PHYLOGENETIC STUDY OF SLC11A1 GENE IN DOMESTIC ANIMALS

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ABSTRACT: Solute carrier family 11-member a1 gene (SLC11a1) previously is a functional member of metal ion-transport protein, earlier known as natural resistance-associated macrophage protein 1 (NRAMP1). The cellular expression of this gene is restricted to phagocytic cells. The function of this gene is to transport bivalent metal cations from the cytosol. The complex reaction of Fenon and Haber-Weiss of this gene reacts toxic antimicrobial radicals against microorganisms. This study investigates the evolutionary divergence of the SLC11a1 gene in domesticated farm animals. SLC11a1 gene sequences of domesticated farm animals were retrieved from NCBI GenBank. The results of this study revealed that there was substantial genetic variation in aligned sequences of the SLC11a1 gene within selected species. One ns-mutation (Q312K) was found in this study which is harmful. The phylogenetic trees showed some form of differentiation in the SLC11a1 gene sequence. The information on SLC11a1 polymorphism might be used to associate with disease resistance of farm animals in Pakistan.

Key words: Disease resistance, divergence, farm animals, polymorphism.

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INTRODUCTION

The Solute Carrier family 11-member 1 (SLC11a1) gene is a functional member of metal ion-transport proteins, earlier known as Natural Resistance Associated Macrophage Protein 1 (NRAMP1). First time discovered by positional cloned of infectious disease (Vidal *et al*., 1993). SLC11a1 gene was found by three independent infectious disease research groups. It was hypothesized that disease resistance or susceptibility was controlled by a single locus. This locus-encoded protein modulates macrophage function (Malo *et al*., 1994). This gene had restricted expression in the spleen, liver, and blood (Blackwell, 1989). Therefore, the gene name was natural resistance-associated macrophage protein 1 or NRAMP1 (Vidal *et al*., 1993). The location of bcg/Lsh/Ity locus in mice was identified on chromosome 1. The point mutation in the coding sequence of NRAMP1 was associated with susceptibility and led to a non-conservative substitute of an amino acid (Glycine). Non-functional protein due to mutation of G169D at position 169 of aspartic acid in trans-membrane domine 4 (Vidal *et al*., 1993; Malo *et al*., 1994; Vidal *et al*., 1996). NRAMP1gene sequence of 27 inbred mice was sequenced and found concordance between wild and mutant. The SLC11a1 gene is associated with the transport of cations with pH-dependent through the phagosome membranes that are essential for cellular function (Forbes and Gros 2003). There is evidence that these cations transport from the phagolysosome to the cytosol in the lumen and prevent acquisition. However, this gene delivers bivalent metal cations from the cytosol into acidic endosomal and lysosomal in normal physiological conditions (Goswami *et al*., 2001). There is also some evidence of pleiotropic effects of the SLC11a1 gene on macrophage function and an increase in tumor necrosis factor-α, inducible nitric oxide synthase, MHC class II expression, chemokine KC, and interleukin-1β (Thomas and Joseph, 2012). There are various studies that the SLC11a1 gene has a significant association with resistance to infectious diseases (Awomoyi, 2007).

However, there is very limited information on the SLC11a1 gene's role and its evolution in domestic animals. It is a matter of fact that identification of SNPs polymorphism from the pool is challenging (George *et al*., 2008). Therefore, computational prediction of gene function is a reliable approach to detecting the disease-related impact of variants (Liu and Kumar 2013; Yakubu, 2014). The aim of this study was to investigate the SLC11a1 gene diversity and differentiation in domesticated farm animals.

MATERIALS AND METHODS

A total of thirty-nine (39) sequences of domestic animal species including cattle (14), Buffalo (6), sheep (5), goat (3), pig (3), camel (3), horses (3), and bison (2) were retrieved from the GenBank (Table 1). ClusterW was used for coding sequences (CDS) alignments, translations, and comparisons (Huerta-Cepas *et al*., 2016). A neighbor-joining (NJ) tree was constructed on the basis of genetic distance using p-distance options. The NJ tree reliability was assessed by bootstrap value (1000 replications) (Felsenstein 1985). The consensus sequence of selected species was used to construct using the UPGMA method (Tamura *et al*., 2011). The variation rate among sites was modeled with a gamma distribution. Amino acid (aa) mutations of selected cattle were performed using the MEGA-MD suite (Stecher *et al*., 2013). MEGA-MD suite was used to obtain changes in coding sequence SNP and protein. MEGA-MD suite is a forecasting tool using multiple methods to develop non-sense SNVs. The graphical interface of this suite enables interactive exploration of nsSNVs on desktop (Fujita *et al*., 2011).

