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SHORT COMMUNICATION

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Myopalladin gene polymorphism is associated with rabbit meat quality traits

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ABSTRACT

The objective of this study was to investigate the effect of the polymorphism in the Myopalladin (*MYPN*) gene on meat quality traits in the Hyla, Champagne, Tianfu Black rabbit breeds using PCR and DNA sequencing. A novel SNP (g.18497416 G > A) was found at 229 bp in exon 13 of chromosome 18. The three rabbit populations had intermediate levels of genetic diversity according to their polymorphism information content values. The statistical analysis indicated that rabbits with the GG genotype had a significantly greater a^*_{24h} and b^*_{24h} than rabbits with the AA genotype ($p < .05$) in the *longissimus dorsi* muscle and that animals with the AA genotype had substantially higher a^*_{0h} and a^*_{24h} than those with the AG genotype ($p < .05$) in the *biceps femoris* muscle. The rabbits with the AA genotype had the highest intramuscular fat (IMF) values and those with the GG genotype had the lowest IMF values in both *longissimus dorsi* and *biceps femoris* muscles. These results indicated that *MYPN* could be considered as candidate genes for genetic improvement of rabbit meat traits.

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Rabbit; MYPN polymorphism; PH; meat colour; intramuscular fat

Introduction

The myopalladin (*MYPN*) gene codes for a multifunctional protein that plays a central role in the organisation and tethering of nebulin and α-actinin in vertebrate Z-lines (McElhinny et al. 2003). This is a signature domain of this protein family and may have a special role in creating the highly ordered cytoskeleton of the sarcomere (Gilbert et al. 1999). Recently, swine research showed that single nucleotide polymorphisms in the *MYPN* gene were associated with lean cuts (LC), visible intermuscular fat (VIF), meat colour and tenderness (Wimmers et al. 2007; Zhai et al. 2010; Braglia et al. 2013). Research in beef cattle indicated that SNP of the MYPN gene affected loin-eye area and water-holding capacity of beef (Jiao et al. 2010) and loin muscle area (Hong et al. 2011).

Rabbit meat has lower fat content and high protein level than meat from other animals, thus it could provide consumers with abundant bioactive compounds (Dalle Zotte 2002; Dalle Zotte & Szendro 2011). Further, rabbit meat has been considered to be a functional food source that can effectively reduce public health costs (Dalle Zotte & Szendro 2011). However, the rabbit MYPN gene has not been thoroughly studied until recently. The gene is located in Chromosome 18 and has118,864 bp length including 22 exons, 21 introns and several unknown sequence gaps (http://asia.ensembl.org/). A large number of researches have conducted studies on rabbit meat quality traits at a molecular level and identified single nucleotide polymorphisms in the MSTN, MC4R, POU1F1, CAST, Myf5 and MYf6 genes that were associated with meat traits (Jiang et al. 2008; Yin et al. 2011; Fontanesi et al. 2013; Qiao et al. 2014; Sternstein et al. 2014; Wang et al. 2015; Yin et al. 2015; Wang et al. 2016, 2017). However, none of these studies considered associations between *MYPN* SNPs and rabbit meat quality traits. Thus, the objective of this research was to investigate a single nucleotide polymorphism in the *MYPN* gene and to explore the association of this variant with meat quality traits in the Hyla, Champagne, Tianfu Black rabbit breeds.

Materials and methods

Animals

We investigated 373 unrelated individuals, each from a separate litter (i.e. number of litters = 373),

