The A Allele of the Cholecystokinin Type A Receptor Gene g.420 C > A Polymorphism Improves Growth Traits in Amakusa Daioh Cross Chicken

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Amakusa Daioh cross chickens are F_1 hybrids of restored Amakusa Daioh sires and Kyushu Rhode dams. In the present study, the association between a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) in the cholecystokinin type A receptor gene and growth traits in Amakusa Daioh cross chicken were investigated. We used 72 male and 72 female birds that had hatched on the same day, were raised in the same chicken house, and were fed the same diet *ad libitum* from day 0 to 17 weeks (wks) of age. Body weight was recorded at weekly intervals and average daily gain of each week interval was calculated from body weight data. Birds were sacrificed at 17 wks and carcass traits were recorded. SNP genotyping was carried out using the mismatch amplification mutation assay. Associations between the SNP and growth traits were analyzed by a generalized linear model. Body weight from 6 to 17 wks was higher in birds with the A allele than in birds with the C allele, although significant differences in average daily gain traits between birds with A and C alleles were not detected during most of the duration of the experiment. Carcass data showed that birds with the A allele had heavier wings and a smaller proportion of the gizzard than those with the C allele. The g.420 C>A SNP will be useful as a selection marker for parent stock lines to increase the growth performance of Amakusa Daioh cross chickens.

Key words: Amakusa Daioh cross chicken, chicken, cholecystokinin type A receptor gene, growth traits, single nucleotide polymorphism

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Introduction

In Japan, fast-growing broilers constitute more than 90% of poultry meat production. However, "Jidori," as defined in the Japanese Agricultural Standard (JAS, Ministry of Agriculture, Forestry and Fisheries of Japan, 1999), still occupies a certain share in the Japanese market, because Japanese consumers recognize and appreciate its rich taste and flavor. The JAS defines Jidori as cross-bred chickens with over 50% of native-bred hereditary. Most Jidori chickens are bred by crossing indigenous Japanese breeds with highly selected lines that exhibit rapid growth and/or high egg productivity.

Of the indigenous chicken breeds in Japan, "Amakusa Daioh," native to the Kumamoto prefecture, was one of the largest. Unfortunately, Amakusa Daioh became extinct in the early Showa era (1926-1981). In 2001, the Kumamoto

Prefectural Government announced that Amakusa Daioh had been restored by the Animal Husbandry Research Institute, Kumamoto Prefectural Agricultural Research Center (Koshi, Japan) (Matsuzaki *et al*., 2001). The procedure used was as follows: first, Shamo (Japanese Game), Kumamoto Cochin (a local variety of Cochin breed native to China), and Langshan (native to China) breeds were crossed in accordance with the documented history of the establishment of the Amakusa Daioh breed. Then, the offspring were selected for body size and shape, including external features that were based on an old painting that had existed for seven generations. F_1 hybrid chickens from the cross between restored Amakusa Daioh cocks and Kyushu Rhode (a synthetic breed resulting from a cross between Rhode Island Red and White Plymouth Rock) hens are currently commercially available as "Amakusa Daioh cross" in the Japanese market. The meat taste of the Amakusa Daioh cross is recognized and is gaining popularity in Japan.

Although Amakusa Daioh cross chickens are the largest among Jidori chickens, their growth is far slower than that of broilers (Nikki *et al*., 2012). Thus, breeding for growth traits

is a major consideration in Amakusa Daioh cross production. Recently, in the Hinai-dori breed native to the Akita prefecture of Japan, a significant association between a single nucleotide polymorphism (SNP; AB604331, g.420 C>A) in the 5′-untranslated region of the cholecystokinin type A receptor gene (*CCKAR*) and growth traits was reported (Rikimaru *et al.*, 2013). The objective of the present study was to verify whether the g.420 C>A SNP in *CCKAR* can be used as a marker to improve growth traits of the Amakusa Daioh cross.

Materials and Methods

Experimental Birds

Experimental birds received humane care as outlined in the Guidelines for Proper Conduct of Animal Experiments (Science Council of Japan, 2006).

One hundred forty-four birds (72 male and 72 female) of Amakusa Daioh cross that had hatched on July 2, 2014 were used. The chicks were raised in a battery cage until 2 weeks (wks) of age and then in solid-floored pens in a conventional poultry research house until 17 wks of age. The chicks were fed a starter diet (ME, 3,050 kcal/kg; CP, 23% (wt/wt)) from 0 to 4 wks, a grower diet (ME, 3,210 kcal/kg; CP, 18%) from 5 to 9 wks, and a finisher diet (ME, 3,260 kcal/kg; CP, 18%) from 10 to 17 wks. The chickens had *ad libitum* access to feed and water throughout the experiment.

