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The molecular characterization and associations of porcine cardiomyopathy asssociated 5 (*CMYA5*) gene with carcass trait and meat quality

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Abstract The cardiomyopathy associated 5 (*CMYA5*) gene was also called *TRIM76*, which was belonged to the tripartite motif super family of proteins (TRIM). It was a direct transcriptional target for *MEF2A* and it played an important role in myofibrillogenesis. In the present study, a 12056 bp cDNA sequence of the porcine *CMYA5* gene was obtained by RT-PCR. The sequence encoded a large protein consisting of 4003 amino acids and the carboxyl terminus of the predicted CMYA5 protein comprised of a B-box coiled-coil, two fibronectin type III (FN3) repeats, and SPRY domains. The porcine *CMYA5* gene was assigned to chromosome 2q21–24 by using the radiation hybrid (IMpRH) panel, and it was significantly linked to microsatellite Sw1602 with LOD scores of 6.74. Semi-quantitative RT–PCR revealed that the porcine *CMYA5* gene was broadly expressed in all seven tissues(heart, liver, spleen, lung, kidney, skeletal muscle and adipose)harvested from different developmental stages(new born, five weeks and adult tongcheng pigs), with a high level in heart and skeletal muscle. One SNP (A7189C), leading to the amino acid alteration from the Ile residue to the Leu residue, was found and detected by *BspT*I PCR-restriction fragment length polymorphism. The association analysis revealed that the substitution of A7189C had significant associations with the percentage of ham ($p < 0.05$), water loss ($p < 0.01$) and intramuscular fat ($p < 0.05$). These results provide the evidence that the porcine *CMYA5* gene can act as a potential candidate gene affecting pig meat quality.

Keywords Pig · *CMYA5* · Radiation hybrid (RH) mapping · Expression pattern · Association analysis

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Introduction

The cardiomyopathy associated 5 (*CMYA5*) gene is also called *TRIM76*, *Myospryn* or *genethonin* 3, and it is drastically decreased in Duchenne muscular dystrophy (DMD) muscles in comparison with the normal muscle tissues [1]. *CMYA5* encodes a novel 413-kDa protein and in the carboxyl terminus, it contains B-box coiled-coil (BBC), fibronectin type III (FN3) repeats, and SPRY domains in a configuration reminiscent of the tripartite motif protein family. *CMYA5* and dysbindin, a major schizophrenia susceptibility factor, co-immunoprecipitate from muscle extracts and are extensively co-localized [2]. *CMYA5* is also identified as a transcript down-regulated in *MEF2A* knock-out mice by microarray analysis [3]. The *CMYA5* promoter contains four *MEF2* cis-elements within 3.5 kb of the transcription start site [3]. CMYA5 localizes to the costamere at the periphery of the Z-disc complex and interacts with sarcomeric α-actinin-2 [3]. At the same time, CMYA5 is an anchoring protein for protein kinase A (PKA) (or AKAP) and serves as a substrate for PKA. CMYA5 co-localizes and interacts with RII alpha (a type II regulatory subunit of PKA) at the peripheral Z-disc/costameric region in striated muscle tissues [4]. The binding of

desmin with CMYA5 is confirmed with glutathione *S*-transferase pull-down assays and co-immunoprecipitation experiments [5]. Further research has shown that CMYA5 co-localizes with desmin at the periphery of the nucleus using an antibody against the COOH terminus of CMYA5. Deletion analysis reveals that desmin binds to CMYA5 through the 24 amino acid-long carboxyl-terminal end of the SPRY domain [5]. Dystrophic muscle exhibits reduced PKA activity resulting, in part, from severely mislocalized CMYA5. Furthermore, CMYA5 and dystrophin also coimmunoprecipitate in native muscle extracts and interact directly in vitro [6].

Much of the available information about the *CMYA5* gene was taken from studies on human and mice muscle disease. However, little has been known about the *CMYA5* gene in the pig. The aim of this study was to clarify the molecular characterization, chromosomal localization, expression profile and associations of the porcine *CMYA5* gene with pig meat quality.

Materials and methods

Isolation and cDNA sequence analysis of the porcine *CMYA5* gene

The human *CMYA5* gene mRNA (GenBank accession Number: NM_153610.3) was applied to compare with all sequences in the EST-others database using standard BLAST (http://www.ncbi.nlm.nih/gov/blast/), and the porcine ESTs which shared at least 85% identity with the corresponding human mRNA were selected to design gene specific primers (Table 1). The porcine *CMYA5* gene mRNA sequence was obtained by reverse transcription PCR (RT-PCR). The PCR products were purified with the 3S Spin DNA Agarose Gel Purification system (Shenergy Biocolor, Shanghai, China) and cloned into the pMD18-T vector (Takara, Dalian, China), then sequenced by commercial service.

