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Full Length Research Paper

Polymorphism and association of a microsatellite SJ01 with birth weight and early growth traits in pigs

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Myostatin is a negative regulator of animal skeletal muscle development and *SJ01* is a microsatellite locus flanking porcine *myostatin* gene. In the present study, the polymorphism of microsatellite *SJ01* in three pig populations, that is, Duroc (n = 420), Yorkshire (n = 414) and Landrace (n = 119), were investigated, and its associations with birth weight and early growth traits were analyzed. The results indicated that genotype BB was predominant in Yorkshire pigs and allele B was predominant in both Yorkshire and Landrace pigs, whereas allele A was more prevalent in Duroc pigs. Among the three pig breeds, Duroc population had the highest heterozygosity and deviated from Hardy-Weinberg equilibrium ($P < 0.05$). The average daily gain from 28 d to 70 d in Yorkshire pigs and the body weight at 70 d in Landrace pigs were significantly different between *SJ01* genotypes ($P < 0.05$). These results suggest that microsatellite *SJ01* is a potential DNA marker for early growth trait selection in Yorkshire and Landrace pigs as well as for refining QTL for early growth traits on SSC15.

Key words: Pig, myostatin, microsatellite SJ01, polymorphism, early growth traits.

INTRODUCTION

Myostatin is a negative regulator of skeletal muscle mass in mammals (McPherron et al., 1997). Natural mutations, such as deletions, point mutations in *myostatin* coding region, intronic region or 3′-untranslated region, bring about double-muscling traits in beef cattle (McPherron and Lee, 1997), sheep (Clop et al., 2006; Boman et al., 2009), human (Schuelke et al., 2004) and dogs (Mosher et al., 2007). In pigs differing in muscular traits, such as wild boar, Pietrain and Laiwu pigs, however, no mutation with similar effect in *myostatin* gene was reported (Jiang et al., 2002a; Stinckens et al., 2008). Recent studies reported associations of polymorphisms in the promoter region of porcine *myostatin* gene with production traits (Jiang et al., 2002b), meat quality traits (Guimaraes et al., 2007) and mRNA level (Stinckens et al., 2008), suggesting that, in pigs, myostatin might also play important roles in the development of skeletal muscle and its related traits.

Microsatellite loci are frequently used as DNA markers to locate quantitative trait loci (QTL), but usually to a relatively wide range of chromosomal fragment, in which many genes reside. To find the functional gene(s) or quantitative trait nucleotide (QTN) fine mapping with more markers is required. Microsatellite *SJ01*, which was identified by our previous study, is a locus located 42 kb downstream of porcine *myostatin* gene (Jiang et al., 2004) whether it is associated with production traits and can be used as DNA marker remain unknown.

Birth weight and early growth traits were two economically important traits in that, on one hand, they directly affect the mortality rate of weanling pigs and on another hand, are associated with growth rate and carcass traits on test. A QTL mapping study based on a F2 pedigree constructed with wild boar ×Yorkshire suggested one QTL for average daily gain from birth to 70 d on SSC15 (50 cM, male; 78 cM, female) (Knott et al., 1998), the mapping interval was partially overlapped with *myostatin* gene and *SJ01* locus.

The production traits associated polymorphisms involve three sites in the promoter region of porcine *myostatin* gene (Jiang et al., 2002a; Guimaraes et al., 2007; Yu et al., 2007; Stinckens et al., 2008), the detection of which is not practical for use in molecular pig breeding. Therefore,

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Table 1. Means and s.d. of birth weight and early growth traits.

Trait	Breed		
	Duroc	**Yorkshire**	**Landrace**
BW0 (kg)	1.44 ± 0.31	1.49 ± 0.31	1.46 ± 0.25
BW28 (kg)	7.12 ± 1.52	7.53 ± 2.05	8.53 ± 2.15
BW70 (kg)	21.13 ± 5.27	21.78 ± 5.53	24.77 ± 5.65
ADG1 (kg)	0.20 ± 0.05	0.20 ± 0.06	0.25 ± 0.08
ADG2 (kg)	0.36 ± 0.10	0.50 ± 0.13	0.35 ± 0.07

BW0, BW28 and BW70 represent birth weight, body weight at 28 d and body weight at 70 d, respectively.