The body weight (BW) of the birds was measured at weekly intervals from day-of-hatch (0 d) to 17 wks. The average daily gain (ADG) of each weekly interval was calculated from the BW at each week of age. At 17 wks, 67 birds were randomly selected, fasted overnight, and slaughtered. After they were bled, each carcass was scalded at 63℃ for 2 min, and feathers were removed with an automatic picker. The carcass was cooled in ice-cold water for 1 h. Evisceration was done by hand and the head was separated. The carcass was dissected, and the legs and breast were deboned. Each part (breast, thigh, tender, wings, bones, abdominal fat, liver, heart, gizzard) was weighed. The percentage weight of each carcass part was calculated as its weight relative to live body weight at 17 wks.

CCKAR Genotyping

Blood was collected from the ulnar vein at 6 wks, spotted onto an FTA filter paper (WB120028; GE Healthcare, Buckinghamshire, UK), and air-dried at room temperature. Genomic DNA was extracted from the FTA paper as described in Rikimaru *et al.* (2013). The SNP (AB604331: g.420 C>A) in the 5′-untranslated region of *CCKAR* was genotyped using the mismatch amplification mutation assay as described in Rikimaru *et al.* (2013).

Statistical Analysis

SNP–trait association analysis was conducted in R (R Core Team, 2016) using the following generalized linear model:

$$y = \mu + C_s s + C_a a + C_d d + e,$$

where y is the response variable of each phenotype; μ is the intercept; the sex effect (s) is a covariate coefficient with C_s having values of 0 and 1 for male and female, respectively; the additive effect (a) is a covariate coefficient with C_a having values of 2, 1, and 0; and the dominance effect (d) is a covariate coefficient with C_d having values of 0, 1, and 0, for genotypes A/A, A/C, and C/C, respectively; and e is the residual standard error.

The percentage of haplotype variance explained by the model was calculated as described in Rikimaru *et al.* (2012), using the following formula:

variance percentage $= 100 \times (1 - F_{var} / R_{var})$,

where R_{var} is the residual variance from the reduced model, omitting the additive effect but including sex and dominance effects; F_{var} is the residual variance from the full model, including sex, additive, and dominance effects.

Results

In the population of Amakusa Daioh cross chickens, the genotype frequencies of AA, AC, and CC at the g.420 C>A SNP were 0.347, 0.563, and 0.090, respectively. The distribution of the genotypes in the population did not contradict Hardy–Weinberg equilibrium. Allele frequencies of A and C were 0.628 and 0.372, respectively.

Sex, additive, and dominance effects of the SNP on BW traits from 0 d to 17 wks are shown in Table 1. Males were significantly heavier than females from 1 to 17 wks. As for the additive effect, the significant differences between chickens carrying the A allele and those having the C allele from 6 to 17 wks show that the A allele is significantly superior to the C allele in these traits. As for the dominance effect, there were no significant differences between birds with the A allele and those with the C allele throughout the experiment.

Sex, additive, and dominance effects on ADG in each one-week interval are shown in Table 2. As for the sex effect, there were significant differences in ADG between males and females from the 0-d–1-wk interval to the 13−14-wk interval. As for the additive effect, there was a significant difference between the A and C alleles in ADG at the 5−6-wk interval. For the other ADG traits, there were no significant differences between the A and C alleles, whereas the A allele tended to be superior in ADG traits, except for ADG at the 14−15-wk interval. In terms of the dominance effect, there were no significant differences between birds with the A and those with the C allele throughout the experimental period.

Sex, additive, and dominance effects on the weight of each carcass part at 17 wks are shown in Table 3. Data on the sex effects show that the weights of breast, thigh, tender, wings, bones, abdominal fat, heart, and gizzard in males were significantly higher than those in females. Data of the additive effects showed that birds with the A allele had heavier wings than those with the C allele. As for the dominance effect, there were no significant differences between the weights of carcass parts in birds with the A and those with the C allele.

