



Asian Journal of **Poultry Science**

ISSN 1819-3609



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Research Article

Genetic Polymorphisms of ApoB2, TGFβ4, LITAF, TRAIL and IAP1 Genes and their Association with Growth Trait in Thai Native Chicken

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Abstract

Objectives: The present study attempts to classify genetic polymorphism of apolipoprotein B 2 (ApoB2), Transforming Growth Factor β4 (TGFβ4), lipopolysaccharide-induced tumor necrosis factor (TNF), a factor (LITAF), TNF Related Apoptosis Inducing Ligand (TRAIL) and Inhibitor of Apoptosis Protein 1 (IAP1) genes and their relationship with growth trait in Thai native chickens. **Methodology:** Genetic variability in the genes and Hardy-Weinberg equilibrium were analyzed in Luenghangkhao (80 animals), Pradhuangdum (100 animals), Chee (60 animals) and the Red Jungle Fowls (40 animals) by PCR-RFLP technique. **Results:** Only LITAF gene was monomorphic but 3 genotypes were identified in ApoB2, TGFβ4, TRAIL and IAP1 genes. Genotypic frequencies of homozygous 11 in ApoB2, TGFβ4, TRAIL and IAP1 genes were 0.29, 0.75, 0.39 and 0.59, respectively. Genotypic frequencies of heterozygous (12) were 0.46, 0.12, 0.46 and 0.28, respectively and genetic frequencies of homozygous 22 were 0.25, 0.13, 0.15 and 0.13, respectively. Moreover, only genetic polymorphisms of ApoB2 gene had significantly influenced to body weight at 12 weeks. Indigenous chickens with heterozygous genotype were heavier than other genotypes ($p < 0.05$). **Conclusion:** Therefore, this gene could be developed to genetic marker for growth improvement in the future.

Key words: Thai native chicken, genetic polymorphisms, growth

Received: January 11, 2016

Accepted: April 22, 2016

Published: June 15, 2016

Citation: C. Supakorn, 2016. Genetic polymorphisms of ApoB2 TGFβ4, LITAF, TRAIL and IAP1 genes and their association with growth trait in Thai native chicken. Asian J. Poul. Sci., 10: 141-146.

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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

Thai native chicken are becoming an increasing and important food source to those who live in rural area. Due to their uniqueness in meat quality, these chickens have become more popular among consumers increasing their market prices to 2 or 3 times higher than the commercial broiler chickens¹. However, most of them have not been improved and have lower productivity than the commercial and improved foreign breeds. There were several attempts to characterize these chickens using their physical properties but only few study efforts have been undertaken a genetic characterization of these indigenous chickens²⁻⁴. Glickman *et al.*⁵ reported that ApoB gene possesses important function in the process of energy absorption, transportation and metabolism. The TGFβ4 gene serves as well as molecular marker in marker assisted selection programs for growth, skeleton and development of many tissues⁶. The LITAF, TRAIL and IAP1 genes were related genetic resistance to Salmonella in relation to pathogen load, survival and frequency of colonization in chicks^{7,8}. They may increase immune responses in chickens and reduce the use of antibiotics and drug residues in food products. Analysis of the genetic control of rapidly growing chickens indicated that these genes may use as candidate genes for growth and fitness traits. Therefore, this study was carried out to identify genetic polymorphisms of ApoB2, LITAF, TGFβ4, TRAIL and IAP1 genes by PCR-RFLP and their association with growth trait in Thai native chickens.

MATERIALS AND METHODS

Chicken DNA isolation and PCR-RFLP technique: Blood samples were collected from a total of 280 individual chickens from 4 different breeds. Thai native chicken breeds, consisting of Luenghangkhao (LK, n = 80 animals), Pradhuangdum (PD, n = 100 animals), Chee (CH, n = 60 animals) and the Red Jungle Fowls (RJF, n = 40 animals) were obtained from southern of Thailand, such as Surat Thani and Nakhon

Si Thammarat. They have been Thai people's way of life in this area. Although, the RJF are generally raised under free-range conditions, production of the fighting cocks (LK, PD and CH) is much more intensive system. These chickens are from parent stocks consisting of 1 cockerel and 3-5 hens/household. They are fed twice daily, in the morning and evening. Data recording of growth trait for the investigate chickens was Body Weight (BW) measured on 12 weeks.

