Polymorphism of the porcine *CGA* gene and its association with growth and carcass traits

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Abstract

Glycoprotein hormones in the pituitary gland affect a myriad of biological processes such as development, growth, metabolic control and gametogenesis. The quantitative trait loci (QTL) near their common glycoprotein α subunit gene (*CGA*) have been reported inconsistently. The aim of this study was to dissect potential genetic factors for these unstable results and validate the association or linkage relationship of *CGA* gene with growth and carcass traits. By resequencing all the exons and part of the introns of the porcine *CGA* gene, 22 polymorphisms in total were identified in this study. Five single nucleotide polymorphism (SNP) markers were chosen and evaluated in six pure-bred pig breeds (n = 228). Breed-specific haplotypes were found and a map of the porcine *CGA* polymorphisms' evolution history was inferred. A resource family (n = 365) with different genetic backgrounds from those used in other papers was used to perform an association study. The resource family was created based on crosses of Pietrain and Jinhua pigs (Central China type pigs). Results indicated that a low correlation between haplotype blocks may abolish each other's effects. Moreover, a significant association of SNP C-925T with growth rate and back-fat thickness in this study confirmed the existence of previously reported QTL. SNP C-925T and SNP A+15599G could be useful linkage markers and SNP C-925T may also be a candidate causative SNP for the corresponding traits. Further investigation for variants within of the *CGA* promoter region and their association with growth rate and back-fat traits is suggested.

Keywords: Common glycoprotein α subunit gene, haplotype, SNP, tetra-primer ARMS, swine
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Introduction

The glycoprotein hormones form a family consisting of the luteinizing hormone (LH), follicle-stimulating hormone (FSH) and thyrotrophin (TSH) in the anterior pituitary. The general biological roles of LH and FSH are the stimulation of testicular and ovarian functions via the regulation of gametogenesis and steroid hormone synthesis in the gonads, whereas TSH stimulates the thyroid gland to produce and release the thyroid hormones, 3,3',5,5'-tetraiodothyronine (T4) and 3,3',5-triiodothyronine (T3), which affect a myriad of biological processes such as skeletal maturation, linear growth, lipid synthesis and lipolysis, and carbohydrate metabolism (Yen, 2001). The thyroid hormones also potentiate the effects of many other hormones such as insulin, the growth hormone (GH), glucocorticoids and glucagon, and has been frequently called a permissive hormone (Bolander, 2004).

Each of the glycoprotein hormones is a heterodimer, formed by the non-covalent association of an α subunit that is common in all the members of the family with distinct β subunits that confer hormone specificity (Bolander, 2004). The common α subunit of these hormones, encoded by the unique, single-copy gene, *CGA* (also named as GSUα), is important to receptor binding and signal transduction (Szkudlinski *et al.*, 1996). No inactivating mutations of the *CGA* gene have been detected in humans and mice yet (Kendall *et al.*, 1995; Huhtaniemi & Alevizaki, 2007), for its widespread consequences in the form of

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hypogonadism, hypothyroidism and so on. Mice with the *CGA* gene knockout were viable, but exhibited severe growth insufficiency and infertility (Kendall *et al.*, 1995).

The porcine *CGA* gene is located on the p arm of *Sus scrofa* chromosome 1 (SSC 1) and consists of four exons and three introns (Kato *et al.*, 1991). A microsatellite marker in the intron 1 (named as PGHAS or ALPHA) has been identified (Moran, 1993) and used in genetic studies. Considering the extensive effects of the glycoprotein hormones, especially TSH, their common *CGA* gene shows to be a promising candidate gene for economic important traits. However, comparing with the large number of Quantitative trait loci (QTL) that have been mapped on SSC 1, there are only a limited number of QTL adjacent to the *CGA* gene, and they have only been reported in some studies (Malek *et al.*, 2001; Beeckmann *et al.*, 2003; Evans *et al.*, 2003; Liu *et al.*, 2007) and were undetectable in others (Rohrer & Keele, 1998; Bidanel *et al.*, 2001; De Koning *et al.*, 2001; Beeckmann *et al.*, 2003; Rohrer *et al.*, 2006; Liu *et al.*, 2007; Edwards *et al.*, 2008).

