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ASSOCIATION OF THE 65-BP INDEL POLYMORPHISM IN *GOGLB1* GENE WITH BODY WEIGHT OF VIETNAMESE NOI CHICKENS

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ABSTRACT

Noi chicken is one of the indigenous fighting-cock breeds of Vietnam with many valuable properties. In chicken, the Golgin subfamily B member 1 (*GOLGB1*) gene, which locates on chromosome 1, encodes the coat protein 1 vesicle inhibiting factor. A previous study showed that the 65-bp insertion/deletion in the *GOLGB1* gene was significantly associated with chicken body weight, neck weight, abdominal fat weight, abdominal fat percentage and the yellow index b of the breast in Chinese indigenous chicken (N409-breed). In this study, 65-bp indel polymorphism in the *GOLGB1*gene was evaluated in association with body weight in Noi chickens. The live body weight of 170 chickens (90 females, 80 males) at continuous ages of 7-day intervals (from 28 to 84 days) was recorded. A 65-bp indel polymorphism in the *GOLGB1* gene was analyzed using the PCR method. The results of PCR and sequencing revealed two alleles (*I* and *D*) corresponding to three genotypes: *II*, *ID*, *DD*, in which, the *I* allele appears with the highest frequency (0.79) in the studied population. An association study using Minitab software showed that genotype was significantly associated with body weight in Noi chickens at days 35 to 84 ($P < 0.05$). Individuals with more allele "*I*" have higher live body weight than others. Both factors (sex and genotype) simultaneously affected the body weight of Noi chickens. The results of this study suggested that the 65-bp indel in the *GOLGB1* gene could be considered a potential marker for Noi chicken breeding.

Keywords: Association study, live body weight, Noi chickens, 65-bp indel, *GOLGB1* gene.

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INTRODUCTION

GOLGB1 (Golgin subfamily B member 1) gene encodes the coat protein 1 vesicle inhibiting factor. It is not only a widely expressed large coiled-coil protein, but also a Golgi-associated large transmembrane protein (Linstedt & Hauri, 1993). Several variations in the *GOLGB1* gene are associated with dozens of human developmental disorders and diseases (Smits et al., 2010; Freeze & Ng, 2011). The *GOLGB1* gene belongs to chromosome 1 in the chicken and consists of 22 exons. A previous study showed that a large number of QTLs on chicken chromosome 1 (where *GOLGB1* is located in chickens) were related to important economic traits (Xie et al., 2012). A novel 65-bp indel polymorphism was detected in the chicken *GOLGB1* intron 5. The polymorphism of *GOLGB1* 65-bp indel in eight different Chinese local chickens and the correlation of this indel polymorphism with growth and carcass traits in the yellow chicken population were investigated (Fu et al., 2020). The result showed a significant association of 65-bp indel with chicken body weight, neck weight, abdominal fat weight, abdominal fat percentage, and the yellow index b of the breast. The expression profile of the chicken *GOLGB1* gene revealed the significantly differential gene expression of three genotypes of 65-bp indel. The mRNA expression level of the *DD* genotype was significantly higher than in the *II* and *ID* genotypes ($P < 0.01$).

Indels (insertion-deletions with lengths from 1 to 10,000 nucleotides) are widely distributed in the genome of an organism and the first indel map of the human genome had been created by Mills et al. (2006). In domestic animals, a number of investigations were reported about the effects of indel's polymorphism on several traits and diseases, such as double-muscle trait in cattle (Grobet et al., 1997), immotile short-tail sperm defect in pig (Sironen et al., 2006), growth traits in goat (Wang et al., 2019). Many indels located in functional genes in chicken confirmed the association with the different traits. For example, 31-bp indel in the *PAX7* gene (Zhang et al., 2014; Thuy et al., 2022), 65-bp indel in the *GOLGB1* gene (Fu et al., 2020), 51-bp indel in the *PTH1R*, 86-bp indel in the *MLNR* gene (Liu et al., 2019), 80-bp indel in the *PRLR* gene (Liang et al., 2019), 62-bp indel in the promoter region of the *TGFB2* gene (Tang et al., 2011) were associated with growth and carcass traits, 99-bp indel of the *CEL* gene promoter was associated with phenotypic traits in chicken (Wang et al., 2020); 24-bp indel in the *PRL* gene was associated with egg production (Cui et al., 2006), a 9-bp indel polymorphism in the *PMEL17* gene was related to plum color (Kerje et al., 2004).

