Poultry Science Journal
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Candidate Gene Polymorphism for IL-Rγ and ChB6 Genes in the Indigenous Chicken of North Western Himalayan State of Himachal Pradesh, India

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Poultry Science Journal 2013, 1 (2): 87-92

Article history:
Received: April 26, 2013
Accepted: November 18, 2013
Available online: December 1 , 2013

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Keywords:
Polymorphism
IL-Rγ gene
ChB6 gene
Indigenous Chicken

Abstract

Present investigation was carried out to identify DNA polymorphism of IL-2Rγ and ChB6 genes. Sixty five birds belonging to the indigenous chicken of Himachal Pradesh were utilized. Good quality DNA samples were subjected to PCR-RFLP analysis using chicken specific primers. Overnight restriction enzyme digestion was carried out at 37°C with IU Hph I and Pvu II for IL-2Rγ and ChB6 genes, respectively. Amplification of IL-2Rγ gene resolved a 600 bp amplicon in all samples, which upon digestion with Hph I RE yielded three patterns i.e. Hph I aa , Hph I a/b and Hph I bb. Hph I aa genotype revealed 465 and 42 bp fragments while Hph I bb genotypes revealed 454, 134, 104 and 42 bp fragments. Hph I a/b genotype resolved 465, 454, 134, 104 and 42 bp fragments. The frequencies of these patterns were 0.47, 0.23 and 0.30 for Hph I aa, Hph I a/b and Hph I bb, respectively. The amplification of Chicken B cell marker (ChB6) generated 215 bp amplicon in all the samples, which upon digestion with Pvu II generated two pattern i.e. Pvu II aa and Pvu II bb respectively. Pvu II aa generated 215 bp fragments while Pvu II bb generated 215, 147 and 68 bp, respectively. The frequencies of the PCR-RFLP pattern of ChB6 gene were 0.57 and 0.43, respectively.

Please cite this article as: Reen JK, Sankhyan V, Katoch S & Thakur YP. 2013. Candidate Gene Polymorphism for IL-2R γ and ChB6 Genes in the indigenous chicken of North Western Himalayan State of Himachal Pradesh, India. Poult. Sci. J. 1 (2): 87-92.

Introduction

Immune system plays an important role in protecting poultry from diseases. Interleukins, an important group of cytokines, play a significant role in elicitation of the immune response. Interleukin-2Rγ (IL-2Rγ) is shared by a receptor complex of many interleukins and it induces proliferation and differentiation of T, B, and NK cells. The chicken B-cell marker (ChB6) gene has been proposed as a candidate gene in regulating B-cell development (Tregskes *et al.*, 1996). ChB6 alleles are associated with the expression level of MHC class II, regression of Rous sarcoma, and resistance to Marek's disease (Gilmour *et al.*, 1986; Tregaskes *et al.*, 1996). With regard to the poultry production, the most important poultry production system prevailing in the hill agro ecosystem of the state is rural poultry farming, which is dominated by indigenous chicken resources (Katoch *et al*, 2010). There is limited information on the genetic architecture of indigenous chicken population. Hardiness/resistance to disease is one of the unique features of these indigenous chickens. This investigation was carried out to identify DNA polymorphism of IL-2Rγ and ChB6 genes by PCR-RFLP in the indigenous chicken of Himachal Pradesh.