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representing three rabbit breeds from the rabbitry of the Institute of Animal Genetics and Breeding, Sichuan Agricultural University in China: Hyla (137), Champagne (147) and Tianfu Black (89). Rabbits were all slaughtered together (i.e. on the same day) at 70 days of age and samples of ear tissue were collected for DNA extraction. Carcasses were kept at 4 °C for 24 hours. Tissue samples were taken from the longissimus dorsi and biceps femoris muscles. Measurements of pH ($pH_{0\,h}$) and colour ($L^*_{0\,h}$, $a^*_{0\,h}$, $b^*_{0\,h}$) were taken within 15 min after slaughter. Next, after carcases had been chilled for 24 at 4 °C, measurements were taken for pH ($pH_{24\,h}$), colour ($L^*_{24\,h}$, $a^*_{24\,h}$, $b^*_{24\,h}$) and intramuscular fat (IMF). The traits of the longissimus dorsi and biceps femoris muscles including the pH, colour and IMF were measured following the methods described in Van Laack et al. (2000) and AOAC (1980) on the same two days (i.e. on the day of slaughter and 24 hours postmortem). All experimental procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University.

Detection of SNP and genotyping

Genomic DNA was extracted using AxyPrep Genomic DNA Miniprep Kit (Axygen, Union City, CA) and stored at −80 °C. The PCR primers were designed by the Primer Premier 6 software based on the rabbit gene sequence (Ensembl accession NO. ENSOCUG00000009939). The PCR primers for the *MYPN* gene were as follows: *MYPN*-F: GGTGGTAAC ACATTTCCCTCCTC, *MYPN*-R: AAGGAATCCTCTGATGGA GAATCC. These PCR primers were used to amplify a 591 bp fragment of exon 13 in the *MYPN* gene. The 25 μL reaction mixture contained 50 ng genomic DNA, 1 μM of each primers, 1.5 mM $MgCl_2$, 200 μM dNTPs (dCTP, dGTP, dATP and dTTP), and 0.3 units of Taq DNA polymerase (MBI). The PCR protocol involved an initial denaturation at 95 °C for 5 min, 38 cycles of denaturing at 95 °C for 40 s, annealing at 55.0 °C for 45 s, extension at 72 °C for 40 s, with a final extension at 72 °C for 10 min. The PCR products were directly sequenced on a 3700 DNA sequencer in both directions. Lastly, sequences were analysed with software DNAStar-Seqman (version 7.10).

Statistical analysis

Genotype and allele frequencies for all rabbit populations were calculated using standard procedures. Briefly, Hardy–Weinberg equilibrium (*HWE*) was tested for different locus-population combinations using numbers of observed and expected alleles and a likelihood ratio test with POPGENE software (Ver. 3.2). Population genetic indexes (*He*, *Ne* and *PIC*) were obtained using methodology described by Nei and Roychoudhury (1974) and Botstein et al. (1980).

The linear model used to analyse the meat quality traits was as follows:

$$Y_{ijkl} = \mu + B_i + G_j + S_k + e_{ijkl}$$

where Y_{ijkl} was a meat quality trait, μ was the overall mean for each trait, B_i was the breed effect, G_j was the genotype effect, S_k was the fixed sex effect and e_{ijkl} was the residual effect. Litter effect was not included in the model because each rabbit came from a separate litter. A random polygenic animal effect was not included in the model because rabbits were unrelated, thus random animal genetic effects were confounded with random residual effects. Initial versions of the statistical analyses included interactions between breed and genotype and between sex and genotype. None of these interactions were significant, thus they were excluded from the final model. Least squares means and their standard errors were computed for all genotype effects, and pairwise comparisons among them were made using the Bonferroni *t*-tests. Computations were carried out using the general linear model (GLM) procedure of SPSS 21 (IBM, Armonk, NY).

Results and discussion

We detected one single nucleotide polymorphism (g.18497416 G > A, in chromosome 18) located at 229 bp in exon 13. The SNP resulted in amino acid changes from serine to glycine. The genotype and allele frequencies, Chi-square test, heterozygosity (*He*), effective number of allele (*Ne*), and polymorphism information content (*PIC*) were calculated and summarised in Table 1. Chi-square tests showed that genotypic frequencies agree with the Hardy–Weinberg equilibrium in the three rabbit populations ($p > .05$). The three rabbit populations had intermediate levels of genetic diversity according to their PIC values.