Sex, additive, and dominance effects on the percentage weight of each carcass part at 17 wks are shown in Table 4. Data on the sex effect show that while the percentage weight of thigh, wings, bones, and heart was significantly higher in males than in females, the percentage weight of breast, tender, abdominal fat, liver, and gizzard was significantly

Table 1. **Effects of g.420 C>A in *CCKAR* on BW in Amakusa Daioh cross chickens**

Body weight (BW, g)	Intercept	Sex	Additive	Dominance	R_{var}	F_{var}	Variance (%)
0 d	44.3±1.0	−0.6±0.6	0.4±0.5	−0.3±0.6	1573.8	1566.1	0.5
1 wk	118.4±2.5	−7.5±1.4***	2.2±1.3	2.3±1.6	9818.2	9627.1	1.9
2 wks	238.5±5.0	−21.8±2.9***	5.4±2.6*	1.9±3.3	41486	40294	2.9
3 wks	409.9±8.9	−52.9±5.1***	8.5±4.7	2.3±5.8	130648	127648	2.3
4 wks	630.8±13.8	−83.1±7.9***	10.9±7.3	0.2±9.0	310661	305725	1.6
5 wks	873.3±20.9	−156.6±11.9***	20.7±11.0	−6.9±13.6	714854	697182	2.5
6 wks	1128.0±29.0	−213.0±16.5***	36.2±15.3*	−7.1±18.9	1400657	1346500	3.9
7 wks	1450.4±33.1	−297.0±18.8***	39.7±17.4*	−13.5±21.5	1810545	1745458	3.6
8 wks	1763.6±39.6	−393.4±22.5***	44.8±20.8*	−24.2±25.8	2589666	2506772	3.2
9 wks	2068.7±44.7	−486.6±25.4***	57.5±23.5*	−26.7±29.1	3324404	3188028	4.1
10 wks	2395.1±52.4	−592.1±29.7***	71.0±27.6*	−26.5±34.1	4589994	4382028	4.5
11 wks	2724.2±56.9	−711.8±32.3***	74.2±29.9*	−25.0±37.1	5398083	5170993	4.2
12 wks	3009.5±61.4	−820.5±34.9***	85.4±32.3**	−26.3±40.0	6322498	6021976	4.8
13 wks	3238.4±64.6	−899.8±36.7***	88.2±34.0*	−21.6±42.1	6984011	6663441	4.6
14 wks	3507.9±73.0	−1017.8±41.4***	101.3±38.4**	−35.0±47.5	8925085	8502412	4.7
15 wks	3684.9±74.8	−1025.5±42.5***	85.9±39.4*	−41.7±48.7	9244574	8940468	3.3
16 wks	3849.7±81.6	−1035.7±46.4***	93.4±42.9*	−56.5±53.2	11003424	10643990	3.3
17 wks	4008.1±88.5	−1053.1±50.3***	104.0±46.6*	−36.9±57.7	12969202	12522910	3.4

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

Table 2. **Effects of g.420 C>A in *CCKAR* on ADG in Amakusa Daioh cross chickens**

Average daily gain (ADG, g)	Intercept	Sex	Additive	Dominance	R_{var}	F_{var}	Variance (%)
0 d–1 wk	10.6±0.3	−1.0±0.2***	0.2±0.2	0.4±0.2	198	195	1.3
1–2 wks	17.2±0.5	−2.0±0.3***	0.5±0.2	−0.1±0.3	359	350	2.4
2–3 wks	24.5±0.8	−4.4±0.5***	0.5±0.4	0.1±0.5	1018	1010	0.8
3–4 wks	31.6±1.0	−4.3±0.6***	0.3±0.5	−0.3±0.6	1546	1541	0.3
4–5 wks	34.6±1.5	−10.5±0.9***	1.4±0.8	−1.0±1.0	3816	3735	2.1
5–6 wks	36.4±2.1	−8.1±1.2***	2.2±1.1*	0.0±1.3	7043	6840	2.9
6–7 wks	46.1±1.8	−12.0±1.0***	0.5±1.0	−0.9±1.2	5252	5242	0.2
7–8 wks	44.7±1.9	−13.8±1.1***	0.7±1.0	−1.5±1.2	5809	5787	0.4
8–9 wks	43.6±2.1	−13.3±1.2***	1.8±1.1	−0.4±1.4	7013	6878	1.9
9–10 wks	46.6±2.1	−15.1±1.2***	1.9±1.1	0.0±1.4	7268	7114	2.1
10–11 wks	47.0±2.5	−17.1±1.4***	0.5±1.3	0.2±1.6	9895	9886	0.1
11–12 wks	40.8±2.8	−15.5±1.6***	1.6±1.5	−0.2±1.8	12392	12288	0.8
12–13 wks	32.7±2.6	−11.3±1.5***	0.4±1.4	0.7±1.7	10544	10537	0.1
13–14 wks	38.5±2.8	−16.8±1.6***	1.9±1.5	−1.9±1.8	12744	12601	1.1
14–15 wks	25.3±3.5	−1.1±2.0	−2.2±1.9	−1.0±2.3	19971	19772	1.0
15–16 wks	23.5±3.8	−1.5±2.2	1.1±2.0	−2.1±−0.9	23270	23223	0.2
16–17 wks	22.6±4.5	−2.5±2.5	1.5±2.4	2.8±2.9	32002	31906	0.3