Materials and Methods

Sixty five (65) birds belonging to a random bred population of the indigenous chicken maintained at University poultry farm were utilized in the present study. These chickens were produced by collecting unrelated birds from different locations of the state. DNA samples were isolated using Phenol: Chloroform extraction method (Kagami *et al.*, 1990). Good quality DNA samples were subjected to PCR-RFLP analysis using chicken specific primers, as the earlier reports of Zhou and Lamont, 2003. The forward and reverse primer sequence for IL-2Rγ were 5′-CCA AGC CTG GAC TAT GAG AA-3′ and 5′-CAT CTT TAG GAC TCC GAC CCA-3′, respectively, whereas for ChB6 were 5′-GCT TCC CCA ATG GAA CTG-3′ and 5′-GAG CAC AAT GGG CCT AGT C-3′, respectively. The PCR was performed in a total volume of 25 µL, containing 50 ng of genomic DNA, 1 µM of each oligonucleotide primers, 2.5 µL of 10 × PCR reaction buffer, 1.5 mM $MgCl_2$, 200 µM of each dNTP, and 1 U of Taq DNA polymerase. Cycle parameters for IL-2Rγ were 94°C for 5 min, then 35 cycles of 94°C for 1 min, 52°C for 1 min, and 72°C for 1 min, with a final extension step of 15 min at 72°C. Cycle parameters were similar for ChB6 except the annealing temperature (55°C). Amplified products were checked on agarose (0.7%) and overnight restriction enzyme digestion was carried out at 37°C with IU Hph I and Pvu II for IL-2Rγ and ChB6 genes, respectively. The restriction digest products were electrophoresed for 1 hour at 100V on a 2% agarose gel. Individual PCR-RFLP fragment sizes in each sample were determined, based on standard DNA molecular weight markers for each gene using Gel Doc system by computer software "Quantity One" of Biorad USA.

Results

PCR-RFLP analysis of IL-2Rγ and ChB6 genes was carried out. The digested fragment with each enzyme for IL-2Rγ and Ch B6 are presented in Table 1. Amplification of IL-2Rγ gene resolved a 600 bp amplicon in all samples, which upon digestion with Hph I RE yielded three patterns i.e. Hph I aa , Hph I a/b and Hph I bb (Figure 1). Hph I aa genotype revealed 465 and 42 bp fragments while Hph I bb genotypes revealed 454, 134, 104 and 42 bp fragments. Hph I a/b genotype resolved 465, 454, 134, 104 and 42 bp fragments. The frequencies of these patterns were 0.47, 0.23 and 0.30 for Hph I aa, Hph I a/b and Hph I bb, respectively (Table 2) . The percentages of homozygous and heterozygous animals were 77 and 23 respectively.

The amplification of Chicken B cell marker (ChB6) generated 215 bp amplicon in all the samples, which upon digestion with Pvu II generated two pattern i.e. Pvu II aa and Pvu II bb respectively (Figure 2). Pvu II aa generated 215 bp fragments while Pvu II bb generated 215, 147 and 68 bp, respectively. The frequencies of thee PCR-RFLP pattern of ChB6 gene were 0.57 and 0.43, respectively (Table 2). The percentages of homozygous and heterozygous were 57 and 43, respectively.

Table 1. PCR-RFLP pattern of IL-2Rγ with Hph I restriction enzyme and their frequency in the indigenous chicken of Himachal Pradesh

Observed Pattern	Reported Pattern	Fragment sizes (bp)	Genotype frequencies
Hph I aa	LL/AA	465, 42	0.47
Hph I ab	LF/AB	465, 454, 134, 104, 42	0.23
Hph I bb	FF/BB	454, 134, 104, 42	0.30

Table 2. PCR-RFLP pattern of ChB6 with Pvu II restriction enzyme and their frequency in the indigenous chicken of Himachal Pradesh

Observed Pattern	Reported Pattern	Fragment sizes (bp)	Genotype frequencies
Pvu II aa	LL/AA	215	0.57
Pvu II ab	FF/BB	215, 147, 68	0.43