To dissect potential genetic factors for these inconsistent results and validate the association or linkage relationship of the *CGA* gene with the growth and carcass traits, a resource family with different genetic backgrounds from those used in other studies was utilized. The resource family was created based on crosses of Pietrain and Jinhua pig (Central China type pigs). First, DNA variations of the sequence of the *CGA* gene were identified by resequencing all exons and part of the introns. Single nucleotide polymorphisms (SNP) were chosen to develop new SNP markers for association analyses. Prior to the association analyses, the SNP markers were evaluated in six pure-bred pigs including pure-bred Pietrain and Jinhua pig breeds to construct breed-specific haplotypes and investigate their evolution history. Both porcine *CGA* haplotypes and single SNP were analyzed in the association study to examine the association of variants with growth and carcass traits.

Materials and Methods

Genomic DNA samples were obtained from 622 pigs: 228 belonged to six pure-bred breeds (57 Yorkshire, 30 Duroc, 27 Landrace, 24 Pietrain, 49 Jinhua pigs, 41 Jiaxing Black pigs) and the others to a three-generation resource family from crosses of Pietrain and Jinhua pigs. The resource population was created by first mating three Pietrain boars and three sows to two Jinhua pig sows and three boars, respectively. Seven litters of F_1 individuals were produced. From six of the seven F_1 litters, six boars and 23 sows were chosen to produce 39 litters of F_2 animals. Besides, two of the F_1 boars were also mated back to the two founder Jinhua sows, and three of the F_1 sows were mated back to the Pietrain boars. DNA samples were extracted from whole blood or ear samples using the traditional phenol and chloroform method (Sambrook *et al.*, 2001). The concentrations of DNA samples were measured by spectrophotometer and each was diluted to 10 ng/μL, exactly.

Growth and carcass traits of 34 F_1, 36 backcross and 295 F_2 individuals were used in the association study. Growth traits analyzed included birth weight and average daily gain (ADG) from birth to weaning, from weaning to 180 days of age and from birth to 180 days of age. The carcass traits analyzed were head weight, carcass weight, ham weight, ham-muscle weight, ham external fat weight, carcass length, leaf-fat weight, back-fat thickness (BFT) at shoulder, between the 6^{th} and 7^{th} ribs, at last rib and last lumbar, and average BFT of these four positions; colour parameters (CIE L^*, a^*, b^*, c^*, h°) of *longissimus dorsi* muscle (LM), pH and conductivity of ham muscle and LM at 45 min *post mortem*, LM area, water-holding capacity and intramuscular protein, fat and water content.

To scan the porcine *CGA* gene for DNA sequence variations, the direct sequencing method was employed using DNA from 4 F_1 boars, 1 Pietrain and 1 Jinhua pig. The porcine *CGA* sequence was obtained from the Ensembl database (http://www.ensembl.org/Sus_scrofa/Info/Index) using the *Sscrofa*9 assembly. Upstream sequence of the gene was also obtained and aligned to the sequence published by Kato *et al*. (1991) to target the 5'-untranslated exon 1. Figure 1 describes the gene structure and the polymorphisms identified by alignment (shown as solid lines). Five sets of primers were designed to amplify all exons and part of the introns. The amplicons were sequenced in both directions. Sequence traces were assembled with the Seqman program (DNAStar, USA) and analyzed to search for SNP.

The tetra-primer amplification refractory mutation system (ARMS) PCR procedure (Ye *et al.*, 2001) was utilized here for SNP genotyping. This method employs four primers to amplify fragments representing each of the two allelic forms and a larger amplicon containing the SNP as control. Primers were designed by using the online computer program made accessible by Ye *et al*. (2001) (http://cedar.genetics.soton.ac.uk/public_html/primer1.html) for five SNP loci: C-925T, C+14547T,

C+15065T, A+15599G and T+15944A. Genotyping PCR was performed in a total volume of 10 μL containing 20 ng of template DNA, appropriate concentration of each primer (see Table 1), 200 μM dNTPs, 1 μL 10 x buffer and 0.4 U of Taq polymerase (TaKaRa, Dalian, China). The PCR profile was the following: an initial step of denaturation of 3 min step at 94 °C; then 35 cycles of 30 seconds at 94 °C, 1 min at respective annealing temperature and 1 min at 72 °C; and the final extension step was for 10 min at 72 °C. The PCR products were separated by standard electrophoresis on 2% agarose gels.

Genotype and allele frequencies of the five SNPs in six pig pure-breds were calculated using PopGen32 software (Yeh *et al*., 1999). Then, haplotypes were constructed by program PHASE (Stephens & Donnelly, 2003) and visualized in Haploview software (Barrett *et al.*, 2005). Linkage disequilibrium between SNPs was estimated with D' and r^2, and the linkage blocks were defined using the four gamete rule (Wang *et al.*, 2002).

