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Association between cholecystokinin type A receptor haplotypes and growth traits in Japanese Hinai-dori crossbred chickens

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Abstract We previously identified quantitative trait loci for body weight and average daily gain in a common region between *MCW0240* (chr 4: 69.9 Mb) and *ABR0622* (chr 4: 86.3 Mb) on chicken chromosome 4 in an F_2 resource population produced by crossing low- and high-growth lines of the Hinai-dori breed. *Cholecystokinin type A receptor* (*CCKAR*) is a candidate gene affecting growth traits in the region. In this study, we genotyped polymorphisms of the *CCKAR* gene and investigated its association with growth traits in a Hinai-dori F_2 intercross population. All the exons of the *CCKAR* gene in the parental population were subjected to PCR amplification, nucleotide sequenced and haplotypes identified. To distinguish resultant diplotype individuals in the F_2 population, a mismatch amplification mutation assay was performed. Five haplotypes (Haplotypes 1–5) were accordingly identified. Six genotypes produced by the combination of three haplotypes (Haplotype 1, 3, and 4) were examined in order to identify associations between *CCKAR* haplotypes and growth traits. The data indicate that Haplotype 1 was superior to Haplotype 3 and 4 in body weight at 10 and 14 weeks of age, average daily gain between 4 and 10 weeks, 10 and 14 weeks, and 0 and 14 weeks of age. It was concluded that *CCKAR* is a useful marker of growth traits and could be used to develop strategies for improving growth traits in the Hinai-dori breed.

Keywords Chicken · Hinai-dori breed · Growth · Marker gene · *Cholecystokinin type A receptor*

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Introduction

The Hinai-dori is a slow-growing breed of chicken native to Akita Prefecture, in northern Honshu Island, Japan. Although Hinai-dori meat has a characteristic taste and the breed has been used for a long time, it has decreased in numbers in recent times owing to the introduction of exotic breeds, and for a while was at risk of extinction. The Hinai-dori breed has been conserved by hobbyists who belong to the Preservation Society (PS) of the Hinai-dori Breed and is now mainly used for exhibition purposes. For effective use of the breed, selection experiments have been performed at the Livestock Experiment Station (LES), Akita Prefectural Agriculture, Forestry, and Fisheries Research Center (since 1973 when fertilized eggs were introduced to LES from PS) with a view towards increasing growth performance. F_1 chickens produced by crossing the improved LES Hinai-dori sires with Rhode Island Red dams have been commercialized as the Hinai-jidori chicken, which is one of the most popular high-quality chickens on the Japanese market [1].

The identification and utilization of potential candidate genes for quantitative trait loci (QTL) with

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significant effects on economically important traits is becoming increasingly important in animal breeding programs. There are currently two main strategies for detecting QTLs: genome scans based on linkage mapping in a cross population and association tests using candidate genes [2]. In the past decade, QTL mapping for chicken growth traits such as body weight has been widely studied [3, 4]. Moreover, there have been numerous association studies investigating the relationship between growth traits and candidate genes, including *thyroid hormone responsive spot 14* α [5] and *pituitary-specific positive transcription factor 1* [6] on chromosome 1; *insulin-like growth factor binding protein* (*IGFBP*) *1* and *3* [7] on chromosome 2; *ornithine decarboxylase* (*ODC*) [8] on chromosome 3; *calpain 3* [9] on chromosome 5; *IGFBP 2* [10–12] on chromosome 7; *growth hormone secretagogue receptor* (*GHSR*) [13] on chromosome 9; and *insulin-like growth factor 1 receptor* [14] on chromosome 10.

The LES line was developed from PS chickens: hence, these individuals possessed genes that influence growth traits. To identify QTLs associated with growth traits in the Hinai-dori breed, an F_2 resource population produced by crossing low- and high-growth lines of the breed was analyzed. A highly significant QTL for body weight at 10 and 14 weeks of age and average daily gain between 4 and 10 weeks and between 10 and 14 weeks of age were accordingly mapped in a common region between *MCW0240* (chr 4: 69.9 Mb) and *ABR0622* (chr 4: 86.3 Mb) on chromosome 4 [15]. Thus, the above-mentioned genes, e.g., *IGFBP 1–3*, *ODC* and *GHSR*, are not objects of our interest, since they are not located on chromosome 4.

