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Determination of TLX/*PstI* polymorphism in Japanese quail (*Coturnix japonica*)

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Abstract

The T-cell leukemia homeobox protein (TLX) gene is essential in vertebrate brain development and cognition in vertebrates. Polymorphisms in TLX can influence productive traits in quail. This study analyzed TLX gene variation in Japanese quail (*Coturnix japonica*). Blood samples were collected from 100 quails. The 544 bp of the TLX gene was amplified by PCR. The PCR-RFLP methods were used for genotyping. The PCR product was digested with *PstI* and three genotypes were obtained. AA (544 bp), AB (544, 402 and 142 bp) and BB (402 and 142 bp). Allele frequencies were 0.60 (A) and 0.40 (B). Genotype frequencies were 0.50 (AA), 0.19 (AB) and 0.31 (BB). The observed genotype proportions were in Hardy-Weinberg equilibrium. The genetic variability at the TLX locus represents an opportunity to incorporate this gene into molecular breeding strategies focused on enhancing performance traits related to cognition, growth, and carcass yield in Japanese quail.

Keywords: Coturnix japonica, TLX, Polymorphism, Allele frequency

1. Introduction

Quail farming has recently gained popularity as an alternative poultry meat source to chicken. The Japanese quail (*Coturnix japonica*), in particular, has emerged as an essential agricultural poultry species due to its rapid growth rate, high egg production, early sexual maturity, and resistance to common poultry diseases [1]. As the global population grows, quail farming provides a sustainable protein source to meet increasing food demands [2]. However, improving productivity traits such as growth rate, feed efficiency, egg production, and meat quality in quail remains a vital breeding objective [3].

The development of molecular markers has enabled marker-assisted selection (MAS) programs in livestock and poultry breeding [4]. MAS allows for the selection of animals based on genotype rather than phenotype alone. This is advantageous because molecular markers are detectable early in life, have higher heritability than polygenic traits, and are unaffected by environmental factors [5]. Various molecular marker techniques, including microsatellites, single nucleotide polymorphisms (SNPs), and copy number variants (CNVs), have been utilized in quail to detect quantitative trait loci (QTLs) and candidate genes associated with economic traits [6]. High-density SNP panels have also been developed using next-generation sequencing to enable genomic selection in quail [7]. Applying MAS in breeding programs allows for more accurate and rapid genetic improvement of quail populations.

Several candidate genes influencing quail productivity, growth, carcass, and egg quality traits have been identified [8]. One such gene is the chicken-like T-cell leukemia homeobox (TLX) gene, an essential regulator of neural stem cell proliferation and self-renewal [9]. While TLX has been well studied in mice and chickens [10], limited research exists on gene function and polymorphisms in Japanese quail. Given its role in cell proliferation and growth, TLX represents a strong candidate gene that may impact muscle development and growth rate in quail [11]. Understanding the genetic mechanisms that control growth and carcass characteristics will enable molecular marker-assisted selection to improve these economically essential traits in quail breeding programs genetically [12]. Characterizing TLX gene polymorphisms provides novel insights into a critical regulator of cell proliferation and its effects on growth and muscle development in quail [13]. Furthermore, the identified SNPs can serve as useful molecular markers for growth rate and carcass quality, advancing MAS efforts to enhance the productivity and profitability of quail farming [4].

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The objective of the present study was to detect the allele and genotype distribution of the TLX/*PstI* polymorphism in Japanese quail.

2. Material and Method

Animals and Sample Collection

Our study has been granted permission number 9-2-2022 by the ethics committee. The study population consisted of 100 Japanese quail (*Coturnix japonica*) obtained from the research and application farm of Çukurova University. The quail was reared under standard conditions with unlimited access to feed and water. At 5 weeks of age, blood samples were collected from each quail after slaughter for subsequent DNA isolation.

DNA Extraction

Genomic DNA was extracted from the blood samples using a salting-out procedure. Briefly, the blood samples were lysed with lysis buffer (10 mM Tris, 400 mM NaCl, 2 mM EDTA) and SDS. Proteins were precipitated using saturated NaCl and removed. DNA was recovered by ethanol precipitation, washed in 70% ethanol, air-dried, and dissolved in TE buffer. DNA quality and quantity were assessed using a NanoDrop spectrophotometer.

PCR Amplification

A 544 bp fragment of the TLX gene was amplified by polymerase chain reaction (PCR) using the primers listed in Table 1. The PCR reaction was carried out in a 20 µL volume using 5 µL (50 ng) DNA, 5 µL of PCR Master Mix (Solis Biodyne, Estonia), 0.5 µL for each primer (forward and reverse), and 9 µL of distilled water. The thermal cycling conditions were as follows: initial denaturation at 94°C for 5 min; 32 cycles of 94°C for 40 s, 60°C annealing for 1 min, and 72°C extension for 1 min; final extension at 72°C for 5 min. The PCR products were visualized by electrophoresis on a 2% agarose gel stained with ethidium bromide.

RFLP genotyping

The 544 bp TLX amplicons were genotyped by restriction fragment length polymorphism (RFLP) analysis using the *PstI* restriction enzyme. The PCR products were digested using *PstI* FastDigest restriction enzymes at 37°C, for a duration of 5-15 minutes, sourced from Thermo Scientific, USA. The reaction mixture consisted of 8 µL of PCR products, 4 µL of distilled water, 2 µL of 10X buffer, and 1 µL of the restriction enzymes. The digested PCR products were separated by electrophoresis on a 3% agarose gel stained with ethidium bromide and visualized under UV light.

Calculation of allele and genotype frequencies

Allele and genotype frequencies for the TLX/*PstI* gene polymorphisms were calculated using the POPGENE (version 1.32) population genetics software program.