Haplotypes of the resource family were inferred by program, PedPhase 3.0 (Li & Li, 2009). Polymorphism C+14547T was omitted from further analyses because of its serious amplification failure. Afterwards, associations between the haplotypes or genotypes of the *CGA* gene and the investigated traits were assessed using the SAS MIXED procedure (SAS Institute Inc.). The model used was:

$$Y_{ijkl} = \mu + H_i + S_j + L_k + \beta * X_{ijkl} + e_{ijkl}$$

Where Y_{ijkl} = phenotypic value of traits; μ = population mean; H_i = fixed effect of haplotype or genotype; S_j = fixed effect of sex; L_k = random effect of litter; β = regression coefficient; X_{ijkl} = live weight at slaughter as a covariate for carcass traits; and e_{ijkl} = random residual error. Multiple comparisons of least square means were adjusted with Bonferroni correction.

Results

The complete *CGA* gene was located on SSC 1 from position 58,190,063 to 58,206,411. A schematic representation of the porcine *CGA* gene and the identified SNPs are shown in Figure 1. Eleven potential polymorphisms were identified by comparison of the sequence obtained from the Ensembl database with that published by Kato *et al*. (1991). Fourteen polymorphic loci were detected by direct sequencing in this study, three of which were identical to the ones identified by sequence comparison.

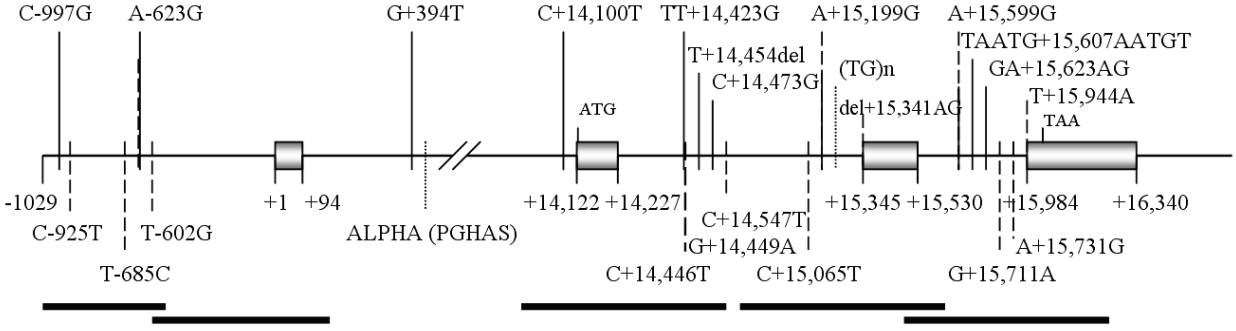

Figure 1 Schematic illustration of the porcine *CGA* gene and its sequence polymorphisms. Exons are presented by boxes and the transcription starting site is denoted as +1. Sequence variations identified by comparing the publicly available sequences are marked with solid lines, while the 14 polymorphisms detected by resequencing in this study are marked with dashed lines. Dotted lines represent the two microsatellites of *CGA* gene, i.e. ALPHA and $(TG)_n$. The positions of the polymorphisms are assigned according to the DNA sequence of *Sscorfa*9 assembly. Resequenced regions are shown with bold horizontal lines in the lower panel.

Five SNPs: C-925T, C+14547T, C+15065T, A+15599G and T+15944A were selected to be genotyped by the tetra-primer ARMS PCR procedure (representative genotyping results are shown in Figure 2). Genotypic and allelic frequencies in six pure-bred pig breeds at these five loci are presented in Table 2. SNP C+14547T segregated in Chinese breeds, while SNP loci C+15065T and A+15599G were the only polymorphic in Western breeds.

Haplotypes were constructed by the program, PHASE, and the haplotype frequencies of each breed were calculated and presented in Table 3. Haplotype CCCGT existed nearly in all breeds, whereas haplotype CTCGT and TTCGT are only shared in Chinese breeds. Haplotype CCCAT, CCTGT and CCTAT are only shared in Western breeds. CCCGA and TCCGA are common in Jinhua pig, but are also found in Yorkshire and Pietrain breeds. Haplotype CCTGA was detected only in the Pietrain breed.