Chromosomal localization of porcine *CMYA5* gene

The INRA/University of Minnesota porcine radiation hybrid panel (IMpRH) was used to assign *CMYA5* gene to the porcine genome [7]. The primers of porcine-specific (Table 1) were designed to amplify porcine genomic DNA within the porcine IMpRH panels. The PCR reaction was performed in a mixtures (10 μl) contained 1× PCR buffer (Promega), 0.25 μM each primer, 1.5 mM $MgCl_2$, 150 μM each dNTPs, 0.5 U *Taq* DNA polymerase (Promega, Madison, WI, USA) and 25 ng porcine genomic DNA. The PCR conditions were performed after an initial 5 min denaturation step at 95°C followed by 34 cycles of 94°C 30 s, 60°C for 30 s, 72°C for 20 s, and then 72°C for 5 min. PCR products were scored on a 1.5% agarose gel after ethidium bromide staining and the data were analyzed with the IMpRH mapping tools on http://www.toulouse.inra.fr/lgc/pig/RH [8].

Table 1 Primer pairs designed for the porcine *CMYA5* gene

Primer name	Primer sequences (5′-3′)	PCR (T_m)	Size (bp)
*CMYA5*P-1F	GTTCAACGGGAAGATAGTGG	60	4,108
*CMYA5*P-1R	GGTACAGGTGGTTCATGCTCAA		
*CMYA5*P-2F	TCACCAGGAGTGGAAAAGGA	57	3,557
*CMYA5*P-2R	TCAGTGTAACGGGGCTCAAT		
*CMYA5*P-3F	TTTGCCTGTAGAAGAATC	53	1,212
*CMYA5*P-3R	CCTTGGAAACCACCTCAT		
*CMYA5*P-4F	TCCGTGCTGATGAGGTGG	54	2,210
*CMYA5*P-4R	CAGGGTGTCCTTGGCAGT		
*CMYA5*P-5F	ATGGATACTGCCAAGGACA	56	1,344
*CMYA5*P-5R	CCCACCCCAATTTGAATG		
*CMYA5*M-F	GACTACACCACCCAGAGGCT	60	260
*CMYA5*M-R	AAACAGACACCCCGAAGG		
*CMYA5*E-F	CTGAGAGGAGACGCCTGACA	60	266
*CMYA5*E-R	GACATCGCTCACAATGCCAC		
pRPL32-F	TAAGCGGAACTGGCGGAAAC	60	284
pRPL32-R	TGGGATTGGTGACCCTGATG		
*CMYA5*SF	ACATAATACCAGAGCCCAAAC	60	458
*CMYA5*SR	CTTTTGTGGCACTTTATCTACT		

Temporal and spatial expression patterns of porcine *CMYA5* gene

Seven tissue samples for expression profile analysis were collected from different stages (new born, five weeks and adult) of Tongcheng (a typical indigenous Chinese breed) pigs' heart, liver, spleen, lung, kidney, skeletal muscle and adipose tissues. The detailed method for cDNA preparation was described previously by Xu et al. [9]. The expression pattern of the porcine *CMYA5* gene mRNA in different tissues from different stages was detected by semi-quantitative RT–PCR with the porcine *RPL32* (*pRPL32*) gene as positive control. *CMYA5* gene specific primers (*CMYA5*E-F and *CMYA5*E-R in Table 1) and *pRPL32* specific primers (*pRPL32*-F and *pRPL32*-R in Table 1) amplified products of 266 bp and 284 bp, respectively, were used to detect the expression pattern.

SNP identification

Pooled genomic DNA from Landrace pigs, Large white pigs, Small Meishan pigs and Duroc pigs was amplified and sequenced directly for the identification of single nucleotide polymorphism (SNP) using gene specific

primers (CMYA5SF and CMYA5SR in Table 1). The polymorphism site was analyzed by sequence comparisons using the DNAstar software (DNAstar Inc., Madison, WI, USA) and was further identified by the PCR-restriction fragment length polymorphism (PCR-RFLP) method.

Breeds used for testing the allele frequencies of the porcine *CMYA5* gene

The allele frequency analysis included 202 unrelated animals from six breeds (Table 2): Qingping pigs (n = 26), Tongcheng pigs (n = 27), Small Meishan pigs (n = 36), Duroc pigs (n = 26), Landrace pigs (n = 40) and Large White pigs (n = 47) [10, 11]. A chi-squared test on the allele frequencies for the six pig breeds was performed using SAS V8.0.