ADG1 and ADG2 represent average daily gain from birth to 28 d and from 28 to 70 d, respectively.

It is necessary to find another closely linked marker. Given the important role of *myostatin* in meat traits and the short physical distance between *myostatin* and *SJ01*, we speculate that *SJ01* is likely associated with some production traits in pigs and therefore is a candidate marker. In this study, the polymorphism at *SJ01* locus and its associations with porcine body weight and early growth traits were analyzed in three pig breeds (Duroc, Yorkshire and Landrace) which are currently used for worldwide pork production.

MATERIALS AND METHODS

Population and traits

Three western pig breeds, including 420 Duroc, 414 Yorkshire and 119 Landrace individuals were randomly sampled from Pig Breeding Center of Shandong Academy of Agricultural Sciences. Animals were reared under the same environment and had free access to feed and water. Data of the traits including birth weight (BW0), body weight on day 28 (BW28) and body weight on day 70 (BW70) of each individual were collected on farm. Sampling was performed by taking a notch from the ear of pigs and put into a 1.5-mL Eppendorf tube containing 70% ethanol and stored at −20ºC. The total number of production records was 2757 and the mean and standard deviation (s.d.) of the five traits are presented in Table 1.

Isolation of genomic DNA

Genomic DNA from ear notch was isolated with phenol/chloroform method (Sambrook and Russell, 2001), dissolved in Tris-EDTA buffer (pH 8.0) and stored at −20ºC. The concentration was checked by running 0.7% agrose electrophoresis and using UV spectrophotometer.

Genotyping

Primers for *SJ01* were from STS database of National Centre for Biological Information (UniSTS accession number: 81993): Forward 5′-CAGAACATAAATGCCAAGAG-3′ and Reverse 5′-AGTATTTA GTGAACACCTCG-3′. PCR was performed by mixing 0.3 µL (50 - 100 ng) of genomic DNA, 1.6 µL of dNTPs (2.5 mM each), 1.2 µL of $MgCl_2$ (25 mM), 0.5 µL each of primers (10 µM), 0.1 µL of r*Taq* polymerase (5 U/µL, TaKaRa, Dalian China) and 2.0 µL of 1×r*Taq* buffer in a 20 µL volume and running on a Mastercycler gradient (Eppendorf, Germany) according to the following program: 95ºC for 3 min, 32 cycles of 95ºC for 30 s, 55ºC for 30 s and 72ºC for 30 s and final extension at 72ºC for 5 min. The PCR products were electrophoresed on 1.5% agarose gel to check amplification efficiency.

Genotyping of *SJ01* was carried out by running 12% polyacrylamide gels (arc : bis = 19:1) with 1×TBE as electrophoresis buffer at 180 V for 10 h. After electrophoresis, the gels were silver-stained and the genotype was determined according to the electrophoresis band patterns.

Sequencing

The PCR products were purified with Gel Extraction System B (BioDev, China), inserted into pMD-18T vector and was used to transform *E. coli* DH5 α competent cells. At least four clones were sequenced using the BigDye v3.1 kit (Applied Biosystem) by Shanghai Songon Co. Ltd (Shanghai, China).

Statistics

The genotype frequency, allele frequency, observed heterozygosity (Ho), expected heterozygosity (He), average heterozygosity (Ha), number of available alleles (na), number of effective alleles (ne) and polymorphic information content (PIC) were obtained and the deviation from Hardy-Weinberg equlibrium was tested with Popgen 32 software.

Associations of *SJ01* genotypes with BW0, BW28, BW70, average daily gain from birth to day 28 (ADG1) and average daily gain from day 28 to day 70 (ADG2) were performed separately in Duroc,Yorkshire and Lanrace pigs with the PROC GLM procedure of SAS 8.2 software (SAS Institute Inc, 1998). In the statistics model, genotype, farm-year-season and sex were treated as fixed effects, litter size as co-variable to eliminate the influences of litter size on birth weight (Model 1), birth weight as co-variable to eliminate the influences of birth weight on body weight (Model 2) and start body weight as co-variable to eliminate the influences of start body weight on average daily gain (Model 3).

