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RESEARCH ARTICLE

Genetic effect of *Myf5* gene in rabbit meat quality traits

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Abstract. The objective of this study was to investigate the effect of the polymorphism in the *Myf5* gene on meat quality traits in the Ira and Tianfu Black rabbit breeds using polymerase chain reaction and DNA sequencing. A total of six SNPs and four haplotypes were found in Ira rabbits and only two SNPs were found in Tianfu Black rabbits. The two rabbit breeds had intermediate levels of genetic diversity according to their polymorphic information content values. The SNP association analysis in Ira indicated that SNP1-6 had a significant association with redness, yellowness and intramuscular fat values in the *biceps femoris* muscle, and also a significantly effect on redness in the *longissimus dorsi* muscle. The haplotype association analysis indicated that some haplotypes could be selected to get higher or lower meat redness, yellowness and intramuscular fat content in *longissimus dorsi* in Ira rabbits. Several SNPs and haplotypes of *Myf5* identified here could be considered as molecular markers to improve the meat quality of Ira and Tianfu Black rabbits.

Keywords. intramuscular fat; meat colour; potential of hydrogen; Myf5 polymorphism; rabbit.

Introduction

Myogenic factor 5 (*Myf5*) is a member of the muscle regulatory factors (MRFs) family that plays an important role in the formation of muscle fibres and transcription of muscle specific genes, and it also has an effect on meat deposition capacity and intramuscular fat level (Fujisawa-Sehara *et al.* 1990; Te Pas *et al.* 2007; Verner *et al.* 2007). Tatusova and Madden (1999) revealed that *Myf5* is involved in the myogenic process especially in the muscle differentiation stage. Moreover, Klosowska *et al.* (2004) found that the *Myf5* gene appeared to regulate the proportion of fast-twitch oxidative fibres which may affect meat production and meat quality in pigs. According to NCBI Reference Sequence NC_013672.1, the *Myf5* gene in rabbits is 2702-bp long with three exons and two introns. The lengths of exons 1, 2, and 3 are 501, 76 and 191 bp, respectively, whereas intron 1 is 773 bp and intron 2 is 418-bp long.

Polymorphism of *Myf5* has been found to be associated with growth performance and meat quality. In cattle, Seong *et al.* (2011) reported that *Myf5* genotypes were significantly associated with back fat and live weight at six month of age in Hanwoo, and that this gene could significantly affect carcass weight, *M. longissimus dorsi* area, back fat thickness and marbling score together with the *POU1F1* gene. Further, *Myf5* SNP significantly influenced live weight, loin eye height, loin eye area and water holding capacity in indigenous Chinese cattle breeds (Ujan *et al.* 2011). Evidence showed that the *Myf5* gene affected the myosin heavy chain (MyHc) isoform expression which further influenced the expression of muscle fibre types and the *Myf5* mRNA selectively expressed in slow muscle fibres (lingual muscles, masseter and diaphragm) or satellite cells (Muroya *et al.* 2002). Recent studies showed that variants in gene intron regions could affect growth and carcass traits in cattle (Sherman *et al.* 2008). SNP g.1911A>G located in intron 2 of the *Myf5* gene was reported to be associated with live weight and carcass weights in Korean cattle (Bhuiyan *et al.* 2009) and with withers height and height at hip cross in Chinese Qinchuan cattle (Zhang *et al.*

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2007). In pigs, several studies found that *Myf5* had an effect on carcass traits (Te Pas *et al.* 1999; Cieslak *et al.* 2002; Liu *et al.* 2007) instead of meat quality traits. However, Khang and Ngu (2013) reported that *Myf5*/*Hin1II* was significantly associated with dressing percentage, loin weight, meat pH_{45min} and compression force in vietnamese Mong Cai pigs. In addition, a *Myf5*/*Hsp92II* polymorphism that produced an amino acid substitution was significantly associated with changes in intramuscular fat and meat moisture content (Liu *et al.* 2008).