Association analysis of the porcine *CMYA5* gene with economic traits

Animals, traits and models used for analysis were described previously by Xu et al. [9] and Liu et al. [12]. Briefly, the linear model with the fixed effects is:

$$Y_{ijklmn} = \mu + G_i + B_j + S_k + C_l + F_m(C) + \varepsilon_{ijklmn}$$

where Y_{ijklmn} is the ijklmn th traits observation value; μ is the mean; G_i is the effect of the ith genotypes; B_j is the effect of jth batch; S_k is the effect of jth sex; C_l is the effect of lth population; $F_m(C)$ is family effects within breed and ε_{ijklmn} is the random residual corresponding to the traits observation value with var $(\varepsilon) = I\sigma_e^2$.

Results and discussion

Molecular cloning and sequence analyses of porcine *CMYA5* gene

The 12,056 bp partial porcine *CMYA5* cDNA (GenBank accession nos.FJ208850) was successfully obtained by RT-PCR procedures. The sequence contained an open reading frame of 12,009 bp, and encoded a protein of 4003 residues with an isoelectric point (p*I*) of 4.56 and calculated molecular mass of 442 kDa. It also contained a 47 bp of 3′-untranslated region (UTR). The analysis of CMYA5 amino acid sequences indicated that the protein contains BBC,FN3 and a SPRY domain in the C-terminal. Multiple alignment of the eight *CMYA5* gene homology sequences indicated that these three domains are conservative among the species (Fig. 1). The three motifs comprise the TRIM region; they all have the ability to interact with the α-actinin independently [3]. TRIM proteins are involved in diverse cellular processes, including cell proliferation, differentiation, development, oncogenesis and apoptosis and, in some cases transcriptional regulation [13]. Mutations or rearrangements of the B-box family members cause abnormal cellular growth or aberrant cell function resulting in a variety of metabolic or growth regulatory dysfunctions [14].

Table 2 Allele frequencies of different pig breeds at the *CMYA5* gene exon2 A383C locus

Breeds	Phenotypes	No. of animals	Genotype			Allele frequency	
			AA	AC	CC	A	C
Duroc	Lean	26	2	16	8	0.38	0.62
Landrace	Lean	40	17	20	3	0.67	0.33
Large White	Lean	47	4	12	31	0.12	0.88
Tongcheng	Obese	27	1	12	14	0.26	0.74
Qingping	Obese	26	0	0	26	0	1
Small Meishan	Obese	36	0	1	35	0.01	0.99

Temporal and spatial expression patterns of porcine *CMYA5* gene

RT-PCR was applied to detect the tissue distribution in different developmental stages (new born, five weeks and adult tongcheng pigs) of the porcine *CMYA5* gene. The internal control, *pRPL32*, displayed a basically identical signal in each tissue. The *CMYA5* gene was broadly expressed in all seven tissues, and significantly high expressed in the striate muscle tissues (Fig. 2). Our result was consistent with previous reports [2, 3]. Meanwhile, CMYA5 localized directly downstream of MEF2A at the costamere in striated muscle, so whether it plays a role in myofibrillogenesis needs further confirmation.

Chromosomal localization of porcine *CMYA5* gene

Using the porcine RH panel, we assigned the porcine *CMYA5* gene to porcine chromosome 2q21–24. It was significantly linked to microsatellite Sw1602 (distance 59 cR, LOD score value 6.74 and retention frequency 22%). The human *CMYA5* gene has been mapped to 5q14.1 (http://www.ncbi.nlm.nih.gov/Louslink). Our assignment of the porcine *CMYA5* gene to SSC2 is in agreement with previous comparative data [15, 16].

SNP identification and allele frequencies of the porcine *CMYA5* gene in different breeds

The amplification of one pair of primers (*CMYA5*SF and *CMYA5*SR in Table 1) on exon2 yielded to a 458 bp partial genomic DNA sequence. Sequencing and comparative