Table 1 Tetra-primers ARMS-PCR genotyping

Primer Name	Primer Sequence (5'-3')	Conc*	T_A	Products (bp)
C-925T-inF	GGAACGGAAAGAAATCAACTTATAAC	0.4		C: 122
C-925T-inR	AATCAGAGTTGTTCTGTGATTTATTATATA	0.4		T: 95
C-925T-ouF	AAACTATGGAAGAAGATAATGGAAAT	0.08	57	Control: 161
C-925T-ouR	GAGAGTTTACAACAACTCTGTAATACAAA	0.08		
C+14547T-inF	TATTCAGTTTAGCTCATTTTGTTTGGTGTT	0.4		C: 221
C+14547T-inR	ATGTAAAAAAAAAGCAAACAAAAGACCG	0.4		T: 165
C+14547T-ouF	CATCTAGGCACTTTCAGATTGTGAACAT	0.04	58	Control: 328
C+14547T-ouR	CAAAACAAATTTACTGCATGAAATGGTT	0.04		
C+15065T-inF	AGTCATGCTTGAAGAAAGAGAAACGAT	0.8		C: 151
C+15065T-inR	AGATATGCATAGGATGTCTCAACAGAAG	0.8		T: 209
C+15065T-ouF	GAGAATATTTTTGCCTCTGGGTAAAATT	0.08	59	Control: 305
C+15065T-ouR	ATGGATTTTTCCTTATTATGAGATGGTAAA	0.08		
A+15599G-inF	AAAGAAATGTCCCCAGAGCACATACG	0.4		A: 162
A+15599G-inR	AGTCGACACCTCTGGCATTAACCCTTAT	0.4		G: 205
A+15599G-ouF	ACAATGTTGGTTCCAAAGAACATCACCT	0.06	63	Control: 313
A+15599G-ouR	CCCCCTCATCACCTCCATTCTAAATAAA	0.06		
T+15944A-inF	TCAATTTTGTCTGCCTATCTATCCGT	0.4	58	T: 199
T+15944A-inR	AGGAAAACAAAGATAGACGATAGATAGGTT	0.4		A: 134
T+15944A-ouF	ATCAGCTGAAAAGAAGAATGTGTGAATA	0.1		Control: 227
T+15944A-ouR	TTAATTTTTCATTCTGAGAAATCAGCAG	0.1		

*Conc: final primer concentration, μM; T_A: annealing temperature, °C.

Linkage disequilibrium between SNPs (D' and r^2) was estimated by Haploview software. The linkage blocks defined using the four gamete rule are shown in Figure 3. SNP A+15599G and T+15944A appeared to be in complete linkage disequilibrium (D' = 1), but their correlation coefficient is quite low (r^2 = 0.043), indicating that one could not substitute the other one and both of them were required for providing the haplotype information. A similar relationship existed between SNP C+14547T and C+15065T. Though values of D' and r^2 between SNP C-925T and others are low, lack of recombinant gamete types caused them to be correlated into one block. A recombination hot spot appears to exist between SNP C+15065T and SNP A+15599G.

Because of the serious amplification failure of SNP C+14547T in the resource family, this variant was omitted from further analyses. Haplotypes of the resource family were inferred by program PedPhase 3.0, and the results indicated that the founder Jinhua pigs possessed haplotypes C-CGT (i.e. CCCGT/CTCGT) and T-CGT (i.e. TTCGT), while the founder Pietrain had haplotypes C-CAT (i.e. CCCAT) and C-TGA (i.e.

CCTGA). Recombination between the two blocks was observed in the pedigree at a rate of approximately 0.06, and resulted in the emergence of another three haplotypes: C-CGA, C-TGT and T-CAT.