Model 1: $Y_{ijklm}=\mu+D_i+C_j+S_k+Z_l+e_{ijklm}$;

Model 2: $Y_{ijklm}=\mu+D_i+C_j+S_k+B_l+e_{ijklm}$;

Model 3: $Y_{ijklm}=\mu+D_i+C_j+S_k+P_l+e_{ijkml}$;

where Y_{ijklm} is the birth weight (BW0), body weight (BW28, BW70)

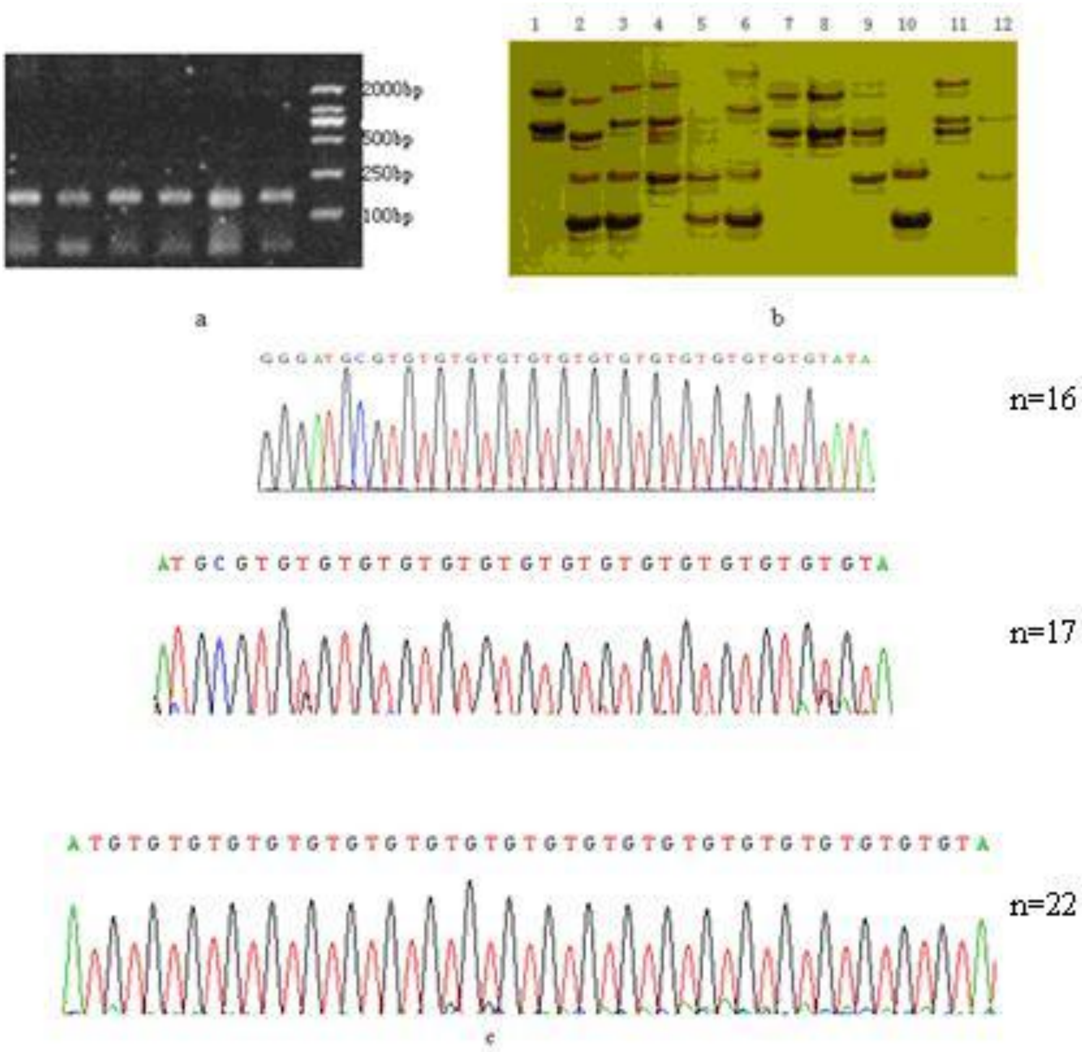

Figure 1. Genotyping and identification by sequencing of microsatellite *SJ01* locus. a. PCR amplification; b. genotyping. Lanes 1-11 represent genotypes AA, BD, AB, AC, BC, BE, DD, DD, CD, BB, AD and CC, respectively. c. Sequencing result, indicating dinucleotide (TG) repeats of 16, 17 and 22, respectively.

