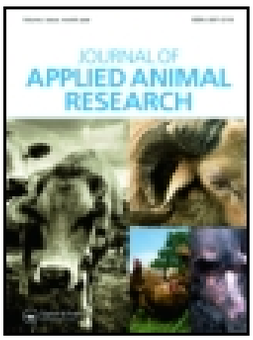

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Calpastatin gene polymorphism is associated with rabbit meat quality traits

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ABSTRACT

The objective of this study was to investigate single nucleotide polymorphisms in the calpastatin (CAST) gene and to test their association with meat quality traits in Hyla, Champagne, and Tianfu Black rabbit breeds. We detected one single nucleotide polymorphism (g.16441502 C > T) located at 67 bp in intron 3 of the CAST gene in Chromosome 11. The three rabbit populations had intermediate levels of genetic diversity in the CAST gene. The statistical analysis indicated that rabbits with the TT genotype had a significantly greater yellowness at 0 and 24 h postmortem than those with the CC genotype ($P < .05$) in the *longissimus dorsi* muscle. Rabbits with CC genotype had higher intramuscular fat content than those with CT and CC genotypes in both *longissimus dorsi* and *biceps femoris* muscles in the three breeds ($P < .05$). Our results indicated that the CAST gene may be used as a possible candidate for marker-assisted selection in rabbit meat breeding programmes.

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Rabbit; CAST; SNP; pH; meat colour; IMF

1. Introduction

Calpastatin (CAST) was first identified in 1978 and exists widely in muscle cells (Nishiura et al. 1978; Waxman & Krebs 1978). CAST is an endogenous inhibitor which controls the activities of calpains with addition of Ca^{2+} (Kwak et al. 1993). Calpastatins are rich in proline and glutamate but poor in aromatic amino acids (Murachi 1983). Koohmaraie et al. (2002) showed that the rate of protein degradation postmortem affected the meat quality. Variation in CAST abundance influences postmortem aging rates in different muscles (Ouali & Talmant 1990; Geesink et al. 1992). Recently, some CAST SNPs have been used as commercial genetic markers by livestock industries (Johnston & Graser 2010). Calvo et al. (2014) showed that a new single nucleotide polymorphism in the CAST gene is associated with beef tenderness. Cafe et al. (2010) provided further evidence that selection based on CAST gene markers may improve meat tenderness in Brahman cattle. Further, Castro et al. (2016) reported that several SNP in the CAST gene showed significant effects on the b* and hue* parameters of the *longissimus thoracis et lumborum* and semitendinosus muscles in Brahman and Brahman crossbred cattle. The CAST gene has also been found to have a large effect on pork quality (Rohrer et al. 2012) and muscle fibre traits in chicken (Liu et al. 2008; Zhang et al. 2012). However, there has been little research on association between SNPs and rabbit meat quality traits.

The hybrid Hyla breed has shown high performance for daily weight gain, feed efficiency, and dressing per cent (Chiericato et al. 1993). The Champagne breed has displayed a high growth rate in young rabbits and was the most productive among medium-sized breeds (Bolet et al. 2004). The Tianfu Black is a Chinese indigenous breed that is popular among Chinese breeders. The aim of this study was to discuss a single nucleotide polymorphism in the CAST gene and to test its association with meat quality traits in the Hyla, Champagne, and Tianfu Black rabbit breeds.

2. Materials and methods

2.1. Animals

A total of 372 rabbits of both sexes (185 males and 180 females) from 3 breeds (including 138 Hyla, 139 Champagne, and 88 Tianfu Black) were used in this study. Rabbits were reared in individual cages after weaning at 6 weeks of age. Rabbits were managed following standard practices and had free access to a commercial diet. Slaughter occurred at 70 days of age. Ear tissue samples were collected for DNA extraction. Carcasses were kept at 4°C for 24 h. All experimental procedures were approved by the Institutional Animal Care and Use Committee of Sichuan Agricultural University.

