《Research Note》

Genetic Variation in the Growth Hormone Promoter Region of *Anas platyrhynchos*, a Duck Native to Myanmar

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Genetic variations were detected in duck growth hormone (*GH*) gene promoters at 8 positions in the genome of ducks from 3 regions (Mandalay, Khayan, and Sittwe) in Myanmar. Different frequencies were detected in the 3 regions. Higher frequencies were detected at positions, 244, 294, 586, and 665 of the *GH* promoter from both Mandalay and Khayan than in the ducks from Sittwe. The duck populations of Mandalay and Khayan have been partially selected for egg production by introducing a layer strain of Thailand native ducks, whereas that of Sittwe has been maintained without introduction of any other strain. The differences in the frequency of variations reflect that the breeding method in each region is unique. Since most of the variations were located in sequences similar to the promoters found in chickens and turkeys, variations in the promoter may affect laying performance by changing the expression of *GH* mRNA levels in the anterior pituitary gland.

Key words: duck, growth hormone, Myanmar, promoter, variation

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Introduction

In South Asia the domestic ducks have descended from the wild mallards (*Anas platyrhynchos*). Due to the climatic reason, domesticated ducks are preferred than chicken in the Asian countries and play an important role for egg and meat production. Selective breeding of domesticated ducks produces large body strains and layer strains. Pekin ducks are of greater body weight compared to wild mallards. Furthermore, layer strains lay over 300 eggs per year, whereas wild mallard lays 8 to 12 eggs. These differences indicate that a Pekin duck is a highly selected strain or breed. Likewise, domesticated chickens lay a high number of eggs. The differences in egg-laying performance in chickens are partially explained by genetic variations in the growth hormone (*GH*) axis (*GH*: Kühnlein *et al.*, 1997; Kansaku *et al.*, 2003; *GH* receptor: Feng *et al.*, 1997; Insulin-like growth factor I: Nagaraja *et al.*, 2000). Similarly, genetic variations in the duck *GH* gene promoter have been reported in Pekin ducks

(Kansaku *et al.*, 2008). However, investigation of detection of variations in *GH* promoter was only conducted in Pekin ducks so far. Thus, it is unknown whether other breed or local strain maintains the variations in duck *GH* promoter.

Because systematic breeding based on genomic DNA information has not yet been applied, the local breeds such as the Asian native duck are expected to maintain their genetic variation. Recently, breeding program and improvement of traits are applied in Myanmar. For example, to increase the economical traits, Thailand duck is introduced into Khayan. Furthermore, improved strain of duck in Khayan is transported to Mandalay to improve the traits. On the other hands, no such approaches have been applied in Sittwe. Since no studies have been conducted that focus on the detection and/or analyses of genetic variation in the promoter of *GH* gene of Myanmar native duck, the relationships between variation and breeding program or variation and economical trait were unknown.

Accordingly, this study was aimed at identifying genetic variations in the *GH* gene promoter using a duck breed native to Myanmar and comparing the frequency of variations among native ducks.

Materials and Methods

Preparation of Genomic DNA

Samples were collected from 3 different regions (Mandalay, Khayan, and Sittwe) in Myanmar by the Research Group on Native Farm Animals in Eastern Asia. Blood sam-

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ples were obtained from the wing vein, collected in a heparinized syringe, and stored on ice until centrifugation. Blood samples were centrifuged at 3000 rpm for 10 min and separated into blood cells and plasma. The precipitated blood cells were used for DNA extraction. Genomic DNA was extracted as described by Kansaku *et al.* (2005).

PCR Amplification and Detection of Genetic Variation

Genomic DNA (50 ng) (Mandalay, n=48; Khayan, n=56; and Sittwe, n=53) was subjected to PCR amplification to examine the genetic variation of the duck *GH* promoter. The *GH* promoter was amplified using primers PromS and PromA, which were previously described (Kansaku *et al.*, 2008). PCR products were purified and directly sequenced by dideoxy method on both the strands using ABI 3710 sequencer (Sanger *et al.*, 1977). Obtained sequences were analyzed and aligned using FASTA and DNASIS Pro (Hitachi Software Engineering, Tokyo, Japan). Haplotype analysis was conducted using PHASE v2.2.2 (Stephens *et al.*, 2001; Stephens and Scheet, 2005). To gauge genetic differentiation, F_{st} values between pairs of the three populations were obtained using Arlequin v3.1 (Excoffier *et al.*, 2005) based on haplotype frequency listed in Table 2.

