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Research Article
The PAX7's 31-bp Indel Associated with Several Carcass Traits in Vietnamese Local Noi Chickens

[1]Huynh Thi Phuong Loan, [1]Nguyen Thi Hong Tuoi, [2]Nguyen Thi Dieu Thuy, [3,4]Nguyen Tuyet Giang, [5]Nguyen Thu Quyen, [6]Takeshi Shimogiri and [5]Do Vo Anh Khoa

[1]Can Tho University, Vietnam
[2]Institute of Biotechnology, Vietnam Academy of Science and Technology, Vietnam
[3]An Giang University, An Giang, Vietnam
[4]Vietnam National University Ho Chi Minh City, Vietnam
[5]Thai Nguyen University of Agriculture and Forestry, Vietnam
[6]Kagoshima University, Japan

Abstract

Background and Objective: PAX7, a member of the paired box family of transcription factors, plays a critical role in forming skeletal muscles and regulating the satellite cell in vertebrates, including chickens. In this study, the genetic association of the PAX7's 31-bp indel with carcass traits of Noi chicken, a recognized Vietnamese native breed was analyzed. **Materials and Methods:** A total of 355 Noi broilers (164 males and 191 females) were slaughtered at 91 days old to evaluate carcass characteristics. Blood samples were collected for DNA extraction. Genotyping the PAX7's 31-bp indel was based on the length of different fragments such as 588 and 557 bp corresponding to E and F alleles. **Results:** Its was owed that the genotypes of PAX7 were significantly associated with wing weight and drumstick weight of the Noi chicken ($p<0.05$), whereas chickens with the FF genotypes always displayed the highest values. Moreover, the interaction between these genotypes of the PAX7 and bird sex was statistically significantly associated with most of the observed traits ($p<0.05$), except the weight of abdominal fat ($p>0.05$). **Conclusion:** These results suggested that the 31-bp indel PAX7 may be a potential molecular marker for breeding Noi chicken.

Key words: Carcass traits, genotype, noi chicken, PAX7, molecular marker

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Corresponding Author: Do Vo Anh Khoa, Thai Nguyen University of Agriculture and Forestry, Vietnam Tel: +84-918026653

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Growth and carcass characteristics are economically important in livestock production. The development of genetic and breeding programs has recently been examined to meet the rising demand of consumers, regarding carcass yield and meat quality of poultry products. Therefore, genetic improvement has been focused on in recent studies[1]. Marker-Assisted Selection (MAS) is currently performed in the poultry industry as a supplement to traditional methods. The association between critical genetic variants and economic phenotypes has, then, been explored[2]. Breast muscle is the most valuable part of the chicken carcass due to its high economic value[3]. However, the development of skeletal muscle is a complex process including myofiber formation and hypertrophy which is regulated by multiple genetic and environmental factors. Therefore, a greater understanding of polymorphisms influences will enable us to select relevant breeding programs[4]. The identification of indels (insertions and deletions) in recent years has contributed to the progress of animal genetics and breeding and provided a better knowledge of indel polymorphism in chicken genome[5,6]. Over a million indels obtained, the vast majority (92.48%) was novel. The validation assays indicated that most (88.00%) of the randomly selected indels represented true variations associated with the economically important traits of chicken[7]. Over 2.1 million indels (1-71 bp) by whole-genome resequencing mixed chicken samples from a full-sib family with feathered and unfeathered legs and 76.52% of them were novel[7].

Dozens of genes are involved in the myogenesis (muscle development and growth) of chickens, especially during the myoblast proliferation and differentiation processes. They were classified as myogenic genes. They mostly came from the groups of transcription factors (TFs), myostatin (MSTN) and growth axis related genes (GH, IGF-1)[4,8]. Therefore, they regulate the expression of MyoD genes, a myogenic regulatory factor (MRFs) among vertebrate species, including chickens[9].

Additionally, the 31-bp indel in intron 3 of the PAX7 gene was associated with growth rate, carcass and meat quality traits of the F2 population between Gushi and Anka chickens[10] while the expression pattern of PAX7 was similar in breast and thigh muscles and it was lower during the embryonic stage and reached the highest point when chickens were 8-10 weeks old in Rugao chickens[11].