Genomic DNA was extracted from venous blood collected in EDTA by using a commercial genomic DNA kit (Geneaid) and following the manufacturer's instructions. A PCR was carried out with 50 ng genomic DNA to investigate sequence polymorphisms. The primer sets used to amplify the fragments of the ApoB2, LITAF, TGFβ4, TRAIL and IAP1 genes in Table 1. The PCR reaction was prepared according to the manufacturer's instructions Top Taq master mix kit using 50 ng of genomic DNA and 0.5 uM of primer. The cycling protocol was 5 min at 95°C, 35 cycles of denaturing at 94°C for 30 sec, annealing temperature as indicated in Table 1 for 30 sec, extension at 72 °C for 1 min, with final extension at 72°C for 7 min. A touchdown program was used to amplify TGFβ4, TRAIL and IAP1 fragments.

Polymorphisms of these genes were screened using restriction enzymes (New England Biolabs, Inc.). The PCR product of ApoB2, LITAF, TGFβ4, TRAIL and IAP1 fragments were digested with AclI (10 U), HinfI (10 U), MboII (5 U), StyI (10 U) and BglI (10 U) restriction endonuclease, respectively at 37°C overnight. The RFLP technique was determined by electrophoresis of the digested DNAs on 2% agarose gel for 2.5 h at 60 V and visualized under UV light. Size of the products for each gene was determined, based on standard DNA molecular weight markers.

Statistical analysis: Population genetics measurements, including allelic and genotypic frequencies and deviation from Hardy-Weinberg Equilibrium (HWE) were calculated using the Chi-square (χ^2) test of Hardy-Weinberg package in

Table 1: Primer sets for PCR-RFLP assay of ApoB2, LITAF, TGFβ4, TRAIL and IAP1 genes¹

Gene	Accession No.	Primer sequence	PCR product (bp)	Annealing temperature (°C)/time	Reference
ApoB2		F:5'-CATATTCTAATGGCATCCAG-3' R:5'-TTCCAGCGTTATTTCCG-3'	779	57°C/60s	Zhang and Shi ⁹
LITAF	AI979890	F:5'-TGAGTTGCCCTTCGT-3' R:5'-CAGAGCATCAACGCAA-3'	497	54°C/30s	Malek <i>et al.</i> ¹⁰
TGFβ4	AF459837	F:5'GGGGTCTTCAAGCTGAGCGT-3' R:5'-TTGGCAATGCTCTGCATGTC-3'	240	63-58°C/45s	Kramer <i>et al.</i> ⁷
TRAIL	AF537189	F:5'-GTAAATTAGAGCCTCATCA-3' R:5'-CACCTCAGTTCCTCCGA-3'	786	59-51°C/50s	Malek and Lamont ¹¹
IAP1	AF221083	F:5'-TCACCATCTCTACGTTCCAT-3' R:5'-CATTGAACTTGTTGGTCT-3'	394	62-54°C/60s	Liu and Lamont ¹²

¹ApoB2: ApolipoproteinB 2, LITAF: Lipopolysaccharide-induced tumor necrosis factor (TNF) a factor, TGFβ4: Transforming growth factor 4, TRAIL: TNF-related apoptosis-inducing ligand, IAP1: Inhibitor of apoptosis protein 1, F: Forward, R: Reverse