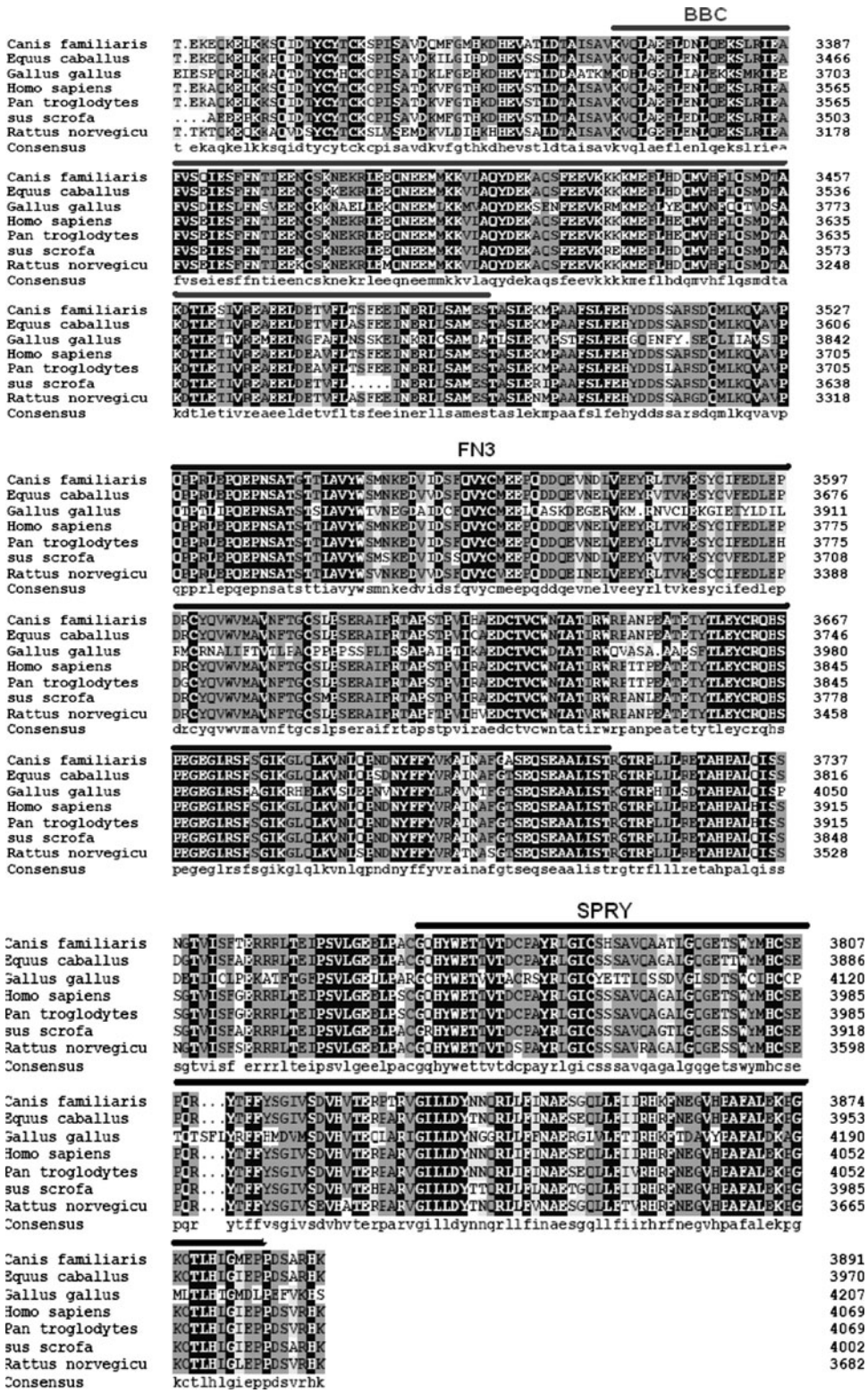

Fig. 1 The amino acid sequence-alignment of *CMYA5* in different species. The protein sequences available from the GenBank were analyzed with ClustralX1.83, and their accession numbers are XP_00113794.7 (*Pan troglodytes*), FJ208850 (*Sus scrofa*), XP_001068814 (*Rattus norvegicus*), XP_536312.2 (*Canis familiaris*), XP_001079985.1 (*Danio rerio*), XP_424765.2 (*Gallus gallus*), XP_001503943 (*Equus caballus*). Different species shared the same domains: C terminus contains B-box coiled-coil *(BBC)*, fibronectin type III *(FN3)* repeats, and domain in SPla and the RYanodine Receptor *(SPRY)*

analysis identified one SNP A383C that was detected by digestion with BspTI, resulting in a 458-bp PCR amplicon produced allele A (458) and allele C (383 and 75 bp) (Fig. 3).

Allele frequencies of the porcine *CMYA5* gene SNP in 202 unrelated pigs indicated that all the breeds were polymorphic with the exception of the A383 C in Qingping pigs and Small Meishan pigs (Table 2).