2.2. Meat quality traits

Meat quality measurements were: (1) pH at 0 and 24 h postmortem, (2) colour at 0 and 24 h postmortem, and (3) intramuscular fat (IMF) at 24 h postmortem. The samples were taken from the *longissimus dorsi* and *biceps femoris* muscles. The pH measurements were taken with a probe using a pH meter (Model PH-STAR CPU, Meister®, Germany). The probe was inserted directly into the muscle to a depth of 3 mm. Colour data were expressed in terms of Lightness (L*), redness (a*), and yellowness (b*) (Van Laack et al. 2000). Lightness (L*) ranged from black (0) to white

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(100), redness (a*) ranged from green (−60) to red (+60), and yellowness (b*) ranged from blue (−60) to yellow (+60). The IMF was analysed using the modified Soxhlet method (AOAC 1980).

2.3. Detection of SNP and genotyping

Genomic DNA was extracted using AxyPrep Genomic DNA Miniprep Kit (Axygen, USA) and stored at −20°C. The polymerase chain reaction (PCR) primers for the *CAST* gene were designed by the Primer Premier 5 software based on the rabbit gene sequence (Ensembl accession NO. ENSOCUG00000007802). The PCR primers for the *CAST* gene were *CAST*-F: CATTAGGCCGTTCCAATCAGC and *CAST*-R: CCTATGTAGCAGCCCGGTTATTC. These PCR primers were used to amplify a 655 bp fragment, including exon 3, intron 3, and exon 4, in the CAST gene. The 25 μL reaction mixture contained 50 ng genomic DNA, 1.5 mM $MgCl_2$, 1 μM of each primer, 200 μM dNTPs (dATP, dTTP, dCTP, and dGTP), and 0.4 units of Taq DNA polymerase (MBI). The PCR protocol was as follows: initial denaturation at 95°C for 4 min, followed by 35 cycles of denaturing at 95°C for 45 s, annealing at 56.0°C for 45 s, extension at 72°C for 45 s, and a final extension at 72°C for 10 min. Then, the PCR products were purified and directly sequenced in both directions with a BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA, USA) on a 3700 DNA sequencer. Sequences were subsequently analysed using program DNAMAN (version 5.2.2).

2.4. Statistical analysis

Genotype frequencies and allele frequencies for Hyla, Champagne, and Tianfu Black were obtained using the usual procedures (Falconer et al. 1996; Yeh et al. 1997). Observed genotype frequencies within each breed were compared with their respective expected frequencies under Hardy–Weinberg equilibrium (HWE) and tested for significant departures from HWE with software POPGENE (Ver. 3.2) (Yeh et al. 1997) using a likelihood ratio test. In addition, gene heterozygosity (He), effective allele numbers (Ne), and polymorphism information content (PIC) were estimated for each of the three breeds using expressions found in Nei and Roychoudhury (1974) and Botstein et al. (1980).

Preliminary statistical analyses included the fixed effects of breed, genotype, sex, and interactions between breed and genotype and between sex and genotype in the linear model for meat quality traits. However, the interactions between breed and genotype and between sex and genotype were non-significant; thus they were excluded from the model. Consequently, the final linear model 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 random error. Least squares means and their standard errors were computed for all genotype effects, and pairwise comparisons among them were made using Bonferroni *t*-tests. Computations were carried out using the general linear model procedure of SPSS 21 (IBM, Armonk, NY, USA).