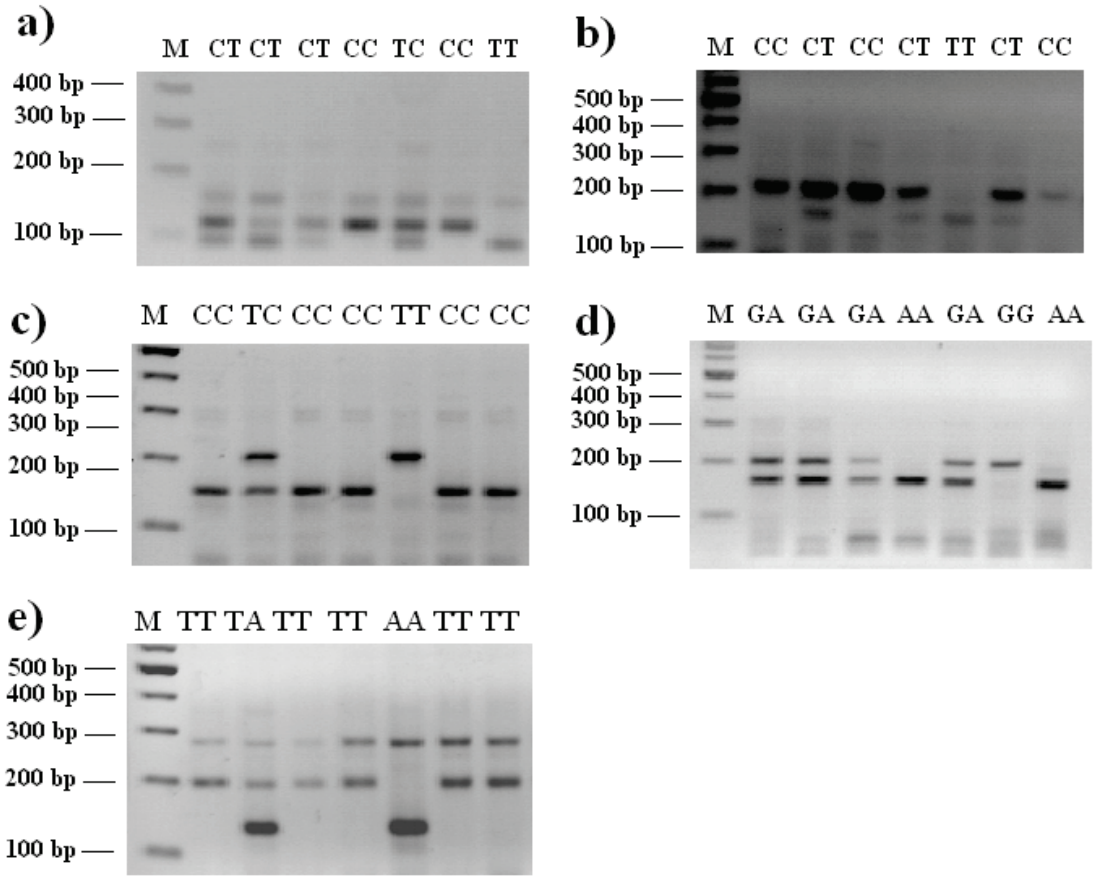

Figure 2 Genotyping results of the five SNPs by the tetra-primer ARMS-PCR procedure. Representative genotyping results for loci a) C-925T, b) C+14547T, c) C+15065T, d) A+15599G and e) T+15944A.

Association analysis showed that no traits were associated (P <0.05) with the *CGA* haplotypes. However, significant association of SNP C-925T with carcass weight, BFT at last rib and ADG, and association of SNP C+15065T with intramuscular water content were observed (P values of genetic effects are presented in Table 4). The haplotype block 1, containing these two SNPs, is significantly associated with ham external fat weight and LEA, besides BFT and ADG. But no significant differences between haplotype block 1 groups were observed in average BFT and BFT at shoulder traits using multiple comparisons adjusted by Bonferroni correction.

SNP A+15599G is significantly associated with ham external fat weight, while SNP T+15944A is significantly associated with intramuscular water content. Significant association of haplotype block 2 with the LM area in addition to the ham external fat weight and intramuscular water content were observed.

Discussion

The porcine *CGA* gene is composed of four exons and three introns spanning 16,349 bp of DNA sequence. The ATG translation starting site of this gene is in exon 2. Except for the two well-reported microsatellites, a total of 22 potential sequence variations were identified. Twelve of them were transition mutations, five were transversion mutations, and two were indel mutations. The remaining three sequence variations (TT+14423G, TAATG+15607AATGT, GA+15723AG) cannot be put under any of the three

classes, and that might have been caused by a sequencing error from the Kato's *CGA* DNA sequence. No coding mutation was detected in this study.

Table 2 Genotypic and allelic frequencies at the five SNP loci in six pig pure-breds

