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A Novel Genetic Polymorphism and Its Genetic Effects of Porcine Myogenin Gene in Intron 1

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Abstract Single-nucleotide polymorphisms of the *MyoG* gene were tested using PCR-SSCP from 73 Landrace pigs, 68 Large White pigs, 57 Yimeng pigs and 83 Laiwu pigs. The effects of the *MyoG* gene on the birth weight, the average daily gain, the meat tenderness, and the backfat thickness were also analyzed. On the basis of the DNA sequence (M14331) of the porcine *MyoG* gene, primers were designed to amplify *MyoG* gene. One polymorphism was found in the amplified region of intron 1, in which two alleles (*A*, and *B*) and three genotypes (*AA*, *AB*, and *BB*) were examined. A G→C transition was detected at 2 943 locus by sequencing the homozygotes. The results show that: (1) The Large White breed differed significantly ($P < 0.05$) in genotype distribution from the Landrace, the Laiwu and the Yimeng breeds; and the Laiwu breed differed significantly ($P < 0.05$) in genotype distribution from the Landrace and the Yimeng breeds; whereas no significant differences ($P > 0.05$) were found in genotype distribution between the Landrace and the Yimeng breeds. (2) On the basis of the fixed effect model, significant differences ($P < 0.05$) were found in the birth weight and the tenderness among the different *MyoG* genotypes, whereas no significant differences ($P > 0.05$) existed in the average daily gain and the backfat thickness. (3) Using least square analysis, it was seen that significant differences ($P < 0.05$) exist in the meat tenderness between the individuals of the *BB* genotypes and those of the *AA* genotypes; and significant differences ($P < 0.05$) were found in the backfat thickness compared the pigs of the *AA* genotypes with the pigs of the *AB*, *BB* genotypes. These results suggest that the MyoG genotype has significant effects on the meat quality, the growth rate, and the backfat thickness, therefore *MyoG* gene can be used in marker-assisted selection to improve meat quality and growth rate, and to accelerate the breeding progress.

Key words pigs; *MyoG* gene; genetic polymorphisms; genetic effects

猪 MyoG 基因内含子 1 的一个遗传多态性及其遗传效应分析

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摘要 以长白猪(73),大白猪(68),沂蒙黑猪(57)和莱芜猪(83)为研究对象,采用 PCR-SSCP 方法对猪 *MyoG* 基因遗传多态性进行分析,并研究基因型与初生重、日增重、肌肉嫩度和背膘厚的相关性. 根据猪 *MyoG* 基因的 DNA 序列(M14331)设计引物,结果在内含子 1 扩增的片段上发现了一个多态性,检测到 2 个等位基因(*A*、*B*),3 种基因型(*AA*、*AB*、*BB*),并对纯合子进行测序,发现2 943位 G→C 突变. 基因型在不同猪种分布的多重比较,结果表明,大白猪与长白猪、沂蒙黑猪和莱芜猪比较差异显著($P < 0.05$),莱芜猪与长白猪和沂蒙黑猪比较差异显著($P < 0.05$),长白猪与沂蒙黑猪比较差异不显著($P > 0.05$). 固定效应模型分析结果表明,初生重及嫩度基因型间差异显著($P < 0.01$),

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而日增重及背膘厚基因型间差异不显著($P > 0.05$) . 最小二乘分析结果表明, *BB* 基因型个体与 *AA* 基因型个体比较肌肉嫩度的差异显著($P < 0.05$) , *AA* 基因型个体与 *AB* 和 *BB* 基因型个体比较背膘厚的差异显著($P < 0.05$) . 因此, 推测 *MyoG* 基因对猪肉品质、生长速度及背膘厚存在一定的影响, 将 *MyoG* 基因应用于猪育种过程中的标记辅助选择将可以改善猪肉品质, 提高生长速度, 加快猪的育种进程.

