SHORT COMMUNICATION

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 a24h and b24h than rabbits with the AA genotype (p < .05) in the longissimus dorsi muscle and that animals with the AA genotype had substantially higher a0h and a24h 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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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 waterholding 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 has 118,864 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),...
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 (pH₀₋₂₄), and colour (L*, a*, b*) were taken within 15 minutes after slaughter. Next, after carcasses had been chilled for 24 at 4°C, measurements were taken for pH (pH₂₄), colour (L*₂₄, a*₂₄, b*₂₄) 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: GTGTTTAGACACATTTGCCCTCCTC, MYPN-R: AAAGAATCCCTCCTGATGGA 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 of genomic DNA, 1 μM of each primer, 1.5 mM MgCl₂, 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, \( \mu \) 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.18497416G>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 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*₂₄ and b*₂₄ 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, $\chi^2$ value test and diversity parameter for the *MYPN* gene in rabbits.

| Breed (number) | Genotype/number/GF | Allele/AF | $\chi^2$ (HWE) | $H$ | $Ne^*$ | PIC |
|---------------|--------------------|-----------|---------------|-----|-------|------|
| Hyla (137)    | AA/56/0.4088       | 0.0361    | 0.0018/p >.05 | 0.4615 | 1.8571 | 0.3550 |
| Champagne (147)| GG/18/0.1314       | 0.4382    | 0.0492        | 1.9967 | 0.3746 |
| Tianfu Black (89)| AG/63/0.4959  | 0.4164/p >.05 | 0.4924 | 1.9699 | 0.3712 |
| Total (373)   | 121/0.3244         | 0.4263    | 0.1416/p >.05 | 0.4891 | 1.9574 | 0.3695 |

$^a$GF: Genotypic frequency.

$^b$Allelic frequency.

$^c$Hardy–Weinberg equilibrium $\chi^2$ value.

$^d$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.

| Trait         | AA       | GG      | AG      | p-Value |
|---------------|----------|---------|---------|---------|
| pH$_{0h}$     | 6.51 ± 0.05 | 6.58 ± 0.06 | 6.46 ± 0.05 | .130    |
| pH$_{24h}$    | 4.42 ± 0.23  | 5.70 ± 0.21  | 4.57 ± 0.25  | .044    |
| 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*$_{0h}$     | 5.08 ± 0.41  | 5.05 ± 0.32  | 4.85 ± 0.58  | .572    |
| a*$_{24h}$    | 4.72 ± 0.11  | 5.63 ± 0.17  | 5.75 ± 0.13  | .026    |
| IMF, %        | 1.83 ± 0.13  | 1.38 ± 0.10  | 1.53 ± 0.11  | .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).

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 carcasses 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 time because of the acidification process in both muscles (Niedziadek 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.

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 < .5). 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).

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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