SNP	Breed		Genotypic Frequency			Allelic Frequency	
			AA	AB	BB	A	B
C-925T	Yorkshire	57	0.983	0.018	0.000	0.991	0.009
	Duroc	30	1.000	0.000	0.000	1.000	0.000
	Landrace	27	1.000	0.000	0.000	1.000	0.000
	Pietrain	24	1.000	0.000	0.000	1.000	0.000
	Jinhua	49	0.816	0.184	0.000	0.908	0.092
	Jiaxing Black	41	1.000	0.000	0.000	1.000	0.000
C+14547T	Yorkshire	44	1.000	0.000	0.000	0.000	0.000
	Duroc	19	1.000	0.000	0.000	0.000	0.000
	Landrace	20	1.000	0.000	0.000	0.000	0.000
	Pietrain	21	1.000	0.000	0.000	0.000	0.000
	Jinhua	42	0.691	0.262	0.048	0.821	0.179
	Jiaxing Black	33	0.091	0.879	0.030	0.530	0.470
C+15065T	Yorkshire	55	0.727	0.218	0.055	0.836	0.164
	Duroc	24	0.792	0.125	0.083	0.854	0.146
	Landrace	25	0.920	0.080	0.000	0.960	0.040
	Pietrain	22	0.044	0.609	0.304	0.364	0.636
	Jinhua	47	1.000	0.000	0.000	1.000	0.000
	Jiaxing Black	39	1.000	0.000	0.000	1.000	0.000
A+15599G	Yorkshire	56	0.054	0.269	0.679	0.188	0.929
	Duroc	30	0.200	0.633	0.167	0.517	0.483
	Landrace	27	0.000	0.222	0.778	0.111	0.889
	Pietrain	24	0.208	0.667	0.125	0.542	0.458
	Jinhua	49	0.000	0.000	1.000	0.000	1.000
	Jiaxing Black	41	0.000	0.000	1.000	0.000	1.000
T+15944A	Yorkshire	56	0.857	0.143	0.000	0.929	0.071
	Duroc	27	1.000	0.000	0.000	1.000	0.000
	Landrace	26	1.000	0.000	0.000	1.000	0.000
	Pietrain	23	0.217	0.783	0.000	0.609	0.391
	Jinhua	49	0.265	0.551	0.184	0.541	0.459
	Jiaxing Black	41	1.000	0.000	0.000	1.000	0.000

Based on the haplotype frequencies among six pig breeds, haplotype CCCGT appeared to be the ancestral type. Then, a mutation of C to T at locus C+14547T occurred and segregated in Chinese pigs, while a mutation of G to A at locus A+15599G and a mutation of C to T at locus C+15065T occurred in Western breeds (Figure 4). The recombination existing between SNP C+15065T and A+15599G generated the new haplotype, CCTAT in Western breeds.

Segregation of SNP T+15944A and SNP C-925T occurred in Jinhua pig. Recombination between SNP C-925T and C+14547T generated the haplotype, TTCGT. The two microsatellites in *CGA* gene may act as

Table 3 Haplotype frequencies of *CGA* gene in six pure-bred pig breeds

Haplotypes[1]	Jiaxing Black	Jinhua	Pietrain	Duroc	Landrace	Yorkshire
CCCGT	0.622	0.365		0.389	0.846	0.579
CTCGT	0.378	0.135				
TTCGT		0.042				
CCCGA		0.406	0.083			0.070
TCCGA		0.052				0.009
CCCAT			0.333	0.482	0.115	0.184
CCTGT			0.063	0.074	0.039	0.158
CCTAT			0.208	0.056		
CCTGA			0.313			

[1] Named after the alleles of the five SNPs in order.

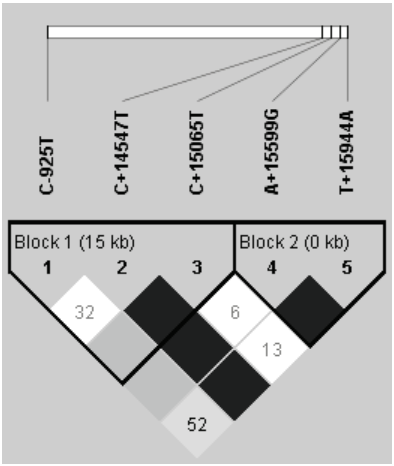

Figure 3 Linkage disequilibrium (LD) matrix plot of the five porcine *CGA* SNP markers. The plots were produced using pair-wise D'/LOD estimates of LD with the computer program, Haploview (Barrett *et al.*, 2005).

Table 4 Association of *CGA* gene with carcass and growth traits in the Jinhua and Pietrain resource families (only traits significantly (P <0.05) associated with SNPs of *CGA* gene are presented)

Trait	C-925T	C+15065T	Block 1	A+15599G	T+15944A	Block 2
	P values					
Carcass weight	0.0261	0.776	0.054	0.752	0.767	0.948
Ham fat weight	0.162	0.772	0.021	0.024	0.771	0.038
Average BFT	0.238	0.664	0.044	0.204	0.685	0.533
BFT at shoulder	0.456	0.203	0.032	0.272	0.214	0.303
BFT at last rib	0.013	0.955	0.06	0.228	0.991	0.704
LM area	0.203	0.927	0.015	0.064	0.941	0.025
Intramuscular water	0.811	0.023	0.140	0.404	0.034	0.011
ADG[1]	0.015	0.319	0.038	0.556	0.396	0.767
ADG[2]	0.021	0.921	0.069	0.183	0.963	0.630

P value of genetic effects <0.05 underlined; BFT – Back-fat thickness; LM - Longissimus dorsi muscle; ADG[1] - Average daily gain from birth to weaning; ADG[2] - from birth to 180 days.

