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Sequence characterization, structural analysis, SNP detection and expression profiling of *SLC11A1* gene in Indian goats

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ABSTRACT

The *Solute Carrier Family 11 Member A1* (*SLC11A1*) is a candidate gene, encodes a divalent cation transporter, SLC11A1 protein, localizes in the phagolysosome membrane in macrophages, essential for immunity and resistance to intracellular pathogens. The indigenous goats of Kerala are known for disease resistance and adaptability, but the underlying genetic causes are to be unraveled. This study comprised of characterization of the caprine *SLC11A1* gene, to detect SNPs and compare the relative abundance of *SLC11A1* mRNA by quantitative-PCR in native goats (Attappady Black and Malabari) and crossbreds. The 1702 bp cDNA of *SLC11A1* comprised of 15 exons, an ORF of 1647 bp, encoding a protein of 548 amino acids. *SLC11A1* amino acid sequence was 83–99% identical to that of other species. SLC11A1 peptide contains 10 serine and 2 threonine phosphorylation loci and 12 transmembrane domains. The study indicated the presence of four synonymous and three non-synonymous mutations (p.Ile132Thr; p.Phe294Leu; p.Gln541His). The amino acid substitution p.Ile132Thr was deleterious and p.Phe294Leu and p.Gln541His were neutral. p.Phe294Leu was located in the 7th transmembrane helix. The relative abundance of the *SLC11A1* mRNA in leukocytes in Malabari and Attappady Black were significantly higher than crossbreds ($P < 0.01$). The significantly higher *SLC11A1* expression in native goats indicates higher disease resistance than crossbreds, gives an insight into the necessity of conservation of native breeds, important role of *SLC11A1* in disease resistance. The detected SNPs would benefit the use of this gene as a candidate gene for disease resistance in goat breeding programs.

1. Introduction

Goat farming is operated worldwide, with goat products having a favorable perspective (Moghadaszadeh et al., 2015). The number of goats has increased globally, even in countries with high and intermediate incomes, despite major changes in agriculture due to industrial combination, globalization, and technological improvements in developed countries (Shamsalddini et al., 2016). Goat is mainly used for meat production. It is also used in dairy production, but to less extent. Most of the goats worldwide are found in Asia and Africa (Mohammadabadi and Tohidinejad, 2017). Goat production is one of the key elements contributing to the economy of farmers living in the arid and semi-arid regions. One of the most important purposes of the genetic improvement of this breed is enhancing the meat production via programmed and accurate selection. On the other hand, determination of gene polymorphism is important in farm animal breeding (Ruzina et al., 2010; Mohammadabadi et al., 2010; Soufy et al., 2009) in order to define genotypes of animals and their associations with economic traits.

Disease occurrence is one of the major threats in the livestock farming as it greatly affects the productivity and survivability of animals. The huge variation among individuals to susceptibility/resistance to numerous infections is due to various factors such as nutritional status, co-infections, exposure to environmental microbes, previous vaccinations and inherited genetic factors (Raszek et al., 2016). Immune system protects the host from invading pathogenic microorganism initially by innate immunity and subsequently by acquired immunity. The host genetic factors regulate the efficiency of the immune response to infectious diseases and forms the important determinants of susceptibility/resistance to various infections. The genes involved in the immune response are numerous and diverse in the genome. One of the candidate gene involved in the innate immunity is *SLC11A1* gene, a member of large family of metal ion-transport proteins and it was the first positional cloned gene related to infectious disease susceptibility in mouse (Vidal et al., 1993). The gene encodes a protein, which expresses exclusively in macrophages (Gruenheid et al., 1997).

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The protein has a pH dependent cation transport activity, acting as a transporter of divalent cations like Fe^{2+}, Mn^{2+}, Zn^{2+}, Mg^{2+} and Co^{2+} ions from the lumen of the phagolysosome towards the cytosol, preventing acquisition of iron by intracellular pathogens. Some studies suggest that the movement of cations may actually occur in the opposite direction, resulting in an increased concentration of iron into the phagolysosome, favoring bacterial killing by generating oxygen intermediates through the Fenton Haberson reaction (Goswami et al., 2001 and Wyllie et al., 2002).

Significant progress has been made to delineate the *SLC11A1* association with disease resistance and susceptibility in man, mouse and other animals. The *SLC11A1* gene has been associated with conferring resistance to numerous intracellular pathogens; in mice against *Mycobacterium sp*., *Leishmania* sp. and *Salmonella* sp. (Vidal et al., 1996); in human againt tuberculosis, Crohn's disease, leprosy, sarcoidosis, and rheumatoid arthritis (Bellamy et al., 1998; Searle and Blackwell 1999; Zaahl et al., 2004; Fitness et al., 2004; Hoal et al., 2004; Sechi et al., 2006; Asai et al., 2008; Jin et al., 2009; Hatta et al., 2010); in cattle with brucellosis, paratuberculosis, tuberculosis and clinical mastitis (Adams and Templeton 1998; Pinedo et al., 2009; Ruiz-Larranaga et al., 2010; Kadarmideen et al., 2011; Cheng et al., 2015; Bagheri et al., 2016) in goats with chemical and hygienic characteristics of milk and paratuberculosis (Korou et al., 2010; Piras et al., 2011; Vacca et al., 2011; Abraham et al., 2017). Thus, *SLC11A1* gene is regarded as the potential candidate gene that confers innate resistance to intra cellular pathogens.