关键词 猪; *MyoG* 基因; 遗传多态性; 遗传效应

中图分类号

Myogenin (*MyoG*) , together with *MyoD*1 (*MYF*3) , *MYF*5 and *MRF*4 (also called *MYF*6 or *herculin*) , belongs to the MyoD family genes. These genes encode basic helix-loop-helix (bHLH) proteins and are involved in muscle cell determination and differentiation *in vitro*[1,2]. The myogenin expression abrogates the myoblast proliferation potential and regulates the differentiation of mononucleated myoblasts into multinucleated myofibers[3]. 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[4]. The knock-out experiments on murine embryos reveal a crucial role of *MyoG* in myogenesis. The null myogenin-mutants in the homozygous states are stillborn and show several muscle and skeletal defects. Muscle tissue is dramatically reduced, mononucleated myoblasts are present instead of muscle fibers, occasional myofibers show a lowered density, disorganized structure and various stages of maturation[5]. The myogenin gene (*MyoG*) , contains three exons and is physically mapped to the porcine chromosome 9q2.1-q2.6[6]. *MyoD*1 is a master regulatory gene for myogenesis that also converts many mesoderm-derived cells into the skeletal muscle phenotype[7]. And a critical myogenic factor induced rapidly upon activation of quiescent satellite cells and required for their differentiation during muscle regeneration[8]. The myogenic factors 5 (MYF5) and MRF4 are integral to the initiation and development of skeletal muscle and to the maintenance of its phenotype[9].

Polymorphic *Nla* Ⅳ and *Msp* Ⅰ sites were discovered in the *MyoG* gene, and genetic relationships between the *MyoG* genotypes and the partial growth traits were analyzed in the previous studies. The different *MyoG* genotypes are related to the variation in the number of the muscle fibers and the growth rate, which lead to a variation in the muscle mass[10,11]. The Southern blot analysis of 105 unrelated pigs reveales three polymorphic *MspI* sites that are located in the promoter region, the second intron, and at the 3′flank of the *MyoG* gene[12]. With respect to the intron 2 *Msp* Ⅰ RFLP site, most of the exotic breeds present as the *AA* genotype, whereas many of the Chinese local pig breeds present as the *BB* genotype. With respect to the 3′flank site, most of the exotic breeds present as the *NN* genotype, whereas many of the Chinese local pig breeds present as the *MM* genotype. The 5′RFLP site is not found in the pig breeds tested[12]. Significant differences are found between the *AA* and *BB* genotypes with regard to the birth weight, the growth rate, and the lean weight, but not the backfat thickness[11].

Based on the studies of the former scientists, *MyoG* as a possible gene affects the muscle phenotype. The candidate gene approach is an effective and fast QTL mapping method. The aim of this study was to analyze the DNA polymorphism of the porcine *MyoG* gene, and correlations between the polymorphism and the pig partial growth traits were analyzed in this study. The results of this study may provide a basis for pure breeding, for crossbreeding, and for the preservation of important genetic resources at a molecular level.

1 Materials and Methods

1.1 Animals

73 Landrace pigs, 68 Large White pigs, 57 Yimeng pigs and 83 Laiwu pigs were used as experimental animals in this study. Ear tissue samples were collected for the extraction of genomic DNA.

1.2 Genomic DNA extraction

Genomic DNA was isolated from 1 mg of ear tissue sample by overnight proteinase K digestion followed by phenol-chloroform extraction and ethanol precipitation, according to standard protocols.

1.3 PCR-SSCP

Based on the published DNA sequence of the porcine *MyoG* gene (M14331) , primers were designed to amplify DNA sequence to detect MyoG polymorphisms. PCR was performed in a total volume of 25 μl of the following mixture: 100 ng porcine genomic DNA, 10 pmol/L of each primer, 200 mmol/L of each dNTP, 1.5 mmol/L $MgCl_2$, 2.5 μL buffer (10 ×concentrate: 200 mmol/L Tris-HCl, pH 8.4, 500 mmol/L KCl) , and 1.0 unit of *Taq* DNA polymerase. The PCR mix was incubated at 94 ℃for 5 min. This step was followed by 30 cycles at 95 ℃45 s for denaturation, 58 ℃30 s for annealing, and 72 ℃45 s for extension. The last PCR step was 72 ℃for 8 min.

Each PCR sample (1 μl) was added to 5 μl of the loading buffer (concentrate: 98 % formamide, 0.025 % bromophenol blue, 0.025 % xylene cyanole, and 10

mmol/L EDTA, pH 8.0). The samples were heated at 98 ℃ for 10 min, cooled on ice, then loaded to 12 % denaturing polyacrylamide gel. Electrophoresis was performed at room temperature for a minimum of 5 hours, then the gel was silver stained. The primer sequence used to amplify MyoG intron 1 to detect polymorphism was F: ACAGGAGGCACCCAGACA and R: CATTTGGCCTTG CCTTTAG.

