0.00</td>
</tr>
<tr>
<td>Torhyb</td>
<td>86</td>
<td>C 0.94 T 0.06</td>
</tr>
<tr>
<td>Stamboek</td>
<td>38</td>
<td>C 0.99 T 0.01</td>
</tr>
<tr>
<td>Zlotnicka Spotted</td>
<td>21</td>
<td>C 0.98 T 0.02</td>
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Table 2. Allele C and T frequency of porcine MYOG gene in different breeds of domestic pig.
a NlaIV polymorphism, predominantly existing in Chinese breeds. From the sequence and RFLP-electrophoresis pattern of the amplified and digested fragment we deduced a possible location of the mutation in intron 1 (data not shown). Taken together, the MYOG gene seems to be polymorphic only in a very low degree, having systems guaranteeing it a high level of conservativeness.

Nevertheless, the Gly amino acid on the 34th position in the myogenin protein can be found in all the analysed species, together with its codon ggc. It is not excluded that the T mutation in allelic codon ggt in some pig breeds may play a role in the posttranscriptional regulation of MYOG expression via siRNA.

In further studies the dependence of carcass and meat quality traits on the particular point mutations should be examined. It will make it possible to distinguish the MYOG gene as potentially useful in MAS (Marker Assisted Selection) or useless because of a high level of conservativeness.

Conclusions

The scan of the promoter and coding sequence of the myogenin gene using the MSSCP (RF-MSSCP) method allowed us to find one silent point mutation in exon 1 in the position of the transactivation domain. The frequency of the mutated T allele appeared to be very rare in the analysed pig breeds. Taken together with the other described MYOG polymorphisms existing in noncoding regions of the gene, it can be stated that myogenin shows a very low level of variability.

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