and average daily gain (ADG1, ADG2) of each individual for models 1-3 respectively; μ is the overall mean of population; D_i is the mean effect of genotype i; C_j is the farm-year-season effect; S_k is the effect of sex; Z_l is the effect of litter size (Model 1); B_l is the effect of birth weight (Model 2); P_l is the effect of start body weight (Model 3); e_{ijklm} is the residual effect.

The difference between genotypes was considered as significant when $P < 0.05$.

RESULTS

Genotypes and alleles

Analysis of *SJ01* polymorphism revealed 13 genotypes (AA, AB, BB, BC, BD, BE, AC, AD, AE, CC, CD, DD and EE) and five alleles (A, B, C, D and E) in Duroc (n = 420), Yorkshire (n = 414) and Landrace (n = 119) pig populations. The electriphoresis result of genotypes AA, BD, AB, AC, BC, BE, DD, CD, BB, AD and CC is shown in Figure 1b. Among the five alleles, alleles A and B were reported by our previous study, which were composed of 13 and 19 repeats of dinucleotide (TG), respectively (Jiang et al., 2004); alleles C, D and E were composed of 16, 17 and 22 repeats of dinucleotide (TG), respectively (Figure 1c) and were reported for the first time by present study.

Population genetic parameters at SJ01 locus

Differences in genotype frequency and allele frequency were found between three pig populations of Duroc, Yorkshire and Landrace (Table 2). For genotypes with frequency exceeding 0.1, genotypes AB, AA and AD in Duroc, AB, BB and BE in Yorkshire and BB and BD in Landrace were identified. Genotypes BC, AE, CC, CD and EE occurred at low frequencies in all of the three pig

Table 2. Genotype frequency of microsatellite *SJ01* in Duroc, Yorkshire and Landrace populations.

Breed (n)	AA	AB	BB	BC	BD	BE	AC	AD	AE	CC	CD	DD	EE
Duroc (420)	0.2167(91)	0.2905(122)	0.0762(32)	0.0048(2)	0.081(34)	0.0024(1)	0.0357(15)	0.2500(105)	0.0000(0)	0.0024(1)	0.0143(6)	0.0262(11)	0.0000(0)
Yorkshire(414)	0.0024 (1)	0.1232(51)	0.6594(273)	0.0000(0)	0.0773(32)	0.1232(51)	0.0000 (0)	0.0024 (1)	0.0048(2)	0.0000(0)	0.0000(0)	0.0024 (1)	0.0048(2)
Landrace(119)	0.0000 (0)	0.0000(0)	0.5210(620)	0.0000(0)	0.4034(48)	0.0084 (1)	0.0000 (0)	0.0000 (0)	0.0000(0)	0.0000(0)	0.0000(0)	0.0672 (8)	0.0000(0)

Table 3. Allele frequency and population genetic parameters of microsatellite *SJ01* in Duroc, Yorkshire and Landrace populations.

Breed	A	B	C	D	E	H-W test	Ho	He	Ha	na	ne	PIC
Duroc	0.5048	0.2655	0.0298	0.1988	0.0012	0.0252	0.6786	0.6351	0.6343	5.0000	2.7347	1.1309
Yorkshire	0.0676	0.8213	-	0.0423	0.0688	0.4226	0.3309	0.3148	0.3144	4.0000	1.4587	0.6618
Landrace	-	0.7269	-	0.2689	0.0042	0.9308	0.4118	0.4010	0.3993	3.0000	1.6647	0.6080

Table 4. Least squares means for performances among genotypes of microsatellite *SJ01* in Duroc populations.