The results of the association analysis between the three genotypes found here and rabbit meat quality traits are shown in Tables 2 and 3. Least squares means listed in Table 2 show that rabbits with the GG genotype had a significantly greater a^*_{24h} and b^*_{24h} in the *longissimus dorsi* muscle than rabbits with the AA genotype ($p < .05$). Further, AA rabbits had significantly higher IMF percentages than rabbits with the GG genotype ($p < .05$). However, no significant differences in IMF percentage in the longissimus dorsi muscle existed between AG rabbits and rabbits with AA and

Table 1. Genotypic and allelic frequencies, χ^2 value test and diversity parameter for the *MYPN* gene in rabbits.

Breed (number)	Genotype/number/GF[a]			Allele/AF[b]		χ^2 (HWE)[c]	He[d]	Ne[e]	PIC[f]
Hyla (137)	AA/56/0.4088	GG/18/0.1314	AG/63/0.4599	A/0.6387	G/0.3613	0.0018/$p>.05$	0.4615	1.8571	0.3550
Champagne (147)	36/0.2449	30/0.2041	81/0.5510	0.5204	0.4796	1.5863/$p>.05$	0.4992	1.9967	0.3746
Tianfu Black (89)	29/0.3258	18/0.2022	42/0.4719	0.5618	0.4382	0.1536/$p>.05$	0.4924	1.9699	0.3712
Total (373)	121/0.3244	66/0.1769	186/0.4987	0.5737	0.4263	0.1416/$p>.05$	0.4891	1.9574	0.3695

[a]GF: Genotypic frequency.
[b]Allelic frequency.
[c]χ^2 (HWE): Hardy–Weinberg equilibrium χ^2 value. Hardy–Weinberg equilibrium ($p > .05$).
[d]He: Gene heterozygosity.
[e]Ne: Effective allele numbers.
[f]PIC: Polymorphism information content.

Table 2. Least square means for *MYPN* genotype effects on meat pH, colour and IMF traits in rabbit longissimus dorsi muscle.

	MYPN Genotype[a]			
Trait[b]	AA	GG	AG	*p*-Value
pH_{0h}	6.51 ± 0.05	6.58 ± 0.06	6.46 ± 0.05	.130
pH_{24h}	5.66 ± 0.03	5.76 ± 0.03	5.74 ± 0.03	.111
L^*_{0h}	48.41 ± 0.71	48.55 ± 0.93	49.57 ± 0.97	.642
L^*_{24h}	58.85 ± 0.76	58.18 ± 0.89	59.55 ± 0.48	.264
a^*_{0h}	5.08 ± 0.41	5.05 ± 0.32	4.85 ± 0.58	.572
a^*_{24h}	4.42 ± 0.23[b]	5.70 ± 0.21[a]	4.57 ± 0.25[b]	.044
b^*_{0h}	2.43 ± 0.11	2.42 ± 0.12	2.76 ± 0.11	.218
b^*_{24h}	4.72 ± 0.11[b]	5.63 ± 0.17[a]	5.75 ± 0.13[a]	.026
IMF, %	1.83 ± 0.13[a]	1.38 ± 0.10[b]	1.53 ± 0.11[ab]	.044

[a]Values with different superscripts within the same row differ significantly at $p < .05$ (a, b) and $p < .01$ (A, B, C).
[b]L* = Lightness from 0 (black) to 100 (white); a* = redness from −60 (green) to 60 (red); b* = yellowness from −60 (blue) to 60 (yellow).

Table 3. Least square means for *MYPN* genotype effects on meat pH, colour and IMF traits in rabbit biceps femoris muscle.

