Gene Reports 11 (2018) 294–298

Contents lists available at ScienceDirect

Gene Reports

journal homepage: www.elsevier.com/locate/genrep

Computational analysis of non-synonymous SNPs in bovine Mx1 gene

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ARTICLE INFO

Keywords:
Bovine
Gene
Mutation
SNP

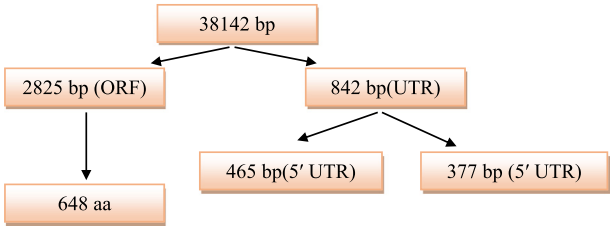
ABSTRACT

The Mx gene encodes an antiviral protein and is induced by type 1 interferons (IFNs). In this study, bovine Mx1B gene was analysed using six online tools to evaluate the effect of single nucleotide polymorphisms (SNPs) that may cause some deleterious effect in the animal. Out of 115 nsSNPs evaluated, 23nsSNPs were classified as highly damaging. The results of computational analysis of the Mx1 gene further widens the scope of research on mutations that can be prioritized in future population and laboratory studies.

1. Introduction

Mx proteins are evolutionarily conserved dynamin-like large GTPases, and GTPase activity is required for their antiviral activity, expressed in the cells of many vertebrates on exposure to several cytokines, mostly type I interferons (IFNs).In addition to GTPase activity, Mx proteins possess leucine zipper motifs that allow self-assembling into higher order structures that resemble rings and helical stacks of rings (van der Bliek and Meyerowitz, 1991). Mx is known to suppress several viruses including the influenza virus and vesicular stomatitis virus (VSV) (Staeheli et al., 1986; Pavlovic et al., 1990; Horisberger and Gunst, 1991; Jin et al., 1999). In cows, two isoforms of Mx are present-Mx1 and Mx2; additionally, bovine Mx1 has alternative splice variants, Mx1-A and Mx1B (Ellinwood et al., 1998; Kojima et al., 2003). The Mx1-A has antiviral activity against VSV, whereas Mx1B does not inhibit VSV proliferation in the cytoplasm, and its own function is unknown (Nakatsu et al., 2004; Yamada et al., 2009). In poultry, Mx1 gene is an interferon induced gene that inhibits single stranded negative sense RNA viruses' proliferation including avian influenza virus (Niraj et al., 2015; Selvaramesh et al., 2018). In fact, Mx1-A localizes in the cytoplasm, but almost all of Mx1B localizes in the nucleus. Mx2 also has antiviral activity against VSV (Babiker et al., 2007), and its intracellular localization is unknown (Sasaki et al., 2013).

The bovine Mx1 gene is comprised of 38,142 nucleotides with an open reading frame of 2825 base pairs encoding 648 amino acids. The remaining 842 nucleotides is untranslated region (UTR), out of which 465 nucleotides lie in 5′ and 377 nucleotides are present in 3′ region (Wang et al., 2009). Bovine Mx1 gene is located on chromosome 1. The 2280 bp region is spread among 17exons which are reported to contain numerous polymorphisms in different bovine populations (Aleksey V Zimini et al., 2009).



Although most of these Mx1 variants are known to be associated with antiviral infections in bovines (Kojima et al., 2003), their exact effect is still unknown. Therefore, identifying the effect of non-synonymous single nucleotide polymorphisms (nsSNPs) is imperative (Stefla et al., 2013; Kucukkal et al., 2015; Petukh et al., 2015). The nsSNPs in the coding region amend the structure as well as the function of the corresponding proteins through altering the catalytic or ligand binding sites, inappropriate protein folding, inaccurate intracellular transportation, decrease in the stability, loss of function of the gene product (Vendruscolo et al., 2003; Thusberg and Vihinen, 2009; Khan and Vihinen, 2010; Dehouck et al., 2011; Worth et al., 2011;Shihab et al., 2013; Valastyan and Lindquist, 2014). Identifying and understanding the molecular variations that are causing changes in phenotype is a challenge in genetic studies (Kim et al., 2014). Genome-wide association studies (GWAS) is a robust

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https://doi.org/10.1016/j.genrep.2018.05.001
Received 9 March 2018; Received in revised form 28 April 2018; Accepted 3 May 2018
Available online 09 May 2018

tool to detect differential phenotypic expression associated with SNPs (Sender et al., 2013; Wang et al., 2015). In-vitro effect assessment of specific variations can be done, however it becomes highly laborious and time-consuming to evaluate the large amount of variation present in the genome (Kumar et al., 2014). In this regard, different tools have been evolved to identify harmful SNPs located within a gene (Thusberg and Vihinen, 2009). Some tools use evolutionary information on amino acid conservation in the gene, based on multiple sequence alignment (MSA) of homologous proteins in related species. Variation in conserved amino acids locations is more detrimental for structure and function of a protein (Ng and Henikoff, 2006; Teng et al., 2008; Barenboim et al., 2008; Li et al., 2013). These tools incorporate the properties of amino acid residues, structural information, evolutionary conservation, and databases with authentic information about the harmful evidence of SNPs (Mooney, 2005; Kumar et al., 2014). The results given by these various tools are combined using consensus predictors that compare different methods (Bendl et al., 2014; Bendl et al., 2016). Studies employing combination of different prediction tools have identified deleterious mutations in genes responsible for numerous biological processes (Brunham et al., 2005; Rajasekaran et al., 2007; George Priya Doss et al., 2008; Hussain et al., 2012; Vanajothi et al., 2012; de Carvalho and De Mesquita, 2013). However, most of the studies restricted to humans and the same in animals are very scanty. Therefore, in this study an attempt has been made to predict the effect of nsSNPs in bovine Mx1 gene (Figs. 1 and 2, Table 1).