Table 1. Primer sequences of the TLX/*PstI* locus

Gene	Primer Sequence	Restriction enzyme	PCR Product (bp)	References
TLX	5'-ACACTAGGAACATAATGGGCT-3' 5'-TCACTGTGGCGTTTCAGATT-3'	*PstI*	544 bp	[14]

3. Results and Discussion

PCR successfully amplified a 544 bp fragment of the TLX gene. PCR products were digested with the *PstI* restriction enzyme, revealing three different genotypes. AA genotype with 544 bp, AB genotype with 544 bp, 402 bp, and 142 bp, and BB genotype with 402 bp and 142 bp (Figure 1). Allele frequencies were calculated to be 0.60 for allele A and 0.40 for allele B. Genotype frequencies were determined to be 0.50 for AA, 0.19 for AB, and 0.31 for BB (Figure 2). The chi-square test found the TLX gene in Hardy-Weinberg equilibrium ($p>0.05$).

The TLX gene codes for a nuclear receptor that functions as a transcription factor and is crucial in vertebrate brain development and adult neurogenesis. Polymorphisms in the TLX gene have been associated with individual differences in learning, memory, cognition, and behavior in different animal species [15-16].

Our genotype and allele frequency results agreed with previous studies in other poultry species. Bozkaya et al. [17] identified similar TLX polymorphisms in Japanese quail, with allele frequencies of 0.55 for A and 0.45 for B. However, they found a lower AA genotype frequency (32%) and higher AB frequency (45%). The reasons for these discrepancies are unclear but may be attributed to differences in the study populations.

The genotype and allele frequencies we observed in Japanese quail differ somewhat from the results of Ahmed and Barzinji [13] in local quail populations. They found a higher AA genotype frequency (56.2% vs. 50%), a lower BB frequency (43.8% vs. 31%), and an additional C allele. The lack of AB heterozygotes in their study is unusual and may indicate a breeding bias or a small sample

size. The divergent TLX genotype distributions between Japanese and local quail could relate to differences in selective pressures, population history, or genetic drift between the two groups. Further comparative analyses between diverse quail populations will elucidate the evolutionary factors shaping TLX gene polymorphism and allow an assessment of how sequence variations relate to phenotypic traits. Our study provides foundational TLX genetic data in Japanese quail against which future studies in local and other quail breeds can be evaluated.

Deef and El-Nabi [18] observed no polymorphisms in the TLX gene across the chicken breeds they analyzed, indicating monomorphism at this locus. These results demonstrate that the genetic diversity of the TLX gene differs between avian taxa and populations. The presence or absence of TLX polymorphisms is likely influenced by environmental pressures, artificial selection imposed by breeding programs, and random genetic drift effects over generations. Further investigation is needed to elucidate the evolutionary and selective forces governing TLX variation among diverse poultry species and breeds. Comparative analysis of TLX genetic diversity across avian populations will provide insight into the molecular evolutionary history of this gene and factors driving divergence in sequence variation between species.

The TLX/*PstI* gene polymorphism was in Hardy-Weinberg equilibrium in our Japanese quail flock. This equilibrium indicates stable allele frequencies between generations and random mating within the population. Violations of equilibrium can signify inbreeding, assortative mating, or selection pressures. Maintaining Hardy-Weinberg proportions will be relevant for future selective breeding programs involving the TLX gene in Japanese quail.

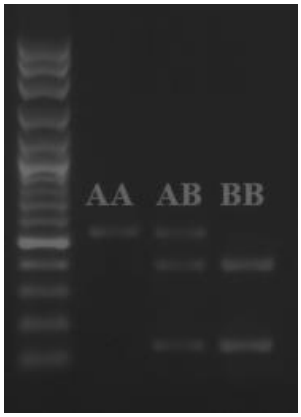

Figure 1. PCR-RFLP results

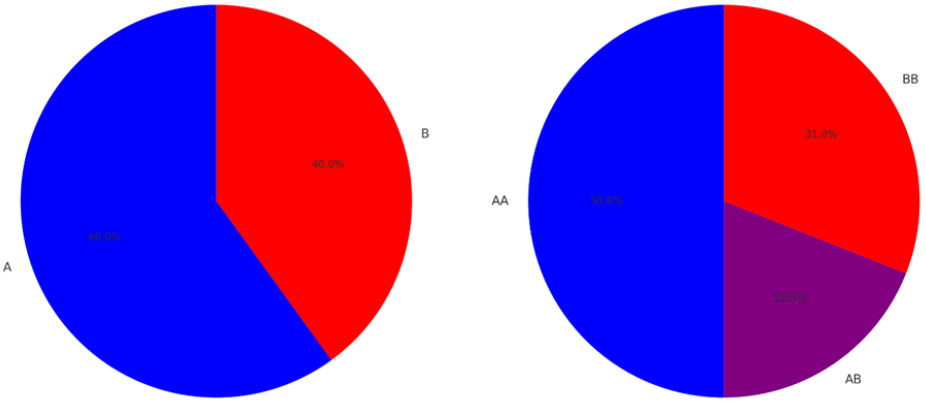

Figure 2. Allele and genotype frequency distribution

4. Conclusion

Identifying polymorphisms in the TLX gene among this Japanese quail flock has important implications for future genetic improvement programs. The polymorphisms identified in the TLX gene among this Japanese quail flock provide an opportunity to improve productive traits related to growth, carcass and influence complex behavioral traits. Elucidating the relationships between specific TLX genotypes and growth rates, feed efficiency, and body composition will enable marker-assisted or gene-editing selection approaches targeting preferred TLX alleles. The genetic diversity found at the TLX locus can be applied to breeding programs

that improve commercially relevant growth and carcass traits in Japanese quail. The TLX polymorphisms represent genetic variation that can be leveraged to enhance productive performance and profitability in quail production.

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