RESULTS AND DISCUSSION

The sequence length of the SLC11a1 gene of species in this study varies from 1647 to 1703 (Table 1). The sequence variation of this gene in different farm animals species might be due to evolutionary differentiation caused by insertions and deletions (Vidal *et al*., 1993). Twenty-eight amino acid (aa) mutations of were analyzed (Table 2). Q325K substitution was deleterious and N141S, V258I, and E35G, and were found neural. The other twenty-four substitutions (aa) were neutral. We found gene diversity between selected species. At the present time, SNPs are being used to insight into the biology of complex traits (Ruiz-Larranaga *et al*., (2010). SNP c.1067C>G (SLC11a1) gene was found a potential variant and causes a change from proline to alanine of codon 356 associated with MAP infection susceptibility in cattle that could alter the protein function of this gene (George *et al*., 2008). In a microsatellite polymorphism association study of SLC11a1 allele (211, 215, and 217) were significantly linked to the minor occurrence of bovine tuberculosis (bTB) in African Zebu (Callaby *et al*., 2020). SLC11a1 gene (3`UTR) is also associated with brucellosis susceptibility in Buffalo (Capparelli *et al*., (2007a, b). In a vitro assay, bovine SLC11a1 3`UTR genotypes were significantly associated with B. abortus resistance (Martinez *et al*., 2008: Kadarmideen *et al*., 2011). Gene polymorphisms of the SLC11a1 gene were also identified in a Merion flock with high MAP infection and related to Johne`s disease (Reddacliff *et al*., 2005). Liandris *et al*. (2009) sequenced the caprine SLC11a1 gene (accession no. FJ388877) and identified potential polymorphisms associated with positive MAP infections. It was also observed in Greece that 3`UTR of the SLC11a1 gene in caprine contains two microsatellites with G-T repeats and was found significantly associated with region B and ELIZA of paratuberculosis (Korou *et al*., 2010; Vacca *et al*., 2011). The findings of this study (aa) need further association investigation to exploit disease-resistant individuals.

The NJ tree (Figure 1) clearly revealed trans-species evolution. The Swine accession no. U55068.1_65-1681, AF132037.1_10-1626, and NM_213821.2_8-1624 clustered more. Phylogeny is a powerful tool to organize diverse information and classify its structure to understand the evolution events (Banum 2008; Holder *et al*., 2020). The numerous alleles present at the locus of the SLC11a1 gene are evidence of long-term evolutionary events.

The nucleotide substitutions per site (Dxy) between selected sequences of species. There is two classification ruminants and non-ruminants. Capra and Bubalus shared the highest Dxy value (0.82). the smallest observed Dxy value was found between ovis and caprine (0.71). In non-ruminants, the Dxy value was found between Equus and Sus (0.72). Dxy is used as a DNA divergence index between and within sequences. The largest Dxy value depicts greater distance and vice versa (Kang *et al*., 2008). Dendrogram (Figure 2) UPGMA drawn from SLC11a1 gene sequence of selected farm animals' species.

Table 1. Retrieved sequence information of domestic animals.

Sequence	Accession No.	Species	CDS
1	NM_174652.2_74-1720	Bos taurus	1647 bp
2	XM_027567381.1_302-2014	Bos indicus X Bos taurus	1713 bp
3	XM_019976599.1_730-2442	Bos indicus	1713 bp
4	XM_005889649.2_217-1929	Bos mutus	1713 bp
5	XM_010861184.1_249-1961	Bison bison	1713 bp
6	U39614.1_74-1720	Bison bison	1647 bp
7	U27105.1_74-1720	Bubalus arnee bubalis	1647 bp
8	XM_006046401.3_221-1933	Bubalus bubalis	1713 bp
9	XM_006046402.3_462-2111	Bubalus bubalis	1650 bp

10	KX610475.1_3-1649	Bos grunniens	1647 bp
11	DQ658151.1_63-1709	Bubalus bubalis	1647 bp
12	KF306239.1	Bos indicus	1647 bp
13	KF306240.1	Bos indicus	1647 bp
14	KF306241.1	Bos indicus	1647 bp
15	KF306236.1	Bos indicus	1647 bp
16	NM_001009345.1_24-1739	Ovis aries	1716 bp
17	KF306237.1	Bos indicus	1647 bp
18	KF306234.1	Bos indicus	1647 bp
19	NM_001285694.1_55-1701	Capra hircus	1647 bp
20	KF306238.1	Bos indicus	1644 bp
21	KF306235.1	Bos indicus	1644 bp
22	MH532569.1	Bos grunniens	1647 bp
23	AF005380.1_22-1668	Ovis aries	1647 bp
24	XM_012111085.4_30-1835	Ovis aries	1806 bp
25	FJ827149.1	Bubalus bubalis	1647 bp
26	KX151137.1_22-1668	Capra hircus	1647 bp
27	KX151136.1_22-1668	Capra hircus	1647 bp
28	XM_042242644.1_123-1577	Ovis aries	1455 bp
29	XM_025278239.2_223-1824	Bubalus bubalis	1602 bp
30	XM_012111094.4_30-1718	Ovis aries	1689 bp
31	XM_010988321.1_146-1792	Camelus dromedarius	1647 bp
32	XM_006186833.2_146-1792	Camelus ferus	1647 bp
33	XM_010948766.2_149-1795	Camelus bactrianus	1647 bp
34	U55068.1_65-1681	Sus scrofa	1617 bp
35	AF132037.1_10-1626	Sus scrofa	1617 bp
36	NM_213821.2_8-1624	Sus scrofa	1617 bp
37	XM_014852531.2_295-1932	Equus asinus	1638 bp
38	NM_001081857.2_48-1682	Equus caballus	1635 bp
39	AF354445.1_48-1682	Equus caballus	1635 bp

Figure 1: NJ tree obtained from SLC11a1 gene using NJ method.