As indicated above, the 31-bp indel functions of PAX7 affecting carcass traits has not been well-documented in native chickens, particularly Noi chicken. Among Vietnamese indigenous chicken breeds, Noi chicken has prominent characteristics of carcass quality such as tender meat and unique taste which its consumers preferred. However, the slow growth rate and low feed utilization are the main constraints of this breed, causing several limits to production. Therefore, further information on PAX7 can be applied to Noi chicken breeding programs and carcass characteristics investigated in this study.

MATERIALS AND METHODS

Study area: This study was conducted from July, 2018-2019 on the farm. The sample was analyzed at the labs during 2019-2020.

Data collection: This study used phenotypic and genotypic data from the previous reports[12-14] to analyze the genetic association of 31-bp indel of PAX7 candidate gene with carcass traits in the Vietnamese indigenous Noi resource population.

Research protocol: A total of 355 broilers (164 males and 191 females) at 28 days old were selected from a population of 1200 Noi chickens and kept in private cages. During the experiment, they were given a diet of 17% crude protein and 3,000 kcal kg^{-1} ME[12]. At 91 days old, they were slaughtered to evaluate carcass characteristics such as Live Weight (LW, g), Killed Weight (KW, g), De-Feather Weight (DFW, g), Carcass Weight (CW, g), Head Weight (HW, g), Weight of Breast Meat (WBM, g), Weight of Thigh Meat (WTM, g), Wing Weight (WW, g), Drumstick Weight (DW, g), Shank Weight (SW, g), Weight of Internal Organs (WIO, g), Gizzard Weight (GW, g), Liver Weight (LRW, g), Heart Weight (HTW, g), Weight of Abdominal Fat (WAF, g), Length of Small Intestine (LSI, mm) and Caeca Length (CL, mm)[14].

Blood samples were collected in a 2 mL EDTA tube and stored at 4°C before DNA was extracted with the instruction of Ausubel *et al.*[15]. Genotyping the PAX7's the 31 bp-indel was based on the length of different fragments such as 588 and 557 bp corresponding to E and F alleles on the 2% agarose gel electrophoresis, respectively[13].

To associate genotypes with phenotypic traits, a General Linear Model (Minitab ver. 16) was used as follows:

$$y_{ijk} = m+a_i+b_j+(a\times b)_{ij}+e_{ijk}$$

Where:

y_{ijk} : Dependent variable
m : Overall population mean

a_l : Effect of sex (I = 1-2)
b_j : Effect of genotype (I = 1-3)
$(a \times b)_{ij}$: Effect of sex and genotype interaction and
e_{ijk} : Random error

RESULTS AND DISCUSSION

In this study, the association of the locus PAX7's 31-bp indel with carcass traits of the Noi chicken population indicated that chickens with FF had the highest WW (123.38 g), DW (193.35 g) and SW (63.85 g), followed by EF (114.77 g, 180.28 g and 58.54 g), respectively) and EE (113.72, 173.72 and 59.65 g, respectively) ($p<0.05$) in Table 1. Interestingly, homozygous genotypes FF (0.18) were higher than EE (0.12) in this population[13].

In Table 2, the statistically significant associations were found in most of the observed traits ($p<0.05$) in the interaction between genotype and sex ($p<0.05$), except the WAF ($p>0.05$). The variables of LW, KW, DFW, CW, HW, WTM, WW, SW and HTW of male chickens with any genotype were higher than those of female chickens. Therefore, it is argued that the combination of PAX7's 31-bp indel and sex has a significant effect on the carcass traits of Noi chicken. The gender effect which enabled males to consume more feed and have a higher growth rate than females in the same genotype is also considered[12].

