

Molecular Characterization of Chicken B Cell Marker (ChB6) in Native Chicken of Poonch Region from International Borders of India and Pakistan

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Abstract : Introduction: Poonch is one of the remotest districts of the Jammu and Kashmir (UT) and situated on international borders. This native poultry population in these areas is quite hardy and thrives well in adverse climatic conditions. Till date, no local breed from this area (Jammu Province) has been characterized thus present study was undertaken with the main objectives of molecular characterization of ChB6 gene in local native chicken of Poonch region located at international borders between India and Pakistan. The chicken B-cell marker (ChB6) gene has been proposed as a candidate gene in regulating B-cell development. Material and Method: RNA was isolated by Blood RNA Purification Kit (HiPura) and Trizol method from whole blood samples. Positive PCR products with size 1110 bp were selected for further purification, sequencing and analysis. The amplified PCR product was sequenced by Sangers dideoxy chain termination method. The obtained sequence of ChB6 gene of Poonchi chicken were compared by MEGAX software. BioEdit software was used to construct phylogenetic tree, and Neighbor Joining method was used to infer evolutionary history. In order to compute evolutionary distance Maximum Composite Likelihood method was used. Results: The positively amplified samples of ChB6 genes were then subjected to Sanger sequencing with "Primer Walking. The sequences were then analyzed using MEGA X and BioEdit software. The sequence results were compared with other reported sequence from different breed of chicken and with other species obtained from the NCBI (National Center for Biotechnology Information). ClustalW method using MEGA X software was used for multiple sequence alignment. The sequence results of ChB6 gene of Poonchi chicken was compared with *Centrocercus urophasianus*, *G. gallus* mRNA for B6.1 protein, *G. gallus* mRNA for B6.2, *G. gallus* mRNA for B6.3, *Gallus gallus* B6.1, *Halichoeres bivittatus*, *Miniopterus fuliginosus* *Ferringtonia patagonica*, *Tympanuchus phasianellus*. The genetic distances were 0.2720, 0.0000, 0.0245, 0.0212, 0.0147, 1.6461, 2.2394, 2.0070 and 0.2363 for ChB6 gene of Poonchi chicken sequence with other sequences in the present study respectively. Sequencing results showed variations between different species. It was observed that AT content were higher than GC content for ChB6 gene. The lower AT content suggests less thermostable. It was observed that there was no sequence difference within the Poonchi population for ChB6 gene. The high homology within chicken population indicates the conservation of ChB6 gene. The maximum difference was observed with *Miniopterus fuliginosus* (Eastern bent-wing bat) followed by *Ferringtonia patagonica* and *Halichoeres bivittatus*. Conclusion: Genetic variation is the essential component for genetic improvement. The results of immune related gene Chb6 shows between population genetic variability. Therefore, further association studies of this gene with some prevalent diseases in large population would be helpful to identify disease resistant/ susceptible genotypes in the indigenous chicken population.

Keywords : ChB6, sequencing, ClustalW, genetic distance, poonchi chicken, SNP

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