Statistical Analyses

Chi-squared tests were used to compare the frequencies of

alleles and genotypes in each group and to determine whether the genotype distributions were in Hardy-Weinberg equilibrium.

Results

By direct sequencing, genetic variations were detected at positions 112, 157, 186, 244, 294, 300, 586, and 665 (Table 1). These substitutions most frequently identified were G/A, T/C, T/A, C/A, A/G, G/A, G/A, and C/T at positions 112, 157, 186, 244, 294, 300, 586, and 665, respectively. Thus, 6 transitions and 2 transversions were identified. The variations at positions 157, 186, 294, and 300 had not been detected in previous studies. Numbers of the genotypes observed at all positions of each variation satisfied Hardy-Weinberg equilibrium (Table 1). Variations at 586 and 665 were completely linked in the samples tested. Furthermore, variations at 244 and 586-665 were generally linked. Variation frequencies at positions 244, 586, and 665 were 0.038, 0.170, and 0.104-0.115 in Sittwe, Khayan, and Mandalay, respectively. Only 1 sample from Mandalay (DM44) showed no linkage between positions 244 and 586-665. On the other hand, the DM44 was heterozygous for variations at positions 112 (A/G) and 186 (A/T). However, variations at positions 112 and 186 were only observed in the DM44.

Table 1. Numbers of variation in the duck *GH* promoter

Nucleotide position of site of variation								Numbers detected by direct-sequencing			
1	1	1	2	2	3	5	6	Khayan	Mandaray	Sittwe	Total
1	3	8	4	9	0	8	6	(n=56)	(n=48)	(n=53)	(n=157)
2	2	6	4	4	0	6	5				
G	T	T	C	A	G	G	C	26	22	45	93
G	T	T	A	A	G	A	T	1	0	0	1
G	T	T	M	A	G	R	Y	14	9	4	27
G	T	T	C	R	G	G	C	6	12	0	18
G	T	T	C	A	R	G	C	3	1	4	8
G	Y	T	C	A	R	G	C	1	2	0	3
G	T	T	M	R	G	R	Y	3	0	0	3
G	T	T	C	R	R	G	C	1	0	0	1
G	Y	T	C	R	R	G	C	1	0	0	1
R	T	W	C	A	G	R	Y	0	1	0	1
G	Y	T	M	A	R	R	Y	0	1	0	1

Table 2. Haplotype sequence and number of each Haplotype

Nucleotide position of site of variation									Area			
Haplotype	1	1	1	2	2	3	5	6	Khayan	Mandaray	Sittwe	Total
	1	5	8	4	9	0	8	6	(NA [†] =112)	(NA=96)	(NA=106)	(NA=314)
	2	7	6	4	4	0	6	5				
Hap1	G	T	T	C	A	G	G	C	79	68	98	245
Hap2	G	T	T	C	G	G	G	C	8	13	0	21
Hap3	G	T	T	C	A	A	G	C	4	1	4	9
Hap4	G	C	T	C	A	A	G	C	2	3	0	5
Hap5	G	T	T	A	A	G	A	T	19	10	4	33
Hap6	A	T	A	C	A	G	G	T	0	1	0	1

[†] Number of copies of all alleles

Thus, the frequencies of variations at 112 and 186 were very low compared to other variations. A total of 6 haplotypes (Hap-1 to Hap-6) were identified by the combination of 8 SNPs in the Myanmar duck population (Table 2). The Hap-2, Hap-3, Hap-4, Hap-5 and Hap-6 has been submitted to GenBank/EMBL/DBJ Database with the accession number AB703150, AB703151, AB703152, AB703153 and AB703154, respectively. Hap-1 showed largest frequency among the Myanmar duck population. Due to the 1 base difference from Hap-1, Hap-2 and Hap-3 likely originated from Hap-1 by one transitional substitution. In Khayan and Mandalay, 5 and 6 haplotypes were observed, respectively. However, only 3 haplotypes were detected in Sittwe. Variation linkage sequence at position 244, 586 and 665 was classified to Hap-5. The F_{st} values based on the haplotype frequencies were 0.00020 ($P=0.30631\pm0.0454$) between Khayan and Mandalay, 0.01824 ($P=0.09009\pm0.0359$) between Khayan and Sittwe, and 0.03319 ($P=0.02703\pm0.0194$) between Mandalay and Sittwe.

