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Original article

Expression pattern and polymorphism of three microsatellite markers in the porcine *CA3* gene

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Abstract – Carbonic anhydrase III (CA3) is an abundant muscle protein characteristic of adult type-1, slow-twitch, muscle fibres. In order to further understand the functions of the porcine CA3 protein in muscle, the temporal and spatial distributions of its gene product were analysed and the association between the presence of specific polymorphisms and carcass traits in the pig was also examined. Real-time PCR revealed that the *CA3* mRNA expression showed no differences with age in skeletal muscles from Yorkshire pigs at postnatal day-1, month-2, and month-4. We provide the first evidence that *CA3* is differentially expressed in the skeletal muscle of Yorkshire and Meishan pig breeds. In addition, the whole pig genomic DNA sequence of *CA3* was investigated and shown to contain seven exons and six introns. Comparative sequencing of the gene from three pig breeds revealed the existence of microsatellite *SJ*160 in intron 5 and microsatellite *SJ*158 and a novel microsatellite marker that includes a tandem repeat of $(TC)_n$ in intron 4. We also determined the allele number and frequencies of the three loci in seven pig breeds and found that they are low polymorphic microsatellite markers. Statistical analysis showed that the *CA3* microsatellite polymorphism was associated with dressing percentage, internal fat rate, carcass length, rib number and backfat thickness in the pig.

pig / carbonic anhydrase III / expression pattern / microsatellite polymorphism

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1. INTRODUCTION

Carbonic anhydrase III (*CA3*) is a member of a gene family encoding proteins, which catalyse the hydration of CO_2 to generate protons and bicarbonate ions for cellular ion transport and pH homeostasis. It is an abundant muscle protein characteristic of adult type-1, slow-twitch, muscle fibres. The protein plays an important role in facilitated CO_2 diffusion and diverse processes involving H^+ and HCO_3^- transport [2]. Pig muscle carbonic anhydrase III is a 30 kDa protein displaying three activities *i.e.* CO_2 hydratase, acetate esterase and p-nitrophenyl phosphatase [10]. The *CA3* gene was first isolated in man [8]. Studies have shown that the expression of the *CA3* gene is strictly tissue-specific and is high in skeletal muscle and much lower in cardiac and smooth muscles [9]. The porcine *CA3* gene has been assigned to pig chromosome SSC4q11-q12 [4]. Previously, we have reported the cDNA sequence of the porcine *CA3* gene and polymorphic sites within this gene. Liu *et al.* [7] isolated the porcine *CA3* gene by mRNA differential display in the *longissimus dorsi* muscle tissues from a Landrace × Large White cross-combination. Wang *et al.* [11] analysed temporal and spatial expression differences of the *CA3* gene in the pig and reported that the CA3 protein is associated with intramuscular fat content and ham percentage.

In this study, we studied the allele frequencies of three microsatellite loci in the *CA3* gene in seven pig breeds. We report the allele frequencies and the results of association analyses of the three microsatellite markers within the *CA3* gene. Additionally, the expression patterns of the porcine *CA3* gene during the porcine skeletal muscle development and in different pig breeds were analysed.

2. MATERIALS AND METHODS

2.1. Analysis of *CA3* expression levels

Total RNA was extracted using TRIzoL reagent (Invitrogen, Carlsbad, CA, USA) from the *longissimus dorsi* of Yorkshire pigs at postnatal day-1, month-2, and month-4 and Meishan pigs at postnatal month-2, respectively. Three animals were sampled for each breed and at each stage. Reverse transcriptase PCR was carried out using M-MLV Reverse Transcriptase (Promega, USA).

Amplification primer CA (Tab. I) was designed to analyse *CA3* expression levels using porcine *CA3* cDNA sequences (AY789514) as templates and Primer Express software. Quantitative PCR reactions were performed in 25 μL volumes containing 12.5 μL 2 SYBR Green Real-time PCR Master Mix

Table I. Primers used in this study.

