

A NOVEL SINGLE NUCLEOTIDE POLYMORPHISM OF THE *POU1F1* GENE ASSOCIATED WITH MEAT QUALITY TRAITS IN RABBITS

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Abstract

The purpose of this research was to investigate the effect of the *POU1F1* gene on meat quality traits in the Hyla, Champagne, and Tianfu Black rabbit breeds. We detected one single nucleotide polymorphism and the SNP was located at 536 bp in intron 5 of this gene. Chi-square tests showed that the genotypic frequencies in the three rabbit populations were not in Hardy-Weinberg equilibrium. The PIC values indicated that the three populations had intermediate levels of genetic diversity. Rabbits with the CC genotype had a significantly greater pH_{0h} than those with the CT genotype in the *biceps femoris* muscle. The least squares means for cooking loss in CT and CC rabbits were significantly higher than those for TT rabbits. Rabbits with the CC genotype had a significantly higher intramuscular fat content in the *longissimus dorsi* and *biceps femoris* muscles than those with genotype TT and CT. Thus, the results here indicate that this *POU1F1* SNP may be of potential use in marker assisted selection for meat quality traits in rabbits.

Key words: rabbit, POU1F1, SNP, meat quality

Rabbit meat is routinely consumed in many European countries (Malta, Cyprus, Italy, Czech Republic, Spain, Belgium, Luxembourg, Portugal, France) and certain north African countries (Egypt, Algeria) (FAOSTAT, 2010). It is highly valued for its nutritional and dietary properties (Hernández, 2008). Compared to meats of other animal species, rabbit meat has lower cholesterol content and high levels of protein with essential amino acids (Dalle Zotte and Szendro, 2011). For consumption, however, the quality of a piece of meat is an important factor influencing a consumer's purchase decision (Grunert et al., 2004). Thus, improving meat quality is of great benefit to the rabbit industry. Color, intramuscular fat (IMF) content, and pH value are all typical meat quality parameters (Li et al., 2013; Dalle Zotte, 2002). Genetic effect is considered to be one of the important factors when trying to improve meat

quality. Genes associated with meat quality have been identified and single nucleotide polymorphisms (SNPs) of many candidate genes have been tested (Li et al., 2013). However, very few of these researches involved rabbits (Zhang et al., 2013; Rafayová et al., 2009; Fontanesi et al., 2013).

POU1F1 (also named *PIT-1*) is the first pituitary-specific transcription factor to be identified in the human and mouse (Turton et al., 2005). As a member of the POU-domain family gene, *POU1F1* is a positive regulator for growth hormone (*GH*), prolactin (*PRL*) and thyroid stimulating hormone β (*TSH* β) by binding to target DNA promoters as a dimer in mammalian animals (Cohen et al., 1997; Jacobson et al., 1997). Mutations of *POU1F1* gene were shown to be associated with mice Snell dwarfism (dw), mice Jackson dwarfism (dw-J), human dwarfism (Li et al., 1990; Pfaffle et al., 1992), swine growth and meat performance (Stancekov et al., 1999), bovine growth (Zhao et al., 2004), goat growth and carcass traits (Lan et al., 2007), cattle milk production traits (Yan et al., 2011), and chicken body weight and egg numbers (Yan et al., 2013). The rabbit's *POU1F1* gene is mapped to chromosome 14 and it contains seven exons and six introns (Ensembl accession NO. ENSOCUG00000017779). No reports on *POU1F1* gene variations in rabbit were found in the literature.

Thus, the objective of this research was to study polymorphisms in the *POU1F1* gene and their associations with rabbit meat quality traits that could provide useful genetic markers for rabbit selection and breeding through marker-assisted selection (MAS). In this paper, we report the identification of a novel SNP in the rabbit *POU1F1* gene and evaluate its effects on meat quality traits.