1.4 Statistical analysis

The genotype frequencies from the examined pigs were calculated and tested for significant differences. An analysis of the genotypic effects of the *MyoG* gene was carried out using the GLM procedure of SPSS. The fixed model was:

$$Y_{ijklm} = u + B_i + S_j + Fys_k + M_l + e_{ijklm}$$

Where, Y_{ijklm} is the observed value of mth individual from the breed i, the sex j, of genotype l, in the kth farm-year-season; u is the least square means of the observed values; B_i is the effective value of the breed i; S_j is the effective value of the sex j; fys_k is the effective value of the kth farm-year-season; m_l is the effective value of the genotype l; and e_{ijklm} is the random residual effect corresponding to the observed value.

2 Results

2.1 PCR-SSCP analysis

The PCR products were tested by agarose gel electrophoresis for size confirmation. The PCR product was shown in Fig. 1. The PCR products were subjected to SSCP analysis. One polymorphism was found in the PCR product of intron 1, which resulted in three genotypes, designated *AA*, *AB*, and *BB*, as shown in Fig. 2. Homozygotes were sequenced using dye terminators on an ABI PRISM 3100 Genetic Analyzer. The Blast software was used to assemble the sequences and a G→C transition was detected, as shown in Fig. 3.

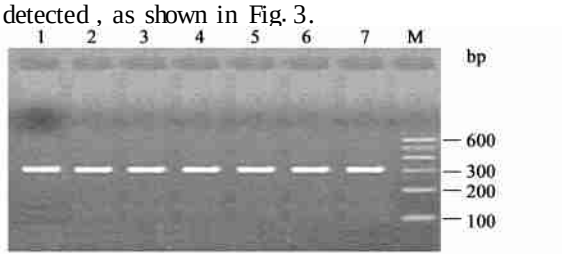

Fig. 1 The PCR product of intron 1 of the myogenin gene (303 bp)

M: 100 bp DNA marker; 1—7: PCR products

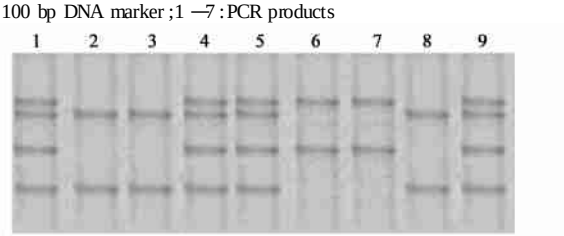

Fig. 2 The SSCP results of the PCR product of intron 1 of the myogenin gene by 12 % PAGE

1, 4, 5, 9: Genotype *AB*; 2, 3, 8: Genotype *AA*; 6, 7: Genotype *BB*

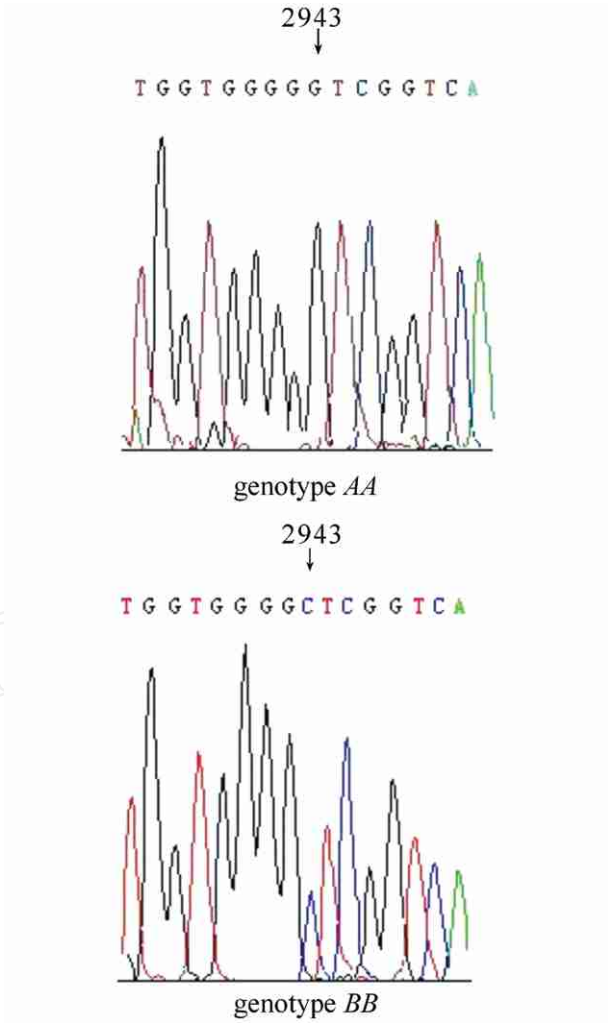

Fig. 3 The sequence comparison of the homozygotes AA **and** BB **of intron 1 of the** MyoG **gene revealed a G→C transition**

