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SHORT REPORT

Conformation polymorphism in myogenin gene in pigs

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Conformation polymorphism (SSCP) in exons 1-3 of myogenin gene was analysed in two groups of fatteners, both out of crossbred sows (Polish Large White × Polish Landrace) sired by Duroc (group I) or Duroc × Pietrain (group II) boars. The total DNA was isolated from a whole blood using phenol/chloroform extraction. Amplifications of exons was carried out using PCR method and primers designed by computer software "Primer 3" (www.genome.wi.mit.edu).
Exon 1 showed low polymorphism with two SSCP patterns: A (two bands) in 98% of group I and 94% of group I, and E (three bands) in 2% of group I and 6% of group II fatteners. Exon 2 in all fatteners was found monomorphic. Exon 3 showed high polymorphism with four SSCP patterns: A, B, C and D (one to three bands). Most frequently observed was pattern A (88% in group I and 82% in group II), while the remaining patterns occurred in 2-10% of fatteners. Sequence analysis of conformation patterns did not show any mutation sites.

KEY WORDS: conformation / myogenin gene / pig / polymorphism

Growth rate and muscle mass gain in pigs correlate with the number of myofibres [Larzul *et al*. 1997]. Formation and further function of muscles in mammals are controlled by genes of MyoD regulatory factors (muscular regulatory factors – MRF),

including myogenin gene [Buchberger *et al*. 1994, Te Pas *et al*. 1994]. The myogenin gene is the only gene of MyoD family that undergoes expression in all skeletal muscle cell lines. The protein coded by the gene is necessary for regulation of skeletal muscles development during embryogenesis [Kitzmann *et al*. 1998].

Soumillion *et al*. [1997] showed that polymorphism within the non-coding region 3' of myogenin gene prevails in all pig breeds, while the promoter region polymorphism appears only in Meishans. There is no information available about the single strand conformation polymorhism (SSCP) within the range of myogenin gene exons.

When mutation type and location are both known, the genotype identification in the *locus* can be carried out with the restriction fragment length polymorphism (RFLP) method.

The results of preliminary studies carried out in Poland by Cieślak *et al*. [2000] showed significant relationship between the myogenin gene genotype and lean meat content of ham, loin and the total carcass-side in Pietrain, Zlotnicka Spotted and Polish Landrace pigs.

In this study an attempt at determining the polymorphism of a myogenin gene in pigs is presented.

Material and methods

Used were two groups of a total of 200 commercial crossbred fatteners from the Experimental Farm of the Warsaw Agricultural University:

– group I – 100 fatteners out of Polish Large White × Polish Landrace sows mated to Duroc boars.
– group II – 100 fatteners out of Polish Large White × Polish Landrace sows mated to Duroc × Pietrain boars.

Genomic DNA was isolated from peripheral blood with phenol/chloroform extraction method.

The conformation polymorphism of myogenin gene exons 1-3 was analysed with SSCP method. The exons were amplified with polymerase chain reaction (PCR), using starters designed by the computer software (Programme "Primer 3" – www.genome.wi.mit.edu). Starter sequences and PCR conditions are shown in Table 1. Amplified fragments were analysed in 1.5% agarose gel, 60 mV, to check whether the required PCR product was obtained.

PCR products were first denaturated for 10 min in 94°C in order to obtain individual conformer strands, and then separated in 10% (1 × TBE buffer) polyacrylamide gel (12 × 12 cm) in non-denaturated conditions at a constant temperature of 10°C, and 35 W using D'Code System (BioRad) device. The total volume of DNA sample subject to electrophoresis was equal to 13.5 μl (SSCP reaction).

After electrophoretic separation (SSCP) the gels were stained with silver nitrate.

