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Mapping, Tissue Distribution and Polymorphism of Porcine Retinol Binding Protein Genes (*RBP5* and *RBP7*)*

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ABSTRACT : The retinoids (vitamin A and its derivatives) play a critical role in vision, growth, reproduction, cell differentiation and embryonic development. Using the IMpRH panel, porcine cellular retinol binding protein genes 5 and 7 (*RBP5* and *RBP7*) were assigned to porcine chromosomes 5 and 6, respectively. The complete coding sequences (CDS) of the *RBP5* and *RBP7* genes were amplified using the reverse transcriptase polymerase chain reaction (RT-PCR) method, and the deduced amino acid sequences of both genes were compared to human corresponding proteins. The mRNA distributions of the two genes in adult Wuzhishan pig tissues (lung, skeletal muscle, spleen, heart, stomach, large intestine, lymph node, small intestine, liver, brain, kidney and fat) were examined. A total of nine single nucleotide polymorphisms (SNPs) were identified in two genes. Three of these SNPs were analyzed using the polymerase chain reaction-restriction-fragment length polymorphism (PCR-RFLP) method in Laiwu, Wuzhishan, Guizhou, Bama, Tongcheng, Yorkshire and Landrace pig breeds. Association analysis of genotypes of these SNP loci with economic traits was done in our experimental populations. Significant associations of different genotypes of *RBP5*-A/G^{63}, *RBP5*-A/G^{517} and *RPB5*-T/C$^{intron1\text{-}90}$ loci with traits including maximum carcass length (LM), minimum carcass length (LN), marbling score (MS), back fat thickness at shoulder (SBF), meat color score (MCS) and hematocrit (HCT) were detected. These SNPs may be useful as genetic markers in genetic improvement for porcine production. (**Key Words** : Retinol Binding Protein, Complete Coding Sequence, Porcine, Reverse Transcriptase Polymerase Chain Reaction, Single Nucleotide Polymorphism, Genotype)

INTRODUCTION

The vitamin A (retinol) and its active derivatives (retinal and retinoic acid) play an essential role in vision, growth, reproduction, cell differentiation and embryonic development. Retinoic acid (RA) regulates multiple biological processes including cell proliferation and differentiation by modulating the transcription of numerous target genes. This function is carried out by retinoic acid receptor (RAR) and retinoid X receptor (RXR) which as homodimer or heterdimer bind to specific response elements in promoter regions of the target genes (Chambon, 1996; Budhu and Noy, 2002). Because of the chemical instability and quite low solubility in aqueous medium, the retinol and its derivatives (retinal and retinoic acid) must be bound to a specific kind of proteins during the process of absorption, transport and excretion. Those proteins include plasma retinol binding protein (RBP), cellular retinol binding proteins (RBPs) and cellular retinoic acid binding proteins (CRABPs). The RBP5 and RBP7 are members of the RBPs family.

Pork quality and production are essentially affected by the number and size of muscle fibers that are determined by prenatal and postnatal stages, respectively (Tang, 2007). Because the RBPs play a pivotal role in retinol and its derivative's absorption, transport, metabolism and homeostasis, those proteins must influence cell differentiation and development of muscle fiber and adipose.

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Table 1. Primers used in PCR and RT-PCR