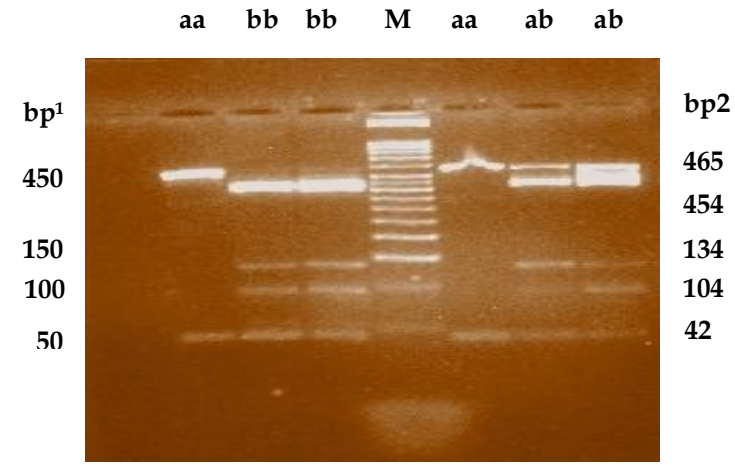

Figure 1. PCR-RFLP pattern of IL-2Rγ with Hph I restriction enzyme. M is 50bp ladder. bp1 and bp2 indicates the size of markers and digested amplicons, respectively.

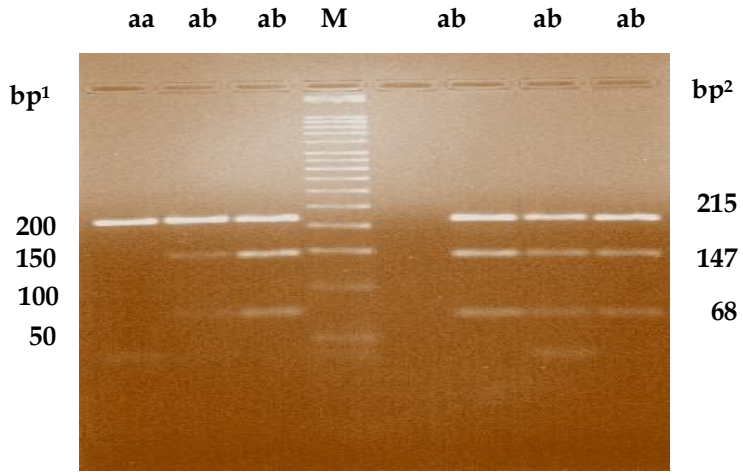

Figure 2. PCR-RFLP pattern of ChB6 with Pvu II restriction enzyme. M is 50bp ladder. bp1 and bp2 indicates the size of markers and digested amplicons, respectively.

Discussion

PCR–RFLP analysis of IL-2Rγ gene in the present study revealed similar pattern as reported earlier by Zhou and Lamont (2003) (LL, LF and FF) in Leghorn /Fayoumi chicken, Jaiswal *et al.* (2009) in Kadaknath native chicken and Kumar *et al.* (2007) in Aseel native Chicken. The frequency of heterozygotes (23%) observed in the present study was lower than those reported earlier by Jaiswal et al. (2009) in Kadaknath native chicken (32%) and Kumar *et al.* (2007) in Aseel native Chicken (36%) which can be attributed to the differences in the population structure.

The Pvu II aa pattern of PCR–RFLP analysis of Chicken B cell marker (ChB6) gene in the present study was similar to the earlier pattern reported by Zhou and Lamont (2003), while pattern Pvu II ab was reported as a new pattern in the present study.

Different population may have various haplotypes indicating their population structure or equilibrium frequency or may be the reservoir of a rare allele. The result of the present findings revealed that individuals were homozygous as well as heterozygous for loci studied and exhibited a moderate to high level of polymorphism. The present findings are suggestive of further exploring and utilization of variation observed at the loci studied to carry out association studies targeting the improvement of humoral immune responsiveness in indigenous chicken of the region.

Conclusions

The result of the present findings revealed that individuals were homozygous as well as heterozygous for loci studied and exhibited a moderate to high level of polymorphism. The present findings are suggestive of further exploring and utilization of variation observed at the loci studied to carry out association studies targeting the improvement of humoral immune responsiveness in indigenous chicken of the region.

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