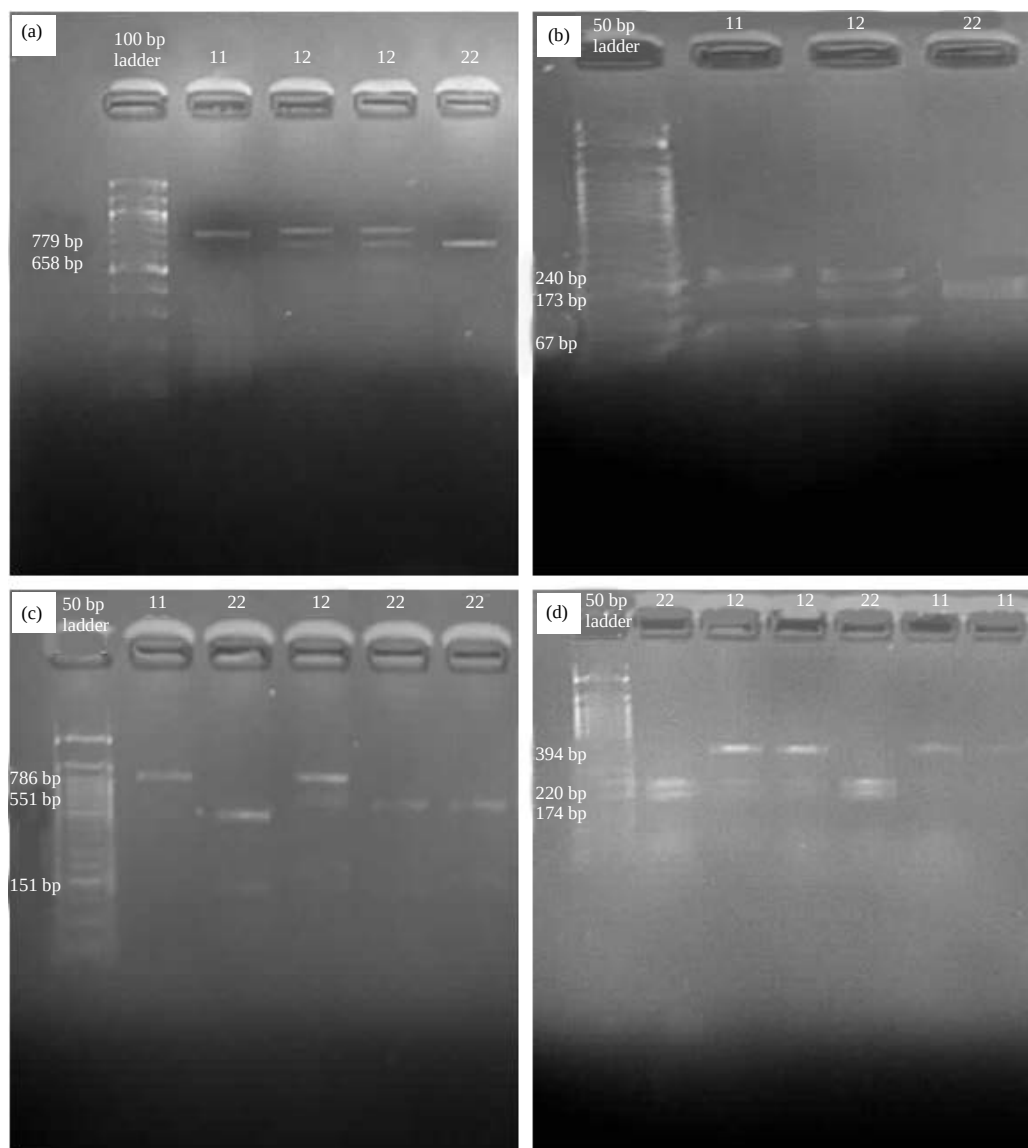


Fig. 1(a-d): Polymerase chain reaction-RFLP patterns for 4 candidate genes including (a) Apolipoprotein B 2 (ApoB2) gene (b) Transforming growth factor β 4 (TGF β 4) gene (c) TNF-related apoptosis-inducing ligand (TRAIL) gene and (d) Inhibitor of apoptosis protein 1 (IAP1) gene

R program¹³. Data were subjected to the GLM procedures separating in each breed group by using R program¹³. However, LITAF gene was observed monomorphic in this study, excluding in the model. The statistical model used to test the effects of sex and the genotypes of the genes as a fixed effect:

$$y_{ijklmn} = \mu + \text{sex}_i + \text{ApoB2}_j + \text{TGF}\beta 4_k + \text{TRAIL}_l + \text{IAP1}_m + e_{ijklmn}$$

where, y_{ijklmn} is the dependent variable (BW at 12 weeks) observed on the n th individual of sex i to test the effect of genotypes at j , k , l and m genes whereby μ is the overall mean and e_{ijklmn} is residual effect as random effect. Multiple

comparisons among significantly fixed effects were obtained by using Duncan Multiple Range Tests (DMRT) in function multcomp.test of R program¹³.

