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SNPS and INDELS detection in the chicken Myostation gene and its intergenic regions

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Abstract:

Next Generation Sequencing (NGS) is revolutionizing genomic studies due to the fast sequencing of many individuals for detection of SNPs or INDELs. Myostatin (MSTN) is a negative regulator of skeletal muscle in mammals, and mutations in this gene can cause hypertrophy and hyperplasia. In chicken, MSTN gene is located on GGA7 (between PMS1-HIBCH genes), and has an important role in muscle development. So far only 12 SNPs and 5 INDELs have been reported in the MSTN gene in chickens on NCBI database (2013). Grade et al. (2009) identified a 260 bp evolutionary conserved region upstream of MSTN gene which can regulate MSTN expression. The objective was to detect SNPs/INDELs by NGS in the MSTN gene and its intergenic regions of 68,210 bp (168,116-236,324 bp) from two chicken lines (TT broiler and CC layer) developed by Embrapa Swine and Poultry to further investigate their potential genomic effects. Three chickens per line were sequenced by Illumina technology. The initial coverage was 18X/chicken. The quality trimming was performed by Seqyclean (v.1.3.12) with quality of 24 and minimal fragment size of 65 bp. Alignment was performed by Bowtie2 (v.2.1.0) against the Gallus_gallus-4.0 reference genome. Variant calling was performed by SAMtools (v.0.1.19) with mapping and base qualities ≥ 20. The filtration of the SNPs/INDELs was based on variant quality score ≥30 and coverage ≥5. Initially we detected 652 SNPs and 48 INDELs from TT broiler and 747 SNPs and 60 INDELs from CC layer line. After filtration 570 SNPs and 37 INDELs were retained from TT and 691 SNPs and 48 INDELs from CC line. This indicates a greater level of variation in the layer MSTN gene and its intergenic regions. In the promoter region (Grade et al. 2009: 195,364-195,619 bp), we identified one SNP (G/T) at the position 195,403 which was present only in layers. After the filtration we obtained 1,205 unique SNPs and 98 INDELs including 37 insertions, 60 deletions and one insertion from CC line or the same INDEL as a deletion from TT line. The deletions and insertions had a maximum size of 17 and 9 bp, respectively. Annotation of the SNPs (ANNOVAR tool) classified 6 as synonymous in exon 1, 39 as intronic, 16 as in upstream, 3 as downstream, and 1,093 as intergenic: 578 in the HIBCH/MSTN and 515 in the MSTN/PMS1 region. Moreover, 14 SNPs were annotated to be in the UTR3' region of the gene PMS1, 28 to be in the PMS1 downstream region and 16 in the HIBCH downstream region. From the 1,205 unique filtered SNPs, ~28% (n=337) were specific to the layer and ~22% (n=269) to the broiler line. This study reports a characterization of good quality variants present in the MSTN gene and its intergenic regions in different chicken lines. Since MSTN gene in chickens has effects on weight, abdominal fat and breast muscle, this study may lead to causal mutations responsible for the observed phenotypes.

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