Gene name	Primer name	Primer sequences	Binding region	Size (bp)	Tm (°C)
RBP5[a]	Mapping PL	CAAAAGAACCTGGAGGACTA	Exon 1	-	-
	Mapping PR	CTGTCTCTTAGCAAGGGTCC	Intron 1	315	63
	CDSPL	CTCCCCTGTCAGACAACACT	5'UTR	-	-
	CDSPR	TCATCCAGGAGACCAGACTT	3'UTR	728	61
	Distribution PL	CTCCTCACTCTGGCTCCTAC	5'UTR	-	-
	Distribution PR	CTATGGTCTGGCACTTTCGT	Exon 3	315	61
	[b]SNP-A/G^{63}, -A/G^{39}, -A/G^{42}, -A/G$^{intron\ 1\text{-}91}$, -T/C$^{intro1\text{-}90}$ PL	ACCCTCCTGAAGCCTTGACC	3'UTR	-	-
	SNP-A/G^{63}, -A/G^{39}, -A/G^{42}, -A/G$^{intron1\text{-}91}$, -T/C$^{intro1\text{-}90}$ PR	AAACCCGAGCCTTTCCACGC	Intron 1	227	61
	SNP- A/G^{517} PL	AAAGGATGCCGTGTGCGAGC	Exon4	-	-
	SNP- A/G^{517} PR	GCTTGGGGGCTGCAAGTTAT	5'UTR	209	61
	SNP- A/G^{649}, T/C^{653} PL	CCAAAGTCCTTTCTCACACT	5'UTR	-	-
	SNP- A/G^{649}, T/C^{653} PR	CCGAATGAATTAAAAGAGGT	5'UTR	138	53
RBP7[a]	Mapping PL	GTATTGACTTTGCCACTCGT	Exon 2	-	-
	Mapping PR	GTCGCAGACATTGCTAACAT	Intron 2	430	57
	CDSPL	AGCACTTGCCACCAAGTCCC	5'UTR	-	-
	CDSPR	CCCCATCAGCAAACCAGGAC	3'UTR	558	61
	Distribution PL	GTATTGACTTTGCCACTCGT	Exon 2	-	-
	Distribution PR	AGCCTCTGTTCTTCTTTTCC	Exon 3	246	61
	SNP- T/C^{6} PL	ACCCGCACTTGCCACCAAGT	3'UTR		
	SNP- T/C^{6} PR	TCGCTGCTGACCAGGTTCCA	Exon 1	103	63
β-action	β-action PL	GGACTTCGAGCAGGAGATGG	Exon3		
	β-action PR	GCACCGTGTTGGCGTAGGG	Exon4	233	61

[a] The primer pairs mapping PL and mapping PR, CDS PL and CDS PR, and distribution PL and distribution PR were employed for radiation hybrid mapping, amplifying the CDS, and mRNA tissue distribution analysis of each gene, respectively. The primer pair SNP-N/N^{n} PL and SNP-N/N^{n} PR was used for SNP locus assaying and RFLP analysis of respective SNP locus.

[b] The GenBank accession no. of porcine *RBP5* intron 1 sequence is EF-208121 and nucleotide position is numbered according to the first base of this intron. The other nucleotide positions are numbered according to the first base (A) of each gene's CDS.

So the *RBPs* may be candidate genes affecting pork quality, production and fatty deposit.

MATEREALS AND METHODS

Samples

Seven breeds of Laiwu pig (n = 43, Laiwu Black Pig Conservation Farm, Shandong Province), Guizhou Miniature pig (n = 44, Experimental Pig Farm of Guiyang University for Chinese Medicine, Guizhou Province), Bama Miniature pig (n = 49, Experimental Pig Farm of Guangxi University, Guangxi Province), Wuzhishan Miniature pig (n = 44, Wuzhishan Miniature Pig Conservation Farm of Chinese Academy of Agricultural Sciences, Beijing), Tongcheng pig (n = 43, Tongcheng, Hubei Province), Landrace (n = 12, sampled from Tongcheng, Hubei Province) and Yorkshire (n = 16, sampled from Tongcheng, Hubei Province) were included for SNP analysis in this study. Two experiment populations of three-way crossbreds of Landrace×(Yorkshire×Tongcheng) (n = 38) and Yorkshire×(Landrace×Tongcheng) (n = 36) collected from Tongcheng, Hubei Province were added for association analysis together with the three breeds of Tongcheng, Landrace and Yorkshire. The detailed management and data collection procedures were described previously (Wang et al., 2005).