2. Materials and methods

2.1. Database

The data on bovine Mx1 gene was collected from Entrez gene on National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nlm. nih.gov), including genomic DNA accession number (AB060171) and mRNA accession number (NM_173940). The Swissprot database (http://expasy.org) was accessed to retrieve amino acid sequence of Mx1 protein (Uniprot IDP79135). The information on 242nsSNPs in bovine Mx1 was amassed from dbSNP (http://www.ncbi.nlm.nih.gov/snp) that contains SNP ID, alleles and functional consequences as and when available.

These nsSNPs were appraised using five prediction tools namely PANTHER (http://www.pantherdb.org/tools/csnpScore.do), PROVEAN (http://provean.jcvi.org/), SIFT (http://sift.jcvi.org/), SNAP2 (https://www.rostlab.org/services/SNAP/) and the consensus prediction tool PredictSNP (http://loschmidt.chemi.muni.cz/predictsnp). The amino acid sequence of bovine Mx1gene (DQ839567), Uniprot accession number (P79135), wild and mutated residue of the nsSNPs along with their position within protein were used according to the program requirements.

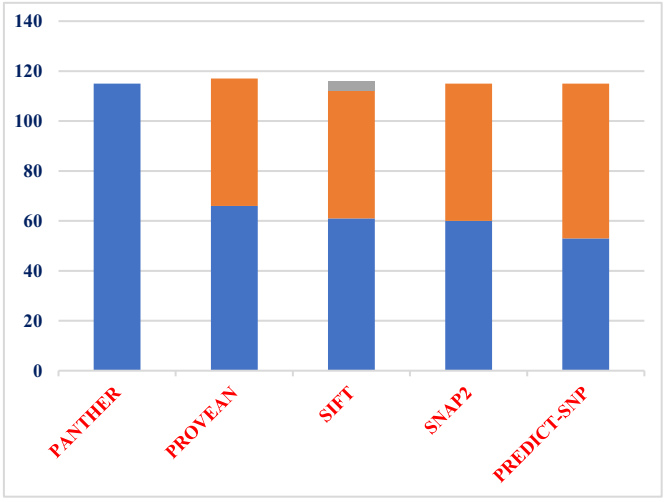

Fig. 2. Computational prediction of nsSNPs effect.

2.2. Validation and functional characterization predicted nsSNPs by PANTHER

PANTHER (Protein Analysis THrough Evolutionary Relationships) assess the likelihood that a particular nsSNP causing a functional alteration of the protein (www.pantherdb.org/tools). It calculates the length of time (in millions of years) a given amino acid has been preserved in the lineage leading to the protein of interest. The longer the preservation time, the greater is the likelihood of functional impact. It determines the PANTHER-PSEC (PANTHER position-specific evolutionary conservation) score by employing a hidden Markov model alignment of related proteins (Thomas et al., 2003; Thomas and Kejariwal, 2004). Substitutions with PANTHER-PSEC score of 0 indicates functionally neutral effect and the scores with negative values show deleterious effect of substitutions. A cut off of -3 subPSEC score imply a 50% probability that an nsSNP as destructive to the protein and the probability of causing a harmful effect on the functional activities of proteins (Pdeleterious) is 0.5. Amino acid sequence in FASTA format was uploaded.

2.3. Prediction of functional impact of nsSNPs

PROVEAN (Protein Variation Effect Analyzer) is a tool which predicts the impact of an amino acid substitution or indel on the biological function of a protein (http://provean.jcvi.org/index.php) and evaluates

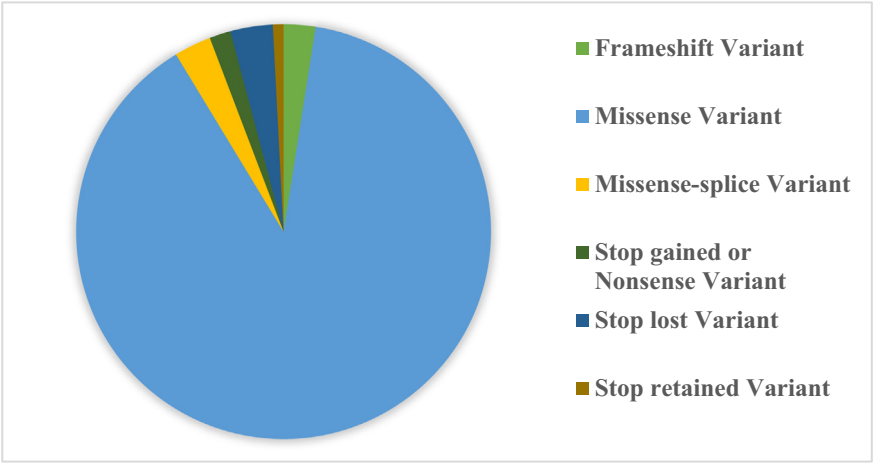

Fig. 1. Classification of nsSNPs found in the coding region of bovine Mx1 gene.