The indigenous goat breeds of Kerala, the Southern state of India, are known for their disease resistance, adaptability, prolificacy and meat production. Kerala has two native goat breeds namely, Attappady Black and Malabari. Malabari is a dual purpose goat originated from North Kerala, well known for high prolificacy, milk yield and adaptability to the climatic conditions prevalent in the state. Attappady Black, a meat type breed originated in the hilly terrains of Palakkad district of Kerala, famous for disease resistance and sturdy nature. (Stephen et al., 2005). Malabari crossbreds maintained the in University Goat and Sheep Farm, Mannuthy, Kerala, were produced by crossing Malabari goats with exotic milch breeds, Saanen and Alpine, subsequently with meat breed, Boer. They have resulted from four decades of breeding and selection, and have turned out to be a productive group. Crossbreds although possess high productivity, lacks superiority in other economically important traits like adaptability to hot and humid tropical conditions and resistance to various diseases (Thomas et al., 2011; Abraham et al., 2017). The present investigation was done to characterize and to detect SNPs of the *SLC11A1* gene in native goat breeds of Kerala. In addition, to compare the expression profile of this gene in crossbred goats and native goat breeds, which are more disease resistant than crossbred goats, so as to find out the significant influence of the *SLC11A1* gene on disease resistance.

2. Materials and methods

2.1. Experimental animals and sample collection for RNA and DNA isolation

All experimental procedures were conducted according to the guidelines of the Institutional Animal Ethics Committee of Kerala Veterinary and Animal Sciences University. Adult Malabari (n = 12) Attappady Black (n = 12) and crossbred goats (n = 12) maintained in the University Goat and Sheep Farm, Mannuthy, Kerala, India were included for RNA isolation. All the animals were of 3–4 years of age, maintained in the same farm with similar environment, nutritional status and with normal health conditions. Blood samples (6 ml) were collected from jugular vein using 18 G blood collection needle into BD Vacutainers with spray coated 5.4 mg ethylene diamine tetra acetic acid (EDTA) as anticoagulant under aseptic conditions and stored at 4 °C until RNA isolation. To analyze the allelic variations of the caprine *SLC11A1* gene, six millilitre of venous blood was collected from the jugular veins of a total of 260 goats belonging to the Malabari (n = 100), Attappady Black breeds (n = 100) and crossbred goats (n = 60) maintained at the University Goat and Sheep Farm, Mannuthy, Thrissur district and the Department of Animal Husbandry Goat Farm, Attappady, Palakkad district, Kerala. The genomic DNA was extracted by the standard phenol chloroform method and stored at −20 °C until use.

2.2. RNA isolation and cDNA synthesis

The total RNA was isolated from blood using TRIzol LS reagent (Sigma, USA) according to the manufacturer's instructions. The isolated RNA sample was treated with *DNase* enzyme and concentration, purity and integrity of RNA samples were measured by Nanodrop™ 1000 spectrophotometer (Thermo Scientific, USA) and 1% agarose gel electrophoresis (Supplementary Fig. 1). The total RNA was reverse transcribed using Revert Aid First strand cDNA synthesis kit (Thermo Scientific, USA) following the manufacturer's instructions. The cDNA was synthesized from 500 ng total RNA using random hexamer primers to a final volume of 20 µl. The resultant first strand of cDNA was stored at −80 °C until use.

2.3. Amplification of coding region of SLC11A1 gene

The complete coding region of *SLC11A1* gene was amplified using a pair of *SLC11A1* specific primers (forward 5′ GTGCCCGAGTCTGCAGT CCT 3′ and reverse 5′ GTGCCCGAGTCTGCAGTCCT 3′) based on the mRNA sequence of caprine *SLC11A1* gene obtained from NCBI (GenBank Accession No: NM_001285694.1) using Primer 3 (V.0.4.0). The PCR reaction was carried out in 25 µl mixture containing 10pM of each primer, 200 µM of dNTPs, 1.25U of JumpStart™ AccuTaq™ DNA polymerase (Sigma Aldrich, India) with proof reading activity and 1 µl of cDNA as template. The thermal cycling protocol includes initial denaturation at 94 °C for 7 min; followed by 34 cycles of denaturation at 94 °C for 1 min, annealing at 62 °C for 30 s and extension at 68 °C for 2 min. The final extension was carried out at 68 °C for 7 min. The amplified PCR products were resolved in 2% agarose gels and visualised by gel documentation system (BioRad, USA) after staining with ethidium bromide (Supplementary Fig. 2). The PCR products were gel purified sequenced in an automated sequencer using Sanger's dideoxy chain termination method, at SciGenom Labs Pvt. Ltd., Cochin.

2.4. Sequence analysis

The derived sequence was subjected to BLASTn analysis (www.ncbi.hlm.nih.gov/BLASTn) to retrieve similar sequences of mammalian origin. Multiple sequence alignment of *SLC11A1* was conducted using the Clustal Omega (http://www.ebi.ac.uk/Tools/msa/clustalo/) and DNASTAR Lasergene MegAlign program (DNASTAR CoreSuit 10 software, Inc. USA). The Secondary structure of deduced amino acid sequence was predicted by SOPMA (https://npsa-prabi.ibcp.fr/t). The conserved domain, open reading frame, transmembrane regions and phosphorylation sites were predicted using Conserved Domain Prediction (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), ORF Finder (http://www.ncbi.nlm.nih.gov/gorf/gorf.html), HMMTOP (http://www.enzim.hu/hmmtop/index.php) and NetPhos2.0 Server (http://www.cbs.dtu.dk/services/NetPhos-2.0/). The Provean tool was used to predict the impact of an amino acid substitution (http://provean.jcvi.org/index.php).

2.5. Validation of SNPs by PCR-SSCP

In order to perform PCR-SSCP, five pairs of primers were designed from the caprine *SLC11A1* gene sequence available in GenBank (Accession No: DQ493965.1) using primer 3 software to amplify exons

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