However, no studies regarding associations between *Myf5* SNPs and meat quality traits were found in rabbits. Thus, the objective of this research was to explore SNPs and haplotypes in the *Myf5* gene and to evaluate associations between these variants and meat quality traits in the Ira and Tianfu Black rabbit breeds. Ira hybrid rabbits were bred for white colour by a French breeding company during the last century and it was composed of four lines (A, B, C and D). Ira rabbits have a high survival rate, a fast rate of growth, high feed conversion rate, and high meat production, hence they have been introduced to many countries. Tianfu Black rabbits were bred for black colour in China based on Californian rabbit, Belgian rabbit and German checkered giant rabbit breeds; now they are famous in China for their fast growth, meat flavour and high disease resistance.

Materials and methods

Animals

We investigated 188 rabbits from two breeds in a single farm in China: Ira ($n = 106$) and Tianfu Black ($n = 82$). Rabbits were all slaughtered together at 70 days of age. Carcasses were kept at 4°C for 24 h. The meat quality traits were pH, colour (L*, a*, b*) and intramuscular fat (IMF). These traits were measured in the longissimus dorsi and biceps femoris muscles following methods described in Van Laack *et al.* (2000) and AOAC (1980). 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 from rabbit ear tissue using AxyPrep Genomic DNA Miniprep Kit (Axygen, USA) and stored at −80°C. The PCR primers were designed by the Primer Premier 6 software based on the rabbit gene sequence (GenBank accession no. NC_013672.1). Primers and fragment sizes are given in table 1. 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

Table 1. Primers for amplification and distribution of genetic variants in the rabbit *Myf5* gene.

Primer sequences F/R ($5' \rightarrow 3'$)	Primer location	SNPs	Variant type	T_a (°C)	SAF (bp)	DPS (nt)
F: CAGGCAACTGCCCTTGTTAATT	Exon 1			55.1	1296	
R: TGCTTGGTTTAGGAAGGCTCAG	Part of intron 1	SNP 1	g.59988451, A>G			0
F: CTGCTGCTCTCCTCTTCATCAAG	Part of intron 1	SNP 2	g.59988905, G>A	55.0	931	454
R: GTAACAGGCCTGGAAGAACTGATC		SNP 3	g.59988997, G>A			92
	Exon 2	SNP 4	g.59989092, C>T			95
	Intron 2	SNP 5	g.59989112, T>A			20
		SNP 6	g.59989135, G>A			23
	Exon 3					

T_a, annealing temperature; SAF, size of amplification fragment; DPS, distance from previous SNP (nt).

at 95°C for 5 min, 40 cycles of denaturing at 95°C for 40 s, annealing at 55.1/55.0°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 the DNAStar-Seqman software (ver. 7.10).

Statistical analysis

Genotype and allele frequencies of the Ira and Tianfu Black rabbit breeds were calculated using standard procedures. Briefly, Hardy–Weinberg equilibrium (HWE) for various locus-breed combinations was tested with a likelihood ratio test using numbers of observed and expected alleles; computations were carried out with software PopGene (ver. 3.2). Population genetic indexes (H_e, N_e and PIC) were obtained with procedures described by Nei and Roychoudhury (1974) and Botstein *et al.* (1980).

Associations among and between SNP, and meat quality traits were analysed by determining haplotype patterns, computing linkage disequilibrium, and performing statistical association analyses. Haplotypes for the Ira and Tianfu Black breeds were identified using Bayesian procedures in software PHASE (ver. 2.1; Stephens *et al.* 2001). Associations between SNP/haplotypes and meat quality traits were analysed separately for Ira using the model: $Y_{ijk} = \mu + H_i + S_j + E_{ijk}$, where Y_{ijk} is meat quality trait; μ, overall mean for each trait; H_i, SNP/haplotype effect; S_j, the sex effect; E_{ijk}, the error. Least squares means (LSM) and their standard errors were computed for all SNP/haplotype effects. Bonferroni *t*-tests were used to evaluate the significance of comparisons between pairs of SNP/haplotype effects. Software SPSS 21 (IBM, Armonk, USA) was utilized to perform the statistical analysis.