In Vietnam, chickens account for about 70% of all poultry, of which 28% are local breeds. Noi chicken is an indigenous breed, raised and distributed widely throughout Vietnam. Noi chickens are characterized by the following features: high legs, long body, crimson crest, sharp heel, and specially, delicious meat quality (Department of Livestock Production, 2009). However, compared to other imported breeds, Noi chickens have slow growth and low fertility. The objective of this study is to analyze whether the 65-bp indel polymorphisms in the *GOLGB1* gene are associated with body weight in Noi chickens.

MATERIALS AND METHODS

Materials

Animals and sampling

This study was conducted on a resource population of 170 Vietnamese Noi chickens (80 males and 90 females) raised at Can Tho University and fed a commercial diet of 17% crude protein and 3,000 kcal/kg ME from 21–91 days old. All the individuals in the experiment were collected with full information on the number of days of age, nutritional indicators, environmental conditions, and health care procedures as well. All the individuals were weighed at 7 am at 28, 35, 42, 49, 56, 63, 70, 77, and 84 days old. About 3 mL of blood samples were taken from the wing vein and collected in anti-coagulant tubes with EDTA and stored at 4 ^{o}C.

Methods

Genotyping

Genomic DNA was extracted by a standard procedure using Proteinase K digestion followed by phenol-chloroform extraction and precipitation with ethanol (Ausubel et al., 1995). The quantity and quality of genomic DNA were checked with a UV spectrophotometer and agarose gel electrophoresis. The 65-bp primer pair F: 5'-TGTGGTAGCTCTCTCCTCCC-3' and R: 5'- AGGCTCTCCTGCTGACCATA-3' (Fu et al., 2020) was used for PCR amplification. PCR was performed using 2x DreamTaq master mix with 10 nM of each primer and 100 ng genomic DNA. A thermal cycle was set as follows: initial denaturation at 95 °C for three minutes followed by 35 cycles of denaturation at 95 °C for 30 seconds, annealing at 60 °C for 30 seconds, extension at 72 °C for 30 seconds, and an additional extension of 72 °C for 10 minutes. A PCR reaction was carried out on the Veriti™ 96-Well Thermal Cycler (Applied Biosystems). The genotype of 65-bp indel was determined according to the size of the PCR fragment generated (the presence or absence of 65-bp Indel) on 2.0% agarose gel electrophoresis. The expected lengths of the amplicon with and without 65-bp indel of the *GOLGB1* gene corresponding to *I* and *D* alleles were 311 bp and 246 bp, respectively. For verification of the amplicon, the PCR product was sequenced using the Sanger method by ABI-3100 Avant Genetic Analyzer (Macrogen, Korea). Obtained nucleotide sequences were identified with the BLAST Tool on NCBI (Alschul et al., 1990).

Data analysis

The indel variant that was detected in Noi chickens by PCR was used to calculate the genotype and allele frequencies. Relationship between genotypes and traits (body weight) were analyzed by General Linear Model (Minitab ver. 16.0) using the model: $y_{ijk} = \mu + \alpha_i + \beta_j + (\alpha*\beta)_{ij} + \varepsilon_{ijk}$, whereas y_{ijk} is the dependent variable, μ is the overall population mean, α_I is the fixed effect of sexes (i = 1–2), β_j is the fixed effect of genotypes (i = 1–3), $(\alpha*\beta)_{ij}$ is the fixed effect of sex and genotype interaction, and ε_{ijk} is the random error. Differences with $P < 0.05$ were considered statistically significant.

RESULTS AND DISCUSSION

Genotypic and allelic frequency

The extracted DNA sample has a clear band with high molecular weight (more than 10 kb) on electrophoresis. The spectrophotometer result also showed good quality and quantity of extract total DNA with the A260 value and the ratio of A260/280 (ranging from 1.8 to 2.0). The genotyping of 65-bp indel of the *GOLGB1* gene was presented in Figure 1.

