Journal of Evolutionary Biology Research Vol. 3(2), pp. 19-21, February 2011
Available online at http://www.academicjournals.org/jebr
ISSN 2141-6583 ©2011 Academic Journals

Short Communication

Genetics of TGF-β_3 gene polymorphism in inbred synthetic white leghorn breed of poultry

A. Ghosh, F. P. Savaliya, D. N. Rank, C. G. Joshi, K. Khanna and S. Taraphder*

Department of Animal Genetics and Breeding, College of Veterinary Science and A.H. Anand Agriculture University, Anand, Gujarat, India, Pin No.-388110, India.

Accepted 17 January, 2011

The experiment was conducted on inbred progeny to confirm the inheritance of *TGF-β_3* gene polymorphism. A total of 124 inbred synthetic white leghorn chicks were generated and reared in deep litter system under standard feeding, management and health care practices. At 16th week of age, 40 females and 40 males were housed in individual California cage system. Genomic DNA was extracted from these 80 inbred birds by John's method with some modifications. Chicken gene specific primers (20 bp) were used for amplification of *TGF-β_3* genes loci. Amplified polymerase chain reaction (PCR) product of TGF-*β_3* gene was digested with *BseI-I r*estriction enzyme for restriction fragment length polymorphism (RFLP) analysis. There was absence of polymorphism at TGF-β_3 locus in both male and female inbred progeny and also in parental generation. *TGF-β_3* gene was monomorphic in both inbred and parental generation.

Key words: White leghorn, inheritance, polymorphism, TGF-β_3, polymerase chain reaction, restriction fragment length polymorphism.

INTRODUCTION

The early embryonic development are mainly regulated by major growth factors such as *IGF-I, IGF-II* and *TGF-β*. The transforming growth factor-*β* (*TGF-β)* family contains a large number of structurally related polypeptide growth factors that play a prominent role in the development and homeostasis of virtually all tissues in organisms. *TGF-β* subfamily molecules are cytokines and consist of four currently identified members: *TGF-β1, TGF-β2, TGF-β3* and, *TGF- β4* (Burt and Law, 1994). Chicken *TGF-β3* locus was physically located on chromosome 5 (Burt et al., 1995; Groenen et al., 2000). The *TGF-β_3* gene contains 7 exons and 6 introns spanning 16 kb of the chicken genome. The biological activities of chicken TGF-*β* isoforms appear to be similar to those of mammals (Cogburn et al., 2000). Biological effects of *TGF-β* isoforms were broad, including effects on cell differentiation, cell proliferation, cell growth, extracellular matrix formation, and immune in processes, such as myogenesis, chondrogenesis, osteogenesis, hematopoiesis, epithelial cell function (Lawrence, 1996; Lechleider and Roberts, 1999). The *TGF-β* genes also played an important role differentiation and adipogenesis (Wall and Hogan, 1994). *TGF-β_3* isoforms have been shown to be present in gastrulating chicken embryo and have functional effects on cell transformation, extracellular matrix deposition and cell proliferation (Sanders, 1994). The *TGF-β_3* gene was, therefore, a logical candidate for investigating effects on chicken growth and development. Using polymerase chain reaction, restriction fragment length polymorphism (PCR-RFLP) technique, the presence of genetic variants in TGF-*β_3* gene can be characterized and confirmed the inheritance pattern in the inbred progeny. The knowledge of inheritance of polymorphism can also be useful in phylogenetic analysis as well as in design of breeding programmes. Hence, the present study was proposed to investigate inheritance of polymorphism of *TGF-β_3* gene in the inbred synthetic white leghorn breed of poultry.

*Corresponding author. E-mail: amritaghosh1983@gmail.com.

MATERIALS AND METHODS

Experimental poultry flocks and sampling

A total of 80 inbred synthetic white leghorn chicks were examined in this present study. The known genotype of parent generation synthetic white leghorn population was further propagated by mating between halfsib or fullsib to produce the next generation inbred progeny. These inbred progenies were utilized as an experimental material in the present study to confirm the inheritance of *TGF-β3* gene polymorphism.

DNA extraction

The blood samples of a total 80 inbred progenies of poultry comprising of 40 males and 40 females were collected from poultry farm maintained at Department of Poultry Science, College of Veterinary Science and A.H. of Anand Agriculture University, Anand, Gujarat, India. Approximately 2 ml of blood sample was collected aseptically from each individual from wing vein in vaccutainer containing 5% EDTA. DNA was extracted from white blood cells using standard phenol-chloroform extraction method as described by John et al. (1990) with minor modifications. The extracted DNA samples were stored at -20°C till further use. DNA samples were dissolved in 0.1XTE buffer (pH 8.0).