* $P<0.05$, ** $P<0.01$, *** $P<0.001$

lower in males than in females. Data on the additive effect showed that birds with the A allele had a smaller percentage weight of the gizzard than birds with the C allele. As for the dominance effect, there were no significant differences between the relative weights of all carcass parts in birds with the A and those with the C allele.

Discussion

The significant association between *CCKAR* haplotypes and growth traits was first reported by Rikimaru *et al.* (2012), who used a resource population produced by crossing low- and high-growth lines of the Hinai-dori breed. Rikimaru *et al.* (2013) reported a significant difference in allele frequency between the low- and high-growth lines, suggesting that the difference was caused by long-term selection for growth performance. Moreover, Rikimaru *et al.* (2014) demonstrated that the A allele of the g.420 C>A SNP in *CCKAR* improves growth traits in the Hinai-dori breed. However, the

Table 3. **Effects of g.420 C>A in *CCKAR* on the weight of carcass parts in Amakusa Daioh cross chickens**

Carcass trait (g)	Intercept	Sex	Additive	Dominance	R_{var}	F_{var}	Variance (%)
Breast	438.2±25.5	−85.8±14.2***	20.0±13.2	−18.1±16.4	218677	210968	3.5
Thigh	845.8±39.5	−287.2±22.1***	30.6±20.4	−35.8±25.4	525881	507780	3.4
Tender	122.5±6.8	−20.4±3.8***	4.3±3.5	1.4±4.4	15437	15085	2.3
Wings	362.7±15.6	−114.3±8.7***	17.9±8.1*	−8.4±10.1	85941	79732	7.2
Bones	1149.5±55.4	−427.7±31.0***	34.7±28.7	1.6±35.7	1024296	1001015	2.3
Abdominal fat	115.5±25.2	38.0±14.1**	−6.5±13.0	−26.4±16.2	207325	206512	0.4
Liver	55.9±5.6	−3.6±3.1	2.4±2.9	−1.5±3.6	10141	10032	1.1
Heart	20.6±1.3	−9.2±0.7***	−0.1±0.7	−1.3±0.8	556	556	0.0
Gizzard	55.8±3.4	−7.8±1.9***	−1.8±1.8	−0.2±2.2	3865	3799	1.7

$*P<0.05, **P<0.01, ***P<0.001$

Table 4. **Effects of g.420 C>A in *CCKAR* on the proportion of carcass parts to BW in Amakusa Daioh cross chickens**

Carcass trait (%)	Intercept	Sex	Additive	Dominance	R_{var}	F_{var}	Variance (%)
Breast	10.8±0.6	1.20±0.31***	0.14±0.29	−0.20±0.36	100.2	99.8	0.4
Thigh	20.8±0.6	−1.76±0.34***	0.11±0.32	−0.43±0.40	123.3	123.1	0.2
Tender	3.0±0.2	0.47±0.09***	−0.02±0.08	0.15±0.10	7.9	7.9	0.1
Wings	8.9±0.3	−0.40±0.17*	0.18±0.16	0.08±0.20	31.2	30.6	1.9
Bones	27.9±1.2	−3.53±0.69***	0.24±0.64	1.09±0.80	498.6	497.5	0.2
Abdominal fat	2.9±0.6	2.02±0.36***	−0.27±0.33	−0.70±0.42	137.7	136.4	1.0
Liver	1.4±0.1	0.38±0.08***	0.01±0.07	0.02±0.09	6.6	6.6	0.0
Heart	0.5±0.0	−0.13±0.02***	−0.02±0.02	−0.02±0.02	0.3	0.3	2.4
Gizzard	1.4±0.1	0.22±0.05***	−0.10±0.05*	0.04±0.06	3.2	3.0	6.5

$*P<0.05, **P<0.01, ***P<0.001$

Hinai-dori breed has not been directly used for commercial purpose; rather, it is used as sires for Hinai-jidori production. Therefore, this is, to our knowledge, the first study showing that the g.420 C>A SNP can be applied to a commercial Jidori chicken.