Primer name	Sequence (5′ to 3′)	Binding region	Annealing temperature (°C)	Size (bp)
CA	F: GCCAAGGGAGACAACCAA	Exon 2	60	234
	R: TGAGGAGCCCCAGTGAAG	Exon 3		
GAPDH-1	F: GAAGGTCGGAGTGAACGGAT	Exon 2	60	251
	R: CTCATTTGATGTTGGCGGG	Exon 4		
CA-1	F: GTCCAGTGCCCACGAAGA	Exon 1	58	918
	R: GGCAGAGCCAGGGTCATA	Exon 2		
CA2	F: CCTGAACAATGGGAAGAC	Exon 2	53	2502
	R: GAAGATGAAACTGGCGAA	Exon 3		
CA-3	F: CACGGATCTGAGCACACT	Exon 3	52	1855
	R: ATGCCAACTACAGCCACT	Exon 4		
CA-4	F: CCCTGATGGAGTGGCTGTA	Exon 4	57	1386
	R: TGGAACTCGCCTTTCTCAC	Exon 5		
CA-5	F: ATAGGACGTGAGAAAGGCG	Exon 5	57	1648
	R: GTTGAAGTTCGTGAAGGGT	Exon 6		
CA-6	F: CTGCGAGGAGTGCATTGTG	Exon 6	57	1130
	R: CTGCCCTTGATAGGCTGTG	Exon 7		
SJ16	F: TGAAGTATTTGAGACAGAAGTTGAGG	Intron 5	55	179/205
	R: TGAGCTACGACTGGAACTCCTAC	Intron 5		
SJ15	F: TCAAGATATTTCTGCTAAGACAAG	Intron 4	55	223/229/231
	R: CCTCGTGGATCAACTCTACTTC	Intron 4		
TC	F: TTGGTTTTCTTTTCCCTCTT	Intron 4	56	100/102/110
	R: GCCTTTCTCACGTCCTATCT	Exon 5		

(TOYOBO, Japan), 1 μL cDNA and 0.3 μM of a terminal concentration of each primer. PCR was run on the Applied Biosystems 7500 Real-Time PCR System. Fluorescent signals were continually monitored at the end of each PCR cycle comprising 1 min at 95 °C for the initial denaturing step, 15 s at 95 °C, 15 s at 60 °C and 50 s at 72 °C for a total of 40 cycles. The relative expression levels of the gene were analysed using the Comparative Ct method, in which glyceraldehyde-3-phosphate dehydrogenase (*GAPDH*) was used as an internal control, to correct for the differences in the mRNA quantities. A specific primer pair (GAPDH-1, see Tab. I) that amplified a 251 bp fragment was designed and the PCR conditions were identical to those described above. A t-test was performed to conclude on the significance of the differences observed.

2.2. Genomic DNA amplification and sequence analysis

The cDNA sequence of the pig *CA3* gene was compared with the human and mouse orthologous mRNA and their genomic sequence in order to

predict the genomic organisation of the pig gene, which was confirmed by PCR amplification and sequencing. Six primer pairs were designed to amplify the genomic sequence including all introns (Tab. I). Three genomic DNA mixture pools from three pig breeds (six Yorkshire pigs, six landrace pigs and six Meishan pigs) were used. PCR was performed in a 25 μL reaction mix containing the following: 200 ng of genomic DNA pool, 200 μM dNTP, 0.4 μmol of each PCR primer, and 1U Taq DNA polymerase in the reaction buffer supplied by the manufacturer. PCR was run as follows: 94 °C for 4 min, 35 cycles of 94 °C for 50 s, optimal temperature (Tab. I) for 50 s, 72 °C for 1 min 30 s and a final extension step at 72 °C for 10 min. The purified PCR products were cloned into the pGEM-T vector (TaKaRa, Dalian, China) and were sequenced using standard M13 primers. DNA sequences were compiled using the DNA star software (Madison, WI, USA). The sequencing results of different pig breeds were compared using BLAST (http://www.ncbi.nlm.nih.gov).