The purity and concentration of DNA samples were estimated by using UV-visible range spectrophotometer. DNA concentration was adjusted to 30 ng/µl before polymerase chain reaction (PCR) amplification. All the DNA samples had 260/280 OD ratios in the range of 1.8 to 2.0, indicating high purity. DNA was also examined by loading samples on 0.8% agarose gel and visualizing the band under gel documentation system.

PCR-RFLP for *TGF-β3* gene

The region of *TGF-β3* in poultry was amplified by forced PCR- RFLP technique using the following primers as described by Li et al. (2003). Primers for *TGF-β3* gene: Forward 5'-TCA GGG CAG GTA GAG GGT GT - 3' Reverse 5' - GCC ACT GGC AGG ATT CTC AC - 3'.

The reverse primer was deliberately introduced a point mutation resulting in PCR products with *TGF-β3* carrier poultry containing *Bsel-I* restriction site (CCNNNNN^NNGG) whereas products from non-carriers lacked this site. N = Any one nucleotide from A, T, G and C.

PCR was carried out in a final reaction volume of 25 µl. A master mix for all samples was prepared and aliquot of 22 µl in each PCR tube. 3 µl genomic DNA (30 ng/ µl) was added in each tube to make the final volume 25 µl. PCR master mix (Cat no. K0171, MBI Fermentas) containing 0.05 U/µl *Taq* DNA polymerase (recombinant) in reaction buffer, $MgCl_2$ (4 mM) and dNTPs (0.4 mM of each).

The amplification for *TGF-β3* gene was carried out using a pre-programmed thermal cycler (Eppendrof Mastercycler) with the following conditions: Initial denaturation of 3 min at 94°C followed by 35 cycles of denaturation at 94°C for 1 min, annealing at 52°C for 1 min and extension at 72°C for 1 min. Lastly the final extension was done at 72°C for 10 min. The PCR product was digested with *Bsel-I* restriction enzyme for over night at 55°C. The resulting product was separated by electrophoresis on 2.5% agarose gel and visualized with ethidium bromide under gel documentation system.

Genotype analysis

The length of amplified PCR product of *TGF-β3* gene was 294 bp. The presence of two restriction sites on both the strand resulted into the appearance of three bands of 145/75/74 bp following digestion with *Bsel-I* restriction enzyme. Fragments of 75 and 74 bp tend to overlap with each other, appeared as single bright band and thus resulted in two bands instead of three bands. The genotype having 145/75/74 bp band pattern could be identified as LL whereas the other genotype could be identified as BB having 124/20/75b/74 bp band pattern as described by Li et al. (2003). The heterozygous were having the both band pattern.

Statistical analysis

Statistical analysis was performed by SPSS 10.0 software package. The following parameters were obtained: the genotype categories and the gene frequency of the alleles that corresponded to the *TGF-β3* genotype. The frequency of genotypes and genes were computed by direct counting method for codominant loci.

RESULTS AND DISCUSSION

The *TGF-β3* gene was amplified using *TGF-β3* gene specific primers. The amplified PCR product (294 bp) was digested with 5 units of *Bsel*-I restriction enzyme at 55°C for overnight and put on electrophoresis on 2.5% agarose gel for 60 to 90 min. The presence of two restriction sites on both the strand resulted into the appearance of three bands of 145/75/74 bp following digestion with *Bsel-I* restriction enzyme (Figure 1). Fragments of 75 and 74 bp tend to overlap with one another, appeared as single bright band and thus resulted in two bands instead of three bands. In the present study this locus was found monomorphic and designated as LL genotype with allelic frequency of 'L'' allele was 1.00 and the similar result was also observed in the parental generation (Table 1).

In the similar type of study was also conducted by Li et al. (2003) and found restriction site for *Bsel-I* restriction enzyme in *TGF-β3* gene present within 4th intron region and alleles were designated as 'B' and 'L' for presence and absence of restriction site, respectively. They identified both genotypes LL and BB in broilers and WLH population. They also observed one *Bsel-I* polymorphisms at position of 283 bp in intron 4 of TGF-β_3 gene. Digestion of the PCR product yielded two restriction patterns named as B (124/20/75 /74 bp) and L (145 /75 /74 bp) in both populations.

Conclusions

In this present study, *TGF-β3* gene was found to be monomorphic with only LL genotype. The frequencies of L and B alleles in both inbred progeny and parental generation were observed as 1.0 and 0, respectively. Thus, there was absence of polymorphism at *TGF-β3* locus in both male and female inbred progeny and also in parental generation. However, this work needs to be continued on a large number of observations.