3. Results and discussion

3.1. Genotypic frequencies, allelic frequencies, and population genetic indexes

The genotyping of the SNP was successfully implemented using PCR and DNA sequencing. Figure 1 shows a sequencing map of the novel SNP (g.16441502 C > T) in the Hyla, Champagne, and Tianfu Black rabbit breeds. We detected one single nucleotide polymorphism located at 67 bp in intron 3 of the CAST gene in Chromosome 11. The genotype and allele frequencies, chi-square test, heterozygosity (He), effective number of allele (Ne), and PIC were calculated and summarized in Table 1. The T allele showed a high prevalence in these breeds. The minor allele frequencies (MAF ranged from 0.2391 to 0.3777) showed that this SNP was polymorphic (MAF > 0.05) in these three breeds. Chi-square tests showed that genotypic frequencies were in HWE ($P > .05$) in three rabbit populations. The three rabbit populations had intermediate levels of genetic diversity according to their PIC values (0.2977 for Hyla, 0.3596 for Champagne, and 0.3522 for Tianfu Black).

3.2. Associations analysis between the SNP and meat quality

The results of the association analysis between the g.16441502 C > T SNP found here and rabbit meat quality traits are shown in Tables 2 and 3. Least squares means in Table 2 showed that rabbits with the TT genotype had a significantly greater b^*_{0h} and b^*_{24h} than rabbits with the CC genotype ($P < .05$), but similar values for pH (0 h, 24 h), L* (0 h, 24 h), and a* (0 h, 24 h) in the *longissimus dorsi* muscle. No significant differences among the three genotypes were found for pH (0 h, 24 h), L* (0 h, 24 h), a* (0 h, 24 h), and b* (0 h, 24 h) in *biceps femoris* muscle (Table 3; $P > .05$). Thus, rabbits with CC genotype had higher IMF than rabbits with CT and TT genotypes in both *longissimus dorsi* and *biceps femoris* muscles among these three rabbit breeds (Tables 2 and 3; $P < .05$).

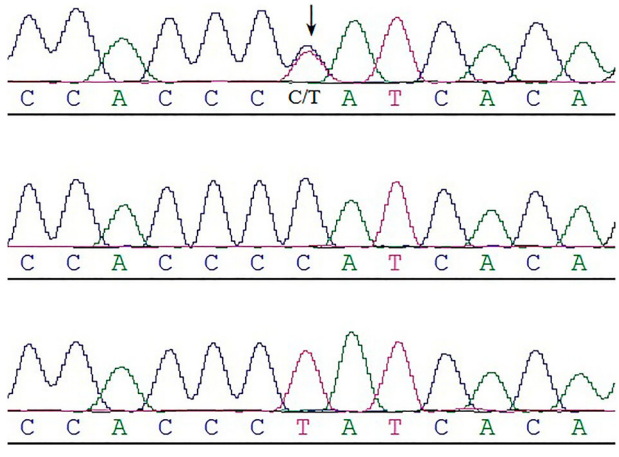

Figure 1. Sequencing map of three genotypes of the rabbit CAST gene in the intron 3 region.

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

Breed (number)	Genotype/number/GF[a]			Allele/AF[b]		χ^2 (HWE)[c]	He[d]	Ne[e]	PIC[f]
Hyla (138)	CC/8/0.0580	TT/80/0.5797	CT/50/0.3623	C/0.2391	T/0.7609	0.3639/$P > .05$	0.3639	1.5721	0.2977
Champagne (139)	18/0.1295	52/0.3741	69/0.4964	0.3777	0.6223	0.4701/$P > .05$	0.4701	1.8871	0.3596
Tianfu Black (88)	14/0.1591	40/0.4545	34/0.3864	0.3523	0.6477	0.4564/$P > .05$	0.4564	1.8394	0.3522
Total rabbit (365)	40/0.1096	172/0.4712	153/0.4192	0.3192	0.6808	0.4346/$P > .05$	0.4346	1.7687	0.3402

[a]GF: genotypic frequency.
[b]Allelic frequency.
[c]χ^2 (HWE): Hardy–Weinberg equilibrium χ^2 value. HWE ($P > .05$), Hardy–Weinberg disequilibrium ($P < .05$).
[d]He: gene heterozygosity.
[e]Ne: effective allele number.
[f]PIC: polymorphism information content.