Performance	AA	AB	AC	AD	BD	P-value
BW0	1.41 ± 0.03 (n = 88)	1.47 ± 0.03 (n = 112)	1.41 ± 0.09 (n=13)	1.47 ± 0.03 (n = 101)	1.49 ± 0.05 (n = 34)	0.7686
BW28	7.18 ± 0.17 (n = 67)	7.28 ± 0.16 (n = 72)	7.12 ± 0.50 (n=8)	7.10 ± 0.18 (n = 57)	7.06 ± 0.26 (n = 27)	0.8664
BW70	21.31 ± 0.72 (n = 57)	21.51 ± 0.64 (n = 71)	19.39 ± 2.01 (n=7)	20.00 ± 0.73 (n = 56)	20.76 ± 1.13 (n = 22)	0.3297
ADG1	0.20 ± 0.01 (n = 65)	0.21 ± 0.01 (n = 66)	0.20 ± 0.02 (n=7)	0.20 ± 0.01 (n = 55)	0.20 ± 0.01 (n = 27)	0.8664
ADG2	0.36 ± 0.01 (n = 43)	0.36 ± 0.01 (n = 43)	0.36 ± 0.07 (n=2)	0.35 ± 0.02 (n = 29)	0.36 ± 0.02 (n = 17)	0.9808

BW0, BW28 and BW70 represent birth weight, body weight at 28 d and body weight at 70 d, respectively.
ADG1 and ADG2 represent average daily gain from birth to 28 d and from 28 to 70 d, respectively.

populations. In Yorkshire pigs, genotype BB was predominant, while in Duroc and Landrace pigs, no predominant genotype was found. As for allele frequencies, five, four and three alleles were detected in Duroc, Yorkshire and Landrace pig populations, respectively and allele B was predominant in Yorkshire and Landrace, whereas allele A was more prevalent in Duroc populations (Table 3). At *SJ01* locus, Yorkshire and Landrace, but not Duroc, were in a state of Hardy-Weinberg equilibrium (P > 0.05). Information obtained from other genetic parameters of Ho, He and PIC (Table 3) indicated that, at this locus, Duroc is genetically more diverse compared to Yorkshire and Landrace pig populations.

Associations of SJ01 genotype with birth weight and early growth traits

The relationship of *SJ01* genotype with birth weight and some early growth traits, that is, body weight at 28 d and 70 d and average daily gain from birth to 28 d and from 28 to 70 d, were analyzed in present study. The results showed that, in Duroc pig populations, differences in birth weight, body weight at 28 and 70 d and average daily gain traits were not significant between genotypes of *SJ01* locus (P > 0.1) (Table 4); whereas in Yorkshire pig populations, the least squares means for average daily gain from 28 to 70 d were significantly different among genotypes of *SJ01* locus (P < 0.05) (Table 5) and in Landrace pigs, the least squares means for body weight at 70 d were significantly different among genotypes of *SJ01* locus (P < 0.05) (Table 6). The effect of *SJ01* on

Table 5. Least squares means for performances among genotypes of microsatellite *SJ01* in Yorkshire populations.

Performance	AB	BB	BD	BE	P-value
BW0	1.44 ± 0.06 (n =51)	1.48 ± 0.02 (n = 272)	1.57±0.07 (n = 32)	1.56±0.05 (n = 51)	0.2361
BW28	7.54 ± 0.34 (n =34)	7.63 ± 0.17 (n = 161)	7.01±0.49 (n = 16)	6.80±0.37 (n = 29)	0.1581
BW70	20.45 ± 1.01 (n = 28)	22.05 ± 0.40 (n = 176)	22.48±1.21 (n = 19)	19.87±0.84 (n = 40)	0.0631
ADG1	0.21 ± 0.01 (n = 30)	0.21 ± 0.00 (n = 149)	0.17±0.02 (n = 12)	0.19±0.01 (n = 28)	0.0910
ADG2	0.42 ± 0.03 (n = 18)	0.49 ± 0.01 (n = 104)	0.53±0.04 (n = 9)	0.47±0.02 (n = 23)	0.0464

BW0, BW28 and BW70 represent birth weight, body weight at 28 d and body weight at 70 d, respectively.
ADG1 and ADG2 represent average daily gain from birth to 28 d and from 28 to 70 d, respectively.