Results and discussion

We amplified the whole sequence of the *Myf5* gene from 188 rabbits and detected six SNPs (table 1). All six SNPs and their genotypes were found in Ira rabbits, but only SNP 1 and its three genotypes were found in Tianfu Black rabbits. Interestingly, for Ira, SNPs 2 and 3, also SNPs 4, 5 and 6 were in complete linkage disequilibrium (figure 1). The frequencies of genotypes, alleles and diversity parameters in the two breeds are provided in table 2. For SNP 1, genotype AA was more frequent than genotypes GG and AG, and A was the predominant allele in the two breeds. For SNPs 2, and 3, only Ira had three genotypes, and AG/G was the prevalent genotype/allele. For SNPs 4, 5, and 6, Ira rabbits had two genotypes and the CC/C was the prevalent genotype/allele. Genotype distributions for SNP 1 and SNPs 4, 5, and 6 deviated from HWE ($P < 0.05$; table 2) perhaps due to artificial selection, migration, and genetic drift. The larger number of variants in Ira than in Tianfu Black rabbits indicate the existence of genetic differences between these two breeds. Thus, breed differences should be accounted for rabbit breeding projects in addition to relationships among animals and inbreeding as all these factors would contribute to differences in animal productivity. Except for SNPs 2 and 3 and SNPs 4, 5, and 6 in Tianfu Black rabbits, the values of H_e (gene expected heterozygosity) approached 0.5 and the values of N_e (effective allele numbers) approached 2.0. All PIC values were within a range 0.25 to 0.50, indicating that the Ira and Tianfu Black breeds had intermediate levels of genetic diversity.

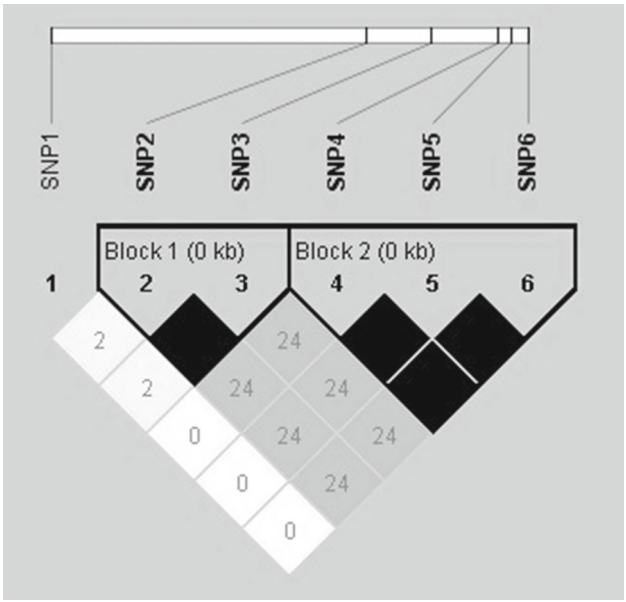

Figure 1. Linkage disequilibrium plot for Ira rabbits. Colour scheme is according to Haploview R^2 scheme. Numbers in each cell represent pairwise R^2 values (%) and empty cells represent pairwise $R^2 = 1$, between the corresponding SNPs.

Associations between SNP/haplotypes, diplotypes (haplotype combinations) and meat quality traits in Ira rabbits are provided in tables 3 and 4. All SNPs had significant effects on redness, yellowness and intramuscular fat in biceps femoris ($P < 0.05$; table 3), and SNPs 4, 5, and 6 were associated only with redness in longissimus dorsi ($P < 0.05$; table 3). This indicated that these Myf5 SNPs could be considered as potential genetic markers for meat quality selection in breeding programmes of Ira and Tianfu Black. Conversely, no significant associations between SNPs 1, 2, and 3 and meat quality traits existed for longissimus dorsi in Ira rabbits (table 3). Similarly, there were also no significant associations between variants and lightness and pH in biceps femoris in Ira rabbits (table 3). However, in biceps femoris Ira rabbits with the AA-AG-AG genotype had higher redness and yellowness LSM values than the other two genotypes ($P < 0.05$), and animals with the GG-AA-AA genotype had the highest intramuscular fat LSM value ($P < 0.05$; table 3). This

Table 2. Genotypic and allelic frequencies, χ^2 tests and diversity parameters for the *Myf5* gene in two breeds of rabbits.