It was reported that at 12 days old the F2 Gushi x broiler cross with EE (1375 g) had LW higher than one with two others EF (1332 g) and FF (1332 g)[10]. This is contrary to the results of the current study. Although there was no significant difference in LW among the three genotypes, chickens with FF genotype (1480 g) showed a higher LW than ones with genotype EE (1394 g). Due to the difference in LW, the CW (EE = 979 g vs. FF = 1051 g) also tended to be similar between the two homozygous genotypes in the two studies. This may be due to population influence (local breed in this study and crossbred in Zhang's study)[10].

Although in the skeletal muscle, the paired box protein, PAX7, a significant transcription factor, plays a critical role in forming skeletal muscles and regulating the satellite cell population[16,17], the housing system (caged and free-range systems) may influence the muscle fibre accretion by coordinating the expression of PAX3 and PAX7 in adult chicken[18]. The PAX7 protein is an early marker for chicken satellite cells during early post-hatch chicken growth[19]. Here, all broilers were kept in private cages with a limited area during the experiment. This could affect the PAX7 expression as well as the development of muscle fibres. Therefore, only three traits associated with the 31-bp indel of PAX7 were found in this study. Interestingly, these associated traits belonged to the active/moving parts of chickens. According to Yablonka-Reuveni and Paterson[20], the PAX7 protein could be expressed at the early stage of age (9 days old) and could prolong to 115 days old in chickens.

Table 1: Effects of genotypes on the carcass characteristics

Traits (g)	EE (n = 43)	EF (n = 267)	FF (n = 65)	SEM	p-value
LW	1394	1422	1480	32.4	0.237
KW	1354	1387	1440	31.6	0.248
DFW	1259	1289	1345	30.1	0.197
CW	979	1004	1051	24.6	0.184
HW	82.0	80.0	79.9	2.56	0.863
WBM	141	147	149	3.62	0.373
WTM	101	102	104	2.80	0.737
WW	114^{a}	115^{a}	123^{b}	2.32	0.007
DW	174^{a}	180^{a}	193^{b}	3.66	0.003
SW	59.6^{a}	58.5^{ab}	63.8^{b}	1.43	0.011
WIO	146	148	154	3.35	0.265
GW	16.4	16.1	15.9	0.41	0.764
LRW	24.5	24.7	26.4	0.72	0.145
HTW	7.20	7.13	7.54	0.25	0.413
WAF	23.2	25.6	23.2	1.79	0.397
LSI	1346	1304	1332	20.8	0.266
CL	309	304	310	4.65	0.437

LW: Live weight, KW: Killed weight, DFW: De-feather weight, CW: Carcass weight, HW: Head weight, WBM: Weight of breast meat, WTM: Weight of thigh meat, WW: Wing weight, DW: Drumstick weight, SW: Shank weight, WIO: Weight of internal organs, GW: Gizzard weight, LRW: Liver weight, HTW: Heart weight, WAF: Weight of abdominal fat, LSI: Length of the small intestine, CL: Caeca length, Means followed by different letters in the same row differ significantly ($p<0.05$)