RESULTS

Genetic polymorphisms of candidate genes: The ApoB2, LITAF, TGF β 4, TRAIL and IAP1 genes were selected for this study in order to classify genetic polymorphisms of Thai native chicken. The results demonstrated that only LITAF gene was monomorphic. The ApoB2, TGF β 4, TRAIL and IAP1 genes had genetic polymorphic sites according to RFLP technique (Fig. 1).

Table 2: Genotypic and allelic frequencies of ApoB2, TGFβ4, TRAIL and IAP1 genes in Thai native chicken

Gene	Thai native breeds ¹	Genotypic frequencies			Allelic frequencies	
		11	12	22	1	2
ApoB2	LK	0.27	0.42	0.31	0.48	0.52
	PD	0.32	0.44	0.20	0.54	0.46
	CH	0.30	0.48	0.22	0.54	0.46
	RJF	0.31	0.38	0.31	0.50	0.50
TGFβ4	LK	0.77	0.10	0.13	0.82	0.18
	PD	0.72	0.16	0.12	0.80	0.20
	CH	0.70	0.15	0.15	0.77	0.23
	RJF	0.70	0.11	0.19	0.75	0.25
TRAIL	LK	0.38	0.47	0.15	0.61	0.39
	PD	0.40	0.50	0.10	0.65	0.35
	CH	0.38	0.46	0.14	0.61	0.37
	RJF	0.33	0.52	0.15	0.59	0.41
IAP1	LK	0.60	0.20	0.20	0.70	0.30
	PD	0.62	0.26	0.12	0.75	0.25
	CH	0.57	0.29	0.14	0.72	0.28
	RJF	0.59	0.37	0.04	0.77	0.23

¹LK: Luenghangkhao, PD: Pradhuangdum, CH: Chee and RJF: Red Jungle Fowls

The restriction enzyme *Acyl* digested PCR products had fragment sizes of 779 bp and 658 bp for homozygous 11 and 22, while, heterozygous (12) had both sizes. For the TGFβ4 gene, sequencing of the PCR products showed C/A mutation at base 632 (accession No. M31160) by detecting *MbolI* endonuclease. The digested products of heterozygous (12) were found 240, 173 and 67 bp. The PCR products of homozygous 11 had fragments of 240 and 67 bp and homozygous 22 were observed a PCR product of 173 bp. For the TRAIL gene, a 786 bp fragment amplified from the TRAIL gene showed an A/G synonymous SNP at base 82 (accession No. AF537189). The restriction enzyme *StyI* produced fragment sizes of 551 and 151 bp. A PCR-RFLP assay was developed to identify a *BglI* SNP of IAP1 gene to characterize the G/A synonymous SNP. The digested products of heterozygous (12) were found 394, 220 and 174 bp. Homozygous 11 was an undigested PCR product of 394 bp and homozygous 22 had fragments of 220 and 174 bp.

Genotypic frequencies of 11, 12 and 22 and allelic frequencies of 1 and 2 in these candidate genes are presented in Table 2. Genotypic frequencies of heterozygous (12) in ApoB2 and TRAIL genes were higher than 2 types of homozygous (11 and 22) but genotypic frequencies of homozygous 11 in TGFβ4 and IAP1 genes were higher in 3 times than heterozygous (12) and homozygous 22. Allelic frequencies of allele 1 in all candidate genes were higher than allele 2. The results of PCR-RFLP assay showed that ApoB2 and TRAIL genes, as well as $c = 0.13$ (p -value = 0.72) and $c = 1.85$ (p -value = 0.17), were consistent with Hardy-Weinberg equilibrium but TGFβ4 and IAP1 genes, as well as $c_b = 36.73$ (p -value = 1.35×10^{-9}) and $c = 9.37$ (p -value = 0.002), were inconsistent with Hardy-Weinberg equilibrium.