All PCR products showing the conformation polymorphism were cloned and sequenced using ALFExpress DNA sequencer (AMERSHAM PHARMACIA. BIO-

Table 1. Primers and conditions of polymerase chain reaction for amplification of exons 1, 2 and 3 of myogenin gene

Exon	PCR product	Primer	PCR conditions
1	431 bp	F 5'tccccctacttctaccaggaa 3' R 5'cctcggtagtggaggtct 3'	1 × 94°C-2'; 62° C-30";72°C-1' 30 × 93° C-30";62°C-30"; 72° C-30'
2	122 bp	F 5'gccttcacctatcttgtag 3' R 5'agggtcctggcctcacttta 3'	1 × 93°C-2'; 64° C-30"; 72°C-30" 30 × 90° C-30"; 64°C-30"; 72°C-30"
3	138 bp	F 5'tcatctgctcacagtgac 3' R 5'catcctggcagaccagtctca 3'	1 × 93°C-2'; 62° C- 30"; 72°C-30" 30 × 90° C-30"; 62°C-30"; 72° C-3"

TECH).

Results and discussion

Three exons of the myogenin gene were amplified using primers designed with the computer software. The PCR products had length of 431, 122 and 138 bp, for exon 1, 2 and 3, respectively (Fig. 1).

The slight conformer polymorphism in exon 1 was shown with two conformer variants identified: A pattern – two conformers, while E pattern – three conformers (Fig.

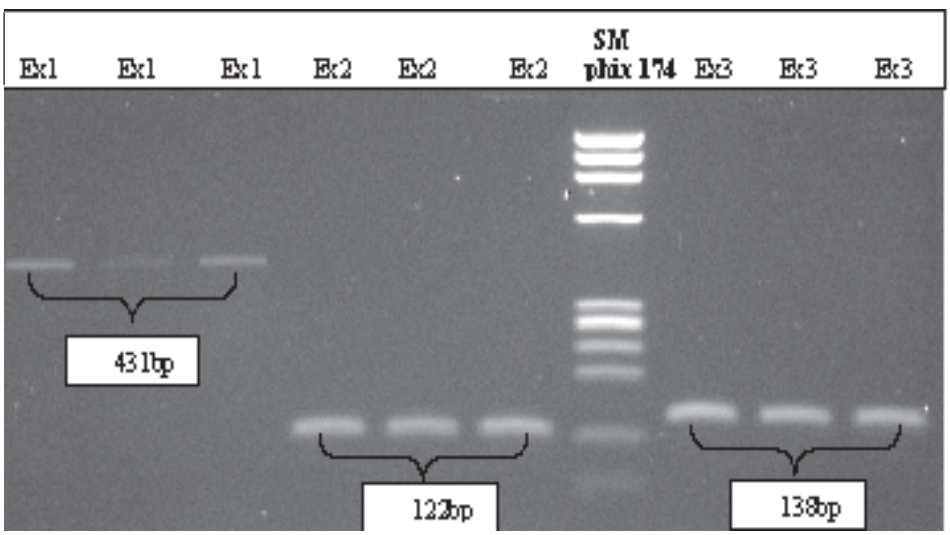

Fig. 1. PCR products of amplification of exons 1-3 of the myogenin gene using original starters (1.5% agarose gel).