2.3. Detection of microsatellite polymorphisms

Based on the BLAST results of the whole genomic sequence of the pig *CA3* gene in Yorkshire, Landrace and Meishan breeds, microsatellite *SJ*160 was identified in intron 5, and microsatellite *SJ*158 and a novel microsatellite marker that includes a tandem repeat of $(TC)_n$ were identified in intron 4. Primers SJ16 and SJ15, designed from the primer sequences of pig *SJ*160 (AB091132) and *SJ*158 (AB091130), were used to detect microsatellite *SJ*160 and *SJ*158 polymorphisms, respectively. Primer TC was designed from porcine *CA3* DNA sequences and used to detect the polymorphism of the novel microsatellite $(TC)_n$ (Tab. I). PCR was performed in a 20 μL reaction mix containing the following: 25 ng of genomic DNA pool, 150 μM dNTP, 0.25 μmol of each PCR primer and 1 U Taq DNA polymerase in the reaction buffer supplied by the manufacturer. PCR was run as follows: 94 °C for 4 min, 30 cycles of 94 °C for 30 s, 55 °C for 30 s, 72 °C for 30 s and a final extension step at 72 °C for 10 min. The denatured PCR products were analysed on 12% polyacrylamide denaturing sequencing gels, which were stained in a $AgNO_3$ solution and then scanned by the Gel Imaging System (Olympia). To ensure the accuracy of the alleles analysed in our study, different samples of DNA alleles were cloned and sequenced.

2.4. Statistical analysis

Allele frequencies were studied in seven different pig populations. The microsatellite markers were genotyped in 330 F2 pigs of a Yorkshire × Meishan

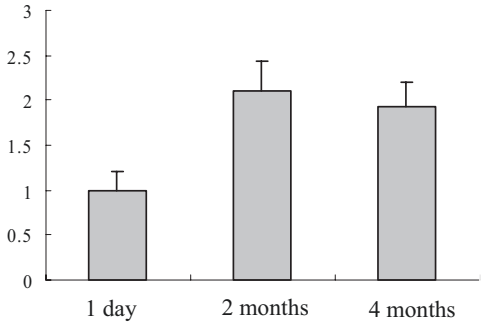

Figure 1. Temporal expression profiles of the porcine *CA3* gene. Relative levels of *CA3* mRNA were calculated using the Comparative Ct method with *GAPDH* as the reference gene in each sample. Bars represent the mean ± SE (n = 3). Postnatal day-1, month-2 and month-4 indicate three stages of skeletal muscle development in Yorkshire pigs. The differences in the levels of expression were not significant ($P > 0.05$) by t-test analysis.

reference family. The association studies between genotype and carcass traits were performed with the least squares method (GLM procedure, SAS® version 8.0). The model used to analyse the data was assumed to be:

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

where, Y_{ijk} is the observation of the trait; μ is the least squares mean; G_i is the effect of the ith genotype; S_j is the effect of the j^{th} sex (j = 1 for male or 2 for female); F_k is the effect of year; b_{ijk} is the regression coefficient of the slaughter weight and e_{ijk} is the random residual.

3. RESULTS

3.1. Expression pattern of porcine *CA3* gene

During the three stages of skeletal muscle development in Yorkshire pigs, we observed no statistically significant differences in *CA3* mRNA expression (Fig. 1) as shown by t-test analysis ($P > 0.05$). An expression pattern was also performed on the skeletal muscle of both Western Yorkshire and Chinese Meishan pigs at month-2. The porcine *CA3* gene was differentially expressed in the skeletal muscle of Yorkshire and Meishan breeds (Fig. 2) with a higher level in the skeletal muscle of Meishan than that of Yorkshire pigs.