SNP	Breed	Genotype/number/GF			Allele/AF		χ^2 (HWE)	H_e	N_e	PIC
1	IR	AA/55/0.52	GG/20/0.19	AG/31/0.29	A/0.67	G/0.33	$P < 0.05$	0.45	1.80	0.35
	TB	44/0.54	12/0.15	26/0.32	0.70	0.30	$P < 0.05$	0.42	1.74	0.33
2, 3	IR	AA/20/0.19	GG/31/0.29	AG/55/0.52	A/0.45	G/0.55	$P > 0.05$	0.49	1.98	0.37
	TB	–	82/1.00	–	0.00	1.00	–	0.00	1.00	0.00
4, 5, 6	IR	CC/59/0.56	TT/47/0.44	CT/0/0.00	C/0.56	T/0.44	$P < 0.05$	0.49	1.97	0.37
	TB	–	82/1.00	–	0.00	1.00	–	0.00	1.00	0.00

SNPs 1 and 2 are in complete linkage disequilibrium. IR, Ira rabbits ($n = 106$); TB, Tianfu Black rabbits ($n = 82$); GF, genotypic frequency; AF, allelic frequency; χ^2 (HWE), Hardy–Weinberg equilibrium χ^2 probability value; H_e, gene heterozygosity; N_e, effective allele number; PIC, polymorphism information content.

indicated that the AA-AG-AG genotype could be regarded as genetic marker for increasing meat redness and yellowness and genotype GG-AA-AA could be used as a genetic marker to increase intramuscular fat in biceps femoris. Ira rabbits with the CC-TT-GG genotype for SNPs 4, 5, and 6 had the higher redness LSM values in the longissimus dorsi muscle than those with the TT-AA-AA genotype. No significant differences in lightness, yellowness, pH and IMF in longissimus dorsi existed in Ira rabbits. Ira rabbits with the CC-TT-GG genotype had higher redness LSM values whereas animals with the TT-AA-AA genotype had higher yellowness and intramuscular fat values in biceps femoris. This indicated that genotype CC-TT-GG could be useful as a genetic marker for increasing redness in longissimus dorsi and biceps femoris, and TT-AA-AA genotype could be used as a genetic marker for increasing yellowness and IMF in biceps femoris. Diplotypes in Ira exhibited significant associations with redness in longissimus dorsi and with redness, yellowness and intramuscular fat in biceps femoris (table 4). Rabbits with haplotype 1 (AAACTG) had the highest redness value in both muscles (table 4). Animals with haplotypes 2 and 4 could help decrease redness in longissimus dorsi and biceps femoris. In biceps femoris, rabbits with haplotype 2 had the highest yellowness value and the haplotype 3 had the lowest yellowness of all haplotypes. Rabbits with haplotype 4 had the highest IMF and those with haplotype 2 had the lowest value of all haplotypes. This indicated that these haplotypes could be potentially useful as genetic markers to increase or decrease meat quality trait values in rabbits.