Chromosome mapping using IMpRH

The INRA-University of Minnesota porcine radiation hybrid (IMpRH) panel (118 clones) was employed to assign porcine *RPB5* and *RBP7* genes to porcine chromosomes. The contigs assembling of *RBP5* and *RBP7* genes were performed as described by Wang et al. (2006a). The Primer Premier 5.0 (PremierBiosoft.com) was employed to design gene specific primers. The primer sequences, primer binding regions and expected PCR product sizes for each gene are shown in Table 1. The PCR was performed in 10 μl volume containing 25 ng template DNA, 2.5 mM $MgCl_2$, 1×PCR buffer, 0.35 units *Taq* DNA polymerase (*TaKaRa Taq*^{r}, TaKaRa Biotechnology, Dalian, China), 50 μM of each dNTP, and 0.25 μM of each primer. Each PCR also contains two positive control DNA samples of pig and hamster each and a negative control without DNA. All PCRs were carried out in PTC-220 DNA Engine Dyad Cycler (MJ Research). The PCR temperature profile was 5 min at 94°C, followed by 30 cycles of 30 s at 94°C, 30 s

annealing (temperatures for each primer set are shown in Table 1) and 30 s at 72°C, and a final extension of 5 min at 72°C. The fragment of each gene was cloned into the pGEM-T Easy vector (Promega Corporation, USA) and sequenced commercially to get an intron sequence of each gene. The PCR products were separated in a 1.5% agarose gel stained with 0.5 μg/ml ethidium bromide. The PCR results were analyzed using the IMpRH mapping tool (http://www.toulouse.inra.fr/lgc/pig/hybrid/htm) and two-point RH analysis was used for identification of linkage groups with a LOD score threshold of 5.0.

CDS sequences and tissue distribution

Based on the contig and its intron/exon boundary of each gene, specific primers to amplify the complete coding sequences (CDS) of each gene were designed. Twelve different tissues: lung, skeletal muscle, spleen, heart, stomach, lymph code, small intestine, liver, brain, kidney and fat were harvested from one adult Wuzhishan Miniature pig, then frozen in liquid nitrogen and stored at -80°C. Total RNAs were extracted using the Trizol reagent kit (Life Technologies, Grand Island, NE, USA) and precipitated with ethanol according to the manufacture's protocol. The syntheses of the first-stand cDNA and the reverse transcriptase polymerase chain reaction (RT-PCR) were performed as described by Yu et al. (2004). As amplifying the CDS of *RBP5* and *RBP7* genes, RT-PCR was carried out using the cDNA template derived from reverse transcription of pig liver and skeletal muscle total RNAs, respectively. The PCR products were purified with TIANGEN Midi Purification Kit [TIANGEN BIOTECH (Beijing) Co., LTD] and cloned into the pGEM-T Easy vector (Promega Corporation, USA). Single clone was randomly selected and sequenced commercially. ORFs of the two genes were identified and the amino acid sequences were deduced with the program Seqman (DNA star, Madison, WI, USA). The PCR was performed as described above except for with 27 cycles. The β-actin was used as an internal control. Five-microlitre PCR products were size-separated in a 1.5% agarose gel stained with ethidium bromide to detect the expression profile.

SNPs identification and association analysis

The SNPs analysis was performed in five indigenous Chinese breeds (Laiwu, Wuzhishan, Bama, Guizhou and Tongcheng) and two commercial breeds (Yorkshire and Landrace). The genomic DNA extraction was according the standard phenol-chloroform method. As assembling the contig of each gene, the potential SNPs loci emerged. Using the Primer Premier 5.0 (PremierBiosoft.com), those potential SNPs were detected if there were restriction enzyme sites. The potential SNPs having restriction enzyme sites were assayed using PCR-RFLP method with 24 individuals randomly selected from Tongcheng (8), Yorkshire (8) and Landrace (8) breeds. For the possible SNPs without restriction enzyme site, the PCR products were directly sequenced in 12 individuals that were randomly selected from Tongcheng (3), Wuzhishan (3), Laiwu (3) and Bama (3) breeds. For the confirmed SNPs showing the RFLPs, the method was further employed to detect genotypes in Laiwu, Wuzhishan, Bama, Guizhou, Tongcheng, Yorkshire and Landrace breeds. Ten-microlitre digested products were electrophoresed in a 3% agarose gel for genotyping.