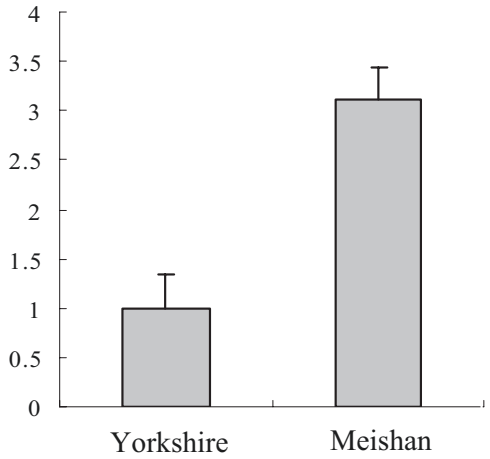

Figure 2. Differential expression analysis of the porcine *CA3* gene in different pig breeds. Real-time PCR analysis of the *CA3* gene in the skeletal muscle of Yorkshire and Meishan pigs at month-2. Relative levels of *CA3* mRNA were calculated using the Comparative Ct method with *GAPDH* as the reference gene in each sample. Bars represent the mean ± SE (n = 3). The difference in the levels of expression was significant ($P < 0.05$) by t-test analysis.

3.2. Genomic sequence analysis of porcine *CA3* gene

The resulting overlapping PCR products were assembled into a single contig revealing the pig *CA3* genomic sequence (Genbank accession number DQ675018). A 9589-bp genomic DNA sequence covering the entire coding region of porcine *CA3* was amplified using six gene-specific primer pairs (Tab. I) and compared with the cDNA sequence to clarify the exon/intron organisation. The porcine *CA3* gene is composed of seven exons and six introns. All splice donor and acceptor sites conform to the typical 5' GT-AG 3' rule. By comparing the sequence of these different pig breeds, we found that microsatellite *SJ*160 which includes a tandem repeat of $(CA)_n$ is located approximately at 431 bp from the beginning of intron 5. Microsatellite *SJ*158 which includes a tandem repeat of $(GT)_n$ is located approximately at 240 bp from the beginning of intron 4 and the novel microsatellite, which included a tandem repeat of $(TC)_n$ is located approximately at 1264 bp from the beginning of intron 4.

3.3. Allele frequencies in different pig breeds

We also studied the allele frequencies of the three microsatellite markers in seven pig breeds. For microsatellite marker *SJ*160, only two alleles were

Table II. Allele frequencies at the *CA3 SJ*160 locus in seven pig breeds.

Breed	Number of pigs	Genotype			Allele frequencies (%)	
		AA	*AB*	*BB*	*A* $(CA)_9$	*B* $(CA)_{22}$
Yorkshire	42	42	0	0	100	0
Landrace	41	41	0	0	100	0
Duroc	26	26	0	0	100	0
Meishan	42	0	3	39	3.6	96.4
Jianli	20	3	9	8	37.5	62.5
Qingping	24	7	8	9	45.8	54.2
Exi	29	19	7	3	77.6	22.4

Table III. Allele frequencies at the *CA3 SJ*158 locus in seven pig breeds.

Breed	Number of pigs	Genotype						Allele frequencies (%)		
		AA	*AB*	*BB*	*AC*	*BC*	*CC*	*A* $(GT)_{18}$	*B* $(GT)_{17}$	*C* $(GT)_{14}$
Yorkshire	34	1	15	18	0	0	0	25.0	75.0	0
Landrace	41	6	12	23	0	0	0	29.2	70.8	0
Duroc	21	0	2	15	0	4	0	4.8	85.7	9.5
Wannan	19	1	2	9	0	0	7	10.5	52.7	36.8
Meishan	39	0	0	0	0	0	39	0	0	100
Jianli	13	0	0	6	0	4	3	0	61.5	38.5
Exi	41	0	8	13	7	5	8	18.3	47.6	34.1