Material and methods

This study was carried out at the experimental rabbitry of the Institute of Animal Genetics and Breeding, Sichuan Agricultural University (China). A total of 372 rabbits, males (n=190) and females (n=182), were used in this study, including 137 Hyla (HY), 144 Champagne (AC) and 91 Tianfu Black (TB). Rabbits were reared in individual cages after weaning at 6 weeks and fed *ad libitum* with a commercial diet using standard feeding and management protocols.

Rabbits were slaughtered by slitting the throat, bled for 3 min, then skinned and eviscerated manually at 70 days of age and samples of ear tissue were collected for DNA extraction. Carcasses were kept at 4°C for 24 hours. Meat quality measurements were: 1) pH at 0 h and 24 h postmorterm, 2) color at 0 h and 24 h postmorterm, 3) cooking loss at 24 h postmorterm, and 4) intramuscular fat (IMF) at 24 h postmorterm. Measurements of pH, IMF and color were taken from the *biceps femoris* and *longissimus dorsi* muscles, whereas cooking loss was measured only in the *biceps femoris* muscle.

Genomic DNA was extracted using AxyPrep Genomic DNA Miniprep Kit (Axygen, USA) and stored at -20°C. The PCR primers for the *POU1F1* gene were designed by the Primer Premier 5 software based on the rabbit gene sequence (Ensembl accession NO. ENSOCUG0000001779). The PCR primers for the *POU1F1* gene were as follows: *POU1F1*-F: GCTGGAGGAAGCTGAGCAAGT, *POU1F1*-R: GAATACCTTATGGTCGTCCTCCG. These PCR primers were used to amplify a 798 bp fragment of intron 5 in the *POU1F1* gene. The 25 μ L reaction mixture contained 50 ng genomic DNA, 1 μ M of each primer, 1.5 mM MgCl₂, 200 μ M dNTPs (dATP, dTTP, dCTP and dGTP), and 0.4 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 45 s, annealing at 56.0°C for 45 s, extension at 72°C for 45 s, with a final extension at 72°C for 10 min. Then, the PCR products were directly sequenced on a 3700 DNA sequencer in both directions using the BigDye Terminator sequencing kit (Applied Biosystems, Foster City, CA, USA) according to the manufacturer's instructions. Next, the sequences were analyzed with DNAMAN software (version 5.2.2).

The genotype and allele frequencies in all breeds were calculated by the standard procedure (Falconer et al., 1996). Hardy-Weinberg equilibrium (HWE) was tested for different locus-population combinations and the number of observed and effective alleles based on the likelihood ratio test with POPGENE software (Ver. 3.2) (Yeh et al., 1997). The population genetic indexes including gene heterozygosity (He), effective allele numbers (Ne) and polymorphism information content (PIC) were estimated by using the following formulas (Nei et al., 1997; Botstein et al., 1980):

$$He = 1 - \sum_{i=1}^{n} P_{i}^{2}$$
$$He = 1 / \sum_{i=1}^{n} P_{i}^{2}$$
$$PIC = 1 - \sum_{i=1}^{m} P_{i}^{2} - \sum_{i=1}^{m-1} \sum_{i=i+1}^{m} 2P_{i}^{2}P_{j}^{2}$$

where:

 P_i was the frequency of the *i*th allele,

n was the number of alleles.

Meat quality traits were analyzed using the following linear model:

$$Y_{ijk} = \mu + G_i + S_j + e_{ijk}$$

where:

 Y_{iik} was a meat quality trait,

 μ was the overall mean for each trait,

 G_i was the genotype effect,

 S_i was the fixed sex effect,

 e_{iik} was the random error.

Least squares means and their standard errors were computed for all genotype effects.

Brought to you by | University of Florida Authenticated Download Date | 8/3/15 8:53 PM Pairwise comparisons among genotype effects were assessed using Bonferroni t-tests. Computations were carried out using the general linear model procedure of SPSS 21 (IBM, Armonk, NY, USA).