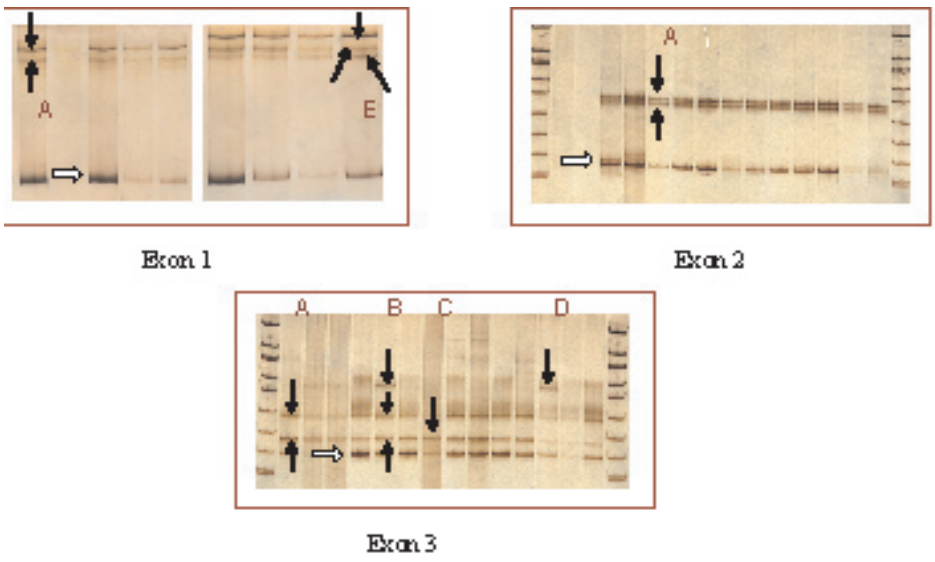

Fig. 2. Analysis of conformation polymorphism in exons 1-3 of the myogenin gene using the SSCP method. White arrows show the renaturated fragments.

Table 2. Frequency of SSCP patterns of the myogenin gene in two groups of fatteners

Exon	SSCP pattern*	Group I	Group II
1	A	0.98	0.94
	E	0.02	0.06
2	A	1.0	1.0
3	A	0.88	0.82
	B	0.07	0.10
	C	0.03	0.06
	D	0.02	0.02

*SSCP patterns in exons 1, 2 and 3 are shown in Figure 2.

2). Pattern A was found in 98% of group I, and in 94% of group II fatteners, whereas E in 2% of group I and 6% of group II. The frequency of individual SSCP patterns for all three exons studied is shown in Table 2.

Analysis of sequences in the exons studied did not show any mutation in myogenin gene fragments considered. Further analysis of families material will allow to conclude whether the polymorphism observed was inherited.

SSCP analysis showed that exon 2 in all fatteners was monomorphic. All conformers migrated with the same rate (type A). Conformation pattern of exon 2 is showed in Figure 2.

In the literature there are neither data on conformation, nor restriction polymorphism of myogene gene. The study by Cieślak *et al*. [2000] on Pietrain pigs showed the presence of two alleles of the myogene gene intron 2. According to Soumillion *et al*. [1997] such polymorphism in Meishan pigs is accompanied by that occurring in a promoter region.

The highest conformation polymorphism was found in exon 3, in which four SSCP patterns (A, B, C and D) were found, including one to three conformers (Fig. 2). Similarly as in exon 1, pattern A appeared with the highest frequency, and was present in 88% of group I and 82% of group II fatteners (Tab. 2). Other conformer patterns were observed in 2-10% of fatteners (Tab. 2).

In summary, the differentiation of SSCP patterns was observed in myogene gene exons. Monomorphism in exon 2 and slight polymorphism (two patterns) in exon 1 were found.

Sequence analysis performed suggests that the conformation polymorphism of DNA fragment is not unquestionable indicator of mutation sites.

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Polimorfizm konformacyjny genu miogeniny świń

S t r e s z c z e n i e

Analizowano polimorfizm konformacyjny (SSCP) eksonów 1, 2 i 3 genu miogeniny w dwóch grupach tuczników mieszańcowych, będących potomstwem matek mieszańcowych wbp × pbz krytych knurami rasy duroc (grupa I) lub knurami mieszańcowymi duroc × pietrain (grupa II). DNA izolowano z pełnej krwi metodą ekstrakcji fenolowo-chloroformowej. Eksony amplifikowano metodą PCR, stosując startery opracowane za pomocą programu komputerowego „Primer 3" (www.genome.wi.mit.edu). Ekson 1 wykazywał mały polimorfizm – stwierdzono dwa wzory SSCP: A (dwa prążki) u 98% zwierząt z grupy I i 94% z grupy II oraz E (trzy prążki) u 2% tuczników z grupy I i 6% z grupy II. Ekson 2 okazał się monomorficzny u wszystkich badanych zwierząt. Ekson 3 wykazywał największy polimorfizm – stwierdzono cztery wzory SSCP: A, B, C i D – z jednym, dwoma lub trzema prążkami. W grupie I i II najczęściej występował wzór A (odpowiednio 88 i 82% zwierząt). Pozostałe wzory występowały u 2-10% tuczników. Analiza sekwencyjna badanych wzorów nie wykazała miejsc mutacyjnych.