The association analysis showed that *Myf5* had a significant effect on meat quality which was consistent with many studies that reported that this gene played an important role in carcass and meat quality (Liu *et al.* 2007; Liu *et al.* 2008; Sherman *et al.* 2008; Bhuiyan *et al.* 2009; Kunhareang *et al.* 2009; Seong *et al.* 2011; Ujan *et al.* 2011). However, the molecular mechanism of how the *Myf5* gene works in animal is unclear. Myf5 was first expressed about 8-d postcoitum and regulated the differentiation of skeletal muscle precursors as a transcription factor in mice (Zammit *et al.* 2004). Moreover, expression of *Myf5* could promote cell proliferation and increase in the number of mononuclear myoblasts (Biressi *et al.* 2013). Zhang *et al.* (2014) reported that the expression of *Myf5* changed with the age of the chicken indicating that the genetic effects of *Myf5* differed at these growth stages. Zammit *et al.* (2004) found that multiple enhancers were needed to generate the full expression pattern of *Myf5* in adult muscle and the controlling elements were genetically separable and possibly distinct from those controlling factors during development. Some researchers reported that satellite cell population and muscle spindles controlled the expression of *Myf5* in adult skeletal muscle (Beauchamp *et al.* 2000; Zammit *et al.* 2004). Braun *et al.* (1989) reported that the *Myf5* protein was a transcription factor in myocyte differentiation and indirectly activated the muscle gene. Consequently, SNPs in *Myf5* was associated with phenotypes for rib eye area, shear force and myofibrillar fragmentation index (Curi *et al.* 2012). However, no QTL associated with fat deposition traits was mapped close to *Myf5* (Cattle QTL database 2010). Kunhareang *et al.* (2009) stated that the substitution of alanine/proline amino acid in pigs may change the protein structure, thus affecting the association between *Myf5* and muscle growth, which was consistent with previous results reported by Komar (2007) and Liu *et al.* (2007).

Many studies had been carried out to investigate *Myf5* gene interaction effects. Seong *et al.* (2011) explored the interaction between *Myf5* and *POU1F1* in Hanwoo cattle and found that the interaction between these two genes significantly affected carcass weight, longissimus dorsi area, back fat thickness, and marbling score. Braun *et al.* (1989) reported that three conserved sequence regions in *MyoD* and *Myf5* were all involved in the myogenic programming of mesodermal cells, and that these two genes determined the muscle lineage (Braun *et al.* 1989, 1990). Zhang *et al.* (2014) studied the interaction between *Myf5* and *MyoG* and found that it had a significant influence on weights at 42 d, 56 d, and 300 d of age in cattle which showed that we should take gene interaction effects into account

Table 3. Associations between SNP/haplotypes and meat quality traits in Ira rabbits.

SNP	Genotype	Number (total = 106)	Traits in the *longissimus dorsi* muscle					Traits in the *biceps femoris* muscle				
			L*	a*	b*	pH	IMF	L*	a*	b*	pH	IMF
1, 2, 3	AA-AG-AG	55	50.66 ± 2.11	5.53 ± 0.25	2.20 ± 0.06	6.44 ± 0.10	1.15 ± 0.06	53.15 ± 1.37	4.94 ± 0.23	2.15 ± 0.14	6.22 ± 0.16	1.70 ± 0.03
	GG-AA-AA	20	48.77 ± 1.01	4.19 ± 0.21	2.22 ± 0.07	6.65 ± 0.06	1.59 ± 0.03	50.20 ± 1.17	2.21 ± 0.05	1.87 ± 0.09	6.75 ± 0.08	2.99 ± 0.24
	AG-GG-GG	31	52.33 ± 1.87	5.26 ± 0.29	2.89 ± 0.11	6.64 ± 0.14	0.89 ± 0.03	51.05 ± 1.10	2.46 ± 0.12	1.45 ± 0.05	6.61 ± 0.13	2.30 ± 0.08
	P value[a]		0.4138	0.0524	0.2894	0.5129	0.2354	0.5042	0.0381	0.0345	0.6732	0.0311
4, 5, 6	CC-TT-GG	59	51.64 ± 1.61	5.48 ± 0.18	2.41 ± 0.15	6.50 ± 0.09	1.07 ± 0.03	52.52 ± 1.00	4.19 ± 0.13	1.94 ± 0.09	6.34 ± 0.13	1.88 ± 0.06
	TT-AA-AA	47	49.04 ± 1.25	3.92 ± 0.14	2.26 ± 0.12	6.65 ± 0.14	1.25 ± 0.07	52.20 ± 1.07	2.61 ± 0.08	2.37 ± 0.15	6.75 ± 0.17	2.45 ± 0.17
	CT-AT-AG[b]	0	–	–	–	–	–	–	–	–	–	–
	P value		0.5132	0.0428	0.1579	0.4327	0.3861	0.4138	0.0314	0.0388	0.5812	0.0478