Results

We detected one single nucleotide polymorphism in the set of animals used in this study. The SNP was located at 536 bp in intron 5 of the POU1F1 gene. The genotyping of the SNP was successfully implemented using DNA sequencing and polymerase chain reaction (PCR). Figure 1 shows a sequencing map of the novel SNP-C>T of rabbits. Three genotypes (CC, TT and CT) were identified, counted, and the genotype and allele frequencies calculated in the Hyla, Champagne, and Tianfu Black breeds (Table 1). The genotype frequency of CC was higher than TT and CT. The C allele frequency (0.5604 to 0.6806) was also much higher than T which showed a high prevalence in these breeds. The minor allele frequencies (MAF) ranged from 0.3194 to 0.4396. Chi-square tests (Table 2) showed that genotypic frequencies were not in Hardy-Weinberg equilibrium (P<0.05) in any of the three rabbit populations. The values of three population genetic indices (H_a, N_a, and PIC) to evaluate diversity of the three rabbit breeds are presented in Table 2. All values of H_a (gene heterozygosity) were above 0.4 and below 0.5, whereas values of N_a (effective allele numbers) approached 0.2. The mean PIC values were 0.3643 for Hyla, 0.3403 for Champagne, and 0.3713 for Tianfu Black.

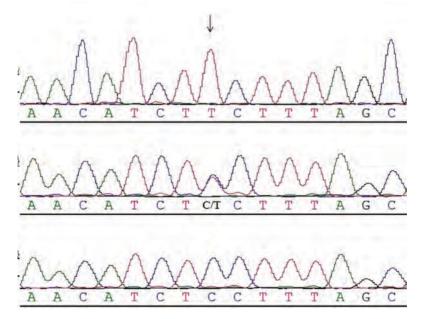


Figure 1. Sequencing map of three genotypes in intron 5 of the POU1F1 gene in rabbit

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		POU1F1 Gen	otype number a	POU1F1 Allele frequency		
Breed	No. rabbits	CC	TT	CT	С	Т
Hyla	137	58 (0.4234)	30 (0.2190)	49 (0.3577)	0.6022	0.3978
Champagne	144	80 (0.5556)	28 (0.1944)	36 (0.2500)	0.6806	0.3194
Tianfu Black	91	41 (0.4505)	30 (0.3297)	20 (0.2198)	0.5604	0.4396
Total	372	168 (0.4516)	99 (0.2661)	105 (0.2823)	0.5927	0.4073

Table 1. Genotypic and allelic frequencies for the rabbit POU1F1 gene

^aIn parenthesis.

Table 2. Chi-square test values and diversity parameters for the rabbit POU1F1 gene

Breed	No. rabbits	$\chi^2 (HWE)^a$	He ^b	Nec	PIC ^d
Hyla	137	8.8032*	0.4791	1.9198	0.3643
Champagne	144	26.0127**	0.4348	1.7693	0.3403
Tianfu Black	91	27.9214**	0.4927	1.9712	0.3713
Total	372	64.1820**	0.4828	1.9335	0.3663

^a χ^2 (HWE) – Hardy-Weinberg equilibrium χ^2 value; * 0.001<P< 0.01; ** P<0.001.

^b He – gene heterozygosity.

 $^{\rm c}$ Ne – effective allele number.

^dPIC - polymorphism information content.

The results of the association analysis between SNP and rabbit meat quality are shown in Tables 3, 4 and 5. Table 3 shows least squares means of L* (0h, 24h), a* (0h, 24h), b* (0h, 24h) for *POU1F1* genotypes CC, TT, and CT in the *longissimus dorsi* muscle whereas Table 4 presents least squares means for these same traits and genotypes for the *biceps femoris* muscle. Table 5 contains least squares means for CC, TT, and CT for cooking loss (*biceps femoris* muscle) and intramuscular fat content for *longissimus dorsi* muscle samples only and for *biceps femoris* muscle samples only.