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

Trait[b]	CAST genotype[a]			*P*-value
	CC	TT	CT	
pH_{0h}	6.47 ± 0.08	6.72 ± 0.08	6.32 ± 0.05	.176
pH_{24h}	5.71 ± 0.03	5.71 ± 0.03	5.74 ± 0.02	.132
L^*_{0h}	47.70 ± 1.04	49.38 ± 1.03	48.63 ± 0.68	.304
L^*_{24h}	56.87 ± 0.75	60.23 ± 1.10	58.32 ± 0.71	.264
a^*_{0h}	4.77 ± 0.41	5.14 ± 0.32	5.67 ± 0.27	.144
a^*_{24h}	4.02 ± 0.23	4.77 ± 0.44	4.40 ± 0.47	.137
b^*_{0h}	1.39 ± 0.19^{c}	3.09 ± 0.11^{a}	2.81 ± 0.13^{ab}	.037
b^*_{24h}	4.62 ± 0.20^{c}	5.40 ± 0.22^{a}	5.20 ± 0.23^{ab}	.048
IMF (%)	2.52 ± 0.11^{a}	1.49 ± 0.09^{b}	1.73 ± 0.21^{ab}	.029

[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).

Colour, IMF content, and pH value are all typical meat quality parameters (Dalle Zotte 2002; Li et al. 2013). The pH values here were similar to those of Hulot and Ouhayoun (1999), who found that pH was almost neutral in live rabbits but it decreased rapidly after slaughter. The higher pH in fresh rabbit meat may imply a higher level of muscle glycogen reserves (Sabuncuoglu et al. 2011). In our study, colour values were similar to values reported in previous research (Trocino et al. 2002; María et al. 2006). The small differences in colour trait values between *longissimus dorsi* and *biceps femoris* muscles here partially agreed with the results reported by Chiericato et al. (1996), who found that yellowness was not consistent across different muscles. The result for IMF indicated that the C > T SNP here was closely associated with QTL, affecting rabbit meat quality. Koohmaraie (1992) indicated that the biological activity of the *CAST* gene played an important role in the tenderization in beef, pork, and lamb because the calpain–CAST system is involved in the degradation of important proteins, and also, the system, a Ca^{2+}-activated protease family, may stimulate more rapid glycolysis and pH decline. Chung et al. (2001) found that CAST genotypes in intron 6 of the CAST gene influenced ($P < .05$) CAST activity in Angus cattle. Thus, it is possible that the g.16441502 C > T SNP found in intron 3 here may be involved in regulation of transcriptional and post-transcriptional levels of gene expression of the CAST gene in rabbits. Further work is necessary with larger rabbit populations to elucidate the mechanisms involved in this gene's effect on rabbit meat quality.

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

Trait[b]	CAST genotype[a]			*P*-value
	CC	TT	CT	
pH_{0h}	6.58 ± 0.14	6.52 ± 0.26	6.18 ± 0.18	.226
pH_{24h}	5.73 ± 0.05	5.72 ± 0.04	5.77 ± 0.02	.125
L^*_{0h}	52.79 ± 0.80	52.70 ± 0.86	52.94 ± 0.77	.312
L^*_{24h}	60.93 ± 1.01	62.39 ± 0.92	62.32 ± 0.87	.208
a^*_{0h}	3.32 ± 0.35	4.15 ± 0.21	3.92 ± 0.33	.114
a^*_{24h}	4.55 ± 0.23	4.89 ± 0.31	4.53 ± 0.33	.272
b^*_{0h}	2.05 ± 0.16	2.51 ± 0.19	2.69 ± 0.21	.119
b^*_{24h}	5.52 ± 0.28	5.17 ± 0.21	5.98 ± 0.31	.071
IMF (%)	3.29 ± 0.31^{a}	1.68 ± 0.13^{b}	2.88 ± 0.23^{a}	.038

[a]Values with different superscripts within the same row differ significantly at $P < .01$ (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).

Disclosure statement

No potential conflict of interest was reported by the authors.

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