Table 5 Least square means of significantly associated traits of SNP C-925T and A+15599G

Traits	Genotype of C-925T		
	CC	CT	TT
Carcass weight (kg)	55.5 ± 0.28[1]	53.8 ± 0.71	51.8 ± 1.48
BFT at last rib (cm)	2.30^{b} ± 0.038	2.38^{ab} ± 0.098	2.97^{a} ± 0.216
ADG - birth to weaning (g/d)	175.5^{b} ± 3.18	201.6^{a} ± 8.37	181.1^{ab} ± 15.69
ADG - birth to 180 d (g/d)	345.4^{b} ± 5.41	392.6^{a} ± 14.36	331.4^{ab} ± 29.97
	Genotype of A+15599G		
	AA	AG	GG
Ham external fat weight (kg)	1.86^{b} ± 0.044	1.96^{ab} ± 0.026	2.02^{a} ± 0.039

[1] Least square means ± standard errors; [a, b] Row means with different superscripts differ significantly at P <0.05.
BFT – Back-fat thickness; ADG - Average daily gain.

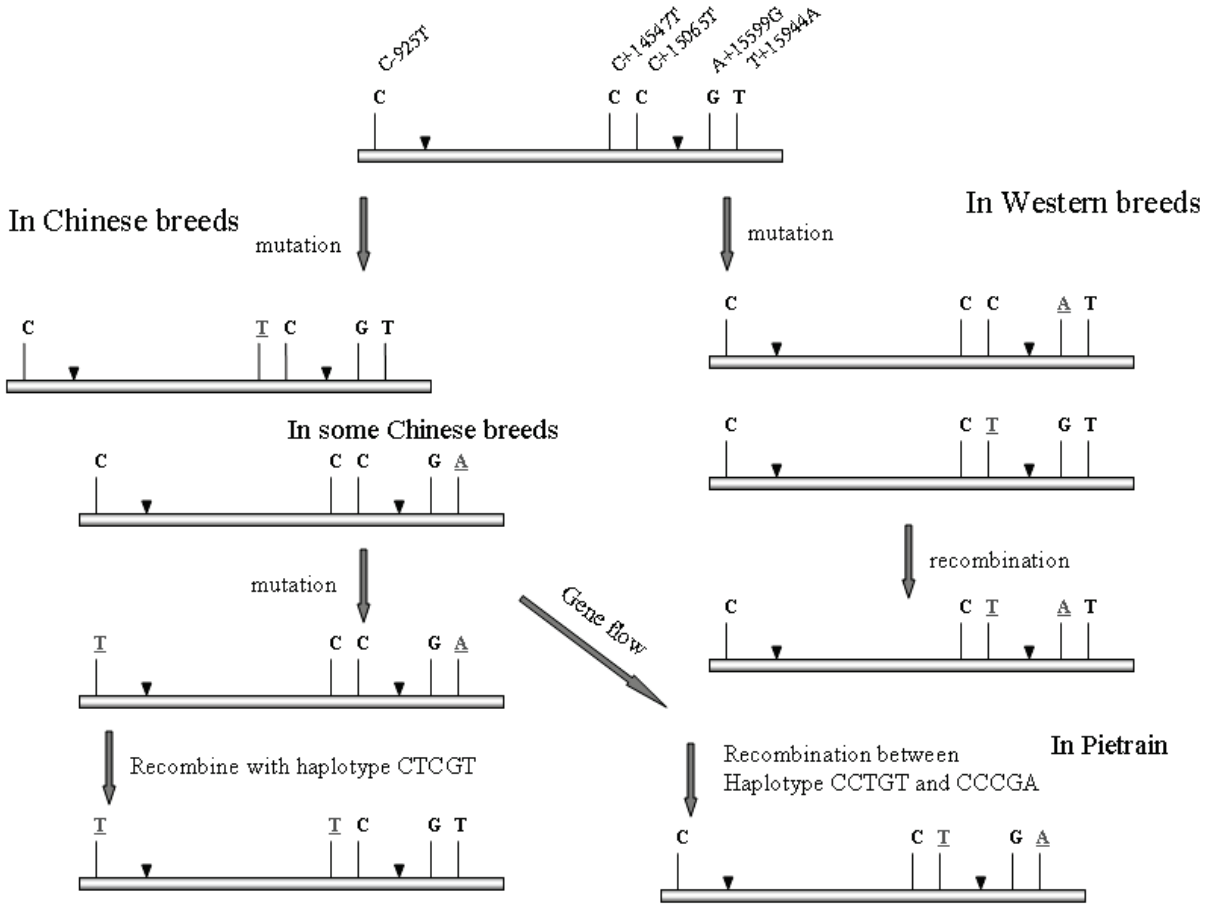

Figure 4 Deduced evolution history of the porcine *CGA* haplotypes. The triangles represent the two microsatellites which may act as recombination hot spots in the *CGA* gene.