Table 6. Least squares means for performances among genotypes of microsatellite *SJ01* in Landrace populations.

Performance	BB	BD	DD	P-value
BW0	1.46 ± 0.03 (n = 61)	1.44 ± 0.04 (n = 48)	1.47 ± 0.10 (n = 8)	0.8384
BW28	8.35 ± 0.25 (n = 49)	8.51 ± 0.36 (n = 25)	7.14 ± 1.22 (n = 2)	0.5400
BW70	24.53 ± 0.83 (n = 27)	26.60 ±1.33 (n = 9)	33.02 ± 2.83 (n = 2)	0.0268
ADG1	0.25 ± 0.01 (n = 48)	0.25 ± 0.01 (n = 25)	0.20 ± 0.04 (n = 2)	0.5400
ADG2	0.38 ± 0.03 (n = 20)	0.35 ± 0.04 (n = 3)	-	0.6554

BW0, BW28 and BW70 represent birth weight, body weight at 28 d and body weight at 70 d, respectively.
ADG1 and ADG2 represent average daily gain from birth to 28 d and from 28 to 70 d, respectively.

other production traits of Yorkshire and Landrace pigs was not significantly different (P > 0.5).

DISCUSSION

Myostatin is an important growth/differentiation factor regulating skeletal muscle development, therefore, is also associated with meat production and quality traits as well as with other related traits. Porcine *myostatin* gene is mapped in a *SW1065*-(53.1)-*SW1263*- (55.0)-*myostatin*-(55.0)-*SWR1533* -(57.9) linkage group on SSC15q2.3 (Sonstegard et al., 1998), the flanking microsatellite loci *SW1263* and *SWR1553* were located at 55.0 and 57.9 cM, respectively. The microsatellite locus *SJ01* is located 42 kb downstream of *myostatin* gene, more close to *myostatin* gene than *SWR1553*. Compared with other microsatellite loci located in nonstructural gene region, the number of genotypes and alleles were rather few (Jiang et al., 2004 and present study) and the heterozygosity and diversity were rather low for *SJ01*, suggesting relatively higher selection force and more conservation in this region than microsatellite loci located in nonstructural gene regions. According to the results of *SJ01* polymorphism, of the three pig breeds used in this study, Duroc pigs were more diverse and its genetic component was more complex.

A QTL for average daily gain from birth to 70 d was mapped on SSC15 (50 cM, male; 78 cM, female) (Knott et al., 1998), where *myostatin* gene and *SJ01* locus also reside. In this study, *SJ01* locus is found to be associated with average daily gain from 28 to 70 d in Yorkshire pigs and with body weight at 70 d in Landrace pigs. Besides, a nearly significant association was also noted for *SJ01* with body weight at 70 d and average daily gain from birth to 28 d in Yorkshire pigs. These data suggest that this region likely harbors a QTL affecting early growth and *SJ01* is a useful marker for refining this QTL.

Growth traits, such as body weight and average daily gain, are controlled by multiple genes. In addition to the aforementioned QTL for average daily gain from birth to 70 d on SSC15 (Knott et al., 1998), other QTLs for body weight of 10 weeks were found on SSC1, 4, 6, 7 and 9 and for average daily gain from 4 weeks to 13 weeks on SSC9 and SSC10, from 3 weeks to 10 weeks on SSC 1, 2, 4 and 7, respectively (http://www. animalgenome.org/ QTLdb /pig.html). The effect of each gene or QTL on early growth traits is somewhat dependent on genetic background and environmental effect, giving rise to the differences between breeds concerning the relationship of *SJ01* genotype with these traits, as shown in this study.

Although three relatively large populations of Duroc, Yorkshire and Landrace were used for association study, some genotypes were present at low frequency, that is, genotype DD in Landrace population. The effect of SJ01 genotype on birth weight at 70 d in Landrace pigs requires further research with a large population containing more individuals with genotype DD, or in some pedigrees segregating at this locus.

In conclusion, *SJ01* is a potential DNA marker for average daily gain from 28 to 70 d in Yorkshire and for body weight at 70 d in Landrace pigs and is useful for refining QTL position for early growth traits.

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