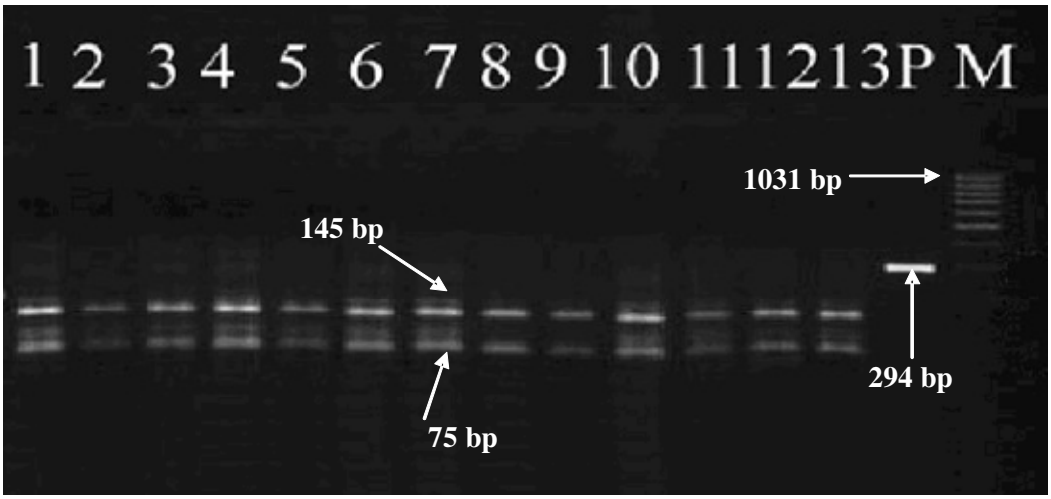

Figure 1. *TGF-β3* fragment of 294bp digested by *Bsel I* restriction enzyme. Lane 1-13: Genotype LL Lane M: 1031 bp ladder Lane P: Undigested control PCR product.

Table 1. Genotypic and Gene frequencies of *TGF-β3* locus in inbred progeny and parental generation.

Generation	Genotypic frequency			Gene frequency	
	LL	BB	BL	L	B
Inbred progeny	1.00 (80)	0	0	1.00	0
Parental generation	1.00 (40)	0	0	1.00	0

Figures within the parenthesis indicate the number of observations.

ACKNOWLEDGEMENTS

The authors are thankful to the Vice Chancellor of Anand Agriculture University, Gujarat, India for necessary permission and funding the project. The authors are also grateful to the Head, Department of Poultry Science, College of Veterinary Science, Anand Agriculture University, Gujarat, India for providing necessary facilities to carry out this research work.

References

Burt DW, Law AS (1994). Evolution of the transforming growth factor-beta superfamily. Progr. Growth Factor Res., 5: 99-118.

Burt DW, Dey BR, Paton IR, Morrice DR, Law AS (1995). The chicken transforming growth factor-beta 3 gene: genomic structure, transcriptional analysis, and chromosomal location. DNA cell Biol., 14(2): 111-112.

.

Cogburn LA, Burnside J, Scanes CG (2000). Physiology of growth and development. Sturkie's Avian Physiol., 5: 635-656.

Groenen MA, Cheng HH, Bumstead N, Benkel BF, Briles WE, Burke TD, Burt W, Crittenden LB, Dodgson J, Hillel JS, Lamont A, Ponce de L, Takahashi MH, Vignal A (2000). A consensus linkage map of the chicken genome. Genome Res., 10: 137-147.

John SWM, Weitzner G, Rozen R, Scriver CR (1990). A rapid procedure for extracting genomic DNA from leukocytes. Nucleic Acids Res., 19(2): 408.

Lawrence DA (1996). Transforming growth factor-beta: A general review. Eur. Cytokine Netw., 7: 363-374.

Lechleider RJ, Roberts AB (1999). Transforming growth factor-β, the Cytokine Network and Immune Functions. J. Thez. ed., 104-110.

Li H, Deeb N, Zhou H, Mitchell AD, Ashwell CM, Lamont SJ (2003). Chicken quantitative trait loci for growth and body composition associated with Transforming Growth Factor-β Genes. Poult. Sci., 82: 347-356.

Sanders EJ, Hu N, Wride MA (1994) Expression of TGF beta1/beta 3 during early chick embryo development. Anat. Rec., 238: 397-406.

Wall NA, Hogan BL (1994). TGF-beta related genes in development. Curr. Opin. Genet. Dev., 4: 517-522.