detected with repeat numbers 9 and 22, for alleles *A* and *B*, respectively. Frequencies of both alleles differ between the two pig breed types, while allele *A* is fixed in three western commercial pig breeds, allele *B* is prevalent in Chinese indigenous pig breeds, with the exception of Exi pigs (Tab. II). For microsatellite marker *SJ*158, three alleles were detected with repeat numbers 18, 17 and 14, for alleles *A*, *B* and *C*, respectively. Allele *B* is prevalent in the two pig breed types, with the exception of Meishan pigs, in which allele *C* is fixed (Tab. III). For the novel microsatellite marker that includes a tandem repeat of $(TC)_n$, three alleles were also detected in six pig breeds with repeat numbers 17, 13 and 12, for alleles *A*, *B* and *C*, respectively. Allele *B* is also prevalent in the two pig breed types, with the exception of Meishan pigs, in which allele *A* is prevalent (Tab. IV).

3.4. Association analysis of the three *CA3* microsatellite polymorphisms

The results of the association analysis between the three *CA3* microsatellite polymorphisms and carcass traits in 330 F_2 offspring (Yorkshire × Meishan) are given in Tables V, VI and VII. At the *SJ*160 locus, statistically significant

Table IV. Allele frequencies of microsatellite marker *CA3*, which includes a tandem repeat $(TC)_n$ in six pig breeds.

Breed	Number of pigs	Genotype						Allele frequencies (%)		
		AA	*AB*	*BB*	*AC*	*BC*	*CC*	*A* $(TC)_{17}$	*B* $(TC)_{13}$	*C* $(TC)_{12}$
Yorkshire	39	0	0	39	0	0	0	0	100	0
Duroc	20	4	10	6	0	0	0	45	55	0
Landrace	39	0	0	35	0	3	1	0	93.6	6.4
Wannan	15	1	0	0	1	2	11	10	83.3	6.7
Meishan	39	35	3	1	0	0	0	93.6	6.4	0
Jianli	16	0	6	8	0	1	1	18.7	71.9	9.4

Table V. Association analysis of the *CA3 SJ*160 polymorphism in 320 pigs. Least squares mean values with different letters are significantly different. Small letters: $P < 0.05$.

Traits	*CA3* genotype ($\mu \pm SE$)		
	AA (120)	*AB* (155)	*BB* (45)
Carcass length (cm)	91.122 ± 0.371^{ab}	90.445 ± 0.326^{a}	92.162 ± 0.605^{b}
Backfat thickness at shoulder (cm)	3.671 ± 0.069^{ab}	3.781 ± 0.061^{a}	3.503 ± 0.112^{b}
Backfat thickness at buttock (cm)	2.118 ± 0.064^{a}	2.017 ± 0.056^{ab}	1.839 ± 0.104^{b}

associations with carcass length, backfat thickness at the shoulder and backfat thickness at the buttock were found, but no significant conclusion could be drawn on other carcass traits. Pigs with the *BB* genotype had significantly higher carcass length and lower backfat thickness when compared with pigs with the *AA* or *AB* genotypes (Tab. V).

At the *SJ*158 locus, statistically significant associations with fat percentage, lean meat percentage, dressing percentage, internal fat rate, rib number, backfat thickness at the 6-7th thorax and backfat thickness at the buttock were found, but no significant conclusion could be drawn on other carcass traits. Pigs with the *AA* genotype had the highest lean meat percentage and rib number, and the lowest fat percentage and dressing percentage, while pigs with the *BC* genotype had the highest fat percentage, internal fat rate and backfat thickness in comparison with pigs with other genotypes. Allele *A* present in Yorkshire pigs is associated with increased lean meat percentage and rib number, and decreased fat percentage (Tab. IV).