Trait ^a				
	CC	TT	CT	P-value
pH _{0h}	6.71±0.06	6.63±0.12	6.49±0.08	0.175
pH _{24h}	5.74 ± 0.03	5.69 ± 0.04	5.76±0.05	0.266
L* 0h	49.44±1.17	$47.40{\pm}1.18$	49.90±1.23	0.452
L*	59.63 ± 0.90	60.05±1.13	56.55±1.13	0.125
a* 0h	4.25 ± 0.38	4.22±0.41	5.92 ± 0.45	0.396
a*	3.92±0.34	4.01±0.37	5.71±0.40	0.202
b*	2.99±0.12	2.17±0.21	2.33±0.16	0.169
b*	5.34±0.23	5.29±0.24	5.32±0.24	0.299

Table 3. Least squares means for *POU1F1* genotype effects on meat pH and color traits in rabbit *longissimus dorsi* muscle

The data were expressed as least squares means \pm standard errors (mean \pm S.E.).

^a L* = lightness from 0 (black) to 100 (white); a* = redness from -60 (green) to 60 (red); b* = yellowness from -60 (blue) to 60 (yellow).

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Trait ^b		P-value		
	CC	TT	СТ	P-value
pH _{0h}	6.63±0.07 x	6.50±0.14 xy	6.26±0.05 y	0.036
pH _{24h}	5.78 ± 0.02	5.77 ± 0.04	5.72 ± 0.05	0.059
L* _{0h}	53.21±0.96	$53.79{\pm}1.06$	52.06 ± 1.12	0.436
$L^{*}_{_{24h}}$	64.20 ± 1.07	$60.14{\pm}1.12$	$60.54{\pm}1.03$	0.146
a* _{0h}	3.76±0.38	4.63±0.41	4.10±0.31	0.223
a* _{24h}	4.06±0.33	4.88 ± 0.35	5.37 ± 0.42	0.203
b*	2.54 ± 0.21	2.78 ± 0.37	2.39±0.37	0.168
b*	6.29±0.32	5.02 ± 0.39	5.68 ± 0.37	0.258

Table 4. Least squares means for *POU1F1* genotype effects on meat pH and color traits in rabbit *biceps femoris* muscle

The data were expressed as least squares means \pm standard errors (mean \pm S.E.).

^a Cells with different letters (x, y) differed significantly (P<0.05).

^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 5. Least square means for *POU1F1* genotype effects on meat cooking loss and intramuscular fat in rabbit

Trait		P-value			
11ait	CC	TT	СТ	r-value	
Cooking loss (%) ^b	33.65±1.23 x	31.28±1.11 y	34.13±1.21 x	0.046	
IMFLD ^c	1.013±0.023 x	0.411±0.018 y	0.601±0.014 y	0.018	
IMFHL ^d	1.002±0.067 x	$0.858{\pm}0.034 \text{ x}$	0.023±0.003 y	0.032	

The data were expressed as least squares means \pm standard errors (mean \pm S.E.).

^a Cells with different letters (x, y, z) differed significantly (P<0.05).

^b Biceps femoris muscle only.

° Longissimus dorsi muscle only.

^d Biceps femoris muscle only.

Table 3 shows that non-significant differences among *POU1F1* genotypes CC, TT, and CT were found for pH (0h, 24h), L* (0h, 24h), a* (0h, 24h), and b* (0h, 24h) in the rabbit *longissimus dorsi* muscle (P>0.05). Fresh *longissimus dorsi* meat pH (0h) and a* (0h) least squares means were higher than those for meat stored for 24 h from rabbits of the three genotypes. Conversely, fresh *longissimus dorsi* meat L* (0h) and b* (0h) least squares means were lower than those of meat stored for 24 h for all genotypes. Thus, lower pH was related to higher L*, b* and lower a* in *longissimus dorsi* in this rabbit population.