Effect of candidate genes for growth trait: Means and standard deviations of sex and genotypes of ApoB2, TGFβ4, TRAIL and IAP1 genes for BW at 12 weeks are presented in Table 3. Descriptive statistics in this study illustrated that BW at 12 weeks in LK was significantly the highest while, RJF had the lowest in this trait ($p < 0.05$). Fixed effects in this study consisted of sex and genotypes of ApoB2, TGFβ4, TRAIL and IAP1 genes. The result of sex effect showed that male chickens in all breeds were significantly heavier than female chickens. Genotypes of investigated genes in this study showed association with the investigated trait only ApoB2 gene. Body weight at 12 weeks of individuals with heterozygous genotype (12) in all breeds was higher than other genotypes ($p < 0.05$).

DISCUSSION

The results of monomorphic site of LITAF gene and polymorphic sites of the investigated genes were accordance with Malek *et al.*¹⁰ and Tohidi *et al.*⁸. They reported an A/T SNP in a *HinfI* site located within an intronic region of LITAF gene in village chickens but homozygous T/T for this locus was observed in the Red Jungle Fowls. However, Mekchay *et al.*⁴ stated that Thai indigenous chicken breeds were more closely related to the Red Jungle Fowls than those of the commercial breeds. Also, LITAF gene in this study was presented in non-genetic variation. The mutation of ApoB2 gene was according to Zhang *et al.*⁹, Buasook *et al.*¹⁴ and Seyedabadi *et al.*¹⁵ who reported genetic polymorphisms of ApoB2 gene in commercial broiler and Thai native chicken crossbreds. The polymorphisms of TGFβ4 gene were in good accordance with those reported by Li *et al.*⁶, Kramer *et al.*⁷

Table 3: Means comparisons of body weight at 12 weeks between sex and genotypes in each gene

Fixed Effects	Thai native breeds ¹			
	LK	PD	CH	RJF
Means $\pm$ SD²	1,304.56 $\pm$ 15.54 ^w	1,120.26 $\pm$ 16.45 ^x	1,040.75 $\pm$ 19.30 ^y	353.40 $\pm$ 7.26 ^z
Sex				
Male	1,350.34 $\pm$ 14.42 ^a	1,231.23 $\pm$ 18.65 ^a	1,135.76 $\pm$ 19.86 ^a	401.13 $\pm$ 7.85 ^a
Female	1,260.45 $\pm$ 16.67 ^b	1,007.29 $\pm$ 12.24 ^b	945.66 $\pm$ 18.70 ^b	305.59 $\pm$ 6.66 ^b
ApoB2 gene				
11	1,250.77 $\pm$ 15.67 ^a	1,007.85 $\pm$ 13.67 ^a	940.05 $\pm$ 18.98 ^a	340.86 $\pm$ 11.23 ^a
12	1,550.76 $\pm$ 20.12 ^b	1,350.88 $\pm$ 19.98 ^b	1,243.66 $\pm$ 14.67 ^b	378.24 $\pm$ 15.67 ^b
22	1,109.47 $\pm$ 18.89 ^c	1,002.05 $\pm$ 14.88 ^a	939.45 $\pm$ 13.67 ^a	341.10 $\pm$ 12.56 ^a
TGFβ4 gene				
11	1,298.08 $\pm$ 13.98	1,110.67 $\pm$ 19.83	1,036.76 $\pm$ 12.56	350.78 $\pm$ 12.11
12	1,303.78 $\pm$ 12.21	1,132.13 $\pm$ 18.56	1,042.67 $\pm$ 17.78	355.98 $\pm$ 11.76
22	1,308.12 $\pm$ 11.76	1,119.78 $\pm$ 17.12	1,042.83 $\pm$ 13.62	354.12 $\pm$ 11.01
TRAIL gene				
11	1,308.13 $\pm$ 14.56	1,124.45 $\pm$ 13.56	1,046.76 $\pm$ 18.00	348.12 $\pm$ 11.86
12	1,304.21 $\pm$ 10.76	1,119.87 $\pm$ 12.67	1,044.54 $\pm$ 15.78	359.18 $\pm$ 13.34
22	1,299.67 $\pm$ 13.68	1,118.56 $\pm$ 11.09	1,030.95 $\pm$ 14.98	352.89 $\pm$ 12.65
IAP1 gene				
11	1,301.02 $\pm$ 12.32	1,112.42 $\pm$ 15.78	1,038.77 $\pm$ 15.56	355.78 $\pm$ 11.00
12	1,300.56 $\pm$ 13.41	1,128.21 $\pm$ 16.56	1,044.67 $\pm$ 18.14	353.34 $\pm$ 12.02
22	1,310.49 $\pm$ 10.01	1,122.37 $\pm$ 14.34	1,038.87 $\pm$ 12.21	352.98 $\pm$ 11.89