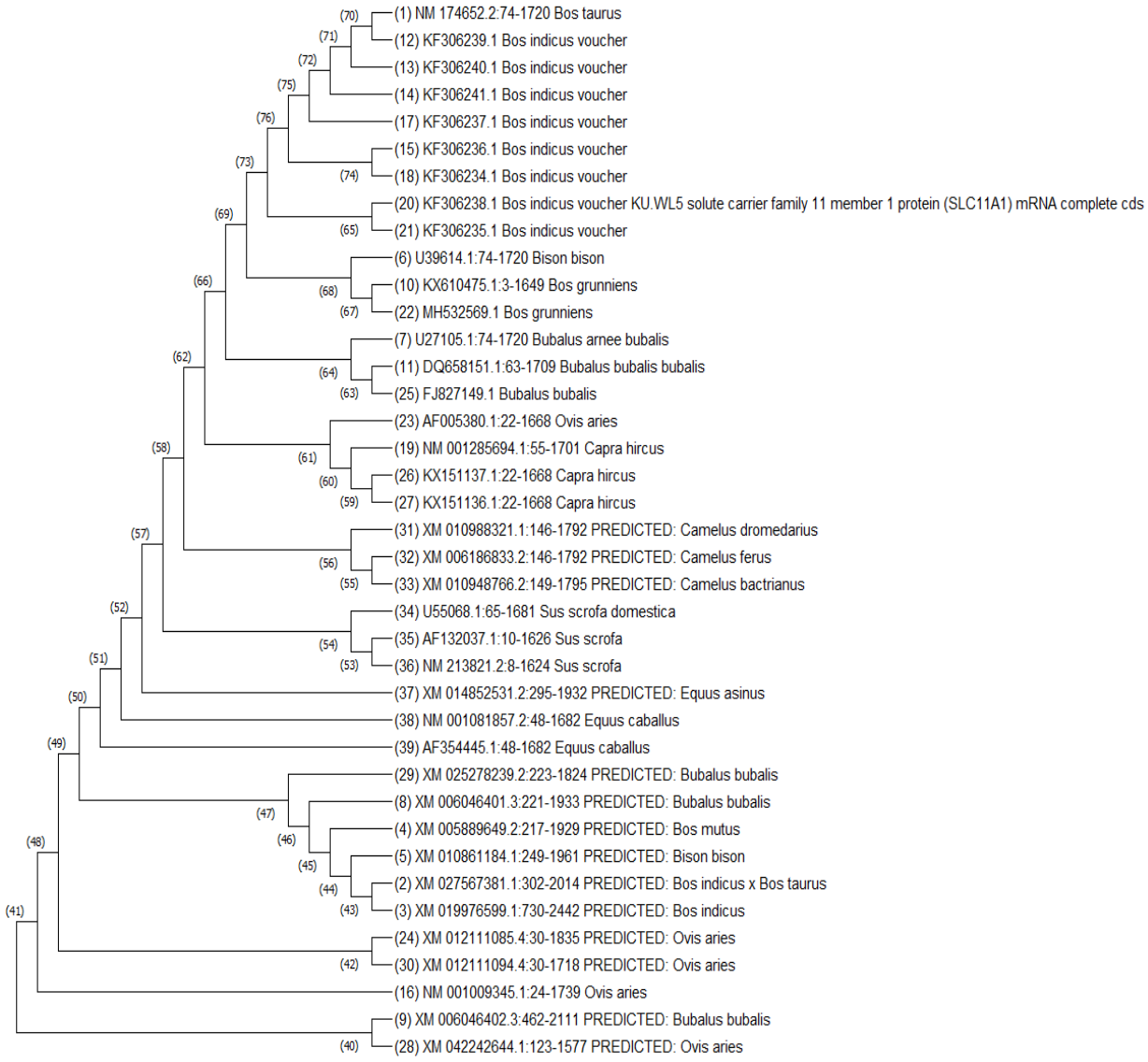

Figure 2: A phylogenetic tree from SLC11a1 gene using UPGMA method.

Conclusion: The present study revealed a great variation and polymorphism in selected sequences of the SLC11a1 gene in domesticated farm animals. Computation analysis of ns-mutations showed one harmful mutation in cattle and twenty-four were beneficial. The results of this study revealed some form of variations between ruminants and non-ruminants. The findings of this study might be used to associate disease resistance, especially tuberculosis in farm animals found in Pakistan.

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