	MYPN Genotype[a]			
Trait[b]	AA	GG	AG	*p*-Value
pH_{0h}	6.40 ± 0.08	6.37 ± 0.05	6.45 ± 0.09	.444
pH_{24h}	5.74 ± 0.03	5.75 ± 0.03	5.77 ± 0.04	.325
L^*_{0h}	51.69 ± 0.57	53.83 ± 0.97	52.40 ± 0.78	.060
L^*_{24h}	60.77 ± 0.96	62.91 ± 0.78	62.83 ± 0.87	.393
a^*_{0h}	4.78 ± 0.25[a]	4.66 ± 0.20[a]	3.52 ± 0.17[b]	.041
a^*_{24h}	5.80 ± 0.22[a]	5.71 ± 0.23[a]	4.65 ± 0.30[b]	.036
b^*_{0h}	2.21 ± 0.13	2.17 ± 0.17	2.05 ± 0.20	.119
b^*_{24h}	5.52 ± 0.28	5.17 ± 0.21	5.98 ± 0.31	.308
IMF, %	3.45 ± 0.25[a]	2.55 ± 0.19[b]	2.69 ± 0.23[bc]	.039

[a]Values with different superscripts within the same row differ significantly at $p < .01$ (A, B).
[b]L* = Lightness from 0 (black) to 100 (white); a* = redness from −60 (green) to 60 (red); b* = yellowness from −60 (blue) to 60 (yellow)..

GG genotypes. For the biceps femoris muscle (Table 3), rabbits with the AA genotype had noticeably higher a^*_{0h} and a^*_{24h} than those with the AG genotype ($p < .05$). Rabbits with the AA genotype had markedly higher IMF than those with the GG and AG genotypes ($p < .05$), but no significant differences in IMF percentages were found between AG and GG rabbits. Lastly, IMF values were noticeably higher in biceps femoris than in longissimus dorsi.

The value of pH, colour and IMF content are all typical meat quality parameters (Dalle Zotte 2002; Li et al. 2013). The pH values in this study were similar to pH values reported by Mazzone et al. (2010) at 15 min after slaughter (6.88) and after chilling rabbit carcases for 24 h (5.79 and 5.81). A pH value lower than 6.0 is important to obtain meat of high quality (Terlouw 2005; Mach et al. 2008). In addition, pH values in different genotypes would decrease with the time because of the acidification process in both muscles (Niedzwiadek et al. 1983; Osman 1991; Lambertini et al. 1996). Our results for L^*_{24h} in *longissimus dorsi* were similar to the L* values of 57.55 and 57.29 reported by Mazzone et al. (2010) for this muscle. However, these authors found values of a^*_{24h} and b^*_{24h} that were two to three times lower (a*=2.61 to 2.65, b*=1.64 to 1.75) than the corresponding values given here. The a^*_{24h} value here was similar to values of a^*_{24h} but L^*_{24h} and b^*_{24h} values were slightly higher than values obtained in *longissimus dorsi* muscles from two rabbit genetic groups (Zeferino et al. 2013). These results indicate that *MYPN* SNP g.18497416 G > A in chromosome 18 affected IMF and colour in the *longissimus dorsi* and *biceps femoris* muscles, indicating that *MYPN* could be an effective candidate gene for genetic selection to help improve meat quality in rabbits. However, genotype values differed for *longissimus dorsi* and *biceps femoris* muscles (Tables 2 and 3). Thus, additional work will be needed to further understand the functional role of this SNP marker on rabbit meat quality traits.

Conclusions

We found one novel SNP (g.18497416 G > A, in chromosome 18) in exon 13 of the *MYPN* gene with 2 alleles (A and G), and allele A had a higher frequency in three rabbit breeds. The three rabbit populations were in the Hardy–Weinberg equilibrium and they had intermediate levels of genetic diversity. The SNP was significantly related to a^*_{24h} and b^*_{24h} in longissimus dorsi, and also significantly related to a^*_{24h} and a^*_{0h} in biceps femoris. Importantly, rabbits with the AA genotype had the highest IMF values and those with

the GG genotype had the lowest IMF values in both longissimus dorsi and biceps femoris muscles.

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Disclosure statement

The authors report no conflicts of interest. The authors alone are responsible for the content and writing of this article.

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