Discussion

This study detected variations at 8 positions in duck *GH* promoters. Although variation at the position 42, which was originally detected in Pekin ducks (Kansaku *et al.*, 2008), was not observed, we detected 4 new positions of variation not previously reported. The detection of new variations and different frequencies in the ducks from the 3 regions is noteworthy. Variation frequencies of Sittwe at positions 244, 586, and 665 were lower than those of Khayan and Mandalay. Furthermore, F_{st} values of Khayan-Mandalay, Khayan-Sittwe and Mandalay-Sittwe indicate that frequency of haplotype in Sittwe statistically different to those in Khayana and Mandalay. This may indicate that there is a greater genetic and livestock diversity of the native ducks in Khayan and Mandalay compared to that in Sittwe. Similar results were obtained by population analyses using duck microsatellites originally reported by Maak *et al.*, (2000). The populations in Khayan and Mandalay showed much higher numbers of microsatellite alleles than Sittwe (data not shown).

The reason for the differences in frequency is unknown. However, this may be partially explained by the differences in breeding and/or approaches to reproduction. In Mandalay and Khayan, the egg-laying performance of ducks is improving due to the introduction of a layer strain of Thailand ducks, whereas the same has not been made in Sittwe. Interestingly, “0.375” and “0.188” were the observed frequencies of variation at positions 244 and 586–665 in Pekin ducks (Kansaku *et al.*, 2008). Scores of variation frequency in Pekin, Mandalay, and Khayan were relatively higher than the variation frequency scores of Sittwe. However, sample of Thailand native duck had not been examined in this experiment. Thus, genetic analysis of Thailand duck is necessary to mention the effects of breeding program on changes of variation frequency in Myanmar native duck.

Since the bases at position 244, 586 and 665 were generally linked, the possibility of simultaneous substitution

including one transversion and two transitions, which changes Hap-1 to Hap-5, were likely very low. Thus, the characterization of origin of genetic variations is very difficult. However, behavior of mallards may explain the reason for the tight linkage of the variations at 244, 586 and 665. Wild mallards migrate seasonally and fly long distances. Migration may increase the genetic diversity due to the resulting reproduction within large populations. Moreover, mallards have hybridized easily with other related species (Browne *et al.*, 1993, Mazourek and Gray, 1994). Recently, the origin and genetic diversity of Chinese domestic ducks were reported (Li *et al.*, 2010). Phylogenetic analyses of mitochondrial DNA suggest that Chinese domestic ducks are primarily derived from mallards and partially derived from spot-billed ducks (*Anas poecilorhyncha*). Interestingly, the same genetic variations at 244, 586, and 665 were observed in the spot-billed duck (data not shown). These results may indicate that genetic variation may be derived from wild mallards and/or other related species such as the spot-billed ducks. Thus, further investigation is needed to identify the origin of genetic variation using Thailand native duck, wild mallards and spot-billed ducks.

It is unknown whether the genetic variation of duck *GH* promoter affects the expression of duck *GH* mRNA. Recently, presence of *GH* mRNA in the developing follicles of the chicken was reported. Furthermore, GHR immunoreactivity has similarly been detected, like GH, in the developing ovary and in all follicles (Hrabia *et al.*, 2011). These results support the possibility that GH affects ovarian function prior to and after the onset of lay in chicken. Since the proximal region (240–760) of the duck *GH* promoter was similar to that found in the chicken and turkey *GH* promoters (78% and 77.3%, respectively) (Kansaku *et al.*, 2008). Moreover, genetic variation at position 665, which was located 1 base ahead of TATA-box, was primarily observed in Mandalay and Khayan. Thus, this variation may affect *GH* mRNA expression levels by changes in TATA box binding protein binding to the TATA-box. To investigate the possibility of changes in *GH* mRNA levels, comparison of levels of mRNA among the genotypes and reporter assay are necessary.

In conclusion, detection of new variations and different frequencies in the ducks from the 3 regions may reflect the genetic diversity of Myanmar native duck. Further study is necessary to investigate the relationship between variations in the *GH* promoter and economically important traits or levels of *GH* mRNA expression.

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