Table 2: Effects of sex and genotype interaction on the carcass characteristics

	EE (n = 43)		EF (n = 267)		FF (n = 65)			
Traits	Male (n = 19)	Female (n = 24)	Male (n = 109)	Female (n = 138)	Male (n = 36)	Female (n = 29)	SEM	p-value
LW	1542^{a}	1277^{b}	1581^{a}	1296^{b}	1632^{a}	1292^{b}	39.7	0.000
KW	1506^{a}	1234^{b}	1542^{a}	1265^{b}	1588^{a}	1256^{b}	38.6	0.000
DFW	1398^{a}	1148^{b}	1437^{a}	1173^{b}	1487^{a}	1170^{b}	36.8	0.000
CW	1091^{a}	891^{b}	1123^{a}	910^{b}	1166^{a}	909^{b}	30.3	0.000
HW	96.5^{a}	70.5^{b}	90.4^{a}	71.9^{b}	88.0^{a}	69.9^{b}	3.30	0.000
WBM	148^{ab}	135^{b}	158^{a}	139^{b}	161^{a}	135^{b}	4.91	0.000
WTM	114^{a}	90.3^{b}	115^{a}	90.9^{b}	114^{a}	91.3^{b}	3.49	0.000
WW	130^{a}	101^{b}	131^{a}	102^{b}	139^{a}	104^{b}	4.00	0.000
DW	197^{ab}	155^{c}	206^{a}	160^{c}	216^{a}	165^{bc}	6.38	0.000
SW	70.8^{a}	50.8^{b}	69.4^{a}	49.9^{b}	73.7^{a}	51.5^{b}	1.69	0.000
WIO	157^{abc}	137^{c}	157^{ab}	141^{c}	165^{a}	141^{bc}	4.56	0.000
GW	16.7^{ab}	16.2^{ab}	16.7^{a}	15.5^{b}	16.8^{ab}	14.8^{b}	0.58	0.034
LRW	27.1^{ab}	22.4^{bc}	27.6^{a}	22.4^{c}	28.2^{a}	24.1^{abc}	0.95	0.000
HTW	8.23^{a}	6.38^{b}	8.08^{a}	6.39^{b}	8.20^{a}	6.72^{ab}	0.33	0.000
WAF	25.4	21.4	24.1	26.8	26.2	19.5	2.53	0.206
LSI	1412^{a}	1293^{ab}	1346^{a}	1271^{b}	1384^{a}	1268^{ab}	28.8	0.000
CL	318^{ab}	301^{ab}	317^{ab}	293^{b}	325^{a}	291^{b}	6.31	0.000

LW (g): Live weight, KW (g): Killed weight, DFW (g): De-feather weight, CW (g): Carcass weight, HW (g): Head weight, WBM (g): Weight of breast meat, WTM (g): Weight of thigh meat, WW (g): Wing weight, DW (g): Drumstick weight, SW (g): Shank weight, WIO (g): Weight of internal organs, GW (g): Gizzard weight, LRW (g): Liver weight, HTW (g): Heart weight, WAF (g): Weight of abdominal fat, LSI (mm): Length of the small intestine, CL (mm): Caeca length, Means followed by different letters in the same row differ significantly ($p<0.05$)

CONCLUSION

In conclusion, the obtained results have provided important evidence for the influence of the predominant genotype FF on phenotypic traits of wing weight, drumstick weight and shank weight in Noi chicken. The F allele should be added to the molecular marker for selecting Noi broilers. These results suggested that the 31-bp indel PAX7 may be a potential molecular marker for breeding Noi chickens.

SIGNIFICANCE STATEMENT

This was an initial basic data on PAX7's 31-bp indel genotypes relating to several traits which were not main cut parts of carcass in the Noi chicken population. However, this was new information about the expression of the PAX7 on carcass which should be studied more on other local chicken breeds for further understandings.

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REFERENCES

1. Li, H. and J. Tian, 2018. Research Progress in Genetic Control of Reproductive Performance in Chicken by High-Throughput Sequencing Technology. In: Application of Genetics and Genomics in Poultry Science, Liu, X. (Ed.)., IntechOpen.
2. Liu, D., R. Han, X. Wang, W. Li and S. Tang *et al*., 2019. A novel 86-bp indel of the motilin receptor gene is significantly associated with growth and carcass traits in Gushi-Anka F_2 reciprocal cross chickens. Br. Poult. Sci., 60: 649-658.
3. Petracci, M. and C. Cavani, 2011. Muscle growth and poultry meat quality issues. Nutrients, 4: 1-12.
4. Luo, W., B.A. Abdalla, Q. Nie and X. Zhang, 2017. The genetic regulation of skeletal muscle development: insights from chicken studies. Front. Agric. Sci. Eng., 4: 295-305.
5. Boschiero, C., G.C.M. Moreira, A.A. Gheyas, T.F. Godoy and G. Gasparin *et al*., 2018. Genome-wide characterization of genetic variants and putative regions under selection in meat and egg-type chicken lines. BMC Genomics, Vol. 19. 10.1186/s12864-018-4444-0.
6. Yang, S., Z. Shi, X. Ou and G. Liu, 2019. Whole-genome resequencing reveals genetic indels of feathered-leg traits in domestic chickens. J. Genet., Vol. 98. 10.1007/s12041-019-1083-4.
7. Yan, Y., G. Yi, C. Sun, L. Qu and N. Yang, 2014. Genome-wide characterization of insertion and deletion variation in chicken using next generation sequencing. PLoS ONE, Vol. 9. 10.1371/journal.pone.0104652.