2.2 Genotype distribution

Genotype distribution varied with the breeds of the pigs as shown in Table 1. Allele *A*, the dominant gene in Large White, had a frequency of 0.77, whereas allele *B*, the dominant gene in Laiwu pigs, had a frequency of 0.83. The results of the multi comparison showed that the Large White pig breeds compared with the Landrace, the Laiwu and the Yimeng pigs, significant difference ($P < 0.01$) existed. The Laiwu pig breeds compared with the Landrace and the Yimeng pigs, significant difference ($P < 0.05$) were found. Whereas no significant differences existed between the Landrace and the Yimeng pigs (Table 2).

Table 1 Genotype frequencies of porcine myogenin gene in the different pig breeds

Genotype	Large White	Landrace	Yimeng	Laiwu
AA	0.72(49)	0.38(28)	0.33(19)	0.10(8)
AB	0.10(7)	0.16(12)	0.19(11)	0.16(13)
BB	0.18(12)	0.45(33)	0.47(27)	0.75(62)
A	0.77	0.47	0.43	0.17
B	0.23	0.53	0.57	0.83

The number of samples were shown in parentheses

Table 2 χ^2 test of MyoG genotypes in the different pig breeds

Breed	Landrace	Yimeng	Laiwu
Large White	13.505 **	19.329 **	31.668 **
Landrace		1.906	7.968 *
Yimeng			9.749 *

2.3 Effects of the different genotypes

The genotypic effects of the *MyoG* gene are summarized in Table 3. Significant differences ($P < 0.05$) were found among the different *MyoG* genotypes for the birth weight and the tenderness, but not for the average daily gain and the backfat thickness. The least square means and the standard errors of the birth weight, the average daily gain, the tenderness and the backfat thickness were analyzed by the GLM procedure using SPSS and were summarized in Table 4. The meat tenderness of the *AA* pigs was significantly higher ($P < 0.05$) than those of the *BB* pigs. The backfat thickness in the *AB* pigs and the *BB* pigs were both significantly lower ($P < 0.01$) than those in the *AA* pigs.

Table 3 Effects of the breed, sex, Fys, and genotype on the birth weight, the average daily gain, the tenderness, and the backfat thickness

Source	df[2)]	F-value			
		Birth weight	Average daily gain	Meat tenderness	Backfat thickness
Breed	3	13.432 **	10.367 **	13.373 **	8.621 *
Sex	1	1.722	6.078 *	7.118 *	6.438 *
Fys1	3	6.031 *	3.577	1.982	1.733
Genotype	2	10.227 *	4.217	7.038 *	3.982

1) Farm-year-season; 2) degree of freedom.
Values with * differ at $P < 0.05$; Values with ** differ at $P < 0.01$

Table 4 Least square means and standard errors for the birth weight, the average daily gain, the meat tenderness, and the backfat thickness, based on three genotypes

Traits	*AA*	*AB*	*BB*
Birth weight	1.403 ±0.041	1.372 ±0.073	1.394 ±0.107
Average daily gain	128.33 ±0.18	131.24 ±0.44	130.44 ±0.16
Meat tenderness	6.537[a] ±0.137	5.738 ±0.152	5.128[b] ±0.382
Backfat thickness	8.931[a] ±0.213	7.132[b] ±0.411	6.744[b] ±0.128

[a,b]within a row, means without a common superscript letter differ ($P < 0.05$)

3 Discussion

Our results have reported in this study provided important evidence that the presence of a new allele of the *MyoG* gene were associated with several important economic traits in pigs.

The genotype distribution varied with the breeds of the pigs. As previous study by Lin[12] shows that significant differences exist between the exotic pig breeds and the Chinese indigenous pig breeds in their genotype frequencies. Therefore, it may be suggested that the significant differences among the different pig breeds with regard to the growth rate and the meat quality are related to the genotypes of the *MyoG* gene, which result from the long-term selection.

The results of the least square analysis show that significant differences ($P < 0.05$) existed among the different *MyoG* genotypes with regard to the birth weight and the meat tenderness. As previous studies by Tepas[11], Lin[13], and Gao[14] show that significant differences exist among the *MyoG* genotypes for the birth weight. However relationships between the *MyoG* genotypes and the meat tenderness have not been reported. The genotypic effects of the *MyoG* on the meat tenderness were firstly analyzed and reported in this study. Therefore, it may be suggested that *MyoG* gene has some effects on the birth weight and the meat tenderness.