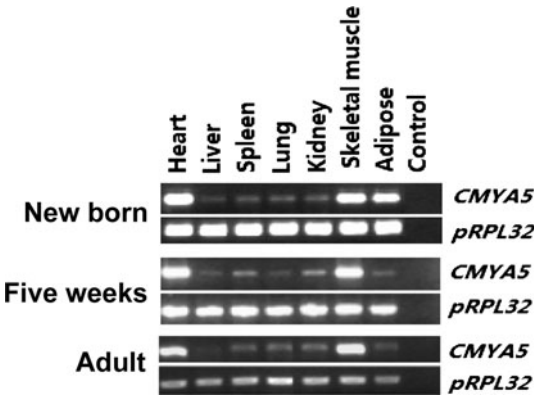

Fig. 2 Expression profile analysis of the porcine *CMYA5* gene

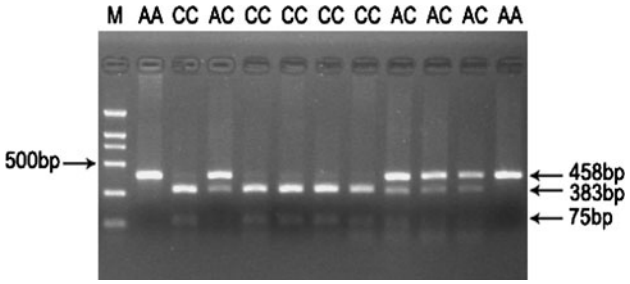

Fig. 3 One A383C SNP was detected in the Exon2. PCR products were digested with BspTI restriction enzyme to distinguish different alleles (458 bp for allele A, 383 and 75 bp for allele C). Two percent agarose gel showing the genotypes was indicated on the top of the column. M DL2000 (Jingmei BioTech Co. Ltd, China); PCR PCR products

Association analysis of the porcine *CMYA5* gene with economic traits

The SNP (A383C) of the porcine *CMYA5* gene was used for association analyses with the carcass and meat quality traits. According to the association analyses results, there were associations between the polymorphism (A383C) and the percentage of ham ($p < 0.05$), drop loss ($p < 0.01$) and intramuscular fat (IMF) ($p < 0.05$) (Table 3). The percentage of ham in pigs with the AA genotype was significantly higher than that of pigs with the CC genotype ($p = 0.039$), the drop loss of pigs with AC genotype was significantly higher than that of pigs with the CC genotype ($p = 0.003$), and IMF of pigs showed a similar trend between two different genotypes ($p = 0.019$).

We have assigned the porcine *CMYA5* gene to porcine chromosome 2q21–24. Some interesting QTLs are located at the area, such as ham percentage QTL [17], IMF QTL [18], off-flavor score QTL [19], pH 24 h post mortem (Loin) [20] and muscle color score [21, 22]. Therefore, the association analyses are in accordance with the QTL distribution surrounding this gene.

In conclusion, the SNP identified in exon2 of the *CMYA5* gene revealed an association between the genotypes and meat quality traits. It is suggested that the porcine *CMYA5* gene could act as a candidate gene for meat quality traits, and thus may be useful as a genetic marker for pig breeding.

Table 3 The effects of breed/breed crosses and association analysis of the *CMYA5* exon2 A383C genotypes with economic traits

	No. of animals	Percentage of ham	Water loss	Intramuscular fat (IMF)
Breed/breed cross				
L	22	32.235 ± 0.425	12.471 ± 1.001	1.754 ± 0.264
LYT	51	30.593 ± 0.266	14.273 ± 0.626	2.093 ± 0.165
T	44	28.009 ± 0.345	12.664 ± 0.813	3.276 ± 0.758
Y	23	31.556 ± 0.378	13.474 ± 0.889	1.768 ± 0.235
YLT	41	30.136 ± 0.304	13.232 ± 0.717	2.200 ± 0.189
Genotypes				
AA	25	31.183 ± 0.354	14.077 ± 0.873	2.207 ± 0.678
AC	69	29.830 ± 0.226	14.732 ± 0.535	2.435 ± 0.841
CC	87	29.961 ± 0.222	12.581 ± 0.552	2.469 ± 0.960
p-value				
AA-CC		0.039*	0.495	0.019*
AA-AC		0.341	0.163	0.169
AC-CC		0.096	0.003**	0.829

* $p < 0.05$; ** $p < 0.01$

L Landrace, *LYT* Landrace × (Yorkshire × Tongcheng), *T* Tongcheng pigs, *Y* Yorkshire, *YLT* Yorkshire × (Landrace × Tongcheng)

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