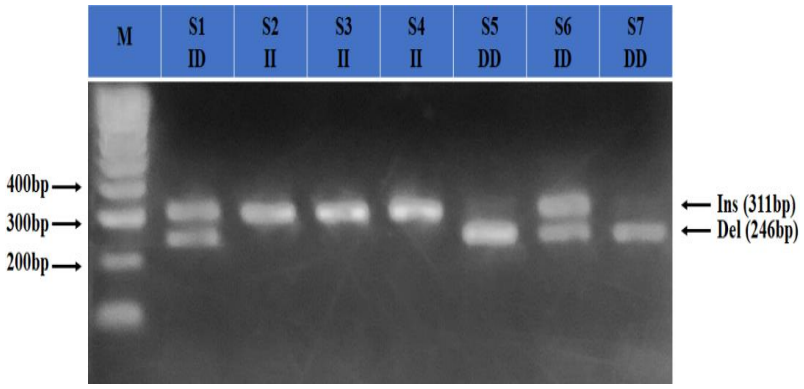

Figure 1. PCR product electrophoresis results in 2% agarose gel. M: DNA ladder 100 bp (Bioline, Germany); S2-4: *II* genotype (311 bp); S1, S6: *ID* genotype (246/311 bp); S5, S7: *DD* genotype (246 bp)

In Figure 1, three genotypes of *II*, *ID* and *DD* were observed in the Noi chicken population, in which the *I* allele was 311 bp in length (insertion indel) and the *D* allele was 246 bp in length (deletion indel).

The PCR product of the *II* genotype was sequenced to confirm the correct amplification of the *GOLGB1* gene fragment. Using the BLAST tool, alignment with the reference sequence on the NCBI database (NC_006088.5) showed a very high identity of 99% (Fig. 2). It was confirmed that the 311 bp sequence region of the *GOLGB1* gene was successfully amplified, in which, the insertion sequence of 65-bp was found. It also confirmed the specificity of the primers.

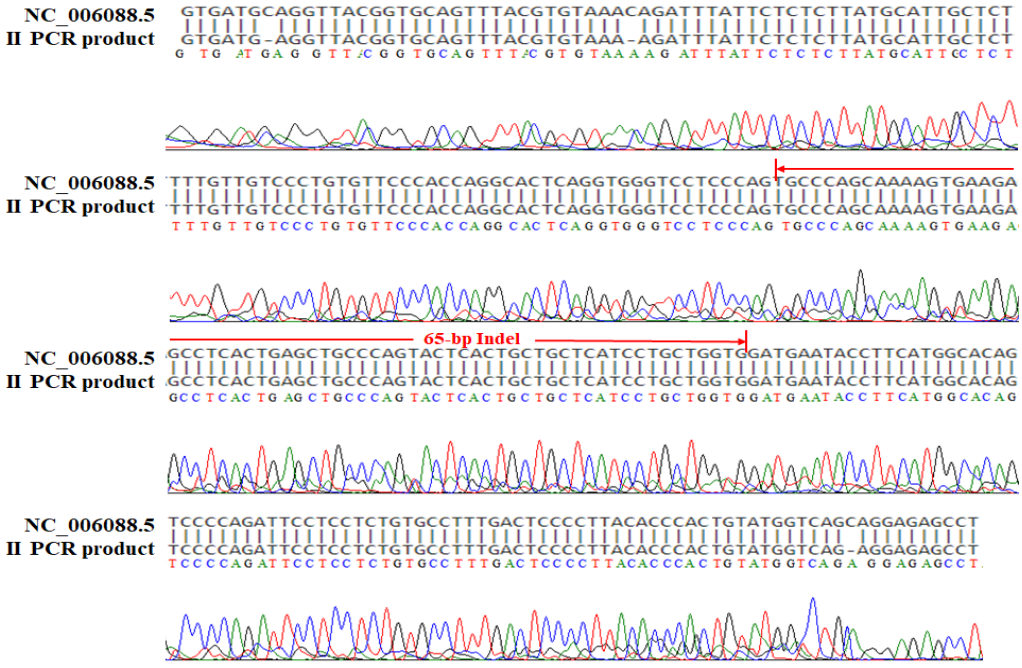

Figure 2. Sequencing results of PCR products with 65-bp indel
(The 65-bp indel sequence was red marked)

The obtained data in Table 1 indicated that all genotypes of *II*, *ID* and *DD* were detected with different frequencies in the Noi chicken population as well as within either males or females. From Table 1, it has been shown that the *II* genotype has the greatest frequency (0.64), higher than the *ID* genotype (0.30). The occurrence of deletion indel on both alleles is quite rare, with the occurrence rate in the population only 6%. The frequency of the *I* allele (0.79) is always greater than the frequency of the *D* allele (0.21). Research results of Fu et al. (2020) also revealed that the frequencies of the allele "*I*" was higher than those of the allele "*D*" in all eight Chinese local chicken breeds, in which, *I* allele appeared with higher frequency (0.51–0.77) than the *D* allele (0.23–0.49).