¹LK: Luenghangkhao, PD: Praduhangdum, CH: Chee and RJF: Red Jungle Fowls, ²SD = Standard deviation, ^w, ^x, ^y and ^z within the same row marked with different letter are significantly different at $p < 0.05$, ^a, ^b and ^c within the same column marked with different letter are significantly different at $p < 0.05$

and Tohidi *et al.*⁸. Li *et al.*⁶ stated that the SNP in this gene caused an amino acid change (Glu/Asp) of the polypeptide chain. The SNPs of TRAIL and IAP1 genes were supported the finding of Kramer *et al.*⁷, Malek and Lamont¹¹ and Liu and Lamont¹². Tohidi *et al.*⁸ reported that the mutation of both genes did not change the amino acid sequence. Moreover, Ye *et al.*¹⁶ reported genetic polymorphisms of TRAIL and IAP1 genes by using FokI and BstXI endonuclease and TRAIL was not in significant Hardy-Weinberg equilibrium test. In this study, the inconsistent with Hardy-Weinberg equilibrium test of TGF β 4 and IAP1 genes indicated that both genes might be selected by natural or artificial selections.

The fixed effects of sex and genotype of the ApoB2 gene had significantly influence to the trait. It was similarity with Buasook *et al.*¹⁴, Abdullah *et al.*¹⁷ and Bekele *et al.*¹⁸ who stated that roosters in both purebreds and crossbreds had higher BW at 4-12 weeks than hens. Genotype effect of ApoB2 gene in this study had significantly associated with the BW at 12 weeks ($p < 0.05$). Zhang and Shi⁹ and Seyedabadi *et al.*¹⁵ reported that effect of ApoB2 gene had significantly influenced on BW at 1, 3 and 6 weeks, breast muscle and wing weight in commercial broiler lines. They stated that heterozygous chickens were heavier than homozygous chickens ($p < 0.05$). Also, it was presumed that it might have a QTL that affected to growth trait in chicken in this study. Innerarity *et al.*¹⁹ supported that ApoB has important function for the energy transportation and metabolism and it can directly or indirectly influence fat accumulation and growth. The result in this study

seemed that polymorphism of ApoB2 gene also acted in a dominant fashion for BW with allele 1 contributing to greater BW.

CONCLUSION

The main objectives of this study were to classify genetic polymorphisms of ApoB2, LITAF, TGF β 4, TRAIL and IAP1 genes and their association with growth trait such as BW at 12 weeks in 4 Thai native chickens. The results demonstrated that only LITAF gene was monomorphic site but the other genes revealed 3 genotypes. Only genetic polymorphism of ApoB2 gene had significantly affected to BW at 12 weeks ($p < 0.05$).

ACKNOWLEDGEMENTS

The author would like to acknowledge Higher Education Research Promotion (HERP) and National Research Universities (NRU) in Thailand for research scholarship. I would like to thanks Dr. Rangsan Chroensook and farmers in Southern of Thailand for chicken samples.

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