We cannot exclude that the associations detected in this study might have resulted from linkage disequilibrium between the SNP and other linked DNA polymorphisms directly involved in the regulation of growth traits. *CCKAR* is located in the distal region of chromosome 4. In this region, *CCKAR* SNPs other than g.420 C>A (Dunn *et al*., 2013) and SNPs in other candidate genes (Lyu *et al*., 2017) have been suggested to affect growth traits by studies on resource populations of White Leghorn×commercial broilers (Dunn *et al*., 2013) and New Hampshire×White Leghorn (Lyu *et al*., 2017), respectively. However, we firmly believe that g.420 C>A is a useful marker and is practicable for improving growth traits based on similar findings in genetically distinct populations, i.e., the Hinai-dori breed and Amakusa Daioh cross chickens.

The candidate gene *CCKAR* and its function merit further discussion. Cholecystokinin (CCK) is well known as a gut peptide implicated in the control of appetite (Gibbs *et al*., 1973). Two receptors for CCK have been identified: CCKAR (Sankaran *et al*., 1980) and CCK type B receptor (CCKBR)

(Innis and Snyder, 1980). CCKAR is predominant in the alimentary tract and CCKBR is more common in the brain (Sankaran *et al*., 1980; Innis and Snyder, 1980). Based on known CCK functions, it has been suggested that *CCKAR* polymorphisms might affect appetite. For example, Dunn *et al*. (2013) reported that birds possessing the high-growth *CCKAR* haplotype had lower *CCKAR* mRNA levels and immunoreactive CCKAR in the brain, intestine, and exocrine organs, which correlated with increased levels of orexigenic AGRP (agouti-related protein) in the hypothalamus, and they were resistant to the anorectic effect of exogenously administered CCK, suggesting that their satiety set point had been altered. Meanwhile, Rikimaru *et al*. (2014) reported that there were no significant differences in feed intake among the three genotypes (A/A, A/C, and C/C) of the g.420 C>A SNP, and the feed conversion ratio between 4 and 10 wks in A/A individuals was significantly higher than that of C/C individuals in the Hinai-dori breed. We think that, depending on the *CCKAR* haplotype, there are differences in metabolic efficiency rather than in appetite, because the data of Rikimaru *et al*. (2014) were produced from within a breed, while the data of Dunn *et al*. (2013) were produced from an interbreed population, and thus, Dunn *et al*. (2013) might have simply detected a breed difference in *CCKAR* haplotypes. In addition, the correspondences of haplotypes re-

ported by Dunn *et al.* (2013) and Rikimaru *et al.* (2012, 2013, 2014) have not been confirmed, because the haplotypes in Dunn's report were constructed from SNPs located in the downstream region of *CCKAR* and did not involve g.420 C>A. In any case, further study is needed to explain why *CCKAR* affects growth traits.

The reasons for the significant associations between the g.420 C>A SNP and gizzard percentage weight and wing weight are unknown. Collins *et al.* (2014) reported that the gizzard percentage of an old-breed broiler strain (Athens Canadian Random Bred) was higher than that of a modern broiler strain (Cobb 500) slaughtered at the same age. Therefore, the phenomenon observed in this study might simply reflect a negative correlation between live weight and gizzard percentage weight. Another possibility is the involvement of other genes affecting gizzard percentage weight. The osteoprotegerin (*OPG*) gene located in the proximal region on chromosome 2 has been reported to affect gizzard percentage (Fornari *et al.*, 2014). *CCK*, *CCKAR*, and *CCKBR* have been listed in the gene network of *OPG* (Fornari *et al.*, 2014). Meanwhile, *CCKAR* and the other genes located in the distal region on chromosome 4 might affect wing weight, as quantitative trait loci of this trait have been reported in this region (Ikeobi *et al.*, 2004; Nassar *et al.*, 2012).

In conclusion, this study revealed significant associations between a *CCKAR* SNP and growth traits in Amakusa Daioh cross chickens. As the parent stock lines of the Amakusa Daioh cross were maintained under restricted feeding conditions to avoid obesity and maintain reproductive performance, growth performance and meat productivity have not been tested in these lines. We will use the information obtained in this study for marker-assisted selection in the parent stock lines of the Amakusa Daioh cross, i.e., the restored Amakusa Daioh and the Kyushu Rhode breeds.

Acknowledgments

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