Table 1. Genotypic and allelic frequency

Number of individual (n)	Genotype frequency (n)			Allele frequency	
	II	*ID*	*DD*	*I*	*D*
Male (80)	0.65 (52)	0.33 (26)	0.02 (2)	0.81	0.19
Female (90)	0.63 (57)	0.28 (25)	0.09 (8)	0.77	0.23
Total (170)	0.64 (109)	0.30 (51)	0.06 (10)	0.79	0.21

Association study

The associations between genotype and body weight in the total Noi chicken population were presented in Table 2. The results of Table 2 showed that genotype was significantly associated with the body weight of the Noi chicken population ($P < 0.05$) at all ages (except day 28). It also showed that individuals with *II* genotypes have the largest body weight, followed by individuals with *ID* and *DD* genotypes. This suggests that body weight seems to be positively correlated with the number of *I* alleles present in the genome. Our result is in agreement with the study of Fu et al. (2020), in which, the 65-bp indel polymorphism in the fifth intron of the *GOLGB1* gene was significantly associated with body weight, abdominal fat weight, and abdominal fat percentage.

Table 2. Effects of genotypes on body weight of Noi chickens

Age (day)	Genotypes (Mean)			SEM	P
	II (n = 109)	ID (n = 51)	DD (n = 10)		
28	280.6	274.5	249.1	7.96	0.082
35	398.2	380.5	358.5	10.34	0.034
42	547.1	523.5	482.0	14.18	0.016
49	701.7	670.5	595.5	18.84	0.004
56	836.7	808.0	726.0	22.46	0.016
63	993.3	963.1	872.5	28.24	0.046
70	1,155.1	1,120.4	1,015.0	33.08	0.049
77	1,291.5	1,263.1	1,124.0	38.21	0.049
84	1,449.3	1,405.9	1,240.0	45.92	0.035

Notes: Mean: The average value, SEM: Standard error, P: The value of the level of statistical significant.

In chickens, some traits such as carcass traits are not affected by sex. On the same day of age, the weight of male and female chickens will be different due to different growth rates, related to puberty factors. Therefore, the association between genotype and body weight by sex was analyzed in female chickens (Table 3) and male chickens (Table 4).

Table 3. Effects of genotypes on body weight of female Noi chickens

Age (day)	Genotypes (Mean)			SE	P
	II (n = 57)	ID (n = 25)	DD (n = 8)		
28	271.5	257.8	248.1	8.48	0.121
35	377.2	356.0	356.3	11.16	0.148
42	519.6	484.0	477.5	14.63	**0.033**
49	654.8	615.0	578.1	19.61	**0.023**
56	779.3	740.0	697.5	24.19	0.067
63	914.1	878.4	834.4	30.14	0.201
70	1063.8	1020.4	963.7	36.22	0.181
77	1174.6	1138.6	1060.0	41.05	0.215
84	1319.6	1266.0	1166.2	51.08	0.160

Notes: Mean: The average value, SEM: Standard error, P: The value of the level of statistical significant.

The obtained results indicated that genotype affected body weight in female Noi chickens at 42 and 49 days of age ($P < 0.05$). Meanwhile, no significant differences were observed between genotype and body weight at all ages of male Noi chickens ($P > 0.05$).

Table 4. Effects of genotypes on body weight of male Noi chickens

Age (day)	Genotypes (Mean)			SE	P
	II (n = 52)	ID (n = 26)	DD (n = 2)		
28	290.5	290.7	253.0	16.27	0.537
35	421.3	404.0	367.5	19.29	0.214
42	577.3	561.5	500.0	27.04	0.310
49	753.0	723.8	665.0	32.78	0.230
56	899.6	873.5	840.0	36.71	0.473
63	1080.0	1044.60	1025.0	44.50	0.466
70	1255.2	1216.5	1220.0	50.40	0.531
77	1419.7	1382.9	1380.0	53.67	0.595
84	1591.3	1540.4	1535.0	67.08	0.528

Notes: Mean: The average value, SEM: Standard error, P: The value of the level of statistical significant.

From the above data, the body weight of individuals with the *II* genotype was higher than that with the *ID* genotype and the lowest was the *DD* genotype in both sexes. However, the difference is not large enough for statistical significance. Therefore, the combined effects of both factors (sex and genotype) on the body weight of Noi chickens were analyzed (Table 5, Fig. 3).