One genetic variation of the porcine *MyoG* gene was detected in this study, which may be associated with the variation in meat quality traits and growth rate. It remains to be further investigated whether the effects are caused by this variation alone or by their linkage disequilibrium with the causative mutations. This identified genetic polymorphism can be used in breeding programs to improve overall meat quality, and thereby the economic value for the pork supply chain and quality products for consumers. Being the limited number of the experimental animals in this study, analyzing more animals may be necessary to confirm the genotypic effects of the *MyoG* gene.

References

[1] Olson E N. MyoD family: a paradigm for development? [J]. Genes Dev, 1990, **4**(9): 1454-1461

[2] Weintraub H, Davis R, Tapscott S, *et al*. The MyoD gene family: nodal point during specification of the muscle cell lineage [J]. Science, 1991, **251**(4995): 761-766

[3] Woodering E W, David A S, Victor K L. Myogenin, a factor regulating myogenesis, has a domain homologous to MyoD [J]. Cell, 1989, **56**(4): 607-617

[4] Montarras D, Chelly J, Bober E, *et al*. Developmental patterns in the expression of Myf-5, MyoD, myogenin, and MRF4 during myogenesis [J]. New Biol, 1991, **3**(6): 592-600

[5] Hasty P, Bradley A, Morris J H, *et al*. Muscle deficiency and neonatal death in mice with targeted mutation in the *myogenin* locus [J]. Nature, 1993, **364**(6437): 501-506

[6] Ernst C W, Mendez E A, Robic A, *et al*. Rapid communication: *myogenin* (*MyoG*) physically maps to porcine chromosome 9q2.1-q2.6 [J]. J Anim Sci, 1998, **76**(1): 328

[7] Van Neck J W, Medina J J, Onnekink C, *et al*. Basic fibroblast growth factor has a differential effect on MyoD conversion of cultured aortic smooth muscle cells from newborn and adult rats [J]. Am J Pathol, 1993, **143**(1): 269-282

[8] Lhonore A, Rana V, Arsic N, *et al*. Identification of a New Hybrid SRF and MEF2-binding element in MyoD enhancer required for *MyoD* expression during myogenesis [J]. Mol Biol Cell, 2007 Mar 21 (Epub ahead of print)

[9] Maak S, Neumann K, Swalve HH. Identification and analysis of putative regulatory sequences for the MYF5/MYF6 locus in different vertebrate species [J]. Gene, 2006, **379**: 141-147

[10] Soumillion A, Erkens J H, Lenstra J A, *et al*. Genetic variation in the porcine myogenin gene locus [J]. Mamm Genome, 1997, **8**(8):

564-568

[11] Tepas M F, Soumillion A, Harders F L, *et al*. Influences of myogenin genotypes on birth weight, growth rate, carcass weight, backfat thickness, and lean weight of pigs[J]. J Anim Sci, 1999, **77**(9): 2352-2356

[12] 林万华,高军,陈克飞,等. 猪 *MyoG* 基因的 PCR-RFLP 多态性分析[J]. 遗传(Lin Wan-Hua, Gao Jun, Chen Ke-Fei, *et al*. Polymorphism analysis of porcine myogenin gene by PCR-RFLP [J]. Hereditas), 2003, **25**(1): 22-26

[13] 林万华,黄路生,艾华水,等. *MyoG* 基因对二花脸猪早期生长性状及肌肉组织学特性的影响[J]. 农业生物技术学报(Lin Wan-Hua, Huang Lu-Sheng, Ai Hua-Shui, *et al*. Influences of *MyoG* genotypes on early growth traits and muscular histological characteristics of Chinese erhualian pigs[J]. J Agricul Biotech), 2002, **10**(4): 367-372

[14] 高勤学,刘梅,杨月琴,等. 猪 *MyoG* 基因的 PCR-RFLP 分型及其与生长性能和肌纤维数目的相关性分析[J]. 中国兽医学报(Gao Qin-Xue, Liu Mei, Yang Yue-Qin, *et al*. Polymorphism analysis of porcine *MyoG* gene by PCR-RFLP and its association with growth traits and fiber number[J]. Chin J Vet Sci), 2005, **25**(3): 330-332

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