A two-step analysis was performed to assess the association between genotype and trait. Firstly, a preliminary generalized linear model was applied to eliminate system effects including sex, combination (different genotypes) and batch (different slaughter groups) using the SAS software package (SAS Inst. Inc., Cary, NC):

$$Y_{ijkl} = \mu+C_i+B_j+S_k+(CB)_{ij}+(BS)_{jk}+e_{ijkl}$$

Where Y_{ijkl} is the phenotypic value of specific trait, μ the population mean, C_i the combination effect, B_j the batch effect, S_k the sex effect, $(CB)_{ij}$ the interaction effect between combination and batch, $(BS)_{jk}$ the interaction effect between batch and sex and e_{ijkl} the residual error effect for each observation. Secondly, the standardized residual value obtained from the model above was then used to partition the effect of the genotype. Significant difference between genotypes was tested after a Bonferroni correction (Wang et al., 2006b).

RESULTS AND DISCUSSION

Chromosome assignment

Using the IMpRH panel, we mapped the porcine *RBP5* being closely linked (two-point-analysis) to the microsatellite marker SW963 (29cR; LOD score 16.78) that is located to q^{21}-q^{24} of SSC5, thus the most likely chromosomal location for *RBP5* is also within this region. The porcine *RBP7* is tightly linked (two-point-analysis) to marker SW1355 (25cR; LOD score 13.01) on SSC6, and its deduced cytogenetic position is assigned to $6q^{12}$-q^{21}. The human *RBP5* and *RBP7* genes were assigned to $12p^{13.31}$ and $1q^{36.22}$, respectively. Our results are completely in accordance with comparative mapping data available for the porcine and human genomes (Goureau et al., 1996).

CDS of porcine *RBP5* and *RBP7* genes

Using the primer pairs of CDS PL and CDS PR of each gene (Table 1), 728 bp and 558 bp cDNA fragments of porcine *RBP5* and *RBP7* genes were amplified by RT-PCR. ORFs were identified and nucleotide sequences of cDNA fragments of porcine *RBP5* and *RBP7* genes were 85% and

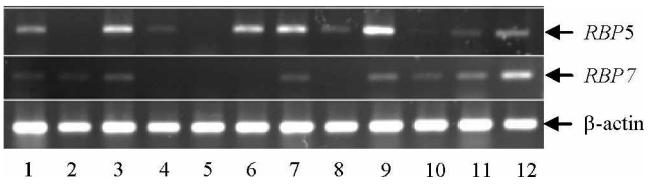

Figure 1. The RT-PCR results of porcine *RBP5* and *RBP7* genes. The number 1 to 12 represent lung, skeletal muscle, spleen, heart, stomach, large intestine, lymph code, small intestine, liver, brain, kidney and fat respectively. The β-actin was used as an internal control.

92% identical to human *RBP5* (GenBank accession no. NM_031491.1) and *RBP7* (GenBank accession no. NM_052960.1) mRNAs, respectively. Analysis of the cDNA sequences of porcine *RBP5* and *RBP7* genes revealed that: (1) The cDNA fragment of porcine *RBP5* contains a 408 bp long ORF that encodes a protein of 135 residues with an estimated molecular mass of 15.8 kDa and an isoelectric point (pI) of 5.76. It contains a 82 bp long 5'-untranslated region (5'-UTR) and a 238 bp long 3'-untranslated region (3'-UTR); (2) The porcine *RBP7* cDNA fragment consists of a 405 bp long ORF, a 56 bp long 5'-UTR and a 97 bp long 3'-UTR. It is predicted to encode a protein of 134 amino acids with an estimated molecular mass of 15.4 kDa and a pI of 6.59. The deduced amino acid sequence of porcine RBP5 is 88% identical to human RBP5 (GenBank accession no. NP_113679.1). The deduced amino acid sequence of RBP7 is 94% and 92% identical to human RBP7 (GenBank accession no. NP_443192.1) and house mouse (*Mus musculus*) RBP7 (GenBank accession no. NP_071303.1), respectively. The CDS sequences of porcine *RBP5* and *RBP7* genes were deposited in the GenBank (GenBank accession nos. EF-208120 and EF-208119, respectively).

Porcine RBP5 and RBP7 belong to intracellular lipid-binding proteins (iLBPs), which contain RBPs, CRABPs and fatty acid-binding proteins (FABPs). In all proteins of the family, 10 β-stands (A-J) and two α-helices (I and II) fold to form a well-defined β-barrel which contains the binding site for the hydrophobic ligand in its internal cavity (Folli et al., 2002). The multiple amino acid alignment of porcine RBP5 and RBP7, human RBP5 and RBP7, and house mouse, Norway rat (*Rattus norvegicus*) and red jungle fowl (*Gallus gallus*) RBP7 is presented in Figure 3. The result demonstrates that porcine RBP5 and RBP7 amino acid sequences perfectly match the characteristics of iLBPs family which all have 10 β-stands (A-J) and two α-helices to form the well characterized β-barrel. In multiple amino acid alignment, there are 51 positions having the identical amino acid residues across the five species (P1,G6, S12, N15, E17, Y19, A22, L23, I25, A28, R30, K31, I32, A33, L36, K37, P38, K40, I42, G46, T53, S55, R58, Y60, F64, G67, E69, F70, E72, D73, D78, R80, K81, C82, V86, L93, C95, Q97, G99, E100, N103, R104, G105, W106, H108, W109, E111, G112, L115, L117 and C126), and these amino acids should be very important in folding the correct tertiary structures for binding the ligands. This shows that RBP5 and RBP7 are extremely conserved in their amino acid sequences.

Within human RBPs, several amino acid residues (K40, T53, R58, W106 and Q108) in close contact with retinol in holo-RBPs are identical or chemically conserved, with the exception of Gln^{108} which is replaced by a His residue in both human RBP5 and RBP7. The side chain of Q108 hydrogen bonds the hydroxyl group of retinol in RPB1and RBP2, and the Q-H replacement at the position might have a functional significance (Folli et al., 2001, 2002). The amino acid residues of porcine RBP5 and RPB7 at positions 40, 53, 58 106 and 108 are identical with the human corresponding RBPs (K40, T53, R58, W 106 and H108).

The tissue distribution

The RT-PCR was performed to detect the porcine *RBP5* and *RBP7* genes' expression patterns in 12 tissues of adult Wzhishan pig. The results are given in Figure 1. The mRNA expression level of porcine *RBP5* was the highest in liver followed by spleen, large intestine and lymph node, whereas expressions in lung, heart, little intestine, kidney and fat were weak. This is not similar with human *RBP5* (alias *CRABIII*) gene expression that its mRNA is most abundant in adult kidney and liver tissues, low in spleen lymph node and appendix (Folli et al., 2001). The porcine *RBP7* mRNA expression level was slightly high in fat, weak in spleen, lymph node, liver, brain and kidney, and very weak in lung and skeletal muscle. This is similar with house mouse *Rbp7* expression pattern that is highly expressed in white adipose tissue and mammary gland, but low in heart, brain and kidney (Conforti et al., 2000). Human *RBP7* (alias *CRBP IV*) mRNA is expressed widely in 61 different tissues with the highest abundance in adult kidney, and the next richest in adult heart, adult transverse colon, fetal heart and fetal spleen (Folli et al., 2002).

In general, porcine *RBP5* and *RBP7* have different expression patterns. In lung, spleen, large intestine, lymph code and liver tissue, the *RBP5* mRNA level is much higher than that of *RBP7*. The *RBP5* expressed in heart, large intestine and small intestine, but no *RBP7* mRNA was detected in these tissues. This reflects the functional differences of the two proteins in transport retinol.

SNPs identification and association analysis

The genomic DNA sequences that contain the possible SNP loci were amplified using the primer pairs of SNP-N/N^{n} PL and SNP-N/N^{n} PR shown in Table 1. The SNP loci A/G^{63}, T/C$^{intron1-90}$ and A/G^{517}of *RBP5* were confirmed by digestion with enzymes Cac8I, Mwo I and Fnu4H I,

Table 2. Results of SNP_S assaying of porcine *RBP5* and *RBP7* genes

Gene	SNP loci	PCR-RFLP		Sequencing	
		Enzyme[1]	Size (bp)	n[2]	Ratio
RBP5	A/G^{63}	Cac8I	26:75:126		
	A/G^{39}			9	6A:3G
	A/G^{42}			9	4A:5G
	$A/G^{intron1-91}$			12	7A:5G
	$T/C^{intron1-90}$	Mwo I	33:63:131		
	A/G^{517}	Fnu4H I	48:150		
	A/G^{649}			10	1A:9G
	T/C^{653}			10	1C:9T
RBP7	T/C^{6}			12	5C:7T

[1] The enzymes were from EEW ENGLAND Biolabs (Beijing), Ltd. [2] Number of individuals sequenced.

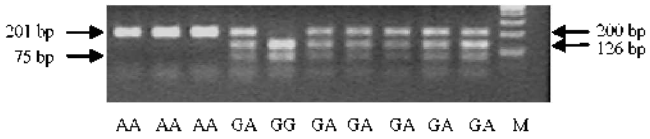

Figure 2. Three genotypes of *RBP5*-A/G^{63} in Chinese indigenous Laiwu breed. The genotypes (AA, GA and GG) are shown in the bottom. M: 100 bp ladder.

respectively, and the others were confirmed by direct sequencing of PCR products. The results are shown in Table 2. Allele A is dominant at locus A/G^{649} and C at T/C^{653}, however, no allele is extremely abundant at other loci among the samples sequenced. With the confirmed SNP loci of A/G^{63}, $T/C^{intro1-90}$ and $A/G^{517}$of *RBP5*, the PCR-RFLP method was employed to further analysis in Wuzhishan, Laiwu, Bama, Guizhou, Tongcheng, Yorkshire and Landrace breeds. The digestion pattern of *RBP5*-A/G^{63} in Laiwu breed is shown in Figure 2. The allele frequencies of the seven breeds are listed in Table 3. It shows that A allele is dominant at A/G^{517} locus in all breeds. At A/G^{63} locus, allele A is dominant in Laiwu, Bama, Yorkshire and Landrace breeds, but allele G is dominant in Wuzhishan, Guizhou and Toncheng breeds. At $C/T^{intron1-90}$ locus, C allele is dominant in Wuzhishan, Guizhou, Tongcheng, Yorkshire and Landrace breeds, but T allele is dominant in Bama and Laiwu breeds.

There are significant associations between different genotypes of *RBP5*-A/G^{517}, -$T/C^{intron1-90}$ and -A/G^{63} and maximum carcass length (LM), minimum carcass length (LN), hematocrit (HCT), marbling score (MS), back fat thickness at shoulder (SBF) and meat color score (MCS) traits. The results are shown in Table 4. The SBF and MCS of pigs with genotype *RBP5*-GA^{517} are significant thicker and better than the pigs with genotype AA. The LN and LM of pigs with genotypes *RBP5*-$CC^{intron1-90}$ and -$TC^{intron1-90}$ are significantly longer than that of pigs with genotype TT. At *RBP5*-A/G^{63}, the means of LN and LM of pigs with genotypes GG and GA are significantly higher than the individual's with genotype AA ($p<0.01$). For the meat quality traits of MCS and MS, pigs with genotypes GG and GA are much better than pigs with genotype AA ($p<0.01$) at -A/G^{517} and -A/G^{63} loci. Furthermore, except for the traits of HCT in genotype *RBP5*-$TC^{intron1-90}$ and of MS in genotype *RBP5*-AG^{63} all remaining traits of pigs with heterozygous genotypes are always the best among the three genotypes at the three SNP loci. This may be great useful in porcine breeding and production. The *RBP5* gene is assigned to SSC $5q^{21}$-q^{24} with the closest marker as of SW963 (position 95.5 cM). On this chromosome (position 107-113 cM), three significant QTLs (average back fat, last rib back fat and lumbar back fat, cm) at the 5% chromosome-wise level associated with back fat thickness were identified (Malek et al., 2001), and a significant QTL (position 135.8 cM) affecting the carcass length (cm) was also detected (Lee et al., 2003). These support our findings although further confirmation in a large population is needed.

As porcine *RBPs* play a profound role in cell differentiation and embryonic development, they probably affect muscle fiber and adipose cell differentiation and development both at prenatal and postnatal stages. This may be the cause of genotypes *RBP5*-A/G^{63}, A/G^{517} and -

Table 3. Allele frequencies of porcine *RBP5*-A/G^{63}, -A/G^{517} and -$C/T^{intron1-90}$ in seven pig breeds

Breeds	*RBP5*-A/G^{63}			*RBP5*-A/G^{517}			*RBP5*-$C/T^{intron1-90}$		
	n	A	G	n	A	G	n	C	T
Laiwu	43	0.66	0.34	41	1	-	43	0.34	0.66
Wuzhishan	38	0.11	0.89	38	0.79	0.21	40	0.89	0.11
Guizhou	38	0.12	0.88	42	0.69	0.31	39	0.91	0.09
Bama	48	0.875	0.125	47	1	-	48	0.125	0.875
Tongcheng	40	0.19	0.81	40	0.575	0.425	39	0.87	0.13
Yorkshire	14	0.96	0.04	14	0.93	0.07	13	0.62	0.38
Landrace	12	0.67	0.33	12	1	-	11	0.55	0.45

Table 4. Association analysis between genotypes of *RBP5*-A/G[63], -A/G[517] and -T/C[intron1-90] and SBP, MCS, LM, LN and HCT traits

A/G[517]			T/C[intron1-90]				A/G[63]			
Genotype	SBF (cm)	MCS	Genotype	LM (cm)	LN (cm)	HCT (%)	Genotype	LM (cm)	LN (cm)	MS
AA	4.33±0.31[a]	3.05±0.04[A]	CC	90.21±0.42[a]	76.12±0.35[A]	37.42±0.89[A]	AA	89.00±0.42[A]	74.60±0.35[Bb]	2.27±0.07[A]
GA	5.96±0.57[b]	3.27±0.07[B]	TC	91.06±0.38[a]	76.41±0.69[AB]	36.70±1.77[AB]	GA	91.50±0.83[B]	76.53±0.70[Ba]	2.40±0.13[AB]
GG	5.03±1.34[ab]	3.11±0.17[AB]	TT	88.86±0.51[b]	74.25±0.42[C]	33.32±1.08[B]	GG	90.15±0.51[AB]	76.25±0.43[bA]	2.55±0.08[B]

The values are presented in mean±standard error.

SBF = Backfat thickness at the shoulder, MCS = Meat color score, LM = Maximum carcass length,

LN = Minimum carcass length, MS = Marbling score, HCT = Hematocrit.

Means within a column with different superscripted capital letters differed at $p<0.01$ and means within a column with different superscripted small letters differed $p<0.05$.

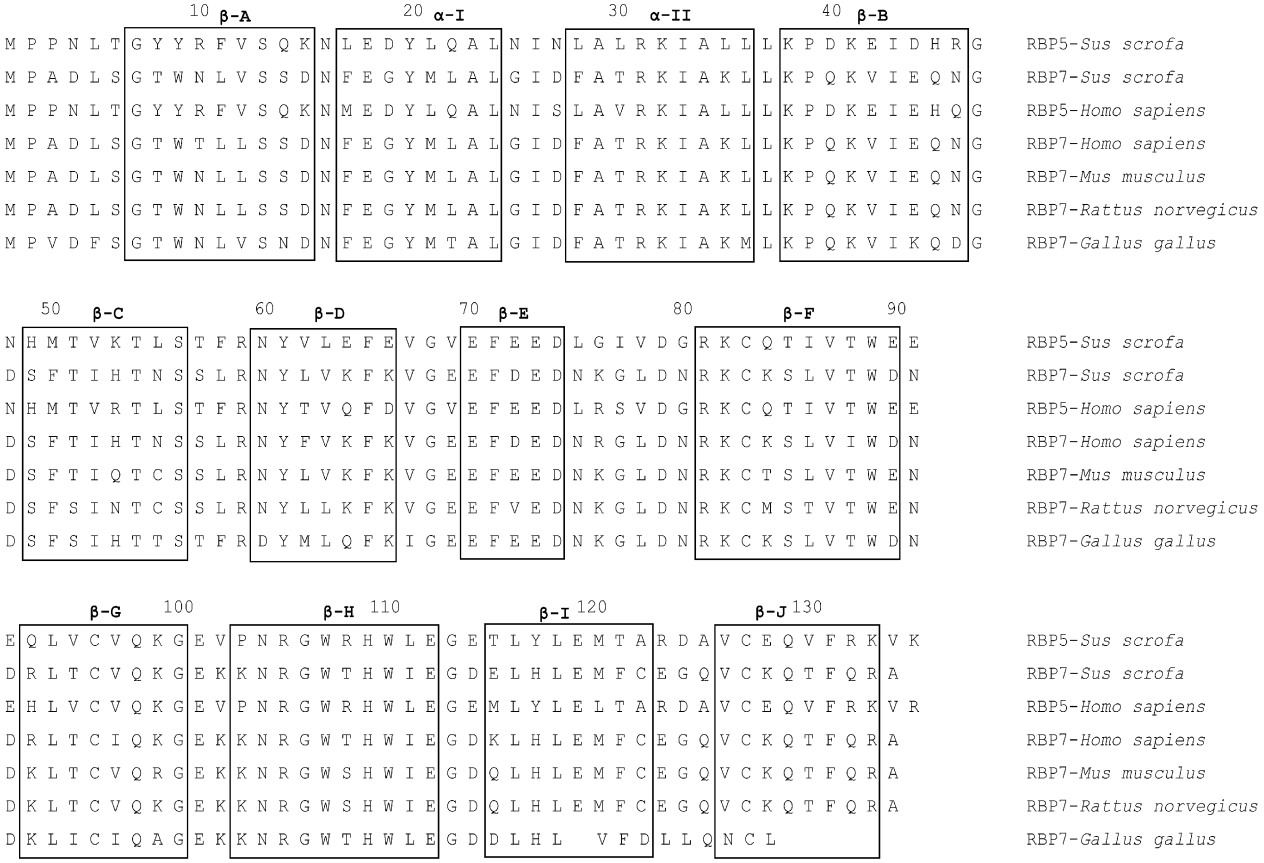

Figure 3. Multiple amino acid sequence alignment of porcine RBP5 and RBP7, human RBP5 and RBP7, and house mouse (*Mus musculus*), Norway rat (*Rattus norvegicus*) and red jungle fowl (*Gallus gallus*) RBP7. The boxed amino acids designated βA-J represent 10 stretches of β-sheet structure and those designated α-I and α-II represent the stretches of α-helix. The positions of β-sheets A-J and α-helices I and II are indicated according to Cowan et al. (1993). GenBank accession nos. Human RBP5: NP_113679.1, RBP7: NP_443192.1; house mouse: NP_071303.1; Norway rat: XP_575960.1; red jungle fowl: XP_417606.2.

T/C[intron1-90] have significant association with economic traits such as SBP, LM, LN, MS, HCT and MCS. Whether these SNPs have really affected some traits associated with biological process or just linked to other major genes need further investigation.

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