Least squares means in Table 4 indicated that rabbits with the CC genotype had a significantly greater pH_{0h} in the *biceps femoris* muscle (P<0.05) than those with the CT genotype. Fresh *biceps femoris* meat pH (0h) was higher than that of meat stored for 24 h, but fresh *biceps femoris* meat L* (0h), a* (0h) and b* (0h) values were lower than those from meat stored for 24 h in all genotypes (CC, TT, and CT).

Table 5 shows that the least squares means for cooking loss from CT and CC rabbits were significantly higher than those from TT rabbits (P<0.05). Rabbits with the CC genotype had a significantly higher intramuscular fat content in the *longis*-

simus dorsi than those with genotype TT and CT (P<0.05), whereas the CC and TT genotypes had similar intramuscular fat in the *biceps femoris* and both of them had higher intramuscular fat in this muscle than the CT genotype.

Discussion

In this work, the result of allele frequencies showed that this SNP was polymorphic (MAF>0.05) in these three breeds. Hardy-Weinberg disequilibrium (P<0.05) in any of the three rabbit populations may have been due to limited sample sizes from these populations and artificial selection of parents for the meat quality traits considered here (e.g., carcass weight, visual meat appraisal, meat taste). According to the classification of PIC values (PIC <0.25 = low polymorphism; 0.25<PIC <0.50 = intermediate polymorphism; PIC >0.50 = high polymorphism), the three rabbit populations had intermediate levels of genetic diversity. This suggested that there was sufficient genetic diversity for selection to be effective in improving meat quality traits in these three rabbit populations.

Meat pH affects many meat quality properties, including protein structure and meat color in rabbits (Hulot and Ouhayoun, 1999). Here, a decrease in pH between 0h and 24h was paired with increases in values of L*, b* and a* between 0h and 24h in *biceps femoris* meat. 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. Conversely, Zeferino et al. (2013) found no difference in pH values in rabbit meat stored for 24 h and 48 h. Lower pH values at 24 h may be due to stimulation of the glycolytic pathway of muscle energy metabolism (Ouhayoun, 2003). The higher pH in fresh rabbit meat may imply a higher level of muscle glycogen reserves (Sabuncuoglu et al., 2011).

Color is the primary attribute influencing consumer choice of chicken meat (Barbut, 2001). The amount of myoglobin in muscle is the main factor that affects the meat color. In our study, color values were similar to values reported in previous research (María et al., 2006; Trocino et al., 2002). The small differences in color 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. Hulot and Ouhayoun (1999) indicated that the brightness of meat rose as the muscle acidified causing the myofibrillary apparatus to shrink and thus increased the reflection of light from the surface. The pattern of least squares means for a* measured at 0h and 24h differed in the *longissimus dorsi* (Table 3) and *biceps femoris* muscles (Table 4). Values of a* decreased in the *longissimus dorsi* but it increased in the *biceps femoris* between 0h and 24h across all genotypes (CC, TT, and CT). This suggested that the *POU1F1* gene may have affected a* in these two muscles differently.

Intramuscular fat and cooking loss can affect the juiciness of meat. The results of Table 5 mean that the *POU1F1* gene may be a candidate gene for rabbit meat breeding. Compared to pig meat, rabbit meat has lower intramuscular fat and higher

cooking loss (Lim et al., 2014; Franco et al., 2014). This may make it less desirable for some consumers. However, lower intramuscular fat would be advantageous for human health.

In conclusion, we found one SNP with 2 alleles (C and T) in intron 5 of the *POU1F1* gene. The T allele had the highest frequency in three rabbit breeds. The SNP was significantly related to pH in the *biceps femoris* muscle and intramuscular fat in the *longissimus dorsi* and *biceps femoris* muscles. Thus, this *POU1F1* SNP is of potential use in marker assisted selection for meat quality traits in rabbits. This assumes that this SNP is closely associated with QTL affecting meat quality traits. Further work is necessary in different, larger rabbit populations to elucidate the mechanisms involved in this gene's effects on rabbit meat quality traits.

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