The results showed that there was a considered difference in body weight under the influence of sex and genotype. In terms of sex, the body weight of male chickens is larger than in female chickens. This can be explained by the differences in development between the sexes, especially after puberty. While males thrive in physical conditions, females focus more on reproductive function. By the time, individuals with the *I* allele (*II* and *ID* genotype) have higher body weight than individuals carrying a 65-bp deletion on both alleles (DD genotype). The expression profiles of the *GOLGB1* gene conducted by Fu et al. (2020) also indicated that the *GOLGB1* gene was widely expressed in the tissues of chickens, in which, the mRNA expression level of the *DD* genotype was significantly higher than in the *ID* and *II* genotypes ($P < 0.01$).

The differential correlation of the two factors (genotype and sex) mentioned above is constant although with increasing age, the difference in body weight increased (Fig. 3).

Table 5. Effects of sex and genotype interaction on body weight of Noi chickens

Age (day)	Genotypes (Mean)						SE	P
	II		ID		DD			
	Male (n = 52)	Female (n = 57)	Male (n = 26)	Female (n = 25)	Male (n = 2)	Female (n = 8)		
28	290.5	271.5	290.7	257.8	253.0	248.1	12.08	0.003
35	421.3	377.2	404.0	356.0	367.5	356.3	15.00	0.000
42	577.3	519.6	561.5	484.0	500.0	477.5	20.40	0.000
49	753.0	654.8	723.8	615.0	665.0	578.1	25.89	0.000
56	899.6	779.3	873.5	740.0	840.0	697.5	30.45	0.000
63	1,080.0	914.1	1,044.6	878.4	1,025.0	834.4	37.47	0.000
70	1,255.2	1,063.8	1,216.5	1,020.4	1,220.0	963.8	43.86	0.000
77	1,419.7	1,174.6	1,382.9	1,138.6	1,380.0	1,060.0	48.45	0.000
84	1,591.3	1,319.6	1,540.4	1,266.0	1,535.0	1,166.3	60.40	0.000

Notes: Mean: The average value, SEM: Standard error, P: The value of the level of statistical significant.

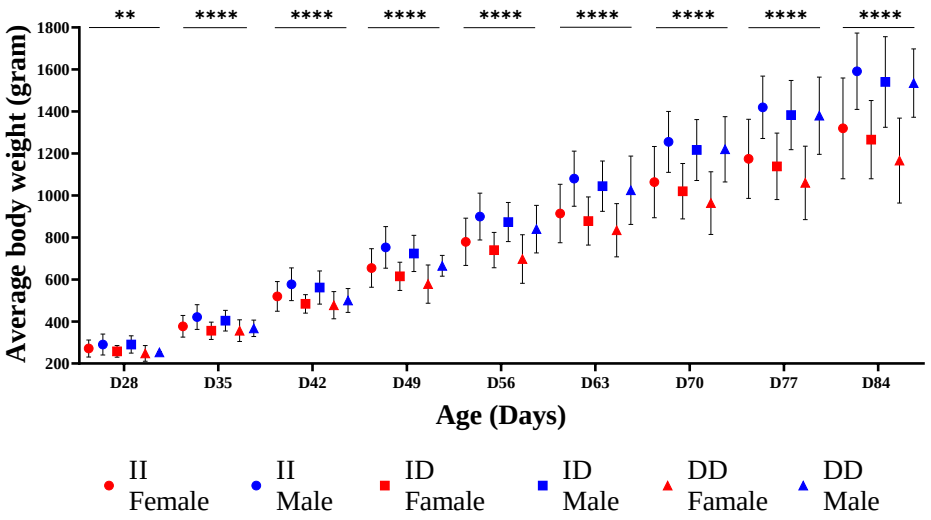

Figure 3. Distribution of body weight of Noi chickens by sex and genotype

Body weight is one of the most important economic traits in broiler breeding. A large number of indels associated with body weight in chicken have been reported in previous studies (Tang et al., 2011; Zhang et al., 2014; Liang et al., 2019; Liu et al., 2019; Thuy et al., 2022). This result supports the positive effects of the 65-bp indel polymorphism of the *GOLGB1* gene on the body weight of chicken.

CONCLUSION

In conclusion, the polymorphism of 65-bp indel of the *GOLGB1* gene was detected in Noi chickens. This 65-bp indel significantly affects Noi chickens body weight and can be considered a candidate molecular marker in poultry breeding programs for Vietnamese local chicken breeds.

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