8. Buckingham, M. and F. Relaix, 2007. The role of *Pax* genes in the development of tissues and organs: *Pax3* and *Pax7* regulate muscle progenitor cell functions. Annual Rev. Cell Dev. Biol., 23: 645-673.
9. Sobolewska, A., G. Elminowska-Wenda, J. Bogucka, M. Szpinda, K. Walasik, M. Bednarczyk and M. Paruszewska-Achtel, 2011. Myogenesis-possibilities of its stimulation in chickens. Folia Biologica, 59: 85-90.
10. Zhang, S., R.L. Han, Z.Y. Gao, S.K. Zhu, Y.D. Tian, G.R. Sun and X.T. Kang, 2014. A novel 31-bp indel in the paired box 7(PAX7) gene is associated with chicken performance traits. Br. Poult. Sci., 55: 31-36.
11. Guobin, C., L. Xiangping, L. Jing, C. Rong and L. Deqin *et al*., 2011. Temporal and spatial expression of the *Pax-7* gene during chicken embryo and postnatal development. J. Anim. Vet. Adv., 10: 1785-1788.
12. Anh, K.D.V., T.N.T. Hong, T.N.T. Dieu, O. Shin and K. Kataro *et al*., 2019. Growth performance and morphology of in 28-84 day-old vietnamese local Noi chicken. Biotechnol. Anim. Husb., 35: 301-310.
13. Thuy, N.T.D., N.T.H. Tuoi, N.T. Giang, H.T.P. Loan, T.T. Hoan, N.T.N. Linh and D.V.A. Khoa, 2022. Association of the PAX's 31-bp-indel polymorphism with some meat quality traits in Vietnamese Noi chickens at 91 days old. J. Anim. Plant Sci., Vol. 32. 10.36899/japs.2022.1.0397.
14. Tuoi, N.T.H., N.T. Giang, H.T.P. Loan, P.T.H. Phuc, V.D. Nguyen, T. Shimogiri and D.V.A. Khoa, 2020. Meat quality traits of vietnamese indigenous Noi chicken at 91 days old. Biotehnol. Stocarstvu, 36: 191-203.
15. Ausubel, F.M., R. Brent, R.E. Kingston, D.D. Moore, J.G. Seidman, J.A. Smith and K. Struhl, 2003. Current Protocols in Molecular Biology. John Wiley and Sons, Inc., New York, pp: 213-214.
16. Seale, P., L.A. Sabourin, A. Girgis-Gabardo, A. Mansouri, P. Gruss and M.A. Rudnicki, 2000. Pax7 is required for the specification of myogenic satellite cells. Cell, 102: 777-786.
17. Kuang, S., S.B. Charge, P. Seale, M. Huh and M.A. Rudnicki, 2006. Distinct roles for Pax7 and Pax3 in adult regenerative myogenesis. J. Cell. Biol., 172: 103-113.
18. Yin, H.D., D.Y. Li, L. Zhang, M.Y. Yang and X.L. Zhao *et al*., 2014. Housing system influences abundance of *Pax3* and *Pax7* in postnatal chicken skeletal muscles. Poult. Sci., 93: 1337-1343.
19. Halevy, O., Y. Piestun, M.Z. Allouh, B.W.C. Rosser and Y. Rinkevich *et al*., 2004. Pattern of Pax7 expression during myogenesis in the posthatch chicken establishes a model for satellite cell differentiation and renewal. Dev. Dynam., 231: 489-502.
20. Yablonka-Reuveni, Z. and B.M. Paterson, 2001. MyoD and myogenin expression patterns in cultures of fetal and adult chicken myoblasts. J. Histochem. Cytochem., 49: 455-462.