At the novel microsatellite $(TC)_n$ locus, statistically significant associations with dressing percentage, internal fat rate, carcass length, backfat thickness at the buttock and average backfat thickness were found, but no significant

Table VI. Association analysis of the *CA3 SJ*158 polymorphism in 320 pigs. Least squares mean values with different letters are significantly different. Small letters: $P < 0.05$, capital letters: $P < 0.01$.

Traits	*CA3* genotype ($\mu \pm SE$)					
	AA (36)	*AB* (47)	*AC* (90)	*BB* (21)	*BC* (45)	*CC* (81)
Fat percentage (%)	22.601 ± 0.787^{a}	24.549 ± 0.685^{ab}	23.084 ± 0.497^{a}	22.891 ± 1.053^{ab}	24.948 ± 0.711^{b}	23.547 ± 0.518^{ab}
Lean meat percentage (%)	59.063 ± 1.079^{a}	55.813 ± 0.956^{b}	56.887 ± 0.645^{ab}	56.541 ± 2.428^{ab}	55.979 ± 0.757^{b}	55.776 ± 0.749^{b}
Dressing percentage (%)	71.591 ± 0.74^{a}	73.125 ± 0.664^{ab}	72.024 ± 0.477^{a}	74.282 ± 0.974^{b}	73.882 ± 0.717^{b}	73.862 ± 0.496^{b}
Internal fat rate (%)	3.101 ± 0.104^{A}	3.103 ± 0.106^{A}	3.099 ± 0.098^{A}	3.105 ± 0.110^{A}	3.497 ± 0.100^{B}	3.035 ± 0.099^{A}
Rib number	14.913 ± 0.106^{Aa}	14.458 ± 0.092^{B}	14.757 ± 0.067^{A}	14.500 ± 0.142^{b}	14.629 ± 0.096^{b}	14.773 ± 0.070^{A}
Backfat thickness at 6-7th thorax (cm)	2.844 ± 0.104^{ab}	2.776 ± 0.090^{a}	2.782 ± 0.065^{a}	2.843 ± 0.139^{ab}	3.039 ± 0.094^{b}	2.851 ± 0.068^{ab}
Backfat thickness at buttock (cm)	1.913 ± 0.118^{A}	2.021 ± 0.103^{a}	1.913 ± 0.075^{A}	1.860 ± 0.158^{a}	2.336 ± 0.107^{Bb}	1.976 ± 0.078^{A}

Table VII. Association analysis of the *CA3* $(TC)_n$ polymorphism in 326 pigs. Least squares mean values with different letters are significantly different. Small letters: $P < 0.05$, capital letters: $P < 0.01$.

Traits	*CA3* genotype ($\mu \pm SE$)		
	AA (51)	*AB* (159)	*BB* (116)
Dressing percentage (%)	74.084 ± 0.632^{a}	73.029 ± 0.355^{ab}	72.575 ± 0.416^{b}
Internal fat rate (%)	2.954 ± 0.097^{a}	3.198 ± 0.055^{b}	3.178 ± 0.064^{ab}
Carcass length (cm)	92.558 ± 0.573^{Aa}	90.644 ± 0.324^{Bb}	91.143 ± 0.380^{ABb}
Backfat thickness at buttock (cm)	1.852 ± 0.098^{a}	2.104 ± 0.056^{ab}	2.078 ± 0.065^{b}
Average backfat thickness (cm)	1.931 ± 0.319^{a}	2.968 ± 0.197^{b}	2.688 ± 0.177^{ab}

conclusion could be drawn on other carcass traits. Pigs with the *AA* genotype had significantly higher dressing percentage and carcass length but lower internal fat rate and backfat thickness when compared to pigs with *BB* or *AB* genotypes (Tab. VII).