recombination hot spots. The Jinhua pig is classified as a Central China pig breed type (Li *et al.*, 2004), while the Jiaxing Black pig, a subpopulation of Taihu pigs which include the well-known Meishan pig, belongs to the lower Changjiang Basin type. Whether these two polymorphisms exist only in some Chinese breed types but not in the others, remains to be investigated. Haplotypes CCCGA and TCCGA in Yorkshire and Pietrain pigs are likely to have originated from Chinese pig breeds, as the use of Chinese breeds with typical European breeds was popular in the 18th century (Cesar *et al.*, 2010). Particularly in Pietrain, recombination between the Chinese pig-originated haplotype CCCGA and the Western pig-originated haplotype CCTGT generated a new haplotype CCTGA, which is common in this breed. Absence of the ancestral haplotype in Pietrain may be due to its low frequency in the population and the small sample size used in this study.

Association analysis in the resource family showed that allele T from the Jinhua pig at locus C-925T was associated with lower carcass weight and higher back-fat thickness at the last rib, and the heterozygotes showed significantly faster growth rates than the homozygotes (Table 5). However, when incorporating the SNP C+15065T, i.e. using block 1 as genetic factor, the association possibility decreased. This suggested that only SNP C-925T, which locates in the promoter region, may be the candidate causative SNP or in linkage at a high r^2 with the causative SNP which is responsible for these traits' variations. Our results confirmed the existence of QTLs adjacent to the *CGA* region for carcass composition, growth rate and back-fat thickness which have been reported previously (Malek *et al.*, 2001; Beeckmann *et al.*, 2003; Liu *et al.*, 2007). Variations in the promoter region of *CGA* gene, but not the other gene components might be the candidates for these QTLs and further investigation is worthwhile.

Allele A at locus A+15599G, which is present in Western breeds, was significantly associated with lower ham external fat weight. A QTL for ham external fat weight on SSC1 in the European wild boar and Pietrain resource family has been reported by Beeckmann *et al*. (2003), and the *CGA* SNP A+15599G could be in linkage with the causative SNP for this QTL. Interestingly, haplotype block 1 also showed significant association with ham external fat weight, but combining the haplotype block 1 and SNP A+15599G decreased the association possibility. This result may be due to the low correlation between these markers.

The A allele of T+15944A and the T allele of SNP C+15065T originated from the same haplotype C-TGA. Thus, it was not surprising that they had similar results in the association analysis. However, after being adjusted with the Bonferroni correction, no significant differences in intramuscular water content existed following comparisons of their least square means. Animals with block 2 diplotype, --GA/--AT, had a lower intramuscular water content. To date, no QTL for intramuscular water content adjacent to the *CGA* region has been reported.

Thyrotropin (TSH), one of the *CGA* gene composing hormones, regulates the synthesis and release of thyroid hormones. The thyroid hormone could stimulate lipolysis directly and indirectly as a result of the potentiation of the effects of GH, glucocorticoid and glucagon. Many of the effects of GH are mediated by a group of hormones called insulin-like growth factors (*IGF's*). *GH* and *IGF'*s secretion will decline when thyroid hormone levels are low (Porterfield & White, 2007). It's known that *IGF'*s and their binding proteins are also associated with growth and fatness traits (Makgahlela *et al.*, 2009; Switonski *et al.*, 2010). Effects of the *CGA* gene on the same characters might be mediated partly by IGFs pathways. Interactions between variations of *CGA* and *IGF'*s were strongly suggested to exist.

Conclusion

Several novel, non-coding polymorphisms were found in the porcine *CGA* gene in this study. Different single nucleotide polymorphisms emerged in specific pig breed populations. At least two linkage disequilibrium blocks were detected in the *CGA* gene. It is suggested that the two microsatellites in this area may act as the recombination hot spots. Association of SNP C-925T with growth rate and back-fat thickness in this study confirmed the existence of previously reported QTL and provides evidence for the roles of SNP C-925T and SNP A+15599G in affecting specific quantitative traits. Further investigation on the *CGA* promoter region with the growth rate and back-fat traits is recommended.

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