Traits were measured 15 min after slaughter; L*, lightness; a*, redness; b*, yellowness; IMF, intramuscular fat. Genotypes AA, GG and AG for SNP 1 were represented in the same set of animals as AG-AG, AA-AA and GG-GG for SNP 2–3, thus a combined analysis involving SNP 1 and SNP 2–3 was conducted. [a]Probability of the *F* test for genotype effect. [b]Because haplotype CT-AT-AG was absent ($n = 0$), only one contrast was estimated, i.e. the difference between haplotypes CC-TT-GG ($n = 59$) and TT-AA-AA ($n = 47$).

Table 4. Associations between diplotypes of six SNPs and meat quality traits in Ira rabbits.

ID	Diplotype	Haplotype (Hap)	Number (total = 106)	Traits in *longissimus dorsi* muscle					Traits in *biceps femoris* muscle				
				L*	a*	b*	pH	IMF	L*	a*	b*	pH	IMF
1	AA-AG-AG-CC-TT-GG	Hap1:AAACTG	39	50.40±1.67	6.70±0.23	2.52±0.13	6.30±0.15	1.25±0.03	52.23±1.71	5.29±1.18	1.95±0.13	6.10±0.19	1.88±0.13
2	AA-AG-AG-TT-AA-AA	Hap2:AAATAA	16	50.43±1.36	3.95±0.15	2.06±0.08	6.05±0.17	1.19±0.06	55.21±1.68	3.52±1.11	2.14±0.10	6.41±0.21	1.51±0.09
3	AG-GG-GG-TT-AA-AA	Hap3:AGGTAA	31	51.79±1.78	5.32±0.17	2.97±0.13	6.57±0.13	1.14±0.08	51.60±2.01	2.49±1.16	1.37±0.11	6.54±0.23	2.24±0.18
4	GG-AA-AA-CC-TT-GG	Hap4:GAACTG	20	48.77±1.82	4.20±0.12	2.22±0.09	6.64±0.16	1.59±0.10	50.99±1.17	2.21±0.09	1.84±0.14	6.75±0.17	2.99±0.21
P value[a]				0.314	0.032	0.518	0.285	0.452	0.0572	0.029	0.041	0.603	0.027

Traits were measured 15 min after slaughter; L*, lightness; a*, redness; b*, yellowness; IMF, intramuscular fat. [a]Probability of the *F*-test for diplotype effects.

when selecting animals for complex traits. Moreover, Yin *et al.* (2011) also found that single SNPs of *Myf5* and *MyoG* genes in chicken had significant associations with carcass traits. All these studies suggest that the mechanism of action of the *Myf5* on the formation of various phenotypes is extensive and complex, thus further research would need to be carried out with larger breed samples of rabbits to confirm the associations found here and to elucidate functional mechanisms.

Conclusions

We found that Ira rabbits had more variants than Tianfu Black rabbits. We found six SNPs in Ira, and SNPs 2 and 3 as well as SNPs 4, 5 and 6 were, respectively, in complete linkage disequilibrium. SNPs 1 to 6 had significant associations with redness, yellowness and intramuscular fat in biceps femoris, and SNPs 4, 5, and 6 had significant associations with redness in longissimus dorsi. Some haplotype combinations were significantly associated with redness in longissimus dorsi, and with redness, yellowness and IMF in biceps femoris. Thus, some novel SNPs and haplotypes could be utilized as molecular markers to improve meat quality in Ira and Tianfu Black rabbits. Because of the complexity of the mechanisms involved between *Myf5* and various phenotypes, further research should include larger breed samples to confirm associations and elucidate functional mechanisms.

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