4. DISCUSSION

The real-time PCR technique is an accurate and sensitive method that has been widely used to determine the relative quantification of mRNA or DNA. In our current study, we employed this technique to assess the changes in the expression of *CA3* mRNA during three important stages of skeletal muscle development in pig and different pig breeds. This provides some information on the functions of *CA3* during muscle development. Liu *et al.* [7] first discovered that the porcine *CA3* gene was differentially expressed in the skeletal muscle from a Landrace × Large White cross-combination. In our study, the porcine *CA3* gene was also differentially expressed in the skeletal muscle of Yorkshire and Meishan pig breeds. The results of expression profiles showed that the expression levels of *CA3* increased in skeletal muscles from prenatal 33- to 65-day-old Chinese Tongcheng pigs and subsequently decreased to a steady state in prenatal 90-day-old, postnatal 2-day-old, postnatal 28-day-old, and pregnant 65-day-old pigs [11]. Therefore, our results are supported by these former reports. We also observed that the *CA3* mRNA expression does not change in the skeletal muscle from postnatal day 1 to 4-month-old Yorkshire pigs.

In our study, we isolated the whole genomic DNA sequence of the porcine *CA3* gene and found that its structural features were identical to those

reported by Wang *et al.* [11]. Moreover, we analysed the polymorphism of microsatellites present in the whole genomic DNA sequence of *CA3*. Previously, microsatellite markers S*J*160, *SJ*158 and *SJ*159 (associated with the *CA3* gene) were located at the position 46.9 cM on the SSC4 linkage map [3]. In our study on the pig *CA3* gene representing about 10 kb, we identified only microsatellites S*J*160, *SJ*158 and a novel microsatellite locus within introns but not the *SJ*159 locus. The presence of SSRs within introns has been documented in a number of species and they are mainly composed of monomers affecting gene transcription, mRNA correct splicing or export to the cytoplasm [6]. In animal breeding studies, microsatellite markers play an important role in constructing genetic maps, QTL mapping and in analysing the function of structural genes. We speculate that these microsatellites represent important markers both for fine QTL mapping of production traits and for the study of the expression of porcine *CA3*.

An assessment of the relationships between the *CA3* functions and the characterisation of its genomic sequence, with its many polymorphisms and repeat elements, was also undertaken. Three microsatellite polymorphic sites were used to further analyse their relationships with important pig economic traits. Moreover, differences between the Chinese and western breeds were revealed by allele frequency analyses of the three microsatellite polymorphisms. Our findings show that the three polymorphic sites, *SJ*160, *SJ*158 and the novel microsatellite $(TC)_n$, can be significantly associated with the following carcass traits *i.e.* carcass length, backfat thickness, dressing percentage, internal fat rate, lean meat percentage, *etc*. We observed a greater carcass quality in individuals with the *BB* genotype at the *SJ*160 locus and with the *AA* genotype at the $(TC)_n$ locus. However, since these genotypes are the most frequent in the Meishan breed, the observation of this gene effect is not consistent with known phenotype differences in this breed. It is possible that Meishan pigs have the favourable recessive allele at the two loci. However, the number of individuals analysed for allele frequencies and association is limited and the genetic background of the Yorkshire × Meishan reference family might influence the observed results and result in a discrepancy. In a previous study [12], we identified another polymorphic site in the *CA3* gene, which is significantly associated with meat production traits *i.e.* backfat thickness, dressing percentage and lean meat percentage. Moreover, the porcine *CA3* gene has been assigned to pig chromosome 4 (SSC4) at position 46.9 cM [3, 4]. SSC4 encompasses several quantitative trait loci that are economically important in pig breeding. QTL for various carcass traits have been detected in the interval between *V-ATPase* and *NGFB* of chromosome 4 [1,5]. In the same F2 individuals

(Yorkshire × Meishan), a QTL affecting backfat thickness was found at position 53 cM, between markers SW835 and SW752 [13]. Therefore, based on previous findings and our present results, the pig *CA3* gene is located close to QTL affecting carcass traits and may be responsible for these QTL. It is also possible that the *CA3* gene may be a candidate gene for fat traits of carcass that regulate skeletal muscle development and consequently affect commercial production traits in the pig.

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