Of all the candidate genes for growth traits on chromosome 4, we herein focused our investigation on the *cholecystokinin type A receptor* (*CCKAR*, chr 4: 75.6 Mb) because it was located just under the QTL peak on chromosome 4 and has also been identified as a candidate gene for human obesity [16]. Therefore, our main objective in this study was to analyze polymorphism of the *CCKAR* gene and test its association with growth traits in Hinai-dori F_2 crossbreeds.

Materials and methods

Resource population

F_1 chickens were produced by crossing three cocks from a low-growth line (obtained from PS) with nine hens from a high-growth line (obtained from LES). In this cross, 1–3 hens were randomly selected to mate with each male. The F_2 individuals were produced by full-sib mating of 17 F_1 cocks and 60 F_1 hens. We accordingly obtained 418 F_2 individuals comprising 206 cocks and 212 hens. The F_2 chickens were hatched on the same day, raised in the same chicken house, and fed the same diet ad libitum for the duration of the experiment.

Traits

Body weight was measured at 4 weeks (BW-4 week), 10 weeks (BW-10 week), and 14 weeks (BW-14 week) of age. Average daily gain between 0 and 4 weeks of age (ADG 0–4 week), between 4 and 10 weeks of age (ADG 4–10 week), between 10 and 14 weeks of age (ADG 10–14 week), and 0 and 14 weeks of age (ADG 0–14 week) was calculated from BW at each week of age.

Identification of *CCKAR* haplotypes

A draft sequence of the chicken genome (May 2006 assembly), available on the University of California, Santa Cruz (UCSC) Genome Browser [17] and the Ensembl Genome Browser [18], was used in this study. The nucleotide sequences of the five exons of *CCKAR* in the parent individuals were determined by polymerase chain reaction (PCR) amplification followed by direct sequencing to determine nucleotide variance of the gene in the resource family. Each genomic DNA of the resource family was purified using a SepaGene kit (Sanko Jyunyaku, Tokyo, Japan). Five PCR primers were designed to amplify the five exons of *CCKAR* and the PCRs were performed (Table 1). For the PCRs, we used 15 μl reaction volumes containing the following: 10 pmol of each primer for each marker, 200 μM of each deoxyribonucleotide triphosphate (dNTP), 1.2 mM magnesium sulfate ($MgSO_4$), 0.5 units of KOD-plus polymerase (Toyobo, Tokyo, Japan), 1× reaction buffer provided by the manufacturer, and 10 ng genomic DNA. Reactions were performed in a 96-well plate in an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following conditions: initial denaturation at 94°C for 2 min; 30 cycles at 94°C for 15 s, at 60.0°C (Exon 1), 54.5°C (Exon 2), or 57.0°C (Exons 3, 4, and 5) for 30 s, at 68°C for 30 s; and a final elongation at 68°C for 4 min 30 s. The PCR products were purified using High Pure 96 UF Cleanup Plates (Roche Diagnostics GmbH, Mannheim, Germany) and subjected to cycle sequencing using a BigDye Terminator Cycle Sequencing FS Ready Reaction kit (Perkin-Elmer Applied Biosystems, Foster City, CA, USA) and the same primers as used for the PCR amplification. Sequencing was performed in both directions and each sequencing product was run in an ABI 3100 Genetic Analyzer (Perkin-Elmer Applied Biosystems).

Table 1 PCR primers for amplification of the five exons in the *cholecystokinin type A receptor* gene

Exon	Forward primer (5′→3′)	Reverse primer (5′→3′)	Product (bp)
Exon 1	TATCACTCTCATCACTCACCG	TTTCTCATCCTAACTTATCAGCA	702
Exon 2	AAAACTAAAACCAGGCAGGC	TGTTCAATAGCAGATAGAAAAAA	464
Exon 3	CAGGAGTATCAGCACGGAGA	CAAGGCAAACATTGTAAAAG	663
Exon 4	CTCCTCCAACCCTCCAGTAG	AACGGAATCACCTCAGTCAA	485
Exon 5	GAACAAACAGTGTCTTCCGT	ACCAGATGATGTCCACTTGA	829

The *CCKAR* gene haplotypes in the F_2 intercross population were then identified.

Statistical Analyses

A mixed-inheritance animal model was used to evaluate the effects of haplotypes on traits. The ld_fix option of the Qxpak software [19] was used for the *CCKAR* haplotypes. For statistical models, the additive effect of each allele and the sex effect were used as fixed effects. In this analysis, the infinitesimal genetic effect was included and treated as a random effect with covariance matrix $A\sigma_u^2$ (where A is the numerator relationship matrix). Likelihood ratio tests were performed by not including the additive effects in the model, and nominal *P* values were obtained by assuming a χ^2 distribution of the likelihood ratio test.

Percentage of haplotype variance explained by the model was calculated as

$$\text{Variance percentage} = 100 \times (R\_\text{variance} - F\_\text{variance}) / R\_\text{variance},$$

where *R* variance is the residual and polygenic variances from the reduced model, omitting haplotype effect but including sex and polygenic effects, and *F* variance is the residual variance from the full model, including haplotype, sex, and polygenic effects.

Results

On sequencing, we found that the sequenced region differed by 11 single nucleotide polymorphisms (SNPs) from the corresponding region in the draft sequence of the chicken genome, and five *CCKAR* haplotypes (Haplotypes 1–5) were identified in the parent individuals (Table 2). The nucleotide sequences of the five haplotypes have been registered in the DNA data bank of Japan (DDBJ) with the following accession numbers: AB604331, AB604332, AB604333, AB604334, and AB604335. The results indicated that these haplotypes might be segregated and individuals with 15 different genotypes (1/1, /2/2, 3/3, 4/4, 5/5, 1/2, 1/3, 1/4, 1/5, 2/3, 2/4, 2/5, 3/4, 3/5, and 4/5) might appear in the F_2 population. Therefore, a mismatch amplification mutation assay (MAMA) PCR protocol was developed that detects the 15 genotypes described by Cha et al. [20]. We designed six PCR primers to distinguish *CCKAR* haplotypes and PCR and genotyping were performed as described in Table 3.

Table 2 Genotypes of parent individuals of the F_2 populations

Individuals	No.	Genotype
Parent male	1	3/5
	2	3/4
	3	3/4
Parent female	1	1/2
	2	1/1
	3	1/1
	4	1/1
	5	1/1
	6	1/1
	7	1/1
	8	1/1
	9	1/1

Of the 15 expected genotypes, seven (1/1, 1/3, 1/4, 1/5, 3/3, 3/4, and 4/4) were detected in the F_2 individuals (data not shown). No individuals with Haplotype 2 were detected in the F_2 population since, by chance, F_1 individuals showing a heterozygous Haplotype 2 were not selected for construction of the F_2 population. In addition, since only one 1/5 individual was detected in the F_2 population, this individual was not included in the statistical analyses.

We observed an association between three *CCKAR* haplotypes (Haplotype 1, 3, and 4) and growth traits (Table 4). A highly significant association was found between these haplotypes and growth traits, i.e., body weight at 10 and 14 weeks of age, average daily gain between 4 and 10 weeks, 10 and 14 weeks, and 0 and 14 weeks of age. Haplotype 1 was superior to Haplotype 3 and 4 in these traits. Percentages of haplotype variance were 7.2%–12.0%.

Table 3 PCR primers for the mismatch amplification mutation assay

Primer sets	Primers (5′→3′)	Product (bp)	Target position (AB214534; Ohkubo, 2005)	PCR amplification Haplotype				
				1	2	3	4	5
CCKAR-220-145	CCCAACAGTAGGCCAGTAACA GGTGCAAGTAAGCTCTTTAACaAt	190	g220. A > G	+	−	−	+	+
CCKAR-220-23	CCCAACAGTAGGCCAGTAACA GGTGCAAGTAAGCTCTTTAACaAc	190	g220. A > G	−	+	+	−	−
CCKAR-767-125	GAGcGTGTCTACATTCAACaTc GTTGGCTGTGCTGTTGTTGT	208	g767. T > C	+	+	−	−	+
CCKAR-749-34	CTATGTGCAGGTATCTCTGTGcGt GTTGGCTGTGCTGTTGTTGT	228	g749. T > C	−	−	+	+	−
CCKAR-1,547-1	GCTGCaCTAAGCAGAAGaCa CCACTTGTAGCCCCTTCTGA	151	g1547. G > A	+	−	−	−	−
CCKAR-1,547-2,345	CTGCgCTAAGCAGAAGaCg CCACTTGTAGCCCCTTCTGA	187	g1547. G > A	−	+	+	+	+

Bases shown in lower case with an underline represent induced mismatches. Bases shown in lower case at the 3′-end represent target SNPs. The haplotypes that can or cannot be amplified by PCR for each primer set are shown as '+' or '–', respectively. For the PCRs, we used 15 µl reaction volumes containing the following: 3 pmol of each primer for each marker, 200 µM of each dNTP, 0.5 units of Paq5000DNA Polymerase (Agilent Technologies, La Jolla, CA, USA), 1×reaction buffer (containing 2 mM $MgCl_2$) provided by the manufacturer, and 10 ng genomic DNA, Reactions were performed in a 96-well plate in an iCycler Thermal Cycler (Bio-Rad Laboratories, Hercules, CA, USA) using the following conditions: initial denaturation at 95°C for 2 min; 35 cycles at 94°C for 30 s, at 62°C (CCKAR-220-145 & -23), 61°C (CCKAR-767-125 & -34), or 56°C (CCKAR-1547-1 &-2345) for 30 s, at 72°C for 1 min; and a final elongation at 72°C for 7 min. The PCR products were electrophoresed on a 2.0% agarose gel with 1× Tris–acetate EDTA (TAE) buffer and stained with ethidium bromide. The combination of these results enabled us to identify diplotypes in the F_2 population

Discussion

Cholecystokinin (CCK) was the first gut peptide to be implicated in the control of appetite [21]. In mammals, CCK promotes gallbladder contraction, increases the secretion of pancreatic enzymes and bicarbonate, inhibits gastric acid secretion, slows gastric emptying, and reduces food intake [21, 22]. Two G-protein-coupled receptors for CCK have also been identified, namely, CCKAR [23] and CCK type B receptor (CCKBR) [24]. Both receptor subtypes are distributed throughout the central nervous system and gut, although CCKAR is predominant in the alimentary tract and CCKBR is more common in the brain. The functions of CCK, CCKAR, and CCKBR have been extensively studied in birds as well as in mammals. For instance, intravenous injection of CCK suppresses food intake in chickens [25]. CCK also modulates intestinal motility [26–28] and biliary flow in the gallbladder [29] of chickens. Furthermore, CCK stimulates amylase secretion from the pancreas in chickens and ducks [30, 31]. Recently, Ohkubo et al. [32] reported that the mRNA of *CCKAR* is mainly distributed in the alimentary tract of chickens (except for the proventriculus and gizzard), whereas that of *CCKBR* occurs predominantly in the brain. Taken together, these reports suggest that CCK, CCKAR, and CCKBR play an important role in the regulation of appetite in birds as well as in mammals.

CCK, CCKAR, and CCKBR gene knockout mice have been produced to study the actions of these three genes [33]. Despite the well-established effect of CCK on satiety, all three knockout mice are viable and exhibit normal body weights. In contrast, Otsuka Long Evans Tokushima Fatty (OLETF) rats, which lack CCKAR because of a genetic mutation [34], are heavier than the corresponding control (Long Evans Tokushima Otsuka) rats from postnatal day 1 until adulthood [35]. The apparent discrepancy in phenotypes between the *CCKAR* knockout mice and OLETF rats may be explained by interspecies differences that influence how the absence of CCKAR manifests as physiologic alterations.

In this study, the association between haplotypes and growth traits cannot be explained by the protein sequences, since *CCKAR* has not been knocked out. Further, the SNPs detected in the coding regions of *CCKAR* do not induce missense mutations, and thus CCKAR proteins are functionally normal. Therefore, at present, it is unknown whether CCKAR haplotypes have any functional significance. The associations detected in this study may be produced by linkage disequilibrium between these haplotypes and another linked gene directly involved in the regulation of these traits. Ankra-Badu et al. [36] proposed positional candidate genes for growth traits in the region, namely, *CCKAR* (chr4: 75.6 Mb); *peroxisome proliferator-activated receptor gamma, coactivator 1 alpha* (chr4:

Table 4 Phenotypic values of growth traits and effects of *cholecystokinin type A receptor* haplotypes on the traits in the F_2 population

Trait	*n*	Phenotypic values			Haplotype 1	Haplotype 3	Haplotype 4	Variance		
		Mean ± SD	LRT	*P* value	Mean ± SE	Mean ± SE	Mean ± SE	*Rvar*	*Fvar*	(*Rvar*−*Fvar*)/*Rvar* (%)
BW-4 week (g)	417*	231.1 ± 38.3	1.5	n.s.						
BW-10 week (g)	418	960.6 ± 163.5	36.6	5.7×10^{-8}	547.9 ± 17	496.8 ± 18.2	502.7 ± 18.9	16554.7	15325.1	7.4
BW-14 week (g)	418	1466.9 ± 260.7	57.7	1.9×10^{-12}	867.1 ± 23.2	779.2 ± 24.8	790 ± 25.7	31852.9	28021.5	12
ADG 0–4 week (g/day)	417*	5.2 ± 1	1.5	n.s.						
ADG 4–10 week (g/day)	417*	17.4 ± 3.3	46.5	4.4×10^{-10}	10.1 ± 0.3	9 ± 0.4	9 ± 0.4	6.8	6.2	9.4
ADG 10–14 week (g/day)	418	18.1 ± 4.4	50.1	7.5×10^{-11}	11.4 ± 0.2	10.1 ± 0.3	10.2 ± 0.3	7.1	6.3	11.5
ADG 0–14 week (g/day)	418	14.6 ± 2.7	57.5	2.2×10^{-12}	8.7 ± 0.2	7.8 ± 0.3	7.9 ± 0.3	3.3	2.9	12

BW-4 week, BW-10 week, and BW-14 week; body weight measured at 4 weeks, 10 weeks, and 14 weeks of age. ADG 0–4 week, ADG 4–10 week, ADG 10–14 week, and ADG 0–14 week; average daily gain between 0 and 4 weeks, between 4 and 10 weeks, between 10 and 14 weeks, and between 0 and 14 weeks of age. SD; standard deviation. SE; standard error. LRT; loglikelihood ratio test statistics, n.s. not significant. *Rvar*; variance by using reduced model that included sex, and polygenic effects. *Fvar*; variance by using full model that included sex, haplotype, and polygenic effects

* Since BW-4 week has a missing datum, ADG 0–4 week and 4–10 week have a missing datum, respectively

76.6 Mb); *extracellular superoxide dismutase 3* (chr4: 76.2 Mb); *C1q and tumour necrosis factor-related protein 7* (chr4: 79.7 Mb); and *fibroblast growth factor binding protein 1* and *2* (chr4: 79.5 Mb). Rubin et al. [37] reported that a further gene, *TBC1 (tre-2/USP6, BUB2, cdc16) domain family, member 1* (chr4: 71.8 Mb), may be associated with growth traits.

One likely possibility to explain the association between *CCKAR* haplotypes and growth traits is that presence of an SNP in the 5′-untranslated region (5′ UTR) of *CCKAR* may affect *CCKAR* gene expression. We actually found an SNP at chromosome 4: 75,630,198 bp in the draft sequence map (2006 assembly) (AB604331:g.420C > A), corresponding to the predicted Yin Yang-1 (YY1) binding site (TCTTC(*C/A*)TAG) [38] in the 5′ UTR of *CCKAR*. Haplotype 1 has an 'A' whereas Haplotype 3, and 4, and the draft sequence of the chicken genome have a 'C' at this binding site SNP. To our knowledge, this SNP has not been previously reported including broilers, layers, and Silkies [18]. YY1 is a zinc finger protein that functions as a transcriptional activator, repressor, or transcription-initiator element-binding protein, depending on the promoter context [39]. In pigs, Houston et al. [40] reported that an SNP in the YY1 binding site in the 5′ UTR of *CCKAR* may affect food intake and growth. We may assume a mechanism similar to that proposed by Houston, which is independent of interspecies differences between pigs and chickens. Therefore, further studies are needed to elucidate the differences in transcriptional and translational efficiencies caused by each SNP.

In conclusion, we observed significant associations between *CCKAR* haplotypes and growth traits in an F_2 population produced by crossing low- and high-growth lines of the Hinai-dori breed of chicken. Our data suggest that haplotype-assisted selection is a potentially useful approach in the Hinai-dori breed. In the future, the associations between haplotypes and growth traits should be studied in other chicken lines and breeds.

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References

1. Rikimaru K, Takahashi H (2007) A method for discriminating a Japanese brand of chicken, the Hinai-jidori, using microsatellite markers. Poult Sci 86:1881–1886
2. Andersson L (2001) Genetic dissection of phenotypic diversity in farm animals. Nat Rev Genet 2:130–138
3. Abasht B, Dekkers JCM, Lamont SJ (2006) Review of quantitative trait loci identified in the chicken. Poult Sci 85:2079–2096
4. Hu ZL, Fritz ER, Reecy JM (2007) AnimalQTLdb: a livestock QTL database tool set for positional QTL information mining and beyond. Nucl Acids Res 35:604–609

5. Cao ZP, Wang SZ, Wang QG, Wang YX, Li H (2007) Association of spot14α gene polymorphisms with body weight in the chicken. Poult Sci 86:1873–1880
6. Nie Q, Fang M, Xie L, Zhou M, Liang Z, Luo Z, Wang G, Bi W, Liang C, Zhang W, Zhang X (2008) The *PIT1* gene polymorphisms were associated with chicken growth traits. BMC Genet 9:20
7. Qu JT, Tang SQ, Sun DX, Zhang Y (2009) Polymorphisms of three neuroendocrine-correlated genes associated with growth and reproductive traits in the chicken. Poult Sci 88:722–727
8. Uemoto Y, Sato S, Ohtake T, Sato S, Okumura Y, Kobayashi E (2011) Ornithine decarboxylase gene is a positional candidate gene affecting growth and carcass traits in F_2 intercross chickens. Poult Sci 90:35–41
9. Zhang ZR, Liu YP, Yao YG, Jiang XS, Du HR, Zhu Q (2009) Identification and association of the single nucleotide polymorphisms in calpain3 (*CAPN3*) gene with carcass traits in chickens. BMC Genet 10:10
10. Lei MM, Nie QH, Peng X, Zhang DX, Zhang XQ (2005) Single nucleotide polymorphisms of the chicken insulin-like factor binding protein 2 gene associated with chicken growth and carcass traits. Poult Sci 84:1191–1198
11. Li ZH, Li H, Zhang H, Wang SZ, Wang QG, Wang YX (2006) Identification of a single nucleotide polymorphism of the insulin-like growth factor binding protein 2 gene and its association with growth and body composition traits in the chicken. J Anim Sci 84:2902–2906
12. Leng L, Wang S, Li Z, Wang Q, Li H (2009) A polymorphism in the 3′-flanking region of insulin-like growth factor binding protein 2 gene associated with abdominal fat in chickens. Poult Sci 88:938–942
13. Fang M, Nie Q, Luo C, Zhang D, Zhang X (2010) Associations of *GHSR* gene polymorphisms with chicken growth and carcass traits. Mol Biol Rep 37:423–428
14. Lei M, Peng X, Zhou M, Luo C, Nie Q, Zhang X (2008) Polymorphisms of the *IGF1R* gene and their genetic effects on chicken early growth and carcass traits. BMC Genet 9:70
15. Rikimaru K, Sasaki O, Koizumi N, Komatsu M, Suzuki K, Takahashi H (2011) Mapping of quantitative trait loci affecting growth traits in a Japanese native chicken cross. Asian-Aust J Anim Sci 24:1329–1334
16. Arya R, Duggirala R, Jenkinson CP, Almasy L, Blangero J, O'Connell P, Stern MP (2004) Evidence of a novel quantitative-trait locus for obesity on chromosome 4p in Mexican Americans. Am J Human Genet 74:272–282
17. UCSC Genome Browser Home (2004) University of California, Santa Cruz, USA. http://genome.ucsc.edu/. Accessed 1 February 2011
18. Ensembl Genome Browser (2004) European Bioinformatics Institute, UK. http://ensembl.genomics.org.cn/. Accessed 1 February 2011
19. Pérez-Enciso M, Misztal I (2004) Qxpak: a versatile mixed model application for genetical genomics and QTL analyses. Bioinformatics 20:2792–2798
20. Cha RS, Zarbl H, Keohavong P, Thilly WG (1992) Mismatch amplification mutation assay (MAMA): application to the c-H-*ras* gene. PCR Methods Applic 2:14–20
21. Gibbs J, Young RC, Smith GP (1973) Cholecystokinin decreases food intake in rats. J Comp Physiol Psychol 84:488–495
22. Kissileff HR, Pi-Sunyer FX, Thornton J, Smith GP (1981) C-terminal octapeptide of cholecystokinin decreases food intake in man. Am J Clin Nutr 34:154–160
23. Sankaran H, Goldfine ID, Deveney CW, Wong KY, Williams JA (1980) Binding of cholecystokinin to high affinity receptors on isolated rat pancreatic acini. J Biol Chem 255:1849–1853
24. Innis RB, Snyder SH (1980) Distinct cholecystokinin receptors in brain and pancreas. Proc Natl Acad Sci USA 77:6917–6921
25. Savory CJ, Gentle MJ (1980) Intravenous injections of cholecystokinin and caerulein suppress food intake in domestic fowls. Experientia 36:1191–1192
26. Rodriguez-Sinovas A, Fernandez E, Manteca X, Fernández AG, Goñalons E (1997) CCK is involved in both peripheral and central mechanisms controlling food intake in chickens. Am J Physiol Regul Integr Comp Physiol 272:R334–R340
27. Martín MT, Fernández E, Rodríguez-Sinovas A, Goñalons E (1995) Effects of cholecystokinin on chicken cecal motility: mechanisms involved. Life Sci 56:601–610
28. Martínez V, Jiménez M, Goñalons E, Vergara P (1995) Modulation of the migrating myoelectric complexes by cholecystokinin and gastrin in the gastrointestinal tract of chickens. Poult Sci 74:563–576
29. Duke GE, Larntz K, Hunter H (1987) The influence of cholecystokinin, vasoactive intestinal peptide and secretin on pancreatic and bilary secretion in laying hens. Comp Biochem Physiol C Comp Pharmacol 86:97–102
30. Satoh S, Furuse M, Choi YH, Okumura J (1994) Cholecystokinin is not a major regulator in the digestive system in the chicken. Cell Mol Life Sci 50:812–814
31. Xiao R, Cui ZJ (2004) Mutual dependence of VIP/PACAP and CCK receptor signaling for a physiological role in duck exocrine pancreatic secretion. Am J Physiol Regul Integr Comp Physiol 286:R189–R198
32. Ohkubo T, Shamoto K, Ogino T (2007) Structure and tissue distribution of cholecystokinin-1 receptor in chicken. J Poult Sci 44:98–104
33. Robinson SW, Dinulescu DM, Cone RD (2000) Genetic models of obesity and energy balance in the mouse. Annu Rev Genet 34:687–745
34. Miyasaka K, Kanai S, Ohta M, Kawanami T, Kono A, Funakoshi A (1994) Lack of satiety effect of cholecystokinin (CCK) in a new rat model not expressing the CCK-A receptor gene. Neurosci Lett 180:143–146
35. Schroeder M, Zagoory-Sharon O, Lavi-Avnon Y, Moran TH, Weller A (2006) Weight gain and maternal behavior in CCK_1 deficient rats. Physiol Behav 89:402–409
36. Ankra-Badu GA, Bihan-Duval EL, Mignon-Grasteau S, Pitel F, Beaumont C, Duclos MJ, Simon J, Carré W, Porter TE, Vignal A, Cogburn LA, Aggrey SE (2010) Mapping QTL for growth and shank traits in chickens divergently selected for high or low body weight. Anim Genet 41:400–405
37. Rubin CJ, Zody MC, Eriksson J, Meadows JR, Sherwood E, Webster MT, Jiang L, Ingman M, Sharpe T, Ka S, Hallböök F, Besnier F, Carlborg O, Bed'hom B, Tixier-Boichard M, Jensen P, Siegel P, Lindblad-Toh K, Andersson L (2010) Whole-genome resequencing reveals loci under selection during chicken domestication. Nature 464:587–591
38. Shrivastava A, Calame K (1994) An analysis of genes regulated by the multi-functional transcriptional regulator Yin Yang-1. Nucl Acids Res 22:5151–5155
39. Liu H, Shi Y (2005) Yin Yang 1. In: Iuchi S, Kuldell N (eds) Zinc finger proteins: from atomic contact to cellular function. Landes Bioscience/Eurekah.com and Kluwer Academic/Plenum Publishers, Dordrecht, New York, pp 182–194
40. Houston RD, Haley CS, Archibald AL, Cameron ND, Plastow GS, Rance KA (2006) A polymorphism in the 5′-untranslated region of the porcine cholecystokinin type A receptor gene affects feed intake and growth. Genetics 174:1555–1563