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Association of a synonymous mutation of the *PGAM2* gene and growth traits in rabbits

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ABSTRACT: Phosphoglycerate mutase (PGAM2) catalyzes the conversion of 3-phosphoglycerate into 2-phosphoglycerate and releases energy during glycolysis in muscle tissues. *PGAM2* has been considered as a candidate gene to influence growth, development, and carcass traits in livestock. The aim of this study was to investigate the association between polymorphisms of *PGAM2* and growth traits in rabbits. Three single nucleotide polymorphisms (SNPs) were identified by direct sequencing in 20 random individuals from three breeds, including c.-10C>T, c.195C>T, and c.414+17C>T. The c.195C>T was genotyped by PCR-RFLP in a total of 222 rabbits of three breeds (Tianfu black, 53 animals; Ira, 91 animals; Champagne, 78 animals). The average allele frequency among the breeds was 0.52 for allele *T* and 0.48 for *C*. The heterozygosity and effective number of alleles were 0.4992 and 1.996, respectively. The association results revealed the *CT* genotype of c.195C>T was associated significantly ($P < 0.05$) with greater body weight at 84 days of age (BW84) and with average daily weight gain (ADG). However, association of the genotypes with other production traits was not observed. The results of this study suggested *PGAM2* is one of the candidate genes affecting BW84 and ADG in the rabbit.

Keywords: SNPs; body weight; average daily weight gain; association analysis; candidate gene

INTRODUCTION

Phosphoglycerate mutase (PGAM) is the glycolytic enzyme that catalyzes the conversion of 3-phosphoglycerate into 2-phosphoglycerate. In mammalian tissues, PGAM is a dimer of two distinct 30 kDa subunits, including the ubiquitously expressed brain form (B form, known also as PGAM1) and the muscle form (M form, known also as PGAM2) expressed only in adult skeletal and cardiac muscles. The two forms consist of three types of PGAM dimers (MM, BB, and MB) (Zhang et al. 2001; Johnsen and Schonheit 2007). The isozyme pattern of human PGAM2 is regulated developmentally during myogenesis. Some mutations have been shown to cause PGAM2 deficiency in humans, which resulted in serious muscle dysfunction with exercise intolerance, cramps, myoglobinuria, scattered atrophic and hypertrophic fibres (Tsujino et al. 1995; Tonin et al. 2009).

The porcine *PGAM2* gene was mapped to SSC18q13-q21 (Davoli et al. 2002; Qiu et al. 2008), where it has been suggested to encompass several quantitative trait loci (QTLs) (the Pig QTL Database is available at http://cn.animalgenome.org/cgi-bin/QTLdb/index) for fat ratio, dressing percentage, diameter of muscle fibre, and lean percentage (Geldermann et al. 2003; Fontanesi et al. 2004; Wimmers et al. 2006). Several studies in pig have described the protein PGAM2 is expressed at a high level in skeletal muscle during all stages of development investigated and related to growth, feed conversion, and slaughter traits (Fontanesi et al. 2004, 2008; Qiu et al. 2008). In cattle, it has

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been associated with beef tenderness and post mortem maturation (Orru et al. 2009; Stella et al. 2011; Dunner et al. 2013). Together, the results of these studies suggested *PGAM2* could be considered as a candidate gene for growth traits due to its fundamental roles in muscle development.

To our knowledge, the association of *PGAM2* polymorphisms and potential association with growth traits in the rabbit have not been reported. Therefore, it is helpful to investigate polymorphisms in the rabbit *PGAM2* gene among different breeds and to study the association between genotype and relative quantitative traits in order to identify breeding selection markers.

MATERIAL AND METHODS

Animals, growth traits, and carcass traits. This study was done with three breeds of rabbits bred commercially for meat (Tianfu black, 53 animals; Ira, 91 animals; Champagne, 78 animals). The rabbits were kept under similar feeding and management conditions. Rabbits were weaned at 28 days of age and fed commercial pelleted food until 84 days of age. Nutritional levels and feeding management were as described by Zhang et al. (2011). In brief, the food (16% crude protein, 10.8 MJ/kg digestible energy) was restricted to ~80% of average *ad libitum* intake and water was available *ad libitum*. Body weight (BW) was recorded for each rabbit at 28 (BW28), 35 (BW35), 70 (BW70), and 84 (BW84) days of age, and average daily weight gain (ADG) was calculated for all rabbits in 28–84 days of age. The animals were slaughtered when 84 days old and the carcass traits, including semi-eviscerated weight, eviscerated weight, semi-eviscerated slaughter rate, and eviscerated slaughter rate were recorded as described by Blasco et al. (1993). All procedures involving animals were done according to protocols approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University.

Genomic DNA extraction and mutation screening. Genomic DNA was extracted from ear tissues using an AxyPrep Genomic DNA Miniprep Kit (Axygen Scientific, Union City, USA) and stored at –20°C. Two polymerase chain reaction (PCR) primer pairs were designed using Primer 5.0 software (Version 5.0, 2000) to amplify all coding regions of the three *PGAM2* gene exons (accession number NC_013678.1) (Table 1). For each primer pair, 20 animals were selected at random for mutational analysis. PCR was done in a final volume of 30 µl containing 15 µl of 2× *Taq* PCR MasterMix (Tiangen Biotech, Beijing, China), 1.2 µl of each primer (10 pmol/µl), 3 µl of DNA template, and 9.6 µl of double-distilled water. The PCR amplification protocol was as follows: 5 min at 94°C; then 34 amplification cycles of 30 s at 95°C, 30 s at the appropriate annealing temperature (Table 1), 50 s at 72°C; and a final extension step at 72°C for 10 min. The purified PCR products were sequenced directly on ABI Prism 3700 DNA Analyzer (Applied Biosystems, Foster City, USA) in both directions using a BigDye Terminator sequencing kit (Applied Biosystems) according to the manufacturer's instructions.

Genotyping using PCR-RFLP. The SNP c.195C>T was genotyped by PCR-RFLP using FastDigest *Csp*6I restriction enzyme (Fermentas, Vilnius, Lithuania). Briefly, 3 µl of PCR product PG1F-PG1R were digested at 37°C for 5 min in a total volume of 10 µl with 3 U of *Csp*6I. The digestion products were separated in 2% (w/v) agarose gel, observed and photographed with a gel documentation system.

Data analysis. The DNA sequences were assembled and analyzed with the DNAstar program (Version 7.1.0, 2006). Genotype and allele frequencies were calculated directly, heterozygosity (He), effective number of alleles (Ne) and polymorphism information content (PIC) were calculated according to Nei and Roychoudhury (1974). The effect of each genotype on the traits was analyzed by the Least-Squares Method as applied in the General Linear Models (GLM) procedure of SAS (Statisti-

Table 1. Primer sequences, PCR amplicon sizes, T_a value and location

Primer names	Primer sequences (5' → 3')	Amplicon (bp)	Annealing (°C)	Location	Note
PG1	F: GAATGCTGATTGGCAGTTGGC R: CCAGTTGTCTGAAACCCCTGTG	855	62	5´ UTR to intron 2	sequencing PCR-RFLP
PG2	F: TGCTTGGTCCCGCCTTGA R: GGAGTCCTGCGTGTCCGTGT	803	63	intron 2 to 3´ downstream	sequencing

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cal Analysis System, Version 9.2, 2002) according to the following statistical model:

$$Y_{ijkl} = \mu + G_i + M_j + B_k + e_{ijkl}$$

where
Y_{ijkl} = record of the trait
μ = overall mean of observations
G_i = gender effect
M_j = fixed genotype effect of *PGAM2*
B_k = fixed breed effect
e_{ijkl} = residual error

Additive genetic effect (a) and dominance effect (d) were calculated as described by Fontanesi et al. (2012). In brief, the additive genetic effect for the *PGAM2* genotypes was estimated as half of the difference between values of the two homozygous groups. The dominance effect at the *PGAM2* locus was estimated as the difference between the values of the heterozygous group and the average of the values of the two homozygous groups. The ratio $|d/a|$ was considered to indicate actual gene effects irrespective of significance (Stuber et al. 1987): $|d/a| < 0.2$, additive; $0.2 < |d/a| < 0.8$, partial dominance; $0.8 < |d/a| < 1.2$, dominance; $|d/a| > 1.2$, overdominance.

RESULTS

We amplified and sequenced the exons and their flanking introns of the rabbit *PGAM2* gene with two PCR primer pairs. Mutational analysis revealed a total of three SNPs: 5′-untranslated region (c.-10C>T), exon 1 (c.195C>T), and intron 1 (c.414+17C>T), by scoring relative to the reference sequence in GenBank (accession number NC_013678.1). The c.195C>T SNP was identified in the coding region of exon 1 and did not cause amino acid change. By *Csp*6I PCR-RFLP and electrophoresis, two alleles were detected in SNP c.195C>T. Allele *C* resulted in fragments of 242 and 613 bp, and allele *T* had fragments 242, 304, and 309 bp (fragments 304 and 309 bp appeared as a single band after electrophoresis) (Figure 1). We genotyped it in a total of 222 animals from three breeds and investigated the association with growth or carcass traits in commercial meat rabbits.

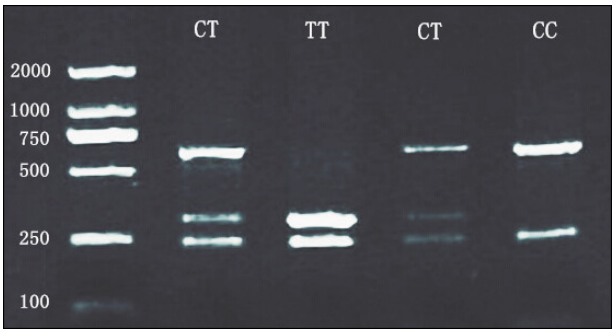

Figure 1. The PCR-RFLP patterns of the rabbit *PGAM2* gene

The genotypes and average allele frequencies in exon 1 of *PGAM2* in the different rabbit breeds are given in Table 2. For these samples, *CT* was the predominant genotype in each breed. *C* was the predominant allele in Champagne, but *T* was the predominant one in Tianfu and Ira. The polymorphic site showed a high degree of heterozygosity (He = 0.4992) and a high effective number of alleles (Ne = 1.996). The genetic diversity was reasonably informative (PIC = 0.3746) in these samples.

Association analysis between this SNP and the recorded traits indicated c.195C>T genotypes are associated significantly with BW84 and ADG (Table 3). Rabbits with genotype *CT* had a higher level of performance compared to those with genotype *CC*. For BW84, the estimated additive genetic effect (a) was −19.15 ± 23.34 g but this was not statistically significant. The estimated dominance genetic effect (d) was 67.50 ± 28.94 g, which was statistically significant ($P = 0.02$). When $|d/a| = 3.5$, therefore, we might assume overdominance at this locus. For ADG, the estimated genetic effects

Table 2. Allele and genotype frequencies of SNP c.195C>T of *PGAM2* in three rabbit breeds

Breeds (n)	Genotype frequency			Allele frequency		Genetic characteristics		
	CC	*CT*	*TT*	*C*	*T*	He	PIC	Ne
Tianfu rabbit (53)	0.11 (6)	0.74 (39)	0.15 (8)	0.48	0.52	0.50	0.37	2.00
Ira rabbit (91)	0.14 (13)	0.46 (42)	0.40 (36)	0.37	0.63	0.47	0.36	1.88
Champagne rabbit (78)	0.22 (17)	0.77 (60)	0.01 (1)	0.60	0.40	0.48	0.36	1.19
Total (222)	0.16 (36)	0.64 (141)	0.20 (45)	0.48	0.52	0.50	0.37	2.00

He = heterozygosity, PIC = polymorphism information content, Ne = effective number of alleles

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Table 3. Least Squares Means of growth and carcass traits of different genotypes

Traits	Genotypes		
	CC (*n* = 36)	*CT* (*n* = 141)	*TT* (*n* = 45)
28-day weight (g)	527.98 ± 15.10	534.49 ± 7.67	509.47 ± 14.87
35-day weight (g)	814.93 ± 40.48	829.61 ± 20.84	773.37 ± 38.89
70-day weight (g)	2159.89 ± 28.10	2145.08 ± 14.31	2154.61 ± 26.63
84-day weight (g)	2476.90 ± 33.74^{b}	2563.55 ± 17.18^{a}	2515.20 ± 31.97^{ab}
ADG (g)	34.75 ± 0.57^{b}	36.16 ± 0.29^{a}	36.17 ± 0.54^{ab}
Semi-eviscerated weight (g)	1384.84 ± 24.49	1433.68 ± 12.47	1382.30 ± 23.56
Semi-eviscerated slaughter percentage	0.56 ± 0.0037	0.57 ± 0.0019	0.56 ± 0.0035
Eviscerated weight (g)	1281.63 ± 22.96	1325.23 ± 11.69	1274.03 ± 22.08
Eviscerated slaughter percentage	0.52 ± 0.0037	0.53 ± 0.0019	0.52 ± 0.0036

ADG = average daily weight gain on days 28–84 of age
data are expressed as Least Squares Means ± standard errors (mean ± SE)
in the same row, different lowercase letters mean significant difference at 0.05 levels

(*a* or *d*) were –0.71 ± 0.39 g and 0.70 ± 0.49 g, respectively, but neither was statistically significant. When $|d/a| = 0.99$, therefore, we might assume dominance at this locus.

DISCUSSION

PGAM2 has key roles in the glycolysis process controlling postnatal development and related meat quality parameters as well as feed conversion, growth rate, muscle mass, and fat deposition traits in other species (Fontanesi et al. 2004; Orru et al. 2009; Dunner et al. 2013). In the present study, there was only one SNP detected within the entire coding region, which suggests the relative low diversity of the rabbit *PGAM2* gene and is consistent with that in porcine (Fontanesi et al. 2008). The exonic SNP c.195C>T was found to be associated significantly with growth traits, lacking consistent association between the *PGAM2* SNPs and carcass and meat quality traits,-which could be owing to species differences, population size, and breed-specific effects.

For c.195C>T, the genetic diversity value (PIC = 0.3746) indicated a high level of genetic variation and a selection potential that could be expected to achieve more genetic progress. We might assume overdominance and dominance for BW84 and ADG at the locus. At the same time, a heterozygous genotype had the highest frequency, which could be due to the advantage of the heterozygous genotype compared to the genotype *CC*; in turn, this might act by maintaining this superiority in a population selected strongly towards increased BW84 and ADG.

The histidine phosphatase superfamily is a functionally diverse set of proteins: cofactor-dependent and cofactor-independent PGAM (dPGM and BPGM, respectively), fructose-2,6-bisphosphatase (F26BP), Sts-1, SixA, and related proteins (Rigden 2008). Genetic variation of human members resulted in many impaired biological functions. In summary, their functions include roles in metabolism, signaling, immune response or regulation (Watkins and Baker 2006; Mikhailik et al. 2007; Marchler-Bauer et al. 2011). Although the superfamily is overwhelmingly composed of phosphatases, the earliest known and arguably best-studied member is dPGM, and a histidine phosphatase domain was found in the N terminus of PGAM2 (Rigden 2008). SNP c.195C>T (Arg65) is located in the sequence coding for N terminus of PGAM2. Although the genetic variation in rabbit did not result in an amino acid change, association analysis revealed it was related significantly with growth traits, suggesting genetic variation in the sequence coding for N terminus of PGAM2 could influence the growth traits. However, we could not exclude the possibility that this synonymous mutation might be able to cause changes in protein expression, conformation, and function (Sauna and Kimchi-Sarfaty 2011). The potential biological effect of the synonymous SNP in *PGAM2* on growth traits in the rabbit requires further investigation.

As post-weaning growth rate is a cost-effective parameter to record in practice, this study shed

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light on the polymorphisms and the association of *PGAM2* with growth traits in the rabbit. These results could be valuable for the guidance of breeding strategies to improve growth efficiency in commercial meat rabbit populations. *PGAM2* might be a good gene for meat and/or carcass traits but further studies are warranted to validate these results in large commercial populations and in other breeds.

CONCLUSION

To summarize, SNP c.195C>T in the rabbit *PGAM2* gene was genotyped in a total of 222 rabbits of three breeds. Association analysis indicated that this polymorphism was significantly linked with BW84 (P = 0.0206) and ADG (P = 0.0251). Individuals with the *CT* genotype reached a higher level of performance on the recorded traits compared to those with the *CC